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Unraveling vascular development-related genes in laticifer-containing tissue of rubber tree by high-throughput transcriptome sequencing

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ABSTRACT

About 90% of natural rubber is obtained from tapping of the rubber tree (*Hevea brasiliensis*) for latex, which is circulated in the laticifers. Nowadays, the world supply of natural rubber is not sufficient for global demand, thus the increased latex yield was significantly underlined. In addition, the demand for wood (as a xylem part) from rubber tree was also increased as renewable resource for various applications. Laticifers are found in the secondary phloem containing tissue of rubber tree trunk-inner soft bark. The number of laticifers varies in consistent with latex yield and in responses to jasmonic acid level. This present study was committed to comparative transcriptome analysis in laticifers containing mature phloem, mature xylem and newly developed stem tissues of high latex-yield clone (RRIT251, with more laticifers) and high wood-yield clone (RRIT402, with less laticifers) of rubber tree to classify the genes and pathways involved with phloem (with laticifers) and xylem cell differentiation. There were 49, 54, 46 and 50 of vascular development-related genes in primary and secondary tissues of phloem and xylem, respectively. Differentially expressed genes in jasmonic acid signaling pathway was established with their highest expression in phloem tissue with laticifer cells of RRIT251, while genes in auxin signaling and secondary cell wall biosynthetic pathways were up-regulated in xylem tissue of RRIT402 for high wood yield. Promoter analysis of candidate-differentially expressed genes suggested the related pathway and putative regulatory elements for gene regulation. This genome-wide exploration of vascular development-related genes unraveled a largely unknown gap of this special vascular development containing laticifers in rubber tree.

1. Introduction

Plants contain a distinct feature which continuously generates organs along their entire life cycle. During plant organ development, the controllable of cell proliferation and cell differentiation are coordinately occurred, when cell division rate is reduced, rate of cell differentiation will be increased instead for organogenesis [1]. The initiation of plant organogenesis is the cell division for high number of cells in meristem zone and then, the cell division will be decreased for undifferentiated cell organogenesis process to maintain plant life [2]. Generally, water, ions, and organic solutes are key factors for plant growth and development. Vascular tissues are responsible to transport these factors to other parts of plants. Plant vascular tissues consist of two major cell types, including xylem and phloem. Xylem serves as a death cell which plays role in water transportation from roots to leaves. In addition, xylem is the main source of wood as plant biomass. By contrast, phloem is a living cell which typically displays for sugar transportation from source to sink. These two vascular tissues are differentiated from vascular cambium as a middle ring between xylem and phloem. Although the xylem and phloem are known to be originated from vascular cambium, but the knowledge of gene regulatory system underlying these two tissue-differentiations is not fully elucidated.

Abbreviations: L, High latex-yield clone (RRIT251; MP, Mature phloem; MX, Mature xylem; NS, Newly developed stem; RRIT251, Rubber Research Institute of Thailand 251; RRIT402, Rubber Research Institute of Thailand 402; W, High wood-yield clone RRIT402

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Plant differentiates itself for huge biomass of wood which participates in xylem tissue. The acquired wood is important as fuel energy for human being activities. The wood formation-regulatory molecular mechanism was extensively elucidated. For xylem cell differentiation, secondary cell wall biosynthesis and programmed cell death were established, respectively. The molecular mechanism exploration of xylem cell differentiation revealed that VASCULAR-RELATED NAC-DOMAIN6 (VND6) and VND7 as NAC domain transcription factors were identified as transcriptional regulator to alternate between metaxylem and protoxylem cell differentiation [3]. MYB46 and MYB83 as downstream regulator of NAC domain transcription factors were characterized as second layer regulators which directly controlled secondary biosynthetic genes [4]. For intensive elucidation, the downstream gene groups of these regulators were associated with secondary cell wall synthetic and programmed cell death pathways. Secondary cell wall of plant is deposited along the xylem vessel to strengthen plant structure during plant primary growth. Cellulose is one of major composition of secondary cell wall which is formed in the hemicellulose and lignin matrix. *CELLULOSE SYNTHASE4/IRREGULAR XYLEM5* (*CESA4/IRX5*), *CESA7/IRX3*, and *CESA8/IRX1* were identified as cellulose biosynthetic genes. On the contrary, *IRX7, IRX8, IRX9*, *IRX10, IRX14* and *IRX15* were responsible for hemicellulose biosynthesis. While, *IRX4* and *IRX12* were characterized in lignin biosynthesis [5–13]. At the end, programmed cell death genes, including *XYLEM CYSTEINE PEPTIDASE1* (*XCP1*) [14] and *METACASPASE9* (*MC9*) [15] were up-regulated for xylem hollow tube formation. Current research emphasized some distinctive points about the auxin responses which concerned to xylem cell differentiation in *Arabidopsis*. For example, ARF5/MONOPTEROS, an auxin-dependent transcription factor, involved directly in attenuating the activity of *WOX4* to stop the stem cell number. Moreover, upon the ARF5 induction, the cell proliferation was decreased, while undifferentiated cells were differentiated to xylem cells in higher ratio than phloem cells. Thus, ARF5 played a dominant role in the transition stage from undifferentiated cell to xylem cell differentiation [16].

Para rubber tree (*Hevea brasiliensis* Muell. Arg.) is the main source of natural rubber which almost utilized for various commercial rubber products. Rubber tree was in *Hevea* genus of *Euphorbiaceae* family. Actually, the origin of rubber tree was firstly found in Amazon rainforests and it is normally cultivated in tropical areas e.g. Southeast of Asia. Natural rubber is the synthesized polymers from various plants and one type of natural rubber is latex which consists of *cis*-1,4-polyisoprene, protein and fatty acid [17]. This specific polymer component provides the flexibility, impermeability and abrasion resistance properties in latex for commercially applicable products [18]. In general, 90% of natural rubber in the world-wide market comes from latex of rubber tree, although the demand of natural rubber has also increased in every year for over 4000 industrial products [19]. Although, the area of rubber tree plantation has gradually increased every year and consequently leading to the higher volume of latex yield to support the global demand, the latex is still not enough to supply the global market. Therefore, extensive studies in molecular latex biosynthesis and its related mechanism are required to pave the way for high latex yield improvement. In rubber tree genome, the diploid chromosome (2n = 36) was identified with 1.47 Gb genome, RRIM600 [20,21], Reyan7-33-97 [22], and BPM24 [23]. Recently, the high-quality genome assembly of *Eucommia ulmoides*, hardy rubber tree, which is an important producer of Eucommia rubber (*trans*-polyisoprene) was performed, and genomic and the gene differences in rubber synthesis were identified and compared with *H. brasiliensis*. There were the differences in the genes responsible for the synthesis of polyisoprene in the format of *cis*-polyisoprene (*H. brasiliensis*) and *trans*-polyisoprene (*E. ulmoides*) suggesting that the polyisoprene biosynthesis has evolved independently between these two rubber trees [24].

Latex production is localized in specific cells of rubber tree called laticifer cells, which were located and differentiated from vascular cambium along with secondary phloem in soft bark area of rubber tree stem. For high latex yield, three main key factors composed of number of laticifer cells in bark, the prolonged time of latex flow after tapping, and the regenerated capacity of latex between tapping were underlined [25,26]. Our research focused on the increasing number of laticifer cells in rubber tree. Previously, exogenous jasmonic acid (JA) was demonstrated as the laticifer cell enhancer which could induce high number of secondary laticifer cells in secondary phloem of rubber tree [27,28]. In JA-signaling pathway, upon JA induction, JAZ was targeted to SCF^{COI1} complex for 26S proteasome degradation [29], and led MYC to bind with JA-responsive gene target for transcriptional enhancement [30]. Later research explored into JA-responsive genes, *COI1, MYB, ARF8* and *HB8,* and provided their expression profiles correlated with more laticifer differentiation upon the methyl-jasmonate treatment [28]. In the meantime, transcriptome analysis in coronatine (as an active jasmonate mimic) treated-rubber tree revealed the induction of secondary laticifer cell differentiation involving in Ca^{2+} signal transduction and CLAVATA-MAPK-WOX signaling pathway as a downstream of MYC in JA-signaling pathways [31]. Normally, CLAVA (CLV) signaling pathway was established in controlling balance of cell proliferation and cell differentiation in plants [32,33], and this signaling pathway manipulated MAPK signaling pathway as downstream gene family [34,35]. During the up-regulated genes in CLV and MAPK signaling pathway, *WOX* was down-regulated to inhibit the cell proliferation and provided laticifer cell differentiation instead in vascular cambia tissue sample upon coronatine treatment [31]. For another independent Ca^{2+} signaling pathway, *CDPK1* as Ca^{2+} signal transduction gene was characterized in development process of *Medicago truncatula* root [36]. In addition, from suppression subtractive hybridization (SSH) and transcriptome sequencing information, *CDPK1* was up-regulated in vascular cambia-containing tissue sample of rubber tree during the coronatine induction, which was supposed to have some roles in secondary laticifer cell differentiation in rubber tree [31,37].

In this study, two different clones with distinct characteristics of rubber tree recommended from Rubber Research Institute of Thailand were used; RRIT251 (selected from illegitimate clonal seed of RRIT, recommended clone for high latex yield with 2,140-2,980 kg/hectare/year) with more laticifers and RRIT402 (Chachoengsao50, selected from illegitimate clonal seed of RRIC110, recommended clone for high wood yield with 113 m^3/hectare of 9-year-old tree) with less laticifers. Transcriptomic analysis of three different tissues, including mature phloem, mature xylem and newly developed stem, of two rubber clones were investigated by RNA-sequencing. Taken together, vascular-development-related genes were acquired and divided into their related tissue groups; primary and secondary phloem and xylem. The differentially expressed genes with their involved pathways had relevance to each distinctive characteristic of rubber clone. The molecular regulatory system of phloem (with laticifers) and xylem cell differentiation processes in rubber tree was demonstrated to fulfill a gap knowledge and may possibly trigger an improvement of rubber tree breeding program for high latex-yield or high wood-yield requirement.

2. Materials and methods

2.1. Plant materials

The high latex/low wood-yield clone, RRIT251 (L) and low latex/high wood-yield clone, RRIT402 (W) were selected for this study. Each rubber tree clone was at the age of 15-year-old and grown at the Rubber Research Center at Chachoengsao Province. In each clone, three mature branches from three trees were used in the experiments to collect RNA samples. Each mature branch contained newly developed stems. Each branch was separated into three main tissues; mature phloem (MP), mature xylem (MX) and newly developed stem (NS). Mature phloem, mature xylem and newly developed stem samples from each branch of each rubber clone were extracted for total RNA to be used in this study.

2.2. Anatomical study of vascular components in mature branch and newly developed stem of rubber tree under microscope

Mature branch samples were cross section-cut by the sharp blade for investigation of their vascular components, consisting of phloem, cambium and xylem under stereo microscope. Newly developed stem at the EU1 position was cross section-cut into 5 mm long and soaked into FAA (FORMALIN-ACETO-ALCOHOL) solution for 48 h at room temperature. Then, the soaked samples were washed in glacial acetic acid, treated with iodine and bromine in glacial acetic acid, and followed with paraffin embedding protocol [28]. The samples were fixed in paraffin and were cut by microtome (Leica Microsystem Inc., Bannockburn, IL, Germany) into 18 μm thick sections. The sample sections were observed under light microscope to notify the laticifers with brown color of latex from iodine-bromine staining.

2.3. Total RNA extraction, mRNA enrichment and purification

Mature phloem, mature xylem and newly developed stem samples from each rubber clone were extracted for total RNA by modified CTAB-LiCl TRIzol method. Each sample was ground in liquid nitrogen. Then, the 65°C-prewarmed extraction buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA pH 8.0, 100 mM Tris-HCl pH8.0, 2% β-mercaptoethanol) and 20% SDS were added and incubated at 65°C for 10 min. The crude extract was extracted by equal volume of chloroform:isoamyl (24:1) for three times and supernatant was separated to be precipitated. The pellet was washed by cold isopropanol alcohol and cold 70% ethanol, respectively. For selective RNA precipitation, 1/3 vol of 8 M LiCl were added to dissolved solution and stored at 4°C for overnight. The crude pellet was dissolved in TRIzol reagent (Invitrogen, USA) and subsequently mixed with chloroform for cleaning up. The attained supernatant was re-precipitated by cold isopropanol alcohol, followed by washing up pellet with cold 70% ethanol. Eventually, total RNA sample was dissolved in 25–40 μl DEPC water. Total RNA quality and quantity were measured by NanoDrop One Microvolume UV–vis spectrophotometer. For RNA-seq, at least 75 μg of total RNA and 1.8–2.0 of absorbance 260/280 ratio for high quality of total RNA sample were required.

Poly (A) mRNA was isolated from total RNA by using Dynabeads™ mRNA Purification Kit (Thermo Fisher Scientific Baltics UAB, Lithuania). Firstly, 75 μg of total RNA was adjusted to final volume 100 μl by DEPC water. Then, Dynabeads Oligo $(dT)_{25}$ was added to 100 μl of total RNA sample for Poly (A) mRNA purification. For elution step, the isolated mRNA was eluted by 20 μl of 10 mM Tris HCl pH 7.5. Fragment Analyzer (Advanced Analytical Technologues, Inc., USA) was committed to quantify the concentration and quality of isolated mRNA by DNF-472M33 kit (HS mRNA 15 nt). According to this kit, mRNA was diluted to 2500 pg/μl and two μl of diluted of mRNA was used to measured. For fragmented mRNA purification, Magnetic Bead cleanup Module (Thermo Fisher Scientific, USA) was performed. Finally, purified mRNA was eluted by 5 μl of pre-heated nuclease-free water.

2.4. RNA library construction

RNA libraries were constructed from the purified mRNA (5 μl) using Ion Total RNA-Seq kit v2 protocol (Life Technologies Corporation, USA) by ligating mRNA with Ion Adapter Mix v2. Ligated mixture was incubated at 30 °C for 1 h on Veriti 96 well Thermal Cycler (Applied Biosystems, USA.). Next, cDNA was synthesized by SuperScript® III Enzyme Mix along with 42 °C for 30 min incubation. Then, magnetic bead cleanup module was utilized for cDNA purification. After that, purified cDNA was eluted by 6 μl of pre-heated nuclease-free water.

In cDNA amplification process, barcodes were mix into 6 μl of purified cDNA samples. The barcoded cDNA libraries were constructed by using Platinum® PCR SuperMix High Fidelity, Ion Express™ RNA 3' Barcode primer and Ion Express™ RNA-Seq Barcode BC primer. The BC primers included BC1-BC9 for RRIT251 samples and BC9, BC11-B14 and BC16 for RRIT402 samples. For cDNA amplification, the thermal cycler was set as the following; at first, the mixture reaction was incubated at 94°C for 2 min. Next, two cycles were committed as followed; 94°C for 30 s, 50°C for 30 s and 68°C for 30 s. After that 14 cycles were amplified at 94°C 30 s, 62°C for 30 s and 68°C for 30 s. At the end, the reaction was hold at 68°C for 5 min. After cDNA amplification, the amplified cDNA was cleanup by magnetic bead cleanup module. Then, 15 μl of 37°C pre-heated nuclease-free water was used to elute the purified cDNA.

For amplified cDNA quality and concentration determination, Fragment Analyzer (Advanced Analytical Technologies, USA) was utilized to measure by using DNF-474-33 kit (HS NGS fragment 1–6,000 bp). To measure, cDNA library was diluted to be 2500 pg/μl. Finally, two μl of diluted cDNA was conducted for measurement.

2.5. Ion-torrent sequencing and differentially expressed genes analysis

Throughout this experiment, Ion proton sequencing was performed based on the Ion 540™ Kit-OT2 instruction. Firstly, each cDNA library with barcode was diluted to 100 pM. All libraries were pooled together. Subsequently, six μl of mixed sample was assigned as a template. For template enrichment, pooled cDNA with template-positive Ion 540™ ion sphere particles (ISPs) were used as described in Ion 540™ Kit-OT2 kit. Then, the mixture reaction was explored by Ion One Touch™2 instrument for amplified enrichment. Eventually, the enriched template was loaded to Ion 540™ Chip and performed the sequencing by Ion S5™ XL semiconductor sequencer.

After sequencing by ion torrent (Ion 540™), the initial data of Ion Sphere Particle (ISP), total bases and total reads were generated. Then, the total reads were automatically filtered polyclonal, low quality and adaptor dimer out by internal Ion Torrent™Suite Software version 5.4.0 and obtained usable reads with their mean, median and mode of read length. Finally, the usable reads were blasted to SILVA (release128) [38] and Rfam (version 14.0) [39] database as rRNA database with criteria of e-value = 10^{-3} to remove rRNA reads out.

Attained reads were mapped to available rubber tree genome database (https://www.ncbi.nlm.nih.gov/assembly/GCF_001654055.1) by STAR version 020201 program with default parameter. Then, mapped reads in gene were counted by HT-seq count version 0.9.1 with union option. Obtained read count was normalized by R package DEseq2 with default parameter. Finally, the differentially expressed genes were determined by the log2 fold-change calculation between tissue and rubber clone comparison with set point criteria p-value < 0.05. The raw transcriptome reads and processed transcript data were submitted to NCBI/SRA database, available at https://www.ncbi.nlm.nih.gov/sra/PRJNA549803, and all identified differentially expressed genes in three different vascular-containing tissues of two different rubber tree clones are available at https://doi.org/10.7910/DVN/EO3QSX.

2.6. Coexpression network analysis of seven candidate genes

Coexpression relationships of seven candidate genes (*MPKKK5, MPKK9, CDPK1, TPR3, ARF5, WOX13* and *MPK3*) were investigated from PlaNet (http://aranet.mpimp-golm.mpg.de/index.html) [40] with default parameters. Since the plant organisms were limited in this program, thus coding sequences of candidate genes in *Arabidopsis thaliana* as plant model were submitted instead to represent the rubber tree candidate gene coexpression network.

2.7. Promoter analysis of seven candidate genes

The 3000 bp upstream promoter regions of *MPKKK5, MPKK9, CDPK1, TPR3, ARF5, WOX13* and *MPK3* genes were retrieved from whole-genome shortgun contigs of *Hevea brasiliensis* of BPM24 rubber

clone from NCBI database [20] (Supplement Table S1). Then, promoter region of each gene was analyzed by PlantPan2.0 (http://plantpan2.itps.ncku.edu.tw/promoter.php) [41] to investigate vascular development-related *cis*-acting elements.

2.8. Validation of candidate genes by quantitative real-time RT-PCR

To validate differentially expressed genes from RNA-sequencing results, seven vascular-development-related candidate genes were performed by qRT-PCR. Total RNAs were extracted using CTAB-LiCl with TRIzol reagent method and converted to cDNA by Super-scriptIII Reverse Transcriptase (Invitrogen, USA) with oligo (dT) primers. The primers for *MPKKK5, MPKK9, MPK3, CDPK1, TPR3, ARF5* and *WOX13* were designed (Supplement Table S2) and validated for specific-gene amplification by RT-PCR. Quantitative real-time PCR was performed on the CFX96 Touch™ Real-Time PCR Detection System (Applied Biosystems, USA) using iTaq™ Universal SYBR® Green Supermix (Bio-Rad, UK). Each gene was explored by nine replicates in total volume of 20 µl PCR system containing 1X SYBR master mix, 100 mM forward and reverse primers, and 2 µl of the 1:2 diluted cDNA. The PCR thermal cycler conditions were included 95°C for 30 s, followed by 40 cycles of 95°C for 15 s, 57°C for 1 min and 72°C for 30 s. At the end, final extension was held on 72°C for 7 min. The relative gene expression levels were calculated by $2^{-\Delta Ct}$ method using *actin* gene as internal control. Significant difference of qRT-PCR results among tissues and two rubber tree clone samples were analyzed by one-way ANOVA (Tukey HSD's compare means) with P-value < 0.05. Throughout statistical analysis was conducted by SPSS (v.18.0.0) software.

3. Results

3.1. Confirmation of xylem and phloem containing tissue sample separation

Mature branches were collected from RRIT251 (high-latex, low-wood-yield clone) and RRIT402 (low-latex and high-wood-yield clone). Each mature branch was separated into two main vascular tissues; phloem and xylem, and the newly developed stems of each branch clone were also collected (Supplement Fig. S1a, S1b and S1c). The mature branch cross sections were observed under stereo microscope to indicate parts of each vascular containing tissue (Fig. 1a). This histological analysis confirmed that each xylem and phloem tissue was correctly separated. From this observation, samples from RRIT251 contained higher number of laticifer cells (132 ± 4 laticifer cells/ 4 mm^2) than those from RRIT402 (70 ± 3 laticifer cells/ 4 mm^2) (Fig. 1b and c). This observation was correlated with each rubber clone characteristic in which RRIT251 provided more latex yield than RRIT402.

To verify the perfect tissue separation of phloem and xylem, RT-PCR by tissue-specific marker genes, such as *HRT* as a specific marker gene for latex (in phloem tissue), *IRX1* as a specific marker for xylem tissue and *actin* as a house keeping gene in both tissues was conducted. The presence of specific transcripts in each specific tissue, *HRT* can only be amplified in phloem tissue while *IRX1* can only be amplified in xylem tissue, confirmed the completed separation between phloem and xylem tissue (Supplement Fig. S2). Thus, sampling phloem and xylem tissues were accurately and completely performed.

3.2. Comparative RNA-seq analysis

In this experiment, nine and six barcoded cDNA libraries from three different tissue RNA samples of RRIT251 and RRIT402 were committed to Ion-torrent sequencing technology, respectively, which were separated into two runs. For first run, the total bases were around 12.5 G with Ion Sphere Particle (ISP) loading 91%. Total reads were 138,122,376 reads. After polyclonal, low quality and adapter dimer filtration, usable reads were remained 91,731,940 reads or around 67%. Mean, median and mode of acquired read length were 136 bp, 162 bp and 164 bp, respectively. For second run, total bases were 11.9 G with ISP loading 84%. The total reads were 127,967,454 reads. The usable reads after filtration were 88,176,581 reads or about 69.4%. And mean, median and mode of read length are 135 bp, 162 bp and 164 bp, respectively. Then, usable reads in each tissue samples were mapped to rRNA database and the mapped rRNAs reads were removed away from usable reads (Supplement Table S4 and S5).

In RRIT251, remained read range of LMP, LMX and LNS libraries was from 601,556 to 5,968,134 reads (Supplement Table S4), while in RRIT402 remained read range was from 1,031,310 to 6,906,039 reads in WMP, WMX and WNS libraries (Supplement Table S5). Obtained reads were mapped to rubber tree genome database [22] and provided percentage of mapping reads in range from 72% to 95% and from 89% to 94% in RRIT251 and RRIT402 samples, respectively. After obtaining mapped reads, the mapped reads were performed the read count. The read count result in each sample was analyzed the differentially expressed genes. In this analysis, Log2FoldChange method was used to determine the differentially expressed genes in each compared condition (Supplemental Table S1 and S2). After differential gene expression analysis in each compared samples, the number of differentially expressed genes (DGEs) were shown as up-regulated and down-regulated genes (Fig. 2). In different tissues of the RRIT251; LMP vs LMX, LMP vs LNS and LMX vs LNS, the number of up and down-regulated genes were 227 vs 204, 399 vs 527 and 102 vs 444, respectively. In different tissues of the RRIT402; WMP vs WMX, WMP vs WNS and WMX vs WNS, the number of up and down-regulated genes were 58 vs 183, 94 vs 601 and 120 vs 452, respectively. In different clones of the same tissue type; LMP vs WMP, LMX vs WMX and LNS vs WNS, the number of up and down-regulated genes were 261 vs 108, 122 vs 260 and 72 vs 79, respectively. After obtaining list of DEGs in each comparable table, these DEGs list were clustered by gene cluster 3.0 software with centroid linkage clustering method. The attained clustering results were performed in graphical heat map by javatreeview (Supplement Fig. S8).

From Log2FoldChange data of each pair of tissue comparison, all highly expressed genes and related genes were classified into their vascular-containing tissue groups, including primary xylem (1X, highly expressed in NS > MX), secondary xylem (2X, highly expressed in MX), primary phloem (1 P, highly expressed in NS > MP), and secondary phloem (2 P, highly expressed in MP), and demonstrated in Venn-Euler diagram (Fig. 3 and Supplement Table S6). There are 421, 514, 309, and 430 of highly expressed genes found only in primary and secondary phloem followed by primary and secondary xylem, respectively. As in this study we aimed to identify tissue specific vascular development-related genes, thus various vascular development-related genes from plants have been retrieved from a number of review literatures [12,31,42–54]. All retrieved vascular development-related genes were used to analyze together with the RNA-sequencing data, which were divided into four main groups based on four different tissues; primary xylem (1X), secondary xylem (2X), primary phloem (1 P), and secondary phloem (2 P) (Fig. 3 in bracket and Supplement Table S7). From Venn–Euler diagram, there were 49, 54, 46, and 50 of highly expressed vascular development-related genes found only in primary and secondary phloem followed by primary and secondary xylem, respectively.

Taken together, the differently expressed genes in each tissue were analyzed and defined their gene product functions by Blast2GO [55]. The enrichment of each annotated function was demonstrated in the bar chart of cellular component function (Supplement Fig. S3), molecular function (Supplement Fig. S3.1) and biological process function (Supplement Fig. S3.2) of highly expressed genes in each different tissue. The obtained bar chart contained the annotated gene functions in phloem and xylem differentiation. According to the biological process enrichment, some xylem-related genes revealed the function in cellulose biosynthetic process, cell wall organization, cell wall biogenesis, regulation of cellulose biosynthetic process, plant-type primary and secondary cell wall biogenesis and xylan biosynthetic process

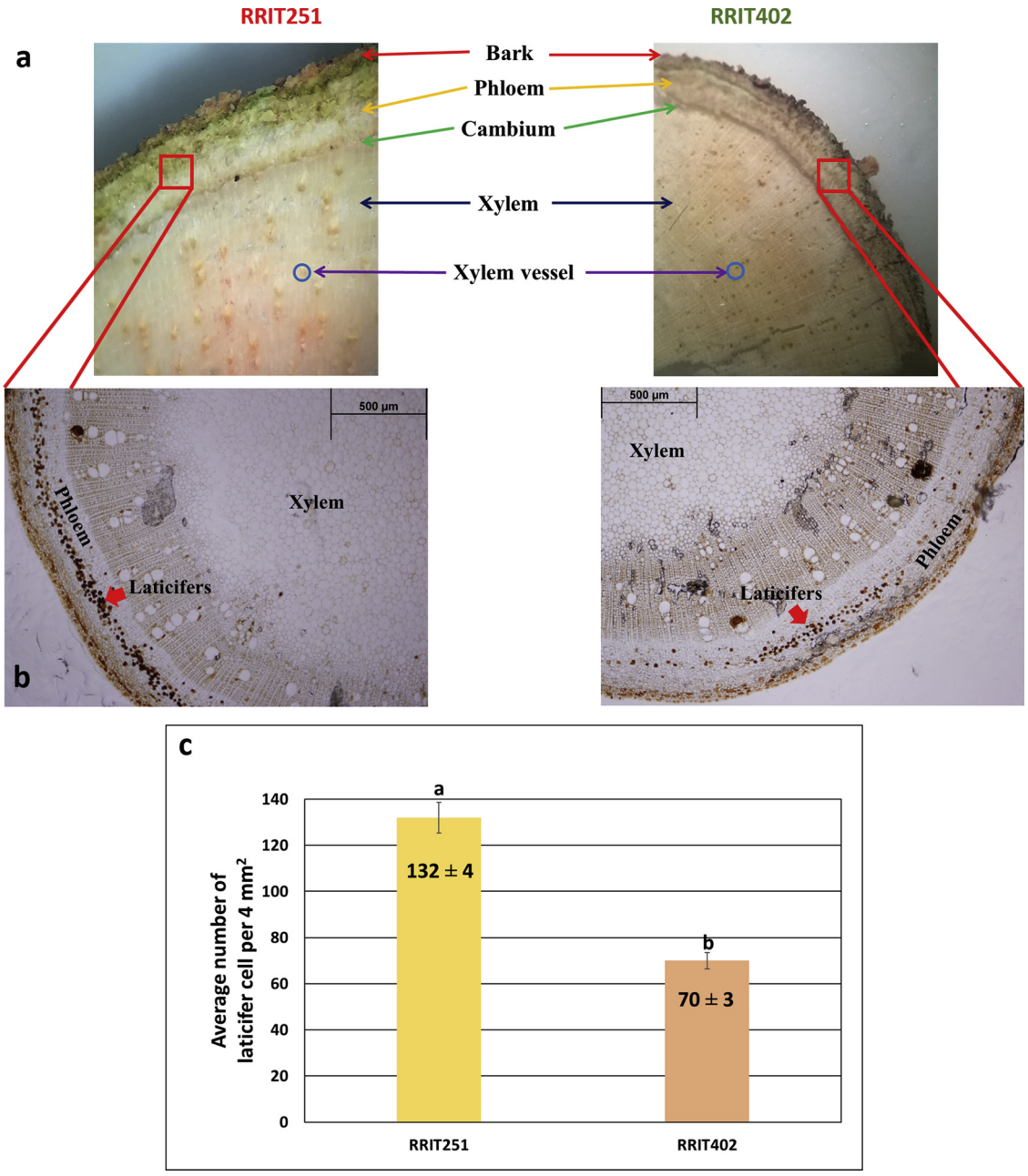

Fig. 1. Cross section of mature branch and newly developed stem at extension unit 1 (EU1) position of two different rubber tree clones. (a) Histological analysis of RRIT251 (high latex-yield clone) and RRIT402 (high wood-yield clone) mature branches under stereo microscope. These cross sections of mature branches showed each part of vascular containing tissues, arrow indicating bark (red), phloem (yellow), cambium (green), xylem (dark blue), and xylem vessel (purple and blue circle). (b) Cross section of newly developed stems and their vascular components and laticifer cells (red arrow) of two rubber clones under microscope. (c) The average number of laticifer cells per 4 mm^2 of newly developed stem at EU1 of two rubber clones. Twenty observed fields were randomly visualized and counted for number of laticifer cells under microscope. Statistical analysis using one-way ANOVA performed the significant differences shown in different letters (a, b) with p value < 0.05.

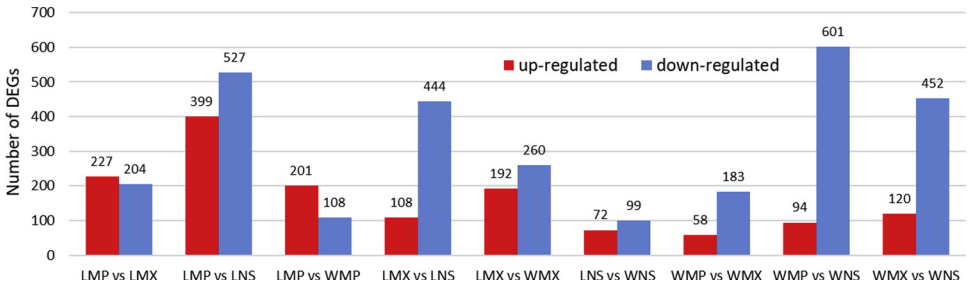

Fig. 2. Number of up and down-regulated genes of each compared couple samples. Red and blue bars are the number of up-regulated genes and down-regulated genes of each couple, respectively. L, High latex-yield clone (RRIT251); W, High wood-yield clone (RRIT402); MP, Mature phloem; MX, Mature xylem; and NS, Newly developed stem.

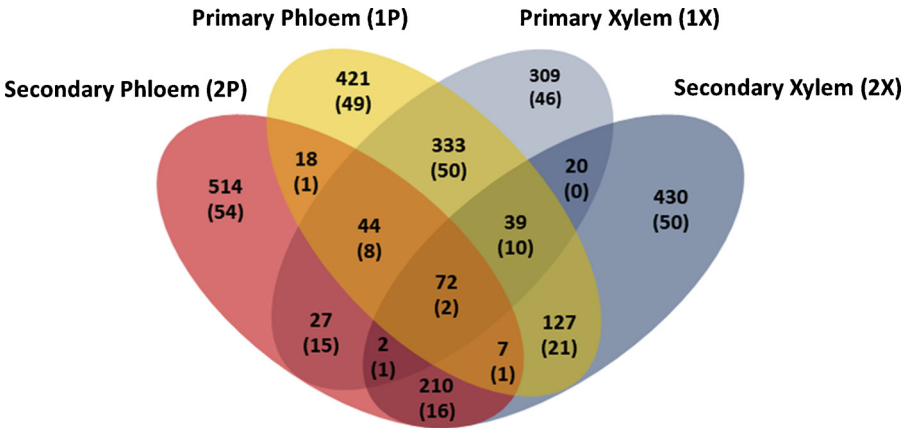

Fig. 3. Venn-Euler diagram of all unigenes in each vascular-containing tissue sample obtained from log2-fold change relative expression in each pair tissue comparison. From all obtained unigenes, the log2-fold change was used to analyze the relative expression in each pair tissue. From log2-fold change information of each pair tissue, each highly expressed and related unigene was categorized into each vascular tissue-containing group, including primary/secondary phloem and primary/secondary xylem (Supplement Table S6). The number of vascular-development related genes based on review literatures which were selected from highly expressed genes in each vascular tissue-containing group was in bracket (Supplement Table S7).

which mainly associated with cell wall biosynthesis. In addition, some genes were established in auxin-related processes, including auxin-activated signaling pathway, auxin polar transport, auxin homeostasis, auxin efflux and positive regulation of auxin metabolic process (Supplement Fig. S3). While, some phloem-related genes were enriched in protein modification processes, including protein stabilization, phosphorylation, O-linked mannosylation, glycosylation, dephosphorylation and autophosphorylation, in various transport processes, including, transmembrane transport, ion transmembrane transport, carbohydrate transport, calcium ion transmembrane transport, borate transmembrane transport, protein transport, maltose transport and sucrose transport, in ATP-related processes, including electron transport chain, ATP metabolic process, ATP hydrolysis coupled proton transport, ATP hydrolysis coupled cation and anion transmembrane transport, in growth and development processes, including gynoecium development, flower development, embryo development, cell division, cell differentiation, positive regulation of organ growth, pollen development, uni- and multi-dimensional cell growth, regulation of shoot system development and regulation of mitotic cell cycle, and in kinase activity, including activation of MAPK activity, MAPK cascade and activation of protein kinase activity (Supplement Fig. S3). As phloem tissue contains laticifer cells which produce latex, some phloem-related genes were also found in isopentenyl diphosphate biosynthetic process as a latex biosynthetic process (Supplement Fig. S3).

3.3. Validation of candidate vascular development-related genes in three different tissues from two different rubber clones by quantitative real-time PCR

In this experiment, the key vascular development-related genes in each vascular containing tissue were targeted, thus the phloem (with laticifer cells)- and xylem-related genes were characterized. Previously, MAPK signaling related genes, Ca^{2+} signal transduction genes and WOX13 were identified as key pathways in phloem with laticifer cell differentiation upon coronatine treatment [31]. Thus, *MPKKK5, MPKK9,* MPK3, *CDPK1* and WOX13 were selected as candidate genes in phloem with laticifer cell differentiation in rubber tree. While, TPR served as corepressor which could directly or indirectly repress transcription process [56] and [57]. Furthermore, WOX was reported that could be interacted with TPR to maintain stem cell division [56]. Accordingly, TPR3 and WOX13 were selected as candidate genes in cell division in rubber tree. In addition, recently, ARF5 as auxin signaling related gene was mainly established in xylem cell differentiation in *Arabidopsis* [16]. Thus, ARF5 was selected as a candidate gene in xylem cell differentiation in rubber tree. From RNA-sequencing result, seven candidate vascular development-related genes, including *MPKKK5, MPKK9,* MPK3, *CDPK1*, TPR3, ARF5 and WOX13 were selected based on their high expression in phloem and xylem tissues. The coding sequences of each candidate gene were obtained from the acquired accession number in RNA-sequencing result (Supplement Table S8). For expression profile of seven vascular development-related genes, the qRT-PCR with specific candidate primers was performed in three different tissues, including xylem, phloem and newly developed stem of

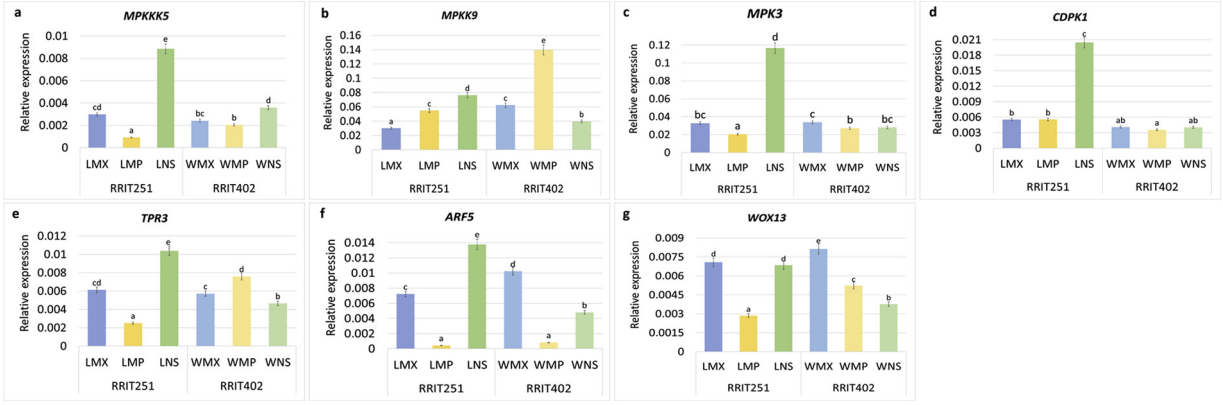

Fig. 4. The relative expression of seven candidate genes in three different vascular-containing tissues of two different rubber tree clones. Two different clones, including RRIT251 (L) and RRIT402 (W) were used to perform the qRT-PCR in three different tissues; mature xylem (MX), mature phloem (MP), and newly developed stem (NS). (a) *MPKKK5*, (b) *MPKK9*, (c) *MPK3*, (d) *CDPK1*, (e) *TPR3*, (f) *ARF5*, and (g) *WOX13.* For relative expression, $2^{-\Delta Ct}$ method was used to calculate and house-keeping gene, *actin*, was used as reference gene to normalize the expression. Significant difference was analyzed by one-way ANOVA (Tukey HSD's compare means) with P-value < 0.05.

RRIT251 and RRIT402 rubber clones. *Actin* was used as a reference gene for relative candidate gene expression profile investigation.

MPKKK5 showed highest expression in LNS of RRIT251 and about 3- and 9-fold higher than LMX and LMP, respectively (Fig. 4a). This observation was consistent with Log2-fold change RNA-sequencing result. Moreover, the relative expression of this gene in WNS of RRIT402 was less than one third when compared to LNS of RRIT251. Apparently, the relative expression of this gene in LMP from RRIT251 was the lowest as compared with other samples. *MPKK9* gene was higher expressed in LMP of RRIT251 than LMX (Fig. 4b). This relative expression result was consistent with the RNA-sequencing data. In RRIT402 clone, this gene remarkably highly expressed in WMP as compared to WMX and WNS. *MPK3* relative gene expression showed the highest expression in LNS of RRIT251 compared with LMX and LMP (Fig. 4c). This result was similar to the RNA-sequencing result. A significant different higher expression of this gene in xylem than phloem tissues was observed in both rubber clones. Apart from MAPK signaling genes, *CDPK1,* as Ca^{2+} signaling gene, provided the higher expression in LMP of RRT251 compared with WMP of RRIT402, which was consistent with RNA sequencing data (Fig. 4d). Noticeably, this gene was highest expressed in LNS of RRIT251. In addition, the overall relative expression of RRIT251 samples was higher than RRIT402 samples when compared between same tissues with different rubber clones. However, *TPR3* served as a negative co-expressor gene which almost involved with various development processes. The relative gene expression of *TPR3* in WMP of RRIT402 was about 3-fold higher than LMP of RRIT251 (Fig. 4e). This qRT-PCR result was similar to the RNA-sequencing result. The highest relative expression of this gene was considerable in LNS of RRIT251, while the lowest expression was apparent in LMP. On the contrary, *ARF5* and *WOX13* were defined in auxin signaling pathway. According to the RNA-sequencing data, *ARF5* gene was highly expressed in WMX of RRIT402 and was also higher than WMP. The qRT-PCR result was also consistent with RNA-sequencing result (Fig. 4f). Interestingly, in both rubber clones, this gene was higher expressed in xylem than phloem tissues. The significantly highest expression was shown in LNS of RRIT251. The relative expression of *WOX13* gene in WMX of RRIT402 was higher than WMP and WNS (Fig. 4g). This finding was also consistent with RNA-sequencing result. It was undeniable that in RRIT251 clone, this gene was highly and equally expressed in LMX and LNS. In RRIT402, this gene was higher expressed in phloem and xylem than in RRIT251. Likewise, WMP of RRIT402 provided the higher gene expression than LMP of RRIT251.

3.4. Prediction of possible regulatory motifs in promoter region of candidate genes

The analysis of regulatory motifs in gene promoter was subjected to gene regulation and gene predicted function in particular tissues or under various stress conditions [58]. The vascular development-related genes were focused in this study. To predict the gene regulation and function of candidate genes, the promoter analysis was used to determine the vascular development-related regulatory elements. The results revealed overall 192 *cis*-regulatory elements, which could be divided into 9 groups based on their functions (Fig. 5a), including defense response, hormone response (stress and growth and development), growth and development, cell division, sugar response, drought stress, light response, transcriptional regulation, and others. For vascular development-related *cis*-regulatory elements, the hormone response (related to growth and development), growth and development, cell division, and transcriptional regulation were focused. The putative vascular development-related regulatory motifs on the promoter regions of all candidate genes from the genome sequence of BPM24 rubber clone were revealed in Supplement Table S1. There are total 48 vascular development-related motifs on seven promoters of candidate genes. From promoter analysis results, all candidate genes contained putative vascular development-related motifs on their promoter region, although the number of putative vascular development-related motifs on promoter region of each candidate genes was varied. There are 156, 110, 134, 102, 121, 92, and 158 vascular development-related motifs on *MPKKK5, MPKK9, CDPK1, TPR3, ARF5, WOX13* and *MPK3* promoter regions, respectively (Fig. 5b). These candidate genes may have some functions associated with vascular and development in rubber tree.

According to number of vascular development-related motif on each candidate gene promoter (Supplement Table S9), G-box variants as MYC2 binding site were notably found to distribute in each candidate gene promoter, suggesting MYC2 as a master regulator in JA-signaling pathway which may bind to these candidate JA-responsive gene promoters for plant growth and development. In addition, coexpression network analysis of these candidate genes in *Arabidopsis* also provided the co-occurrence genes in various pathways, for example defense response, hormone signaling pathway (JA, abscisic acid, salicylic acid, auxin and ethylene), isopentenyl diphosphate biosynthetic process, isoprenoid biosynthesis pathway, defense response, cell growth and development, cell wall metabolic process, cell death regulation, ion transport, oxidative stress, and others (Supplement Fig. S4). Interestingly, *MPKKKK5, MPKK9, MPK3, CDPK1* and *TPR3* were associated with JA-signaling pathways, while *TPR3, ARF5* and *WOX13* were responsible for auxin signaling pathway. This coexpression network analysis was pertained to the promoter analysis results which can be conformed with candidate gene function and their related pathways.

3.5. Differentially expressed genes involved in secondary cell wall biosynthetic pathway

According to the RNA-sequencing results, the vascular development-related genes could be divided base on their involved tissues. Not only from gene ontology analysis, xylem related genes were found to almost associate with secondary cell wall biosynthesis (Supplement Fig. S3). *VNI2* (*NAC083,* LOC110665243) was observed to higher accumulate in newly developed stem than mature xylem tissue in both clones. *VND7* (LOC110635279) in mature xylem tissue of RRIT402 clone was higher expressed than newly developed stem and mature xylem tissue of RRIT251 clone. *REV* (LO110648065) was highly expressed in both mature xylem tissue of two rubber clones as compared with newly developed stem. *MYB46* (LOC110633800 and LOC11065612) was also demonstrated to highly express in mature xylem tissue of RRIT402 compared to RRIT251. Moreover, this study discovered another downstream regulator called *SND2* or *NAC073* (LOC110648261) from RNA-sequencing data which revealed the higher accumulation in WMX of RRIT402 than RRIT251. *IRX9* (LOC110655034) and *IRX15* (LOC110657423) displayed the higher expression in mature xylem tissue of RRIT402 than RRIT251. *MYB52* (LOC110673719), as a downstream regulator of MYB46, was higher accumulated in mature xylem tisssue of RRIT251 than RRIT402. On the other hand, *MYB4* (LOC110662525 and LOC110660138) was higher expressed in newly developed stem compared to mature xylem tissue in both rubber clones.

4. Discussion

Plant cells are consisted of two different cell wall types, including primary and secondary cell wall. To differentiate these two types of cell wall, the strength and thickness was considered. Secondary cell wall is stronger and thicker than primary cell wall. The thin and extensible wall of primary cell wall is defined as the major function in cell elongation and cell expansion [59]. Conversely, the thickness and strength of secondary cell wall is established by the deposition of cellulose, hemicellulose and lignin within the xylem cell compartments. The secondary cell wall provides strength and rigidity in plant tissues and also assists plant water transportation [60]. The molecular genetics of secondary cell wall biosynthesis has been studied for long time in various plants, for example *Arabidopsis thaliana* [12], loblolly pine (*Pinus taeda*, [61,62]), poplar (*Populus*, spp., [63]), white spruce (*Picea*

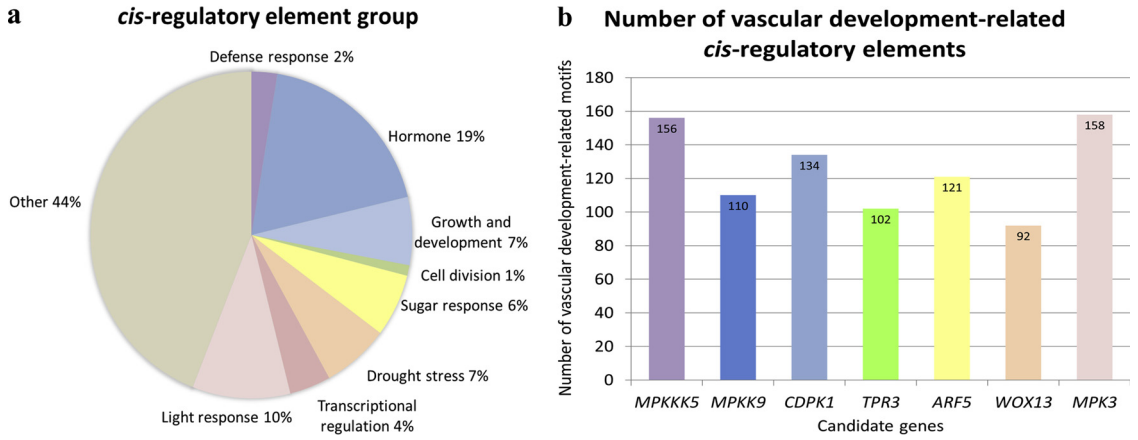

Fig. 5. Determination of vascular development-related *cis*-regulatory elements on promoter regions of seven candidate genes. **(a)** Percentage of *cis*-regulatory elements on promoter region of each candidate gene in their group functions. **(b)** Number of vascular development-related motifs on each promoter region of each candidate gene.

glauca, [64]), and eucalyptus (*Eucalyptus gunnii*, [65]). Indeed, these secondary cell wall-related genes have not been elucidated in rubber tree, yet. In this study, from gene ontology analysis of RNA-sequencing data of rubber tree, differentially expressed genes in xylem was mostly shown in cellulose and primary and secondary cell wall biosynthesis annotated in both biological process and molecular function. Moreover, from cellular component results, the most highly expressed genes in xylem were found to be localized and activated mainly in cell wall. Based on our observation, these related genes were confirmed to highly express in xylem. In contrast to the death vessels of xylem, phloem contains the living cells which have to cooperate with companion cells for food translocated activities. High metabolic activity and respiration rates were found in phloem tissue for ATP production [66]. This generated ATP was facilitated for sucrose translocation from source to sink [67]. Hence, sugar transport and ATP related functions were focused. Considering on biological process identified function, most highly expressed genes were identified in phloem had the function in sugar transport and ATP related functions. Interestingly, in annotated molecular function analysis result demonstrated that most highly expressed genes were determined in sugar transporter and ATP binding, activities and ATPase binding or activities. These highly expressed genes were confirmed as phloem-related genes. The phloem tissue of rubber tree contains specialized laticifer cells to produce natural rubber latex. From the biological process annotated function, most highly expressed genes in phloem were exhibited in latex biosynthesis of isopentenyl-diphosphate biosynthetic process. Additionally, in molecular function annotation, these highly expressed genes were also involved with isopentenyl-diphosphate delta isomerase activity. The cellular component results depicted that most highly expressed genes in phloem were activated in cytoplasm and chloroplast for latex biosynthetic in MVA and MEP pathway. Of note, this observation could confirm that highly expressed genes were organized into the correct groups of xylem- and phloem-vascular development-related genes. Remarkably, most xylem-related genes were associated with cell wall biosynthesis, while most phloem-related genes were involved with sugar transportation, ATP activities and latex biosynthesis.

VNI2 was identified as the inhibitor which targeted to *VND7* (*NAC030*) and caused the xylem cell prohibition [68]. *VNI2* (*NAC083,* LOC110665243) was observed to higher accumulate in newly developed stem than mature xylem tissue in both clones, while *VND7* (LOC110635279) in mature xylem tissue of RRIT402 clone was higher expressed than newly developed stem and mature xylem tissue of RRIT251 clone. REV, member of the Class III HD-Zip proteins, was identified as one of the upstream regulators of *AtVND7* [43,69,70]. *REV* (LO110648065) was highly expressed in mature xylem tissue of both rubber clones as compared with newly developed stem. MYB46 was characterized as downstream regulator of NAC domain master regulators [51,54]. This *MYB46* (LOC110633800 and LOC11065612) was also demonstrated to highly express in mature xylem tissue of RRIT402 compared to RRIT251. Moreover, this study discovered another downstream regulator called *SND2* or *NAC073* (LOC110648261) from RNA-sequencing data which revealed the higher accumulation in mature xylem tissue of RRIT402 than RRIT251. Other researches have reported SND2 as the downstream regulator of MYB regulators, which activated the cellulose, hemicellulose and lignin biosynthesis-related genes [69]. The secondary cell wall biosynthetic genes comprise of *IRX* gene family as a main group because *irx* mutant exhibited the loss of secondary cell wall formation in *Arabidopsis* [12]. *IRX9* and *IRX15* were assigned to be hemicellulose biosynthetic genes [7,10]. *IRX9* (LOC110655034) and *IRX15* (LOC110657423) displayed the higher expression in mature xylem tissue of RRIT402 than RRIT251, suggesting that SND2 may regulate *IRX9* and *IRX15* to promote secondary cell wall formation in xylem tissue of RRIT402 [71]. Thus, from this study, the higher accumulation of *VND7, MYB46* and *SND2* transcripts in mature xylem tissue may manipulate *IRX9* and *IRX15* to promote more secondary cell wall formation in RRIT402, which is consistent to its high wood yielding characteristic. To gain more understanding, we have noticed that *MYB52* (LOC110673719), as a downstream regulator of MYB46, was higher accumulated in mature xylem tissue of RRIT251 than RRIT402. In previous study, AtMYB52 was defined as the repressor in lignin biosynthesis or whole secondary cell wall biosynthesis as the *myb52* insertion lines exhibited the high lignin accumulation phenotypes [72]. Together with our observation, high expression of *MYB52* in mature xylem tissue of RRIT251 inhibited the secondary cell wall biosynthesis was consistent with low wood yielding clone characteristic of RRIT251. On the other hand, *MYB4* (LOC110662525 and LOC110660138) was higher expressed in newly developed stem compared to mature xylem tissue in both rubber clones. This finding was correlated to previous report that MYB4 was identified as a repressor to inhibit secondary cell wall biosynthesis and also served as one of downstream regulator of MYB46 [4,73]. Thus, the high expression of *MYB4* may inhibit the secondary cell wall biosynthesis in newly developed stem of both rubber clones. This finding was correlated with high expression of *VNI2* gene as inhibitor in newly developed stem of both rubber clones. From this observation, we suggested that secondary cell wall was formed more in mature xylem tissue, especially in RRIT402 clone than newly developed stem of both rubber clones. A schematic diagram represents the possible regulation of secondary cell wall biosynthesis in xylem-containing tissues of two different rubber tree clones was shown in Supplement Fig. S5.

Several previous researches discovered that laticifer cell differentiation was induced after wounding or JA treatment in juvenile stem

of rubber tree [27,28,74,75]. Apart from laticifers induction, different endogenous JA levels also correlated with different latex yield [75]. From this observation, RRIT251 with high latex yield may accumulate more endogenous JA for more laticifer cell differentiation than RRIT402 with lower latex yield, less laticifers but higher wood yield. The hypothesis of higher level of endogenous JA in RRIT251 than RRIT402 was consistent to RNA-sequencing result, in which the higher expression of *allene oxide synthase* (*AOS*) (LOC110648229), a rate-limiting gene in JA biosynthetic pathway [76], in both mature phloem and newly developed stem of RRIT251 than RRIT402. JA served as a major hormone for plant to improve their response to wounding and also assist some role in secondary metabolite biosynthesis [77]. COI1 was identified as a JA receptor which formed a complex with the E3 ubiquitin ligase type, SCF^{COI1} to interact with JA-Ile as bioactive form of JA [78,79]. JASMONATE ZIM DOMAIN (JAZ) was the target of SCF^{COI1} complex for 26S proteasome degradation [29]. Without JA-Ile, JAZ will bind to MYC2 to inactivate its transcription process and on the contrary, the increased of JA-Ile level formed complex with SCF^{COI1} -JAZ and brought the JAZ out for degradation by 26S proteasome, leading to release MYC2 transcription factor to bind to JA-responsive genes to promote the transcription process [30]. G-box *cis*-regulatory element on promoter of each JA-responsive genes was signified for MYC2 binding and enhance the transcription process [80–82]. In this study, the candidate genes consisted of *MPKKK5, MPKK9, CDPK1, TPR3, ARF5, WOX13* and *MPK3* were investigated their expression profile in different vascular-containing tissues; xylem, phloem, and newly developed stem of two different rubber clones. Each candidate gene promoter was predicted to contain the G-box motif types, including 5'-CACATG-3' (MYCATRD22), 5'-CACGTG-3' (CACGTGMOTIF), 5'-AACGTG-3' (T/GBOXATPIN2), 5'-CATGTG-3' (MYCATERD1), and 5'-CACGAG-3' (G/A-box, GBOXSORBC1) [78,83–86]. Thus, it can be assumed that MYC2 may be the regulator of these candidate genes. Recent report has been established that MYC2 was a regulator in JA signaling pathway via coronatine treatment (as an active isoleucine conjugate of JA imitation) and manipulate MAPK signaling and Ca^{2+} signal transduction pathways for laticifer cell differentiation in rubber tree [31]. From our RNA-sequencing results, *MYC2* was higher expressed in newly developed stem of RRIT251 than mature phloem tissue. The high latex yield character of RRIT251 consistent to high endogenous JA accumulation which may cause free MYC2 for JA-responsive gene enhancement. Higher expression in newly developed stem of RRIT251 may help in promote more laticifer cell differentiation.

CLAVA (CLV) signaling pathway was identified as homeostasis control in stem cell proliferation and differentiation [33]. MAPK signaling pathway was characterized to mediate CLV signaling pathway in downstream regulation [34,35]. Interestingly, MAPK signaling pathway gene components, including *MPKKKs, MPKKs,* and *MPKs*, were up-regulated in vascular cambia-containing tissue samples treated with coronatine, suggesting that these MAPK cascades may have some roles to dominate the laticifer cells differentiation [31]. *MPKKK5* gene was highly expressed in newly developed stem of RRIT251 than RRIT402. Based on the assumption that there was more endogeneous JA accumulated in RRIT251, thus this gene performed the high expression to induce more laticifer cell differentiation in newly developed stem of RRIT251. Consistently, *MPKK9* and *MPK3* gene were also demonstrated the higher expression in newly developed stem of RRIT251 than RRIT402. This finding was also consistent to RNA-sequencing data on *MYC2* gene expression. All together, this study indicated that more endogeneous JA accumulation in newly developed stem of RRIT251 may play the key role for CLV and MAPK signaling pathway stimulation to increase more laticifer cell differentiation via MYC2 regulator. This was also correlated with the expression of JA-biosynthetic gene, *AOS* gene which highly expressed in newly developed stem of RRIT251 to provide more endogenous JA for JA-signaling pathway activation.

In general *CDPKs* were up-regulated upon wounding to enhance JA accumulation, and *CDPK1* was reported to play role in root development as well as in response to cold, drought and wounding stresses [36,87–89]. Additionally, MAPK signaling pathway was demonstrated to be independent to Ca^{2+} signal transduction pathway. *CDPK5* and *CDPK6* were identified to trigger the ethylene accumulation by wounding, but wounded seedling of *Arabidopsis* double mutant of *cdpk5 cdpk6* was still showed activation of *MPK3* and *MPK6* [90]. Interestingly, the previous results from suppression subtractive hybridization and Illumina sequencing of vascular cambia-containing tissue samples treated with coronatine revealed that *CDPK1* and Ca^{2+} signal transduction genes were up-regulated, suggesting that these genes in Ca^{2+} signal transduction pathway may be associated with secondary laticifer cell differentiation in rubber tree [31,37]. Consistent with qRT-PCR expression results in this study, *CDPK1* was highly expressed in newly developed stem of RRIT251, while low expression was observed in RRIT402, thus this gene may display some crucial roles in phloem and laticifer cell differentiation in RRIT251 clone to promote more phloem and laticifer cell differentiation than RRIT402. Although this gene was shown to lowly express in mature phloem tissue than newly developed stem of RRIT251, it was still higher expressed than mature phloem tissue of RRIT402. This finding suggested that *CDPK1* may mainly regulated in newly developed stem of RRIT251 to promote phloem together with laticifer cell differentiation at early stage. In contrast to RRIT251, this gene was performed the low expression in RRIT402, thus led to lower number of laticifers. *TOPLESS* gene family contains *TPL* and *TPL-related* (*TPR*) genes which can interact with transcription factors directly or indirectly as co-repressors to suppress the transcription processes [56,57]. Moreover, these repressor complexes were related to inhibition of auxin and JA-responsive genes and to stabilize the meristem homeostasis and defense response [56,91–94]. Of note, TPL/TPR was interacted with NINJA to fuse with JAZ and bound on JA-responsive genes for transcriptional inhibition [92]. From qRT-PCR result in this study, *TPR3* was depicted the higher expression level in mature phloem tissue of RRIT402 than in RRIT251, thus the high expression of this gene in RRIT402 may affect the low expression level of JA-responsive genes then alleviate laticifer cell differentiation. Conversely, low expression of *TPR3* in mature phloem tissue of RRIT251 may lead to the low JAZ protein binding to JA-responsive genes and stimulated laticifer cell differentiation. A schematic diagram represents the differential expression of JA-signaling related genes and their possible regulation in phloem with laticifer cell differentiation of two different rubber tree clones was shown in Supplement Fig. S6.

In auxin signaling pathway, AUXIN RESPONSE FACTORS (ARFs) were identified as mediator to motivate or suppress the auxin-responsive genes for transcription process [95]. Interestingly, *ARF5* was defined to reduce the cambium cell proliferation to promote more xylem cell and less phloem cell differentiation instead [16]. Consistent to our study, the lower expression of this gene in MP than MX of both rubber clones, supporting that highly expression of this gene in MX may promote xylem cell differentiation. According to CLAVA (CLV) signaling pathway, there are CLV3/Embryo Surrounding Region-related (CLE), CLV1 and WUSCHEL (WUS) or WUS-related homeobox (WOX) as major components [96–99]. *WUS* and *WOX* gene family was signified as stem cell regulator by feedback loop to enhance CLV signaling gene, *CLV3* [100–103]. On the other hand, CLV signaling pathway was also suppressed *WUS* and *WOX* through MAPK signaling pathway. These MAPK cascades inhibited *WUS* and *WOX* to stop stem cell proliferation, which in turn to start stem cell differentiation for laticifer cell differentiation in rubber tree [31]. *WOX13*, a member of *WOX* gene family, was defined to play role in flowering, root development and fertility [104,105], and *WOX13* was reported to be essential in cellular processes on cellular reprograming during the stem cell initiation and cell growth in moss [106]. From qRT-PCR result in this study, *WOX13* performed the lower expression in mature phloem tissue of RRIT251 than in RRIT402. Consistent with illumina sequencing of vascular cambia-containing tissue, this gene was also down-regulated under coronatine treatment to provide laticifer cell differentiation [31]. Thus,

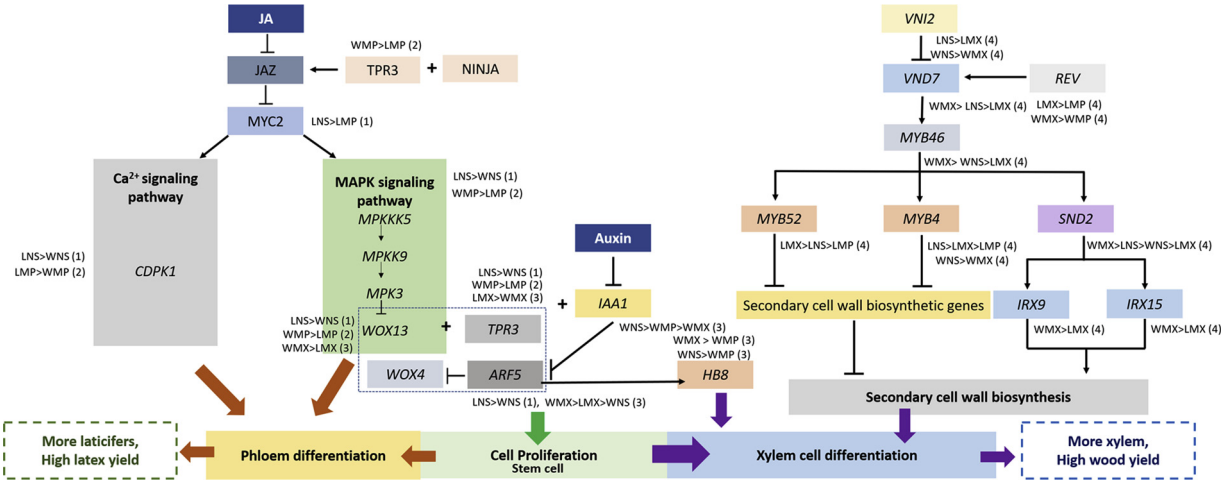

Fig. 6. A schematic diagram represents the possible regulation of JA-signaling related genes and their possible regulation in phloem with laticifer cell differentiation, secondary cell wall biosynthesis in xylem-containing tissues, and auxin-signaling related genes and their possible regulation in xylem cell differentiation. From qRT-PCR analysis and RNA-sequencing data, differential expression of candidate genes depicted the correlation to the different characteristics of more laticifers/high latex-yield and more xylem/high wood-yield rubber tree clones. (1) and (2) Set of differentially expressed candidate genes in newly developed stem and mature phloem tissues underlying JA-signaling and their possible regulation in phloem with laticifer cell differentiation. (3) and (4) Set of differentially expressed candidate genes in mature xylem tissues underlying auxin-signaling and secondary cell wall biosynthesis and their possible regulation in xylem cell differentiation.

the lower expression of this gene may prohibit stem cell proliferation and start to promote phloem as well as laticifer cell differentiation in LMP of RRIT251.

Another point of view is that WOX can interact with TPR as a co-repressor for stem cell maintenance [56]. These two genes exhibited the lower expression in RRIT251 to form a co-repressor to maintain the meristem homeostasis for laticifer cell differentiation. *WOX13* showed the highest expression in mature xylem tissue of RRIT402, suggesting that it may play role stem cell division in xylem. However, the expression of *TPR3* in mature xylem tissue of RRIT402 is lower than RRIT251, whereas *ARF5* performed the opposite expression to *TPR3*. The RNA-sequencing results showed low expression of *IAA1* and *TPR3* in mature xylem tissue of RRIT402. The arrayed transcription factor library together with Arabidopsis Interactome Mapping Consortium reported that TPR3 can interact with IAA1 in *Arabidopsis* [107,108]. Recently, *WOX4* was predicted as the target suppression of *ARF5* to transform the undifferentiated to differentiated more xylem and less phloem influent on wood formation in *Arabidopsis* [16]. Accordingly, low expression of *TPR3* and *IAA1* in mature xylem tissue of RRIT402 may lead to high expression of *ARF5*. The high expression of *ARF5* was forecasted to suppress the *WOX4* gene for promoting xylem differentiation in mature xylem tissue of RRIT402. Thus, while *WOX4* was inhibited, *WOX13* may be assigned to maintain the stem cell proliferation homeostasis instead. On the contrary, *WOX13* gene showed the similar expression level in both tissues, whereas the expression of *TPR3* and *ARF5* was opposite to mature xylem tissue of RRIT402. Thus, the high expressed level of *WOX13* may promote the cell proliferation, but the high level of *TPR3* may lead to low *ARF5* expression level. The low level of *ARF5* triggered *WOX4* to enhance more cell proliferation with undifferentiated xylem along with *WOX13*, so the wood formation was less in mature xylem tissue of RRIT251 but more in RRIT402, which supported character of high wood-yield clone. For newly developed stem, *WOX13, TPR3* and *ARF5* expression in RRIT251 was higher than RRIT402. High expression of *WOX13* and *TPR3* resulted in high cell proliferation (undifferentiated cells), and high *ARF5* expression may contribute to inhibit *WOX4* to induce undifferentiated cells to differentiated phloem or xylem cells in RRIT251. In newly developed stem of RRIT251, high cell proliferation and differentiation may lead to more in phloem and laticifer differentiation than xylem cell differentiation, result in less wood yield in RRIT251. Nevertheless, the low *WOX13* and *TPR3* expression in newly developed stem of RRIT402 led to the less cell proliferation, and to maintain this homeostasis, while low expression of *ARF5* may release *WOX4* for cell proliferation. More cell proliferation was prepared in newly developed stem of RRIT402, then more undifferentiated cells were differentiated to be more xylem, result in more wood yield in RRIT402. A schematic diagram represents the differential expression of auxin-signaling related genes and their possible regulation in xylem cell differentiation of two different rubber tree clones was shown in Supplement Fig. S7.

5. Conclusion

Rubber tree vascular tissues consist of phloem with specialized cells called laticifer cells, and xylem for latex and wood production, respectively. To investigate the gene regulation in these two different vascular tissues in rubber tree, the high latex-yield clone (RRIT251) and high wood yield-clone (RRIT402) were performed the Ion-torrent RNA-sequencing to discriminate the highly expressed genes in each tissue. All differentially expressed genes were classified into their four different tissue groups, including primary and secondary phloem and xylem, and the vascular development-related genes were focused. From gene ontology, most highly expressed genes in phloem were responsible for sugar and ATP transporter as well as latex biosynthesis. Conversely, almost highly expressed genes in xylem were established in secondary cell wall biosynthetic pathway, and remarkably expressed in RRIT402 for more xylem than RRIT251. The experimental validation by qRT-PCR of seven candidate genes was consistent to RNA-sequencing results. In phloem of RRIT251, JA-signaling pathway with its related genes was elucidated to promote more phloem with laticifer cell differentiation. The auxin signaling pathway and its related genes were highly expressed in xylem of RRIT402, for more xylem cell differentiation. These results were correlated with *Arabidopsis* candidate genes coexpression network analysis. Apart from these, promoter analysis of seven candidate genes also revealed the putative hormonal JA and auxin responsive motifs, thus these seven candidate genes should be responsible for JA and auxin signaling pathways. A schematic diagram represents the possible regulation of cell differentiation in phloem (with laticifers) and xylem was proposed in Fig. 6. The obtained RNA-sequencing information in two different clones of rubber tree provided better understanding in molecular mechanism of phloem with laticifer cell and xylem cell differentiation. This acquired information may be benefit to utilize as selectable markers to investigate the suitable clones for rubber

plant-breeding program for high potential of phloem with more laticifers or xylem differentiation. Furthermore, hormonal treatment of JA or auxin in rubber tree may be the optional procedure to promote high phloem with more laticifers and xylem cell differentiation, respectively. For more elucidation, the specific function of each gene in each pathways of phloem with laticifer and xylem cell differentiation in rubber tree should be identified.

Declaration of Competing Interest

Authors have no conflict of interest.

Data availability

The raw transcriptome reads and processed transcript data were submitted to NCBI/SRA database, Bioproject: PRJNA549803, Biosample: SAMN12097879. The submitted data is available at https://www.ncbi.nlm.nih.gov/sra/PRJNA549803, and all identified differentially expressed genes in three different vascular-containing tissues of two different rubber tree clones are available at https://doi.org/10.7910/DVN/EO3QSX.

Author contribution

JN, UV, PK and ST conceived and designed research. PS, CN, TY conducted experiments, analyzed data and all bioinformatics. KN contributed rubber tree from the Rubber Research Institute of Thailand. JN and PS wrote the manuscript. All authors discussed, read and approved the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cpb.2019.100112.

References

[1] J. Hepworth, M. Lenhard, Regulation of plant lateral-organ growth by modulating cell number and size, Curr. Opin. Plant Biol. 17 (2014) 36–42.
[2] D. Inzé, L. De Veylder, Cell cycle regulation in plant development, Annu. Rev. Genet. 40 (2006) 77–105.
[3] M. Kubo, M. Udagawa, N. Nishikubo, G. Horiguchi, M. Yamaguchi, J. Ito, T. Mimura, H. Fukuda, T. Demura, Transcription switches for protoxylem and metaxylem vessel formation, Genes Dev. 19 (2005) 1855–1860.
[4] J.H. Ko, W.C. Kim, K.-H. Han, Ectopic expression of *MYB46* identifies transcriptional regulatory genes involved in secondary wall biosynthesis in *Arabidopsis*, Plant J. 60 (2009) 649–665.
[5] D.M. Brown, L.A. Zeef, J. Ellis, R. Goodacre, S.R. Turner, Identification of novel genes in *Arabidopsis* involved in secondary cell wall formation using expression profiling and reverse genetics, Plant Cell 17 (2005) 2281–2295.
[6] D.M. Brown, Z. Zhang, E. Stephens, P. Dupree, S.R. Turner, Characterization of *IRX10* and *IRX10-like* reveals an essential role in glucuronoxylan biosynthesis in *Arabidopsis*, Plant J. 57 (2009) 732–746.
[7] D. Brown, R. Wightman, Z. Zhang, L.D. Gomez, I. Atanassov, J.P. Bukowski, T. Tryfona, S.J. McQueen- Mason, P. Dupree, S. Turner, *Arabidopsis* genes *IRREGULAR XYLEM* (*IRX15*) and *IRX15L* encode DUF579- containing proteins that are essential for normal xylan deposition in the secondary cell wall, Plant J. 66 (2011) 401–413.
[8] J.K. Jensen, H. Kim, J.C. Cocuron, R. Orler, J. Ralph, C.G. Wilkerson, The DUF579 domain containing proteins IRX15 and IRX15-L affect xylan synthesis in Arabidopsis, Plant J. 66 (2011) 387–400.
[9] L. Jones, A.R. Ennos, S.R. Turner, Cloning and characterization of *irregular xylem4* (*irx4*): a severely lignin- deficient mutant of *Arabidopsis*, Plant J. 26 (2001) 205–216.
[10] C. Lee, M.A. O'Neill, Y. Tsumuraya, A.G. Darvill, Z.H. Ye, The *irregular xylem9* mutant is deficient in xylan xylosyltransferase activity, Plant Cell Physiol. 48 (2007) 1624–1634.
[11] M.J. Pena, R.Q. Zhong, G.K. Zhou, E.A. Richardson, M.A. O'Neill, A.G. Darvill, W.S. York, Z.H. Ye, *Arabidopsis irregular xylem8* and *irregular xylem9*: implications for the complexity of glucuronoxylan biosynthesis, Plant Cell 19 (2007) 549–563.
[12] S.R. Turner, C.R. Somerville, Collapsed xylem phenotype of *Arabidopsis* identifies mutants deficient in cellulose deposition in the secondary cell wall, Plant Cell 9 (1997) 689–701.
[13] A.M. Wu, C. Rihouey, M. Seveno, E. Hornblad, S.K. Singh, T. Matsunaga, T. Ishii, P. Lerouge, A. Marchant, The *Arabidopsis IRX10* and *IRX10-LIKE glycosyltransferases* are critical for glucuronoxylan biosynthesis during secondary cell wall formation, Plant J. 57 (2009) 718–731.
[14] V. Funk, B. Kositsup, C. Zhao, E.P. Beers, The *Arabidopsis* xylem peptidase XCP1 is a tracheary element vacuolar protein that may be a papain ortholog, Plant Physiol. 128 (2002) 84–94.
[15] B. Bollhöner, B. Zhang, S. Stael, N. Denancé, K. Overmyer, D. Goffner, F. Van Breusegem, H. Tuominen, Post mortem function of AtMC9 in xylem vessel elements, New Phytol. 200 (2013) 498–510.
[16] K. Brackmann, V. Jouannet, J. Qi, T. Schlamp, K. Gruenwald, P. Sanchez, T. Greb, Spatial specificity of auxin responses coordinates wood formation, Nat. Commun. 9 (2018) 875.
[17] J.T. Sakdapipanich, Structural characterization of natural rubber based on recent evidence from selective enzymatic treatments, J. Biosci. Bioeng. 103 (2007) 287–292.
[18] K. Cornish, Similarities and differences in rubber biochemistry among plant species, Phytochemistry 57 (2001) 1123–1134.
[19] W. Verheye, Growth and production of rubber, in: W. Verheye (Ed.), Land Use, Land Cover and Soil Sciences. Encyclopedia of Life Support Systems (EOLSS), UNESCO EOLSS Publishers, Oxford, 2010.
[20] A.Y.A. Rahman, A.O. Usharraj, B.B. Misra, G.P. Thottathil, K. Jayasekaran, Y. Feng, S. Hou, S.Y. Ong, F.L. Ng, L.S. Lee, H.S. Tan, M.K. Sakaff, B.S. Teh, B.F. Khoo, S.S. Badai, N.A. Aziz, A. Yuryev, B. Knudsen, A. Dionne-Laporte, N.P. Mchunu, Q. Yu, B.J. Langston, T.A. Freitas, A.G. Young, R. Chen, L. Wang, N. Najimudin, J.A. Saito, M. Alam, Draft genome sequence of the rubber tree Hevea brasiliensis, BMC Genomics 14 (2013) 75.
[21] N.S. Lau, Y. Makita, M. Kawashima, T.D. Taylor, S. Konda, A.S. Othman, A.C. Shu-Chien, M. Matsui, The rubber tree genome shows expansion of gene family associated with rubber biosynthesis, Sci. Rep. 6 (2016) 28594.
[22] C. Tang, M. Yang, Y. Fang, Y. Luo, S. Gao, X. Xiao, Z. An, B. Zhou, B. Zhang, X. Tan, H.Y. Yeang, Y. Qin, J. Yang, Q. Lin, H. Mei, P. Montoro, X. Long, J. Qi, Y. Hua, Z. He, M. Sun, W. Li, X. Zeng, H. Cheng, Y. Liu, J. Yang, W. Tian, N. Zhuang, R. Zeng, D. Li, P. He, Z. Li, Z. Zou, S. Li, C. Li, J. Wang, D. Wei, C.Q. Lai, W. Luo, J. Yu, S. Hu, H. Huang, The rubber tree genome reveals new insights into rubber production and species adaptation, Nat. Plants 2 (2016) 16073–16082.
[23] W. Pootakham, C. Sonthirod, C. Naktang, P. Ruang-Areerate, T. Yoocha, D. Sangsrakru, K. Theerawattanasuk, R. Rattanawong, N. Lekawipat, S. Tangphatsornruang, *De novo* hybrid assembly of the rubber tree genome reveals evidence of paleotetraploidy in *Hevea* species, Sci. Rep. 7 (2017) 41457.
[24] T.-n. Wuyun, L. Wang, H. Liu, X. Wang, L. Zhang, J.L. Bennetzen, T. Li, L. Yang, P. Liu, L. Du, L. Wang, M. Huang, J. Qing, L. Zhu, W. Bao, H. Li, Q. Du, J. Zhu, H. Yang, S. Yang, H. Liu, H. Yue, J. Hu, G. Yu, Y. Tian, F. Liang, J. Hu, D. Wang, R. Gao, D. Li, H. Du, The hardy rubber tree genome provides insights into the evolution of polyisoprene biosynthesis, Mol. Plant 11 (2018) 429–442.
[25] P. Chantuma, A. Lacointe, P. Kasemsap, S. Thanisawanyangkura, E. Gohet, A. Clement, A. Guilliot, T. Ameglio, P. Thaler, Carbohydrate storage in wood and bark of rubber trees submitted to different level of C demand induced by latex tapping, Tree Physiol. 29 (8) (2009) 1021–1031.
[26] S. Raj, G. Das, J. Pothen, S.K. Dey, Relationship between latex yield of *Hevea brasiliensis* and antecedent environmental parameters, Int. J. Biometeorol. 49 (2005) 189–196.
[27] B.Z. Hao, J.L. Wu, Laticifer differentiation in *Hevea brasiliensis*: induction by exogenous jasmonic acid and linolenic acid, Ann. Bot. 85 (1) (2000) 37–43.
[28] T. Laosombut, P. Arreewichit, K. Nirapathpongporn, P. Traiperm, P. Kongsawadworakul, U. Viboonjun, J. Narangajavana, Differential expression of methyl jasmonate-responsive genes correlates with laticifer vessel proliferation in phloem tissue of rubber tree (*Hevea brasiliensis*), J. Plant Growth Regul. 35 (2016) 1049–1063.
[29] J. Moon, G. Parry, M. Estelle, The ubiquitin-proteasome pathway and plant development, Plant Cell 16 (2004) 3181–3195.
[30] P. Fernndez-Calvo, A. Chini, G. Fern?ndez-Barbero, J.M. Chico, S. Gimenez-Ibanez, J. Geerinck, D. Eeckhout, F. Schweizer, M. Godoy, J.M. Franco-Zorrilla, L. Pauwels, E. Witters, M.I. Puga, J. Paz-Ares, A. Goossens, P. Reymond, G. De Jaeger, R. Solano, The *Arabidopsis* bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses, Plant Cell 23 (2011) 701–715.
[31] S. Wu, S. Zhang, J. Chao, X. Deng, Y. Chen, M. Shi, W.M. Tian, Transcriptome analysis of the signalling networks in coronatine induced secondary laticifer differentiation from vascular cambia in rubber trees, Sci. Rep. 6 (2016) 36384.
[32] S.E. Clark, M.P. Running, E.M. Meyerowitz, CLAVATA1, a regulator of meristem and flower development in *Arabidopsis*, Development 119 (1993) 397–418.
[33] G. Wang, G. Zhang, M. Wu, CLE peptide signaling and crosstalk with phytohormones and environmental stimuli, Front. Plant Sci. 6 (2015) 1211.
[34] S. Betsuyaku, F. Takahashi, A. Kinoshita, H. Miwa, K. Shinozaki, H. Fukuda,

S. Sawa, Mitogen-activated protein kinase regulated by the CLAVATA receptors contributes to shoot apical meristem homeostasis, Plant Cell Physiol. 52 (2011) 14–29.
[35] M.A. Butenko, A.K. Vie, T. Brembu, R.B. Aalen, A.M. Bones, Plant peptides in signalling: looking for new partners, Trends Plant Sci. 14 (2009) 255–263.
[36] S. Ivashuta, J. Liu, D.P. Lohar, S. Haridas, B. Bucciarelli, K.A. VandenBosch, C.P. Vance, M.J. Harrison, J.S. Gantt, RNA interference identifies a calcium-dependent protein kinase involved in Medicago truncatula root development, Plant Cell 17 (11) (2005) 2911–2921.
[37] S.X. Zhang, S.H. Wu, Y.Y. Chen, W.M. Tian, Analysis of differentially expressed genes associated with coronatine-induced laticifer differentiation in the rubber tree by subtractive hybridization suppression, PLoS One 10 (2015) e0132070.
[38] C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, F.O. Glo¨ckner, The SILVA ribosomal RNA gene database project: improved data processing and web-based tools, Nucleic Acids Res. 41 (2013) D590–D596.
[39] S. Griffiths-Jones, S. Moxon, M. Marshall, A. Khanna, S.R. Eddy, A. Bateman, Rfam: annotating non-coding RNAs in complete genomes, Nucleic Acids Res. 33 (2005) D121–D124.
[40] S. Proost, M. Mutwil, PlaNet: comparative co-expression network analysesfor plants, in: A.D.J. vanDijk (Ed.), Plant Genomics Databases: Methods and Protocols, Springer, New York, 2017, pp. 213–227.
[41] C.N. Chow, H.Q. Zheng, N.Y. Wu, C.H. Chien, H.D. Huang, T.Y. Lee, Y.F. Chiang-Hsieh, P.E. Hou, T.Y. Yang, W.C. Chang, PlantPAN 2.0: an update of plant promoter analysis navigator for reconstructing transcriptional regulatory networks in plants, Nucleic Acids Res. 44 (2016), https://doi.org/10.1093/nar/gkv1035.
[42] Y. Aoki, S. Takahashi, D. Takayama, Y. Ogata, N. Sakurai, H. Suzuki, K. Asawatreratanakul, D. Wititsuwannakul, R. Wititsuwannakul, D. Shibata, T. Koyama, T. Nakayama, Identification of laticifer-specific genes and their promoter regions from a natural rubber producing plant Hevea brasiliensis, Plant Sci. 225 (2014) 1–8.
[43] A. Carlsbecker, J.Y. Lee, C.J. Roberts, J. Dettmer, S. Lehesranta, J. Zhou, O. Lindgren, M.A. Moreno-Risueno, A. Vatén, S. Thitamadee, A. Campilho, J. Sebastian, J.L. Bowman, Y. Helariutta, P.N. Benfey, Cell signalling by *microRNA165/6* directs gene dose-dependent root cell fate, Nature 465 (2010) 316–321.
[44] Y. Kondo, T. Ito, H. Nakagami, Y. Hirakawa, M. Saito, T. Tamaki, K. Shirasu, H. Fukuda, Plant GSK3 proteins regulate xylem cell differentiation downstream of TDIF-TDR signaling, Nat. Commun. 5 (2014) 3504.
[45] Y. Kondo, A.M. Nurani, C. Saito, Y. Ichihashi, M. Saito, K. Yamazaki, N. Mitsuda, M. Ohme-Takagi, H. Fukuda, Vascular cell induction culture system using *Arabidopsis* leaves (VISUAL) reveals the sequential differentiation of sieve element-like cells, Plant Cell 28 (2016) 1250–1262.
[46] Q. Li, Y.C. Lin, Y.H. Sun, J. Song, H. Chen, X.H. Zhang, R.R. Sederoff, V.L. Chiang, Splice variant of the SND1transcription factor is a dominant negative of SND1 members and their regulation in Populus trichocarpa, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 14699–14704.
[47] Y. Makita, K.K. Ng, G. Veera Singham, M. Kawashima, H. Hirakawa, S. Sato, A.S. Othman, M. Matsui, Large-scale collection of full-length cDNA and transcriptome analysis in Hevea brasiliensis, DNA Res. 24 (2017) 159–167.
[48] J. Pirrello, J. Leclercq, F. Dessailly, M. Rio, P. Piyatrakul, K. Kuswanhadi, C. Tang, P. Montoro, Transcriptional and post-transcriptional regulation of the jasmonate signalling pathway in response to abiotic and harvesting stress in Hevea brasiliensis, BMC Plant Biol. 14 (2014) 341.
[49] B. Xu, M. Ohtani, M. Yamaguchi, K. Toyooka, M. Wakazaki, M. Sato, M. Kubo, Y. Nakano, R. Sano, Y. Hiwatashi, T. Murata, T. Kurata, A. Yoneda, K. Kato, M. Hasebe, T. Demura, Contribution of NAC transcription factors to plant adaptation to land, Science 343 (2014) 1505–1508.
[50] R. Zhong, T. Demura, Z.H. Ye, SND1, a NAC domain transcription factor, is a key regulator of secondary wall synthesis in fibers of Arabidopsis, Plant Cell 18 (2006) 3158–3170.
[51] R. Zhong, E.A. Richardson, Z.H. Ye, The MYB46 transcription factor is a direct target of SND1 and regulates secondary wall biosynthesis in Arabidopsis, Plant Cell 19 (2007) 2776–2792.
[52] R. Zhong, E.A. Richardson, Z.H. Ye, Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of Arabidopsis, Planta 225 (2007) 1603–1611.
[53] R. Zhong, C. Lee, J. Zhou, R.L. McCarthy, Z.H. Ye, A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in Arabidopsis, Plant Cell 20 (2008) 2763–2782.
[54] R. Zhong, Z.H. Ye, MYB46 and MYB83 bind to the SMRE sites and directly activate a suite of transcription factors and secondary wall biosynthetic genes, Plant Cell Physiol. 53 (2012) 368–380.
[55] A. Conesa, S. Götz, J.M. García-Gómez, J. Terol, M. Talón, M. Robles, Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research, Bioinformatics 21 (18) (2005) 3674–3676.
[56] M. Kieffer, Y. Stern, H. Cook, E. Clerici, C. Maulbetsch, T. Laux, B. Davies, Analysis of the transcription factor WUSCHEL and its functional homologue in Antirrhinum reveals a potential mechanism for their roles in meristem maintenance, Plant Cell 18 (2006) 560–573.
[57] J.A. Long, C. Ohno, Z.R. Smith, E.M. Meyerowitz, TOPLESS regulates apical embryonic fate in Arabidopsis, Science 312 (2006) 1520–1523.
[58] M.S. Porto, M.P.N. Pinheiro, V.G.L. Batista, R.C. Santos, P.A. Filho, L.M. Lima, Plant promoters: an approach of structure and function, Mol. Biotechnol. 56 (2014) 38–49.
[59] A. Geitmann, Mechanical modeling and structural analysis of the primary plant cell wall, Curr. Opin. Plant Biol. 13 (2010) 693–699.
[60] A.H. Liepman, R. Wightman, N. Geshi, S.R. Turner, H.V. Scheller, *Arabidopsis* a powerful model system for plant cell wall research, Plant J. 61 (2010) 1107–1121.
[61] I. Allona, M. Quinn, E. Shoop, K. Swope, S.S. Cyr, J. Carlis, J. Riedl, E. Retzel, M.M. Campbell, R. Sederoff, R.W. Whetten, Analysis of xylem formation in pine by cDNA sequencing, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 9693–9698.
[62] W.W. Lorenz, J.F.D. Dean, SAGE Profiling and demonstration of differential gene expression along the axial developmental gradient of lig- nifying xylem in loblolly pine (*Pinus taeda*), Tree Physiol. 22 (2002) (2002) 301–310.
[63] F. Sterky, R.R. Bhalerao, P. Unneberg, B. Segerman, P. Nilsson, A.M. Brunner, L. Charbonnel-Campaa, J.J. Lindvall, K. Tandre, S.H. Strauss, B. Sundberg, P. Gustafsson, M. Uhlén, R.P. Bhalerao, O. Nilsson, G. Sandberg, J. Karlsson, J. Lundeberg, S. Jansson, A populus EST resources for plant functional genomics, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 13951–13956.
[64] N. Pavy, C. Paule, L. Parsons, J.A. Crow, M.J. Morencey, J. Cooke, J.E. Johnson, E. Noumen, C. Guillet- Claude, Y. Butterfield, S. Barber, G. Yang, J. Liu, J. Stott, R. Kirkpatrick, A. Siddiqui, R. Holt, M. Marra, A. Seguin, E. Retzel, J. Bousquet, J. MacKay, Generation, annotation, analysis and database integration of 16,500 white spruce EST clusters, BMC Genomics 6 (2005) 144.
[65] D. Rengel, H.S. Clemente, F. Servant, N. Ladouce, E. Paux, P. Wincker, A. Couloux, P. Sivadon, J. Grima- Pettenati, A new genomic resource dedicated to wood formation in Eucalyptus, BMC Plant Biol. 9 (2009) 36.
[66] A.J.E. van Bel, M. Knoblauch, Sieve element and companion cell: the story of the comatose patient and the hyperactive nurse, Aust. J. Plant Physiol. 27 (2000) 477–487.
[67] N.D. DeWitt, M.R. Sussman, Immunocytological localisation of an epitope-tagged plasma membrane proton pump (H+-ATPase) in phloem companion cells, Plant Cell 7 (1995) 2053–2067.
[68] M. Yamaguchi, M. Ohtani, N. Mitsuda, M. Kubo, M. Ohme-Takagi, H. Fukuda, T. Demura, V.N.D.- INTERACTING2, A NAC domain transcription factor, negatively regulates xylem vessel formation in Arabidopsis, Plant Cell 22 (2010) 1249–1263.
[69] H. Endo, M. Yamaguchi, T. Tamura, Y. Nakano, N. Nishikubo, A. Yoneda, K. Kato, M. Kubo, S. Kajita, Y. Katayama, M. Ohtani, T. Demura, Multiple classes of transcription factors regulate the expression of VASCULAR-RELATED NAC-DOMAIN7, a master switch of xylem vessel differentiation, Plant Cell Physiol. 56 (2015) 242–254.
[70] S. Miyashima, S. Koi, T. Hashimoto, K. Nakajima, Non-cell- aoutonomous *microRNA165* acts in a dose- dependent manner to regulate multiple differentiation status in the *Arabidopsis* root, Development 138 (2011) 2303–2313.
[71] S.G. Hussey, E. Mizrach, A.V. Spokevicious, G. Bossinger, D.K. Berger, A.A. Myburg, SND2, a NAC transcription factor gene, regulates genes involved in secondary cell wall development in Arabidopsis fibres and increases fibre cell area in Eucalyptus, BMC Plant Biol. 1 (11) (2011) 173.
[72] H. Cassan-Wang, N. Goué, M.N. Saidi, S. Legay, P. Sivadon, D. Goffner, J. Grima-Pettenati, Identification of novel transcription factors regulating secondary cell wall formation in Arabidopsis, Front. Plant Sci. 4 (2013) 189.
[73] H. Jin, E. Cominelli, P. Bailey, A. Parr, F. Mehrtens, J. Jones, C. Tonelli, B. Weisshaar, C. Martin, Transcriptional repression by AtMYB4 controls production of UV-protecting sunscreens in Arabidopsis, EMBO J. 19 (2000) 6150–6161.
[74] W. Tian, M. Shi, F. Yu, J. Wu, B. Hao, K. Cui, Localized effects of mechanical wounding and exogenous jasmonic acid on the induction of secondary laticifer differentiation in relation to the distribution of jasmonic acid in Hevea brasiliensis, Acta Bot. Sin. 45 (11) (2003) 1366–1372.
[75] J.H. Yu, S.G. Yang, M.J. Huang, W.M. Tian, Effects of season, exploitation and exogenous jasmonic acid on the laticifer differentiation in mature rubber tree, Chin. J. Trop. Crops 28 (2007) 1–5.
[76] R.A. Creelman, J.E. Mullet, Biosynthesis and action of Jasmonates in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48 (1997) 355–381.
[77] C. Wasternack, B. Hause, Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development: an update to the 2007 review in Annals of Botany, Ann. Bot. 111 (2007) 1021–1058.
[78] J. Yan, C. Zhang, M. Gu, Z. Bai, W. Zhang, T. Qi, Z. Cheng, W. Peng, H. Luo, F. Nan, Z. Wang, D. Xie, The *Arabidopsis* CORONATINE INSENSITIVE1 protein is a jasmonate receptor, Plant Cell 21 (2009) 2220–2236.
[79] L. Xu, F. Liu, E. Lechner, P. Genschik, W. Crosby, H. Ma, W. Peng, D. Huang, D. Xie, The SCF(COI1) ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis, Plant Cell 14 (2002) 1919–1935.
[80] A. Chini, S. Fonseca, G. Fernández, B. Adie, J.M. Chico, O. Lorenzo, G. García-Casado, I. López-Vidriero, F.M. Lozano, M.R. Ponce, J.L. Micol, R. Solano, The JAZ family of repressors is the missing link in jasmonate signaling, Nature 448 (2007) 666–671.
[81] B. Thines, L. Katsir, M. Melotto, Y. Niu, A. Mandaokar, G. Liu, K. Nomura, S. He, G. Howe, J. Browse, JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signaling, Nature 448 (2007) 661–665.
[82] Y. Yan, S. Stolz, A. Chetelat, P. Reymond, M. Pagni, L. Dubugnon, E. Farmer, A downstream mediator in the growth repression limb of the jasmonate pathway, Plant Cell 19 (2007) 2470–2483.
[83] H. Abe, K. Yamaguchi-Shinozaki, T. Urao, T. Iwasaki, D. Hosokawa, K. Shinozaki, Role of *Arabidopsis MYC* and *MYB* homologs in drought- and abscisic acid-regulated gene expression, Plant Cell 9 (1997) 1859–1868.
[84] M. Boter, O. Ruíz-Rivero, A. Abdeen, S. Prat, Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and Arabidopsis, Genes Dev. 18 (2004) 1577–1591.
[85] B. Dombrecht, G.P. Xue, S.J. Sprague, J.A. Kirkegaard, J.J. Ross, J.B. Reid, G.P. Fitt, N. Sewelam, P.M. Schenk, J.M. Manners, K. Kazan, MYC2 differentially modulates diverse jasmonate dependent functions in Arabidopsis, Plant Cell 19

(2007) 2225–2245.
[86] V. Yadav, C. Mallappa, S.N. Gangappa, S. Bhatia, S. Chattopadhyay, A basic helix-loop-helix transcription factor in *Arabidopsis*, MYC2, acts as a repressor of blue light-mediated photomorphogenic growth, Plant Cell 17 (2005) 1953–1966.
[87] R.A. Bressan, C. Zhang, H. Zhang, P.M. Hasegawa, H.J. Bohnert, J.K. Zhu, Learning from the *Arabidopsis* experience. The next gene search paradigm, Plant Physiol. 127 (4) (2001) 1354–1360.
[88] M.L. Lanteri, G.C. Pagnussat, L. Lamattina, Calcium and calcium-dependent protein kinases are involved in nitric oxide-and auxin-induced adventitious root formation in cucumber, J. Exp. Bot. 57 (6) (2006) 1341–1351.
[89] D.-H. Yang, C. Hettenhausen, I.T. Baldwin, J. Wu, Silencing Nicotiana attenuata calcium-dependent protein kinases, CDPK4 and CDPK5, strongly up-regulates wound- and herbivory-induced jasmonic acid accumulations, Plant Physiol. 159 (2012) 1591–1607.
[90] S. Li, X. Han, L. Yang, X. Deng, H. Wu, M. Zhang, Y. Liu, S. Zhang, J. Xu, Mitogen-activated protein kinases and calcium-dependent protein kinases are involved in wounding-induced ethylene biosynthesis in Arabidopsis thaliana, Plant Cell Environ. 41 (2018) 134–147.
[91] A. Gallavotti, J.A. Long, S. Stanfield, X. Yang, D. Jackson, E. Vollbrecht, R.J. Schmidt, The control of axillary meristem fate in the maize ramosa pathway, Development 137 (2010) 2849–2856.
[92] L. Pauwels, G.F. Barbero, J. Geerinck, S. Tilleman, W. Grunewald, A.C. Pe´rez, J.M. Chico, R.V. Bossche, J. Sewell, E. Gil, G. García-Casado, E. Witters, D. Inzé, J.A. Long, G. De Jaeger, R. Solano, A. Goossens, NINJA connects the co-repressor TOPLESS to jasmonate signaling, Nature 464 (2010) 788–791.
[93] H. Szemenyei, M. Hannon, J.A. Long, TOPLESS mediates auxin-dependent transcriptional repression during *Arabidopsis* embryogenesis, Science 319 (2008) 1384–1386.
[94] Z. Zhu, F. Xu, Y. Zhang, Y.T. Cheng, M. Wiermer, X. Li, Y. Zhang, *Arabidopsis* resistance protein SNC1 activates immune responses through association with a transcriptional corepressor, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 13960–13965.
[95] T. Ulmasov, G. Hagen, T.J. Guilfoyle, Activation and repression of transcription by auxin-response factors, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 5844–5849.
[96] U. Brand, J.C. Fletcher, M. Hobe, E.M. Meyerowitz, R. Simon, Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by CLV3 activity, Science 289 (2000) 617–619.
[97] M. Fiers, K.L. Ku, C.M. Liu, CLE peptide ligands and their roles in establishing meristems, Curr. Opin. Plant Biol. 10 (2007) 39–43.
[98] Y. Hirakawa, Y. Kondo, H. Fukuda, Regulation of vascular development by CLE peptide-receptor systems, J. Integr. Plant Biol. 52 (2010) 8–16.
[99] M. Kucukoglu, O. Nilsson, CLE peptide signaling in plants - the power of moving around, Physiol. Plant. 155 (2015) 74–87.
[100] S.E. Clark, R.W. Williams, E.M. Meyerowitz, The *CLAVATA1* gene encodes a putative receptor-kinase that controls shoot and floral meristem size in *Arabidopsis*, Cell 89 (1997) 575–585.
[101] J.C. Fletcher, U. Brand, M.P. Running, R. Simon, E.M. Meyerowitz, Signaling of cell fate decisions by CLAVATA3 in *Arabidopsis* shoot meristems, Science 283 (1999) 1911–1914.
[102] M. Lenhard, T. Laux, Stem cell homeostasis in the *Arabidopsis* shoot meristem is regulated by intercellular movement of CLAVATA3 and its sequestration by CLAVATA1, Development 130 (2003) 3163–3173.
[103] H. Schoof, M. Lenhard, A. Haecker, K.F. Mayer, G. Jurgens, T. Laux, The stem cell population of *Arabidopsis* shoot meristems in maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes, Cell 100 (2000) 635–644.
[104] Y. Deveaux, C. Toffano-Nioche, G. Claisse, V. Thareau, H. Morin, P. Laufs, H. Moreau, M. Kreis, A. Lecharny, Genes of the most conserved *WOX* clade in plants affect root and flower development in *Arabidopsis*, BMC Evol. Biol. 8 (2008) 291.
[105] M. Romera-Branchat, J.J. Ripoll, M.F. Yanofsky, S. Pelaz, The *WOX13* homeobox gene promotes replum formation in the *Arabidopsis thaliana* fruit, Plant J. 73 (2012) 37–49.
[106] K. Sakakibara, P. Reisewitz, T. Aoyama, T. Friedrich, S. Ando, Y. Sato, Y. Tamada, T. Nishiyama, Y. Hiwatashi, T. Kurata, M. Ishikawa, H. Deguchi, S.A. Rensing, W. Werr, T. Murata, M. Hasebe, T. Laux, *WOX13-like* genes are required for re-programming of leaf and protoplast cells into stem cells in the moss *Physcomitrella patens*, Development 141 (2014) 1660–1670.
[107] Arabidopsis Interactome Mapping Consortium, Evidence for net- work evolution in an *Arabidopsis* interactome map, Science 333 (2011) 601–607.
[108] B. Causier, M. Ashworth, W. Guo, B. Davies, The *TOPLESS* Interactome: a framework for gene repression in *Arabidopsis*, Plant Physiol. 158 (2012) 423–438.