A Comparative Analysis of Transcription Networks Active in Juvenile and Mature Wood in Populus

Laifu Luo
Institute of Plant Physiology and Ecology Shanghai Institutes for Biological Sciences
Yingying Zhu
Lanzhou University
Jinshan Gui
Institute of Plant Physiology and Ecology Shanghai Institutes for Biological Sciences
Tongmin Yin
Nanjing Forestry University
Wenchun Luo
Lanzhou University
Jianquan Liu
Lanzhou University
Laigeng Li (lgli@sibs.ac.cn)
Institute of Plant Physiology and Ecology Shanghai Institutes for Biological Sciences
https://orcid.org/0000-0001-6924-4431

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Abstract

Background: Juvenile wood (JW) and mature wood (MW) have distinct physical and chemical characters, reflecting the different wood formation over the tree life-span. However, the regulatory mechanisms that distinguish or modulate the characteristics of JW and MW in relation to each other have not been mapped. Using RNA sequencing (RNA-seq) and whole genome bisulfite sequencing (WGBS), we analyzed wood properties associated with JW and MW forming tissue from *Populus* trees with an identical genetic background.

Results: JW and MW of *Populus* displayed different wood properties as the result of significant differences in transcriptional programs and patterns of DNA methylation. Differences were concentrated in gene networks involved in regulating hormonal signaling pathways responsible for auxin distribution and brassinosteroids biosynthesis as well as genes active in regulating cell expansion and secondary cell wall biosynthesis. An observed correlation between gene expression profiling and DNA methylation indicated that DNA methylation affected expression of the genes related to auxin distribution and brassinosteroids signal transduction, cell expansion in JW and MW formation.

Conclusions: Auxin distribution, brassinosteroids biosynthesis and signaling play critical roles in formation of JW and MW. DNA methylation is involved in formatting the transcriptional programs in different development phases which contribute to JW and MW formation. The study sheds light to better understand the molecular networks underlying regulation of wood properties which could inform improvement of wood formation.

Background

Perennial woody plants are characterized by large size and a long lifespan, in which a long non-flowering period of juvenile phase can last years to decades, for example, 3–5 years in *Populus* and 10–15 years in *Pinus* [1, 2]. Wood produced during juvenile phase is called juvenile wood (JW) which is followed by a mature phase during which trees start flowering and producing mature wood (MW) at outside of JW [3]. Compared to JW, MW is characterized with longer xylem cells, thicker secondary cell walls, lower density of vessels, higher crystallinity of cellulose in fibers and smaller microfibril angles [4]. Thus, MW is more desirable from a processing and utilization perspective for construction wood, wood pulping, and fiber material production. As a matter of fact, to meet the increasing demand for raw wood material, artificial forest plantation aims to reduce the rotation length and enhance productivity, which makes JW with lower wood quality as a major source for wood industry [5]. This seriously affects the utilization and processing of wood. How to make wood to be matured quickly and the proportion of JW to be reduced has become an important aspect of improving wood properties.

Wood formation starts with the cell divisions at vascular cambium and subsequent differentiation into secondary xylem through cell expansion, secondary cell wall thickening and programmed cell death [6]. Plant hormones, such as auxin, brassinosteroids and gibberellin, participate in regulation of wood

formation [7–9]. The size of wood cells depend on cell expansion process while mechanical and chemical properties of wood are largely determined by secondary cell wall thickening [10]. Cell expansion is controlled by extension of the primary cell wall, which is composed of 20–30% cellulose, 30–50% pectins, 20–25% hemicelluloses and 10% glycoproteins [11]. Following cell expansion, wall thickening is initiated with transcriptional programs for secondary cell wall biosynthesis [12]. Secondary cell walls are composed of 40–80% cellulose, 10–40% hemicellulose, 5–25% lignin and glycoproteins [13]. As JW and MW display distinct wood properties, regulation of the secondary cell formation in JW and MW is most likely through different ways.

DNA methylation, a critical epigenetic mechanism among eukaryotes, affects many biological processes. In plant, most of DNA methylation occurs at the fifth carbon of cytosine (including three cytosine contexts, CG, CHG and CHH, where H represents A, C or T) to form 5- methylcytosine by DNA methyltransferase [14–16]. Evidence indicates that DNA methylation can regulate gene expression in numerous biological processes including response to abiotic stresses [17–21], plant development and morphogenesis [22] and wood formation [23]. The degree of DNA methylation is also related to plant development phases. The degree of DNA methylation at mature phase was significantly higher than that at juvenile phase in *Pinus radiate* [24]. DNA methylation increases along with the age extension in some species [25]. It is unclear how DNA methylation is involved in regulation of JW and MW formation.

Despite studies which have shown physicochemical difference of wood properties between JW and MW, the molecular regulatory networks underlying formation of the different wood properties is not fully elucidated. In this study, by employing *Populus* trees with an identical genetic background, we analyzed different physical and chemical characters in association with the transcriptomic profiles and DNA methylation during formation JW and MW. Correlation analysis revealed the transcriptional networks and DNA methylation that are involved in regulation of wood formation with different wood properties. This study provides an array of mechanistic information for understanding of JW and MW formation as well as new clues for genetic manipulation for improvement of wood properties.

Results

Properties of JW and MW in Populus

To examine the properties of the JW and MW produced in *Populus*, plantation-grown trees that were propagated from a single clone were sampled. Three trees at two-years-old and eight-years-old were collected with trunk at breast height, respectively (Additional file 1). In wood anatomical section, difference in the ratio of fiber/vessel, the length and size of fibers and vessels was observed between JW and MW (Fig. 1a-d). MW contained higher ratio of fiber/vessel, lower density of vessel cell in wood section, longer and larger fiber and vessel than those in JW (Fig. 1e-j). Chemical analysis indicated that MW contained higher content of crystalline cellulose and lower content of lignin compared to JW (Table 1). Sugar composition in hemicelluloses also showed difference between JW and MW. MW contained higher xylose, mannose, glucose, arabinose but lower galactose compared to JW (Table 1).

These results indicated that JW and MW in *Populus* displayed different cellular structure and chemical composition.

Table 1
Chemical composition in JW and MW of Populus

Chemical composition (μg/mg AIR)		JW	MW
Cellulose		400.3 ± 24.4	432.4 ± 27.0*
Lignin		223.1 ± 9.7	206.7 ± 18.9*
Hemicellulose	Xylose	49.2 ± 3.5	61.0 ± 4.2**
	Mannose	9.2 ± 1.4	11.7 ± 1.0**
	Galactose	4.2 ± 0.3	3.9 ± 0.2*
	Glucose	34.0 ± 3.3	37.8 ± 3.1*
	Arabinose	1.3 ± 0.1	1.8 ± 0.1**

Significance was determined by Student's t-test (* p□0.05 and **p□0.01).

Dna Methylation In Formation Of Jw And Mw

The differential gene expression in different growth phases prompted us to examine the whole genome bisulfite sequencing (WGBS). The bisulfite sequencing showed that 87.6–91.9% of the reads were qualified for methylation assay against to the *Populus* genome (http://phytozome.jgi.doe.gov/) (Additional file 10). Overall, the methylation level was different within cytosine methylation contexts (CG, CHG and CHH). The context of CG had higher methylation level, while CHG and CHH was lower (Additional file 11). The DNA methylation context patterns displayed a similarity with those previous observed in *Populus* [18, 59]. PCA showed that JW and MW had distinct DNA methylation (Fig. 5a). Comparison of the DNA methylation in JW and MW revealed 12176 differentially methylated regions (DMRs) (with methylation difference ≥ 10, Q-value < 0.05). Majority of DMRs were in the contexts of CG sites (10303) and CHG sites (1663) (Additional file 12 and Additional file 13), Among them, 10237 DMRs were located in gene body and/or flanking regions (± 2 kb), named differentially methylated genes (DMGs). In MW, 5414 DMGs showed higher methylation while JW contained 4849 DMGs with higher methylation (Fig. 5b and Additional file 14), suggesting that different DNA methylations occurred in formation of JW and MW. Analysis of the correlation between DMGs and DEGs indicated that DMRs in gene promoter region were more likely to affect gene expression (Additional file 12). About 20% DEGs (802) displayed different methylation (Additional file 15). These DEGs were closely related to plant hormones signaling and response, cell wall formation and modification, metabolic process, transcription and translation etc. (Additional file 12 and Additional file 16). For example, the homologs of *ARFs*, *BAK1*, *BSK1* and *BZR1*, which are involved in auxin and BR signaling, were differential methylated in their different gene region in JW and MW (Fig. 5c and Additional file 15). Furthermore, several genes in related

to cell wall formation such as *XTH30*, *PAEs*, *WND1B*, *CESA4*, *CESA7* and *CESA8* (Fig. 5c and Additional file 15) showed differential methylation in JW and MW. In addition, several DMRs in intergenic region were neighbored to the homologs of *PILS2*, *AUX1*, *PIN7*, *WND2A*, *MYBs* and *PAL1*, which are involved in auxin distribution and cell wall biosynthesis (Additional file 15). In summary, the results revealed that DNA methylation displayed a clear difference in formation of JW and MW, which may play a role in regulating gene expression in different growth phases, particularly for the genes involved in hormone signal transduction, cell division and cell wall formation in wood formation.

Discussion

At a given point of tree development, wood can be differentiated into juvenile wood and mature wood which have distinct properties [3, 4]. In the present study, we profiled the transcriptome and DNA methylation patterns in JW and MW derived from an identical genetic background in order to uncover the paths involved in wood formation at different developmental phases. Different transcription profiles and DNA methylation were identified in formation of JW and MW. Differences in gene expression were primarily associated with plant hormones including auxin and BR signaling and response, cell wall formation and modification, cell organization and biogenesis, transcription regulation processes. Different patterns of DNA methylation were also detected in genes involved in auxin transport, BR signaling, and cell expansion which suggest a role for the epigenetic regulation of JW and MW formation.

Different expression of auxin transport genes was observed in JW and MW. Relative to JW, we observed the genes related to auxin influx (homolog of *AUX1*) [26] were down-regulated in MW, while the genes related to auxin efflux (homologs of *PINs*, *PILSs*, *ABCB19*) [26, 27] were up-regulated in MW. Meanwhile, that different members of the *AUX1/LAX3* family were expressed in JW and MW implies a possibility that formation of JW and MW involves distinct auxin molecule formats, as *AUX1/LAX* members correspond with different auxin formats [60]. Further characterization of PIN, ABCB, AUX1 and LAX3 proteins in association with auxin in the JW-/MW-forming tissues would be able to provide mechanistic evidence for verification of the findings. However, current results indicate that auxin transport play a role in regulating formation of JW and MW.

Furthermore, we also found that homologs of *DIM* which is a key gene for BR biosynthesis [28] was up-regulated in MW, while homologs of *BAK1*, *BSK1*, *BZR1* which are marker genes for BR signaling [61–64] showed down-regulated expression in MW, suggesting that BR signaling play a role in regulating MW formation. Studies have showed that BR promotes wood formation [65]. It is worthy of studying whether BR manipulates wood properties in wood formation because the properties JW and MW are different.

DNA methylation act as an epigenetic mechanism to regulate gene expression in plants [21, 66–68]. In this study, we found that the methylation level of the auxin transport genes *PILS2*, *AUX1*, *PIN7* was different between JW and MW. In addition, the different degree of DNA methylation was also detected in the BR signaling genes *BAK1*, *BSK1*, *BZR1*. It is likely that the different expression of these genes in JW and MW may be related to their DNA methylation changes over the developmental process. Further

investigation of the DNA methylation effect on the transcription activities of the auxin and BR genes would help understanding the regulation of the hormones signaling during different development phases in perennial trees. In summary, the present results suggest that auxin distribution and transportation, BR biosynthesis and signaling are involved in regulating the wood formation at juvenile and mature phase. DNA methylation plays an important role in regulating the expression of the auxin and BR genes at different development phases.

In consistent with the hormones signaling changes, the downstream biological processes in response to auxin and BR also showed alternation in JW and MW. For instance, TFs such as *ARFs*, *SRSs*, *AP2*, *MYB3R4* and genes related cell loosing and cell expansion such as *HA11*, *XTHs*, *FUC1*, *PAEs*, *PMEs*, *PMRs*, *PIPs*, *TIPs*, of which the expression is responding to auxin signaling [48, 69], showed differential expression in formation of JW and MW. These transcription regulations are in agreement with the MW properties that have significantly longer and larger fiber cells and vessel elements.

Cell wall composition (including lignin, cellulose and hemicellulose) which is closely related to wood properties is rather different in JW and MW (Table 1). Expression of the genes related to lignin biosynthesis were down-regulated and the genes for hemicelluloses biosynthesis were up-regulated in MW, consistent with the result of less lignin content and higher hemicellulose content in MW. Interestingly, expression of the cellulose biosynthesis genes (such as *CesA4*, *CesA7* and *CesA8*) was down-regulated in MW compared to JW. However, the cellulose content was higher in MW. As this discrepancy requires further verification, regulation of the CesA activity at protein level may be considered. It is known that protein phosphorylation plays a crucial role in regulating CesA catalytic activity and motility [53, 70, 71]. More evidence is needed for elucidation of the different cellulose accumulation in JW and MW.

Conclusions

In this study, we analyzed transcription profiles and genome-wide DNA methylation in association with the wood propertied of JW and MW by employing *Populus* trees with an identical genetic background. Results suggest that auxin distribution and BR signaling may act as major mechanisms to modulate the wood formation in different development phases. In response to the hormones signaling alteration, the transcription activities are modulated, leading to the formation of different wood properties in JW and MW. Furthermore, results also indicate that the transcription changes of hormones-related genes may regulated through their DNA methylation. The study outlines a picture of the main transcription networks related to wood formation in JW and MW and a possible role of DNA methylation in tuning the transcriptional network (Fig. 6). These findings shed light towards a better mechanistic understanding of wood formation in different development phases and new evidence to inform the engineering of wood properties.

Methods

Tissue sampling

Populus trees propagated from the same clone (*Populus deltoides×P. euramericana* cv.'*Nanlin895*') were grown in an experimental plantation of the Nanjing Forestry University located at Siyang, Suqian, Jiangsu, China (33° 47' N, 118° 22' E). Wood-forming tissues were sampled from at the trunk in a length of 1 meter at the breast height (1.3 meter from ground) from 2-years-old (formation of JW) and 8-years-old trees (formation of MW). The tissues were directly collected into liquid nitrogen and stored at -80 °C for late analysis [72]. Three biological replicates were sampled from juvenile and mature trees (Additional file 1), respectively. The tree trunks at the breast height were collected and used for wood property analysis.

Analysis of wood properties

JW and MW were sampled according to the year-ring of wood. Wood tissue was sectioned into 20-μm in thickness and stained with 0.5% phloroglucinol in 12% HCl. Cross sections were observed under a microscope (Olympus, BX53). Number of fibers and vessels and their cross area were counted using Image J. Meanwhile, the wood cells were separated after treatment using acetic acid/ hydrogen peroxide (1:1 v/v) solution at 80°C for 6 hours. Then, the separated wood cells were stained with safranine (1% in water) and the length of fiber cells and vessels was measured under a microscope (Olympus, BX53) using Image J.

Cell wall composition determination

Air-dried wood sample was ground into powder and filtered through 60-mesh. According to our previous established protocol [73], alcohol insoluble residues (AIR) was firstly obtained by extracting the wood powder with 70% ethanol, chloroform/methanol (1:1 v/v) and acetone. Amylase and pullulanase in 0.1 M sodium acetate buffer (pH 5.0) were used to treat the extracted AIR overnight. For analysis of the sugar in hemicelluloses, AIR was treated with 2 M trifluoroacetic acid (TFA) at 121°C for 90 minutes. The supernatant was evaporated and incubated in 20 mg/ml fresh sodium borohydride solution at 40° for 90 minutes. The product then was neutralized with acetic acid and mixed with 1-methylimidazole and acetic anhydride for acetylation. After extraction with dichloromethane, the product was mixed with ethyl acetate for GC-MS (6890N GC system and 5975 Mass detector, Agilent Technologies, equipped with a SP-2380 capillary column, Supelco, Sigma-Aldrich) analysis. Meantime, the insoluble precipitate from the AIR treated with TFA was collected for crystalline cellulose content determination. The updegraff reagent (Acetic acid: nitric acid: water, 8:1:2 v/v) was added to the precipitate and incubated at 100 °C for 30 minutes. After washed with H_2O and acetone, the precipitate was incubated with 72% sulfuric acid at room temperature for 1 hour. The content of crystalline cellulose was determined by anthrone assay [74]. For lignin measurement, AIR was incubated with freshly prepared acetyl bromide (25%, acetyl bromide in acetic acid) at 50°C for 3 hours. After cooled, the AIR was mixed with 2M NaOH, 0.5 M fresh hydroxylamine hydrochloride and acetic acid. Lignin content was determined using a microplate reader (Varioskan Flash, Thermo) [75].

RNA isolation and RNA sequencing

Total RNA was extracted from wood-forming tissues using a mirVana miRNA Isolation Kit (Ambion-1561) following the manufacturer's instruction. After treated by RNase-free DNase I (Sigma, 4716728001), the quality of total RNA was assessed on NanoDrop spectrophotometer (NanoDrop 2000, Thermo Scientific) and on agarose gel electrophoresis. For RNA sequencing (RNA-seq), cDNA library was generated from 5 μg of total RNA with TruSeq Stranded mRNA LTSample Prep Kit (Illumina, RS-122-2101) and Agencourt AMPure XP (BECKMAN COULTER, A63881). cDNA library was qualified through length distribution of fragments using Agilent 2100 (Bioanalyzer). The 150 bp paired-end sequencing was performed using platform of Illumina HiSeq X10. About 5 million reads per samples were generated.

DNA isolation and bisulfite sequencing

Genomic DNA was extracted from wood-forming tissues using QIAamp DNA Mini kit (Cat.51306, Qiagen). DNA quantification and integrity were determined by a Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE) and 1% agarose electrophoresis, respectively. Before bisulfite treatment, lambda DNA was added to the purified DNA, which was used as an internal reference to calculate the conversion rate. Then, the mixed DNA was bisulfite treated using a Zymo Research EZ DNA methylaiton-Glod Kit (Zymo, D05005). Bisulfite sequencing (BS-seq) libraries were constructed by TruSeq® DNA Methylation Kit (Illumina, EGMK91396) following the manufacturer's instruction. After libraries were qualified, sequencing was performed on the Illumina HiSeq X Ten platform and 150 bp paired-end reads were generated.

Analysis of transcriptome sequencing data

Raw reads of sequencing were processed using NGS QC Toolkit to remove low-quality reads [76].The cleaned reads were mapped to *Populus trichocarpa*'s genome (http://phytozome.jgi.doe.gov/) using hisat2 with default parameters [77]. Gene expression level was measured as fragments per kilobase per million reads (FPKM) using cufflinks [78, 79]. Read counts for each gene in each sample were obtained using htseq-count and standardized by rlog [80]. Principle component analysis (PCA) was performed by plotPCA of DEseq2 R packge with default parameters. Differential expression genes (DEGs) were identified using the DESeq R package by estimation of Size Factors and nbinomTest. Analysis of DEGs with gene ontology (GO) enrichment and the Kyoto Encyclopedia of Genes and Genomes (KEGG) [81] pathway enrichment was performed using R based on the hypergeometric distribution.

Analysis of genome bisulfite sequencing

The raw reads of BS-seq were cleaned using Fastp [82] by removing adapters, ploy-N and low quality reads. The remaining high-quality clean reads were mapped to the *Populus trichocarpa*'s genome (http://phytozome.jgi.doe.gov/) using Bismark software with default parameters [83]. Methylcytosine (mC) sites were identified using MethylKit [84]. With default parameters, MethylKit was applied for PCA analysis. Differentially methylated regions (DMRs) were identified using MethylKit software with a Q-value (P-value corrected by FDR method) threshold of 0.05 and an absolute delta cutoff of 10% between

the two groups. Analysis of DMGs with GO enrichment and KEGG pathway enrichment was performed according to the same method used for DEGs analysis.

Quantitative real-time PCR

The first-strand cDNA was synthesized from 2 μg of total RNA using a cDNA Synthesis SuperMix (TransGen Biotech, AT311-03). Using cDNA as template, quantitative real-time PCR (qRT-PCR) was performed using *Perfectstart*™ Green qPCR SuperMix (TransGen, AQ601) and a Quantstudio™ 3 Real-Time PCR Detection System (Thermo). The primers used for selected genes are listed in Additional file 17 and *TUB9* was used as an internal control to normalize gene expression.

Abbreviations

JW
Juvenile Wood; MW:Mature Wood; RNA-seq:RNA sequencing; WGBS:Whole Genome Bisulfite Sequencing; BS-seq:Bisulfite sequencing; PCA:Principal Component Analysis; FPKM:Fragments Per Kilobase per Million reads; DEGs:Differential expression genes; GO:Gene Ontology; KEGG:Kyoto Encyclopedia of Genes and Genomes; AIR:Alcohol Insoluble Residues; TFA:Trifluoroacetic Acid; mC:Methylcytosine; DMRs:Differentially methylated regions; DMGs:Differentially Methylated Genes; qRT-PCR:Quantitative real-time PCR; BR:Brassinosteroids; GAs:gibberellins; ER:Endoplasmic Reticulum; TFs:transcription factors; CSC:cellulose synthase complex; UXS:UDP-glucuronic acid decarboxylase; UDP-GlcA:UDP-glucuronic acid

Declarations

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Availability of data and material

The data sets supporting the results of this article are included within the article and its additional file.

Author' Contributions

LFL performed experiments, analyzed data and wrote the manuscript. YYZ analyzed data and wrote the manuscript. JSG conducted RNA-seq analysis and wrote the manuscript. TMY prepared the tree samples and analyzed data. WCL and JQL analyzed data and wrote the manuscript. LGL conceived the project, analyzed data and wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have read and approved the final manuscript

Declarations

The authors declare that they have no competing interests.

References

1. Braatne JH, Rood SB, Heilman PE. **Life history, ecology, and conservation of riparian cottonwoods in North America**; 1996.
2. Owens JN. **The reproductive biology of lodgepole pine**. 2006.
3. Basheer-Salimia R. Juvenility, Maturity, and Rejuvenation in Woody Plants. Hebron University Research Journal. 2007;3:17–43.
4. Barrios A, Trincado G, Watt MS. Wood Properties of Juvenile and Mature Wood of Pinus radiata D. Don Trees Growing on Contrasting Sites in Chile. Forest Sci. 2017;63(2):184–91.
5. Moore JR, Cown DJ. Corewood (Juvenile Wood) and Its Impact on Wood Utilisation. Curr for Rep. 2017;3(2):107–18.
6. Fromm J. Xylem Development in Trees: From Cambial Divisions to Mature Wood Cells. Berlin Heidelberg: Springer. 2013;20:3–39.
7. Demura T, Fukuda H. Transcriptional regulation in wood formation. Trends Plant Sci. 2007;12(2):64–70.
8. Choi H, Dang TVT, Hwang I. Emergence of plant vascular system: roles of hormonal and non-hormonal regulatory networks. Curr Opin Plant Biol. 2017;35:91–7.
9. Israelsson M, Sundberg B, Moritz T. Tissue-specific localization of gibberellins and expression of gibberellin-biosynthetic and signaling genes in wood-forming tissues in aspen. Plant J. 2005;44(3):494–504.
10. Cosgrove DJ. Diffuse Growth of Plant Cell Walls. Plant Physiol. 2018;176(1):16–27.
11. Mcneil M, Darvill AG, Fry SC, Albersheim P. Structure and Function of the Primary-Cell Walls of Plants. Annu Rev Biochem. 1984;53:625–63.

12. Plomion C, Leprovost G, Stokes A. Wood Formation in Trees. Plant Physiol. 2001;127(4):1513–23.
13. Kumar M, Campbell L, Turner S. Secondary cell walls: biosynthesis and manipulation. J Exp Bot. 2016;67(2):515–31.
14. Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. Annu Rev Biochem. 2005;74:481–514.
15. He XJ, Chen TP, Zhu JK. Regulation and function of DNA methylation in plants and animals. Cell Res. 2011;21(3):442–65.
16. Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet. 2010;11(3):204–20.
17. Dowen RH, Pelizzola M, Schmitz RJ, Lister R, Dowen JM, Nery JR, Dixon JE, Ecker JR. Widespread dynamic DNA methylation in response to biotic stress. Proc Natl Acad Sci U S A. 2012;109(32):E2183–91.
18. Su YT, Bai XT, Yang WL, Wang WW, Chen ZY, Ma JC, Ma T. **Single-base-resolution methylomes of Populus euphratica reveal the association between DNA methylation and salt stress**. *Tree Genet Genomes* 2018, 14(6).
19. Ci D, Song YP, Tian M, Zhang DQ. **Methylation of miRNA genes in the response to temperature stress in Populus simonii**. *Frontiers in Plant Science* 2015, 6.
20. Wang WS, Pan YJ, Zhao XQ, Dwivedi D, Zhu LH, Ali J, Fu BY, Li ZK. Drought-induced site-specific DNA methylation and its association with drought tolerance in rice (Oryza sativa L.). J Exp Bot. 2011;62(6):1951–60.
21. Liang XL, Hou X, Li JY, Han YQ, Zhang YX, Feng NJ, Du JD, Zhang WH, Zheng DF, Fang SM. **High-resolution DNA methylome reveals that demethylation enhances adaptability to continuous cropping comprehensive stress in soybean**. *Bmc Plant Biol* 2019, 19.
22. Lafon-Placette C, Faivre-Rampant P, Delaunay A, Street N, Brignolas F, Maury S. Methylome of DNase I sensitive chromatin in Populus trichocarpa shoot apical meristematic cells: a simplified approach revealing characteristics of gene-body DNA methylation in open chromatin state. New Phytol. 2013;197(2):416–30.
23. Wang QS, Ci D, Li T, Li PW, Song YP, Chen JH, Quan MY, Zhou DT, Zhang DQ. **The Role of DNA Methylation in Xylogenesis in Different Tissues of Poplar**. *Frontiers in Plant Science* 2016, 7.
24. Fraga MF, Canal MJ, Rodriguez R. Phase-change related epigenetic and physiological changes in Pinus radiata D. Don. Planta. 2002;215(4):672–8.
25. Fraga MF, Rodriguez R, Canal MJ. Genomic DNA methylation-demethylation during aging and reinvigoration of Pinus radiata. Tree Physiol. 2002;22(11):813–6.
26. Enders TA, Strader LC. Auxin activity: Past, present, and future. Am J Bot. 2015;102(2):180–96.
27. Liu BB, Zhang J, Wang L, Li JB, Zheng HQ, Chen J, Lu MZ. A survey of Populus PIN-FORMED family genes reveals their diversified expression patterns. J Exp Bot. 2014;65(9):2437–48.
28. Klahre U, Noguchi T, Fujioka S, Takatsuto S, Yokota T, Nomura T, Yoshida S, Chua NH. The Arabidopsis DIMINUTO/DWARF1 gene encodes a protein involved in steroid synthesis. Plant Cell.

1998;10(10):1677–90.

29. Youn JH, Kim TW, Joo SH, Son SH, Roh J, Kim S, Kim TW, Kim SK. Function and molecular regulation of DWARF1 as a C-24 reductase in brassinosteroid biosynthesis in Arabidopsis. J Exp Bot. 2018;69(8):1873–86.
30. Jin J, Zhang H, Kong L, Gao G, Luo J. PlantTFDB 3.0: a portal for the functional and evolutionary study of plant transcription factors. Nucleic Acids Res. 2014;42(Database issue):D1182–7.
31. Eklund DM, Staldal V, Valsecchi I, Cierlik I, Eriksson C, Hiratsu K, Ohme-Takagi M, Sundstrom JF, Thelander M, Ezcurra I, et al. The Arabidopsis thaliana STYLISH1 Protein Acts as a Transcriptional Activator Regulating Auxin Biosynthesis. Plant Cell. 2010;22(2):349–63.
32. Pinon V, Prasad K, Grigg SP, Sanchez-Perez GF, Scheres B. Local auxin biosynthesis regulation by PLETHORA transcription factors controls phyllotaxis in Arabidopsis. P Natl Acad Sci USA. 2013;110(3):1107–12.
33. Zhong RQ, Lee CH, Ye ZH. Evolutionary conservation of the transcriptional network regulating secondary cell wall biosynthesis. Trends Plant Sci. 2010;15(11):625–32.
34. Hussey SG, Mizrachi E, Creux NM, Myburg AA. Navigating the transcriptional roadmap regulating plant secondary cell wall deposition. Front Plant Sci. 2013;4:325.
35. Li CF, Wang XQ, Ran LY, Tian QY, Fan D, Luo KM. PtoMYB92 is a Transcriptional Activator of the Lignin Biosynthetic Pathway During Secondary Cell Wall Formation in Populus tomentosa. Plant Cell Physiol. 2015;56(12):2436–46.
36. Wang SC, Li EY, Porth I, Chen JG, Mansfield SD, Douglas CJ. **Regulation of secondary cell wall biosynthesis by poplar R2R3 MYB transcription factor PtrMYB152 in Arabidopsis**. *Sci Rep-Uk* 2014, 4.
37. Zhong RQ, McCarthy RL, Lee C, Ye ZH. Dissection of the Transcriptional Program Regulating Secondary Wall Biosynthesis during Wood Formation in Poplar. Plant Physiol. 2011;157(3):1452–68.
38. Romano JM, Dubos C, Prouse MB, Wilkins O, Hong H, Poole M, Kang KY, Li E, Douglas CJ, Western TL, et al. AtMYB61, an R2R3-MYB transcription factor, functions as a pleiotropic regulator via a small gene network. New Phytol. 2012;195(4):774–86.
39. Haga N, Kobayashi K, Suzuki T, Maeo K, Kubo M, Ohtani M, Mitsuda N, Demura T, Nakamura K, Jurgens G, et al. Mutations in MYB3R1 and MYB3R4 cause pleiotropic developmental defects and preferential down-regulation of multiple G2/M-specific genes in Arabidopsis. Plant Physiol. 2011;157(2):706–17.
40. Seyfferth C, Wessels B, Jokipii-Lukkari S, Sundberg B, Delhomme N, Felten J, Tuominen H. Ethylene-Related Gene Expression Networks in Wood Formation. Front Plant Sci. 2018;9:272.
41. Vahala J, Felten J, Love J, Gorzsas A, Gerber L, Lamminmaki A, Kangasjarvi J, Sundberg B. A genome-wide screen for ethylene-induced ethylene response factors (ERFs) in hybrid aspen stem identifies ERF genes that modify stem growth and wood properties. New Phytol. 2013;200(2):511–22.
42. Genard M, Fishman S, Vercambre G, Huguet JG, Bussi C, Besset J, Habib R. A biophysical analysis of stem and root diameter variations in woody plants. Plant Physiol. 2001;126(1):188–202.

43. Mcqueenmason S, Durachko DM, Cosgrove DJ. 2 Endogenous Proteins That Induce Cell-Wall Extension in Plants. Plant Cell. 1992;4(11):1425–33.
44. Van Sandt VS, Suslov D, Verbelen JP, Vissenberg K. Xyloglucan endotransglucosylase activity loosens a plant cell wall. Ann Bot. 2007;100(7):1467–73.
45. Nishikubo N, Takahashi J, Roos AA, Derba-Maceluch M, Piens K, Brumer H, Teeri TT, Stalbrand H, Mellerowicz EJ. Xyloglucan endo-transglycosylase-mediated xyloglucan rearrangements in developing wood of hybrid aspen. Plant Physiol. 2011;155(1):399–413.
46. Gou JY, Miller LM, Hou GC, Yu XH, Chen XY, Liu CJ. Acetylesterase-Mediated Deacetylation of Pectin Impairs Cell Elongation, Pollen Germination, and Plant Reproduction. Plant Cell. 2012;24(1):50–65.
47. Kato S, Hayashi M, Kitagawa M, Kajiura H, Maeda M, Kimura Y, Igarashi K, Kasahara M, Ishimizu T. Degradation pathway of plant complex-type N-glycans: identification and characterization of a key alpha1,3-fucosidase from glycoside hydrolase family 29. Biochem J. 2018;475(1):305–17.
48. Spartz AK, Ren H, Park MY, Grandt KN, Lee SH, Murphy AS, Sussman MR, Overvoorde PJ, Gray WM. SAUR Inhibition of PP2C-D Phosphatases Activates Plasma Membrane H+-ATPases to Promote Cell Expansion in Arabidopsis. Plant Cell. 2014;26(5):2129–42.
49. McFarlane HE, Doring A, Persson S. The cell biology of cellulose synthesis. Annu Rev Plant Biol. 2014;65:69–94.
50. Song D, Shen J, Li L. Characterization of cellulose synthase complexes in Populus xylem differentiation. New Phytol. 2010;187(3):777–90.
51. Watanabe Y, Meents MJ, McDonnell LM, Barkwill S, Sampathkumar A, Cartwright HN, Demura T, Ehrhardt DW, Samuels AL, Mansfield SD. Visualization of cellulose synthases in Arabidopsis secondary cell walls. Science. 2015;350(6257):198–203.
52. Xi W, Song DL, Sun JY, Shen JH, Li LG. Formation of wood secondary cell wall may involve two type cellulose synthase complexes in Populus. Plant Mol Biol. 2017;93(4–5):419–29.
53. Polko JK, Kieber JJ. The Regulation of Cellulose Biosynthesis in Plants. Plant Cell. 2019;31(2):282–96.
54. Kuang BQ, Zhao XH, Zhou C, Zeng W, Ren JL, Ebert B, Beahan CT, Deng XM, Zeng QY, Zhou GK, et al. Role of UDP-Glucuronic Acid Decarboxylase in Xylan Biosynthesis in Arabidopsis. Mol Plant. 2016;9(8):1119–31.
55. Bernal AJ, Jensen JK, Harholt J, Sorensen S, Moller I, Blaukopf C, Johansen B, de Lotto R, Pauly M, Scheller HV, et al. Disruption of ATCSLD5 results in reduced growth, reduced xylan and homogalacturonan synthase activity and altered xylan occurrence in Arabidopsis. Plant J. 2007;52(5):791–802.
56. Liepman AH, Wilkerson CG, Keegstra K. Expression of cellulose synthase-like (Csl) genes in insect cells reveals that CslA family members encode mannan synthases. Proc Natl Acad Sci U S A. 2005;102(6):2221–6.
57. Verhertbruggen Y, Yin L, Oikawa A, Scheller HV. Mannan synthase activity in the CSLD family. Plant Signal Behav. 2011;6(10):1620–3.

58. Suzuki S, Li LG, Sun YH, Chiang VL. The cellulose synthase gene superfamily and biochemical functions of xylem-specific cellulose synthase-like genes in Populus trichocarpa. Plant Physiol. 2006;142(3):1233–45.
59. Vining KJ, Pomraning KR, Wilhelm LJ, Priest HD, Pellegrini M, Mockler TC, Freitag M, Strauss SH. Dynamic DNA cytosine methylation in the Populus trichocarpa genome: tissue-level variation and relationship to gene expression. BMC Genom. 2012;13:27.
60. Enders TA, Strader LC. Auxin Activity: Past, Present, and Future. Am J Bot. 2015;102(2):180–96.
61. Tang WQ, Kim TW, Oses-Prieto JA, Sun Y, Deng ZP, Zhu SW, Wang RJ, Burlingame AL, Wang ZY. BSKs mediate signal transduction from the receptor kinase BRI1 in Arabidopsis. Science. 2008;321(5888):557–60.
62. Li J, Wen JQ, Lease KA, Doke JT, Tax FE, Walker JC. BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell. 2002;110(2):213–22.
63. Nam KH, Li JM. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell. 2002;110(2):203–12.
64. Wang ZY, Nakano T, Gendron J, He JX, Chen M, Vafeados D, Yang YL, Fujioka S, Yoshida S, Asami T, et al. Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. Dev Cell. 2002;2(4):505–13.
65. Du J, Gerttula S, Li ZH, Zhao ST, Li-Liu Y, Liu Y, Lu MZ, Groover AT. Brassinosteroid regulation of wood formation in poplar. New Phytol. 2020;225(4):1516–30.
66. Fraga MF, Canal MJ, Rodriguez R. Phase-change related epigenetic and physiological changes in Pinus radiata D. Don. Planta. 2002;215(4):672–8.
67. Vining KJ, Pomraning KR, Wilhelm LJ, Priest HD, Pellegrini M, Mockler TC, Freitag M, Strauss SH. **Dynamic DNA cytosine methylation in the Populus trichocarpa genome: tissue-level variation and relationship to gene expression**. *Bmc Genomics* 2012, 13.
68. Matzke MA, Mosher RA. RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. Nat Rev Genet. 2014;15(6):394–408.
69. Guilfoyle TJ, Hagen G. Auxin response factors. Curr Opin Plant Biol. 2007;10(5):453–60.
70. Chen S, Ehrhardt DW, Somerville CR. Mutations of cellulose synthase (CESA1) phosphorylation sites modulate anisotropic cell expansion and bidirectional mobility of cellulose synthase. Proc Natl Acad Sci U S A. 2010;107(40):17188–93.
71. Speicher TL, Li PZQ, Wallace IS. **Phosphoregulation of the Plant Cellulose Synthase Complex and Cellulose Synthase-Like Proteins**. *Plants-Basel* 2018, 7(3).
72. Song DL, Xi W, Shen JH, Bi T, Li LG. Characterization of the plasma membrane proteins and receptor-like kinases associated with secondary vascular differentiation in poplar. Plant Mol Biol. 2011;76(1–2):97–115.
73. Yu LL, Chen HP, Sun JY, Li LG. PtrKOR1 is required for secondary cell wall cellulose biosynthesis in Populus. Tree Physiol. 2014;34(11):1289–300.

74. Foster CE, Martin TM, Pauly M. **Comprehensive compositional analysis of plant cell walls (lignocellulosic biomass) part II: carbohydrates**. *J Vis Exp* 2010(37).
75. Foster CE, Martin TM, Pauly M. **Comprehensive compositional analysis of plant cell walls (Lignocellulosic biomass) part I: lignin**. *J Vis Exp* 2010(37).
76. Patel RK, Jain M. **NGS QC Toolkit: A Toolkit for Quality Control of Next Generation Sequencing Data**. *Plos One* 2012, 7(2).
77. Kim D, Landmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. Nat Methods. 2015;12(4):357-U121.
78. Roberts A, Trapnell C, Donaghey J, Rinn JL, Pachter L. **Improving RNA-Seq expression estimates by correcting for fragment bias**. *Genome Biology* 2011, 12(3).
79. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol. 2010;28(5):511-U174.
80. Anders S, Pyl PT, Huber W. HTSeq-a Python framework to work with high-throughput sequencing data. Bioinformatics. 2015;31(2):166–9.
81. Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, Katayama T, Kawashima S, Okuda S, Tokimatsu T, et al. KEGG for linking genomes to life and the environment. Nucleic Acids Res. 2008;36:D480–4.
82. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34(17):i884–90.
83. Krueger F, Andrews SR. Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. Bioinformatics. 2011;27(11):1571–2.
84. Akalin A, Kormaksson M, Li S, Garrett-Bakelman FE, Figueroa ME, Melnick A, Mason CE. **methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles**. *Genome Biology* 2012, 13(10).

Figures

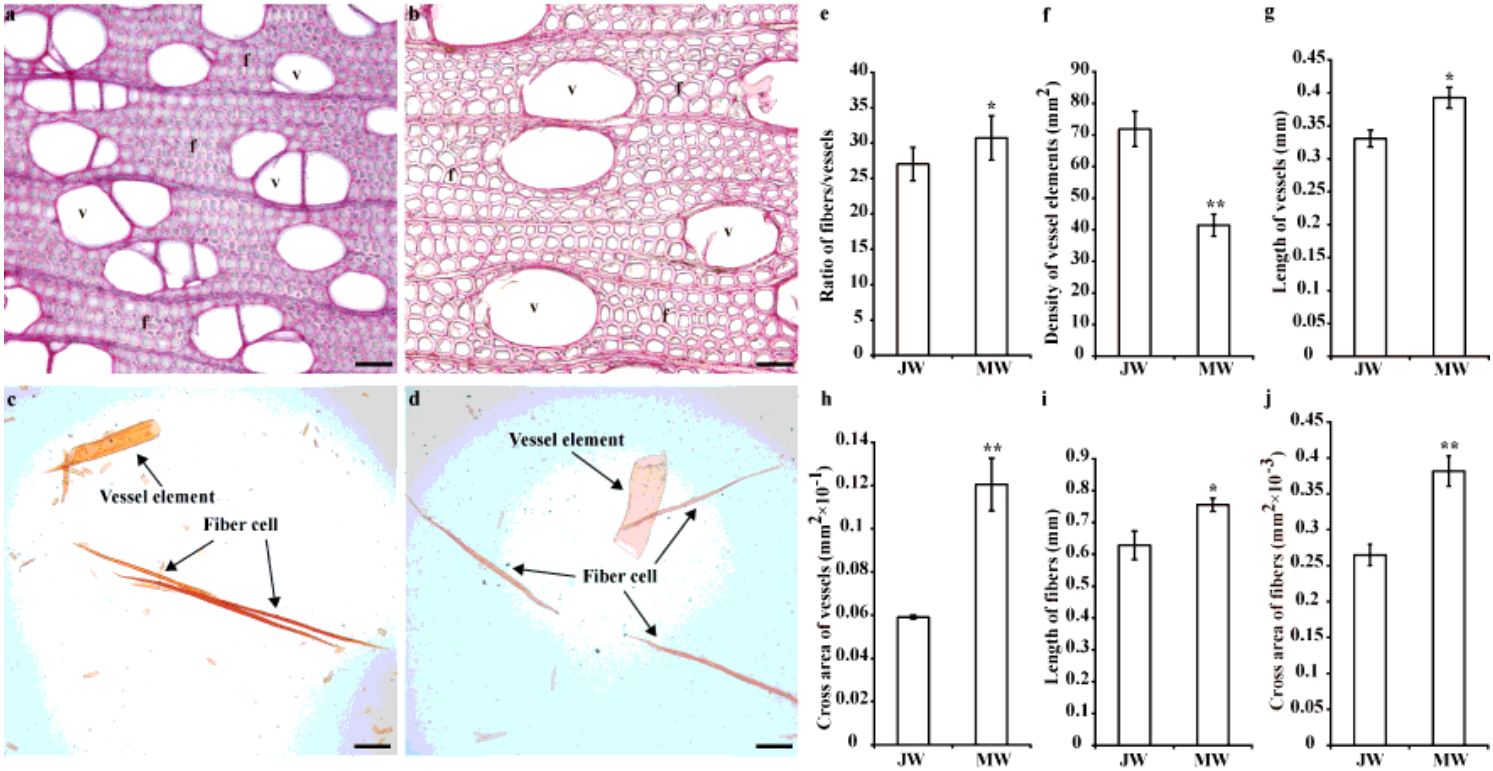

Figure 1

Properties of the wood produced in juvenile phase (JP) and mature phase (MP). (a) and (b) Wood sections stained with phloroglucinol from juvenile wood (JW) (a) and mature wood (MW) (b). Bars=50 μm. (c) and (d) Fibers and vessels from JW (c) and MW (d). Bars=200 μm. (e) Ratio of numbers of vessel/fiber. (f) Density of vessel elements. (g) and (h) Length and cross area of vessel. The values were means ± SE of 500 and 800 independent vessels from JW and MW, respectively. (i) and (j) Length and cross area of fiber. The values were means ± SE of 1000 independent fibers from JW and MW, respectively. Significance was determined by Student's t-test (* p≤0.05 and **p≤0.01). f, fiber cell; v, vessel elements.

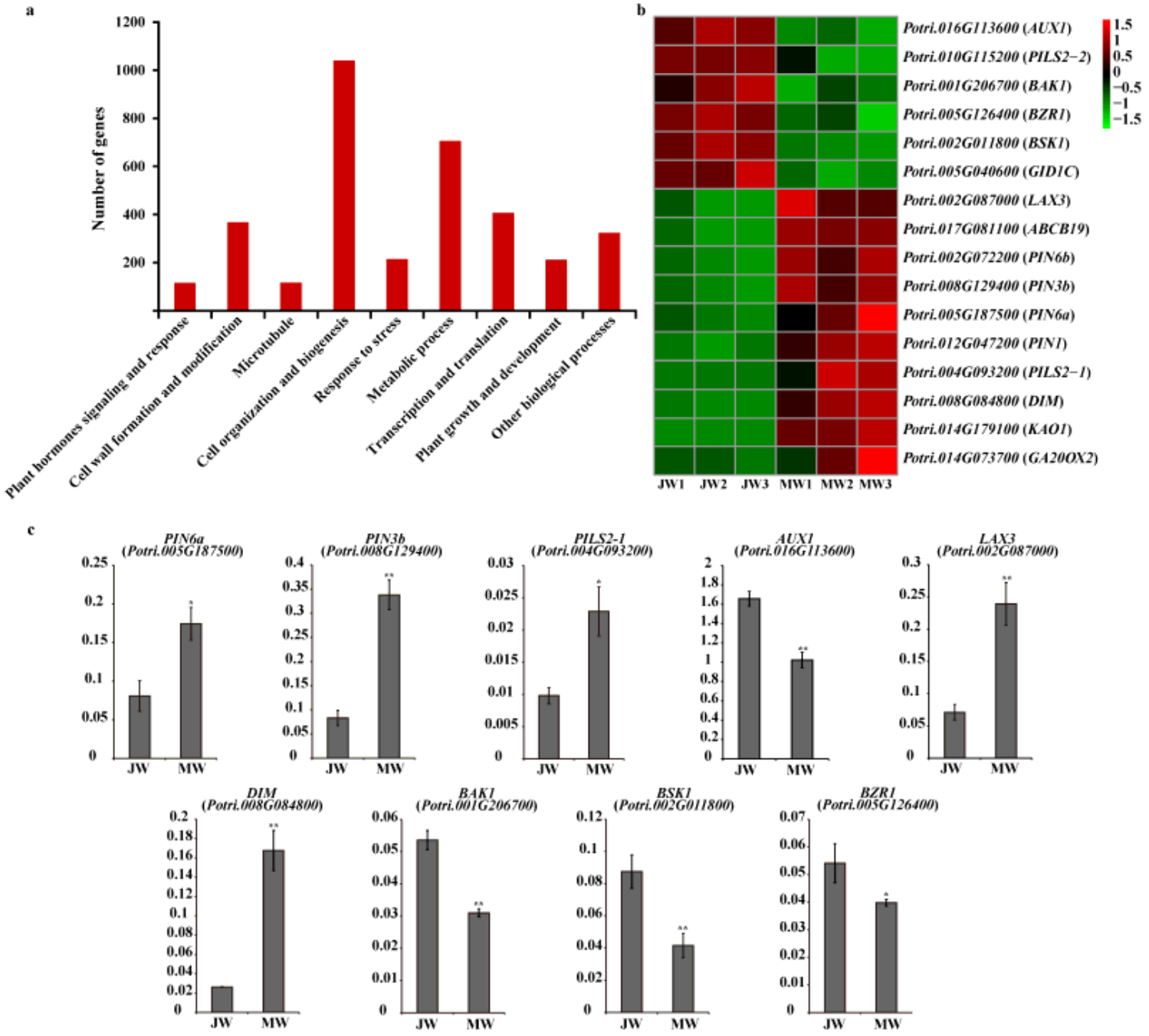

Figure 2

(a) the functional of the differentially expressed genes (DEGs). (b) The heat map of genes related to plant hormones. (c) The results of quantitative real-time PCR of selected genes related to plant hormones in JW and MW. Significance was determined by Student's t-test (* p□0.05 and **p□0.01).

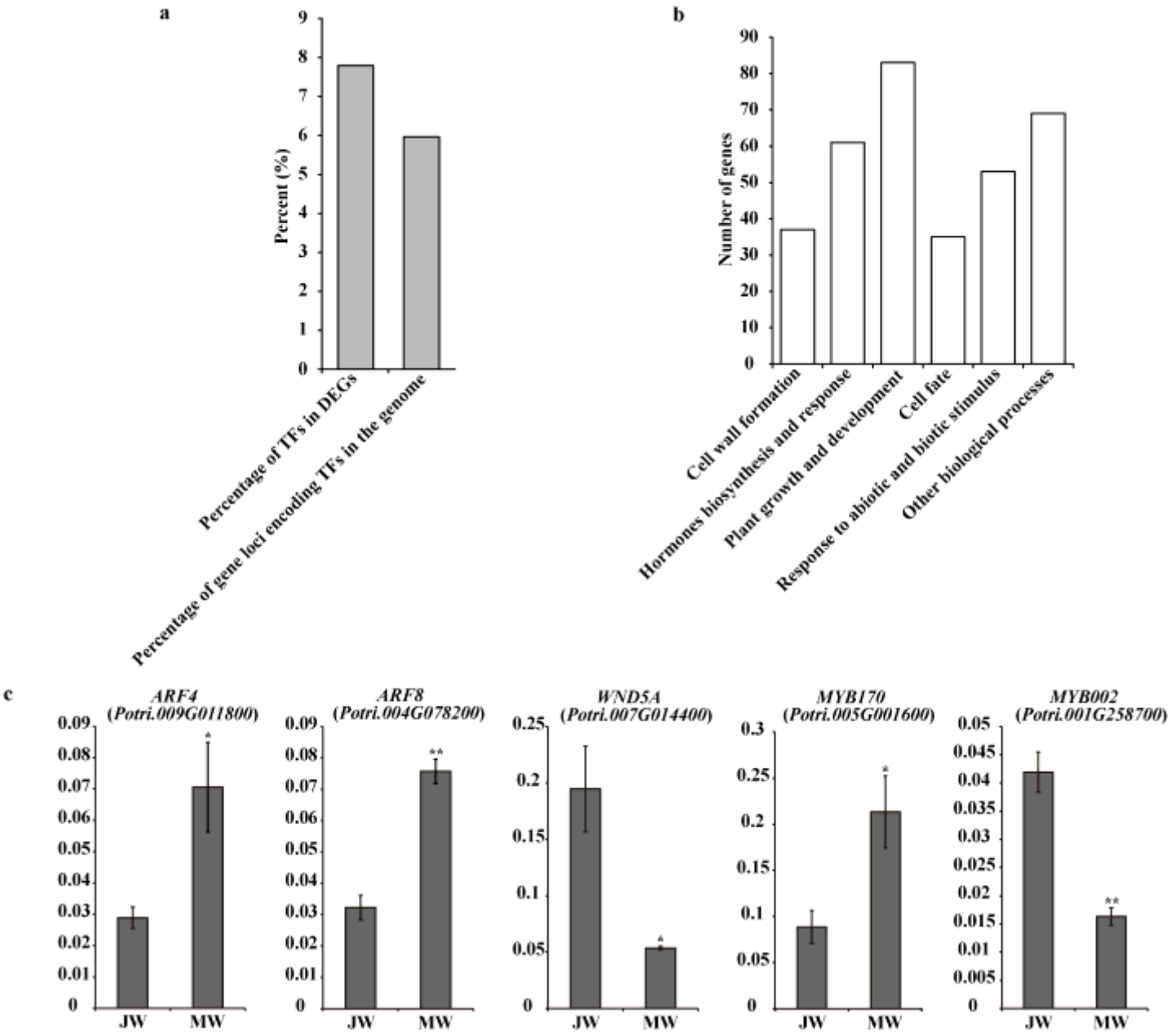

Figure 3

Transcriptional factors in DEGs. (a) Number of TF genes in detected DEGs. (b) Functions of the TFs among DEGs. (c) The results of quantitative real-time PCR of selected TFs in JW and MW. Significance was determined by Student's t-test (* p<0.05 and ** p<0.01).

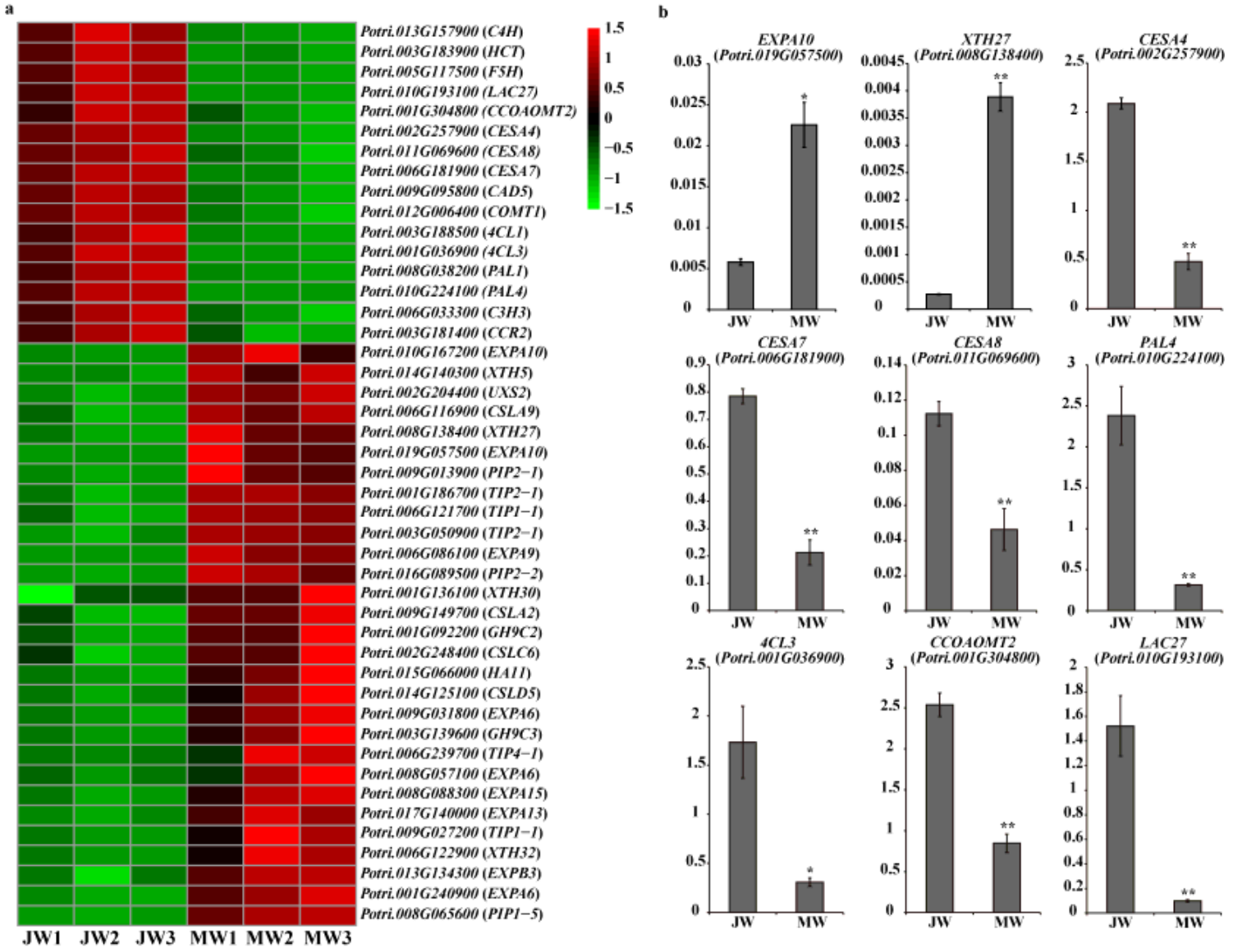

Figure 4

Differentially expressed genes involved in cell wall formation in JW and MW. (a) The heat map of genes related to cell wall formation. (c) The results of quantitative real-time PCR of selected genes related to cell wall formation in JW and MW. Significance was determined by Student's t-test (* p□0.05 and **p□0.01).

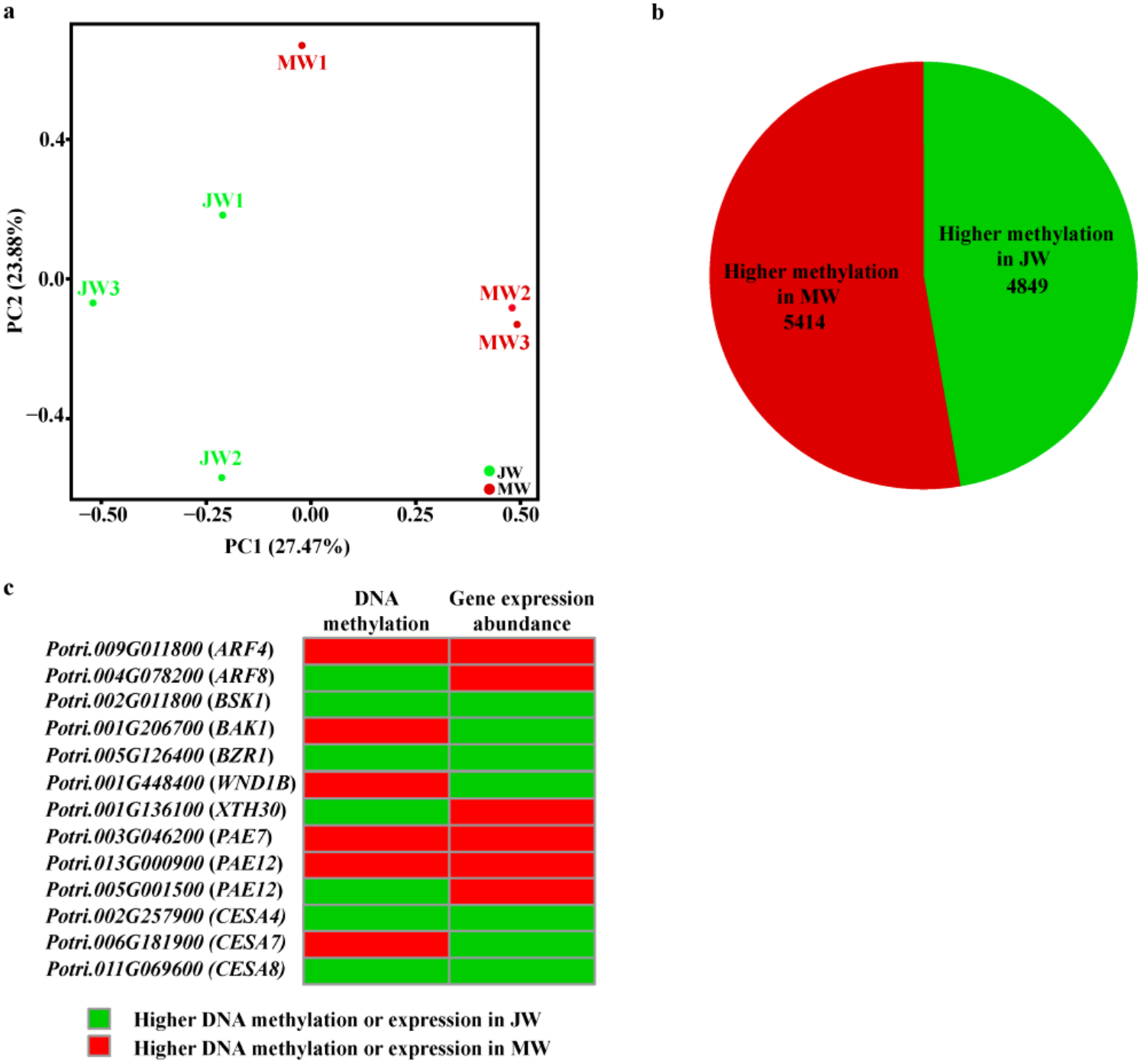

Figure 5

The methylation profile of JW and MW. (a) Principal Component Analysis (PCA) ofDNA methylation in each sample. (b) The number of higher methylation genes in DMGs. (c) Methylation and expression of genes related to wood formation.

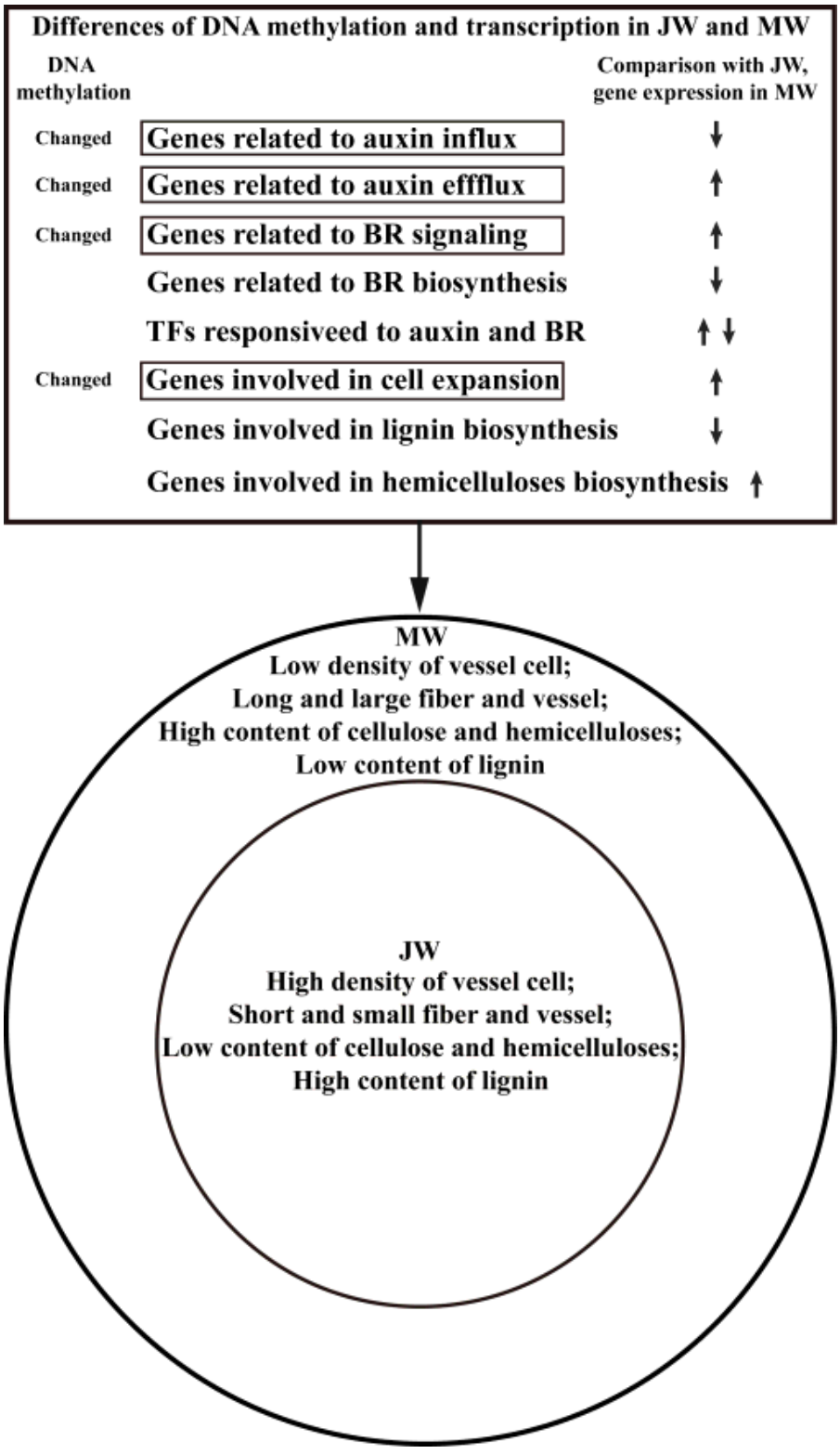

Figure 6

Outline of the DAN methylation and transcription regulation in formation of JW and MW.

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