3 Biotech (2018) 8:315
https://doi.org/10.1007/s13205-018-1344-6

ORIGINAL ARTICLE

Co-expression network of transcription factors reveal ethylene-responsive element-binding factor as key regulator of wood phenotype in *Eucalyptus tereticornis*

Veeramuthu Dharanishanthi[1] · Modhumita Ghosh Dasgupta[1]

Received: 5 May 2018 / Accepted: 9 July 2018 / Published online: 13 July 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Suitability of wood biomass for pulp production is dependent on the cellular architecture and composition of secondary cell wall. Presently, systems genetics approach is being employed to understand the molecular basis of trait variation and co-expression network analysis has enabled holistic understanding of complex trait such as secondary development. Transcription factors (TFs) are reported as key regulators of meristematic growth and wood formation. The hierarchical TF network is a multi-layered system which interacts with downstream structural genes involved in biosynthesis of cellulose, hemicelluloses and lignin. Several TFs have been associated with wood formation in tree species such as *Populus, Eucalyptus, Picea* and *Pinus*. However, TF-specific co-expression networks to understand the interaction between these regulators are not reported. In the present study, co-expression network was developed for TFs expressed during wood formation in *Eucalyptus tereticornis* and ethylene-responsive element-binding factor, *EtERF2*, was identified as the major hub transcript which co-expressed with other secondary cell wall biogenesis-specific TFs such as *EtSND2, EtVND1, EtVND4, EtVND6, EtMYB70, EtGRAS* and *EtSCL8*. This study reveals a probable role of ethylene in determining natural variation in wood properties in *Eucalyptus* species. Understanding this transcriptional regulation underpinning the complex bio-processing trait of wood biomass will complement the *Eucalyptus* breeding program through selection of industrially suitable phenotypes by marker-assisted selection.

Keywords Co-expression network · Ethylene · Regulation · Transcription factor · Wood formation

Introduction

Wood is one of the most abundant renewable biological resources serving multiple functions including mechanical support, water conduction, nutrient storage and defense. Wood property is a highly plastic trait with significant intra- and inter-specific variation in phenotypes. Natural genetic variations in wood phenotypes occur due to multifactorial genetic perturbations which are further confounded by external factors such as photoperiod, nutrient availability, moisture content and temperature (Groover et al. 2010). Hence, genome-wide analysis is presently used to comprehend the genetic basis governing this naturally occurring developmental variation. The present understanding of secondary development is predominantly based on single-gene variations affecting the wood composition (Vanholme et al. 2012). However, systems model which integrates multi-level biological information is an effective strategy to study the spectrum of natural phenotypic variation occurring due to genome-wide perturbations (Mizrachi and Myburg 2016; Ingvarsson and Street 2011).

Transcription factors (TFs) are one of the critical regulators of meristematic growth and development (Long and Benfey 2006). Molecular and genetic analyses of xylem development have uncovered master transcriptional regulators that control the expression of secondary cell wall biosynthetic genes. The major TFs associated with this developmental stage include members of NAC, MYB, HB,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-018-1344-6) contains supplementary material, which is available to authorized users.

✉ Modhumita Ghosh Dasgupta
modhumitaghosh@hotmail.com; ghoshm@icfre.org

[1] Division of Plant Biotechnology and Cytogenetics, Institute of Forest Genetics and Tree Breeding, R.S. Puram, Coimbatore 641002, Tamil Nadu, India

zinc finger, LIM domain, MADS-box, WRKY, ARF, AP2-EREBP, ARID, AUX-IAA, bHLH, AS2/LOB and bZIP. Several reviews have summarized the transcriptional regulation of secondary wall biogenesis (Ohashi-Ito and Fukuda 2010; Zhong et al. 2010; Wang and Dixon 2012; Zhang et al. 2011; Schuetz et al. 2013; Zhong and Ye 2014; Ye and Zhong 2015; Jokipii-Lukkari et al. 2018).

Eucalyptus is one of the widely planted hardwood species because of its superior growth, adaptability and amenable wood properties for pulp, bioenergy and lignicellulosic products (Mizrachi et al. 2017). Molecular regulation of wood formation in Eucalypts has been widely documented (Kirst et al. 2005; Barros et al. 2009; Salazar et al. 2013; Thavamanikumar et al. 2014; Hefer et al. 2015; Shinya et al. 2016; Dharanishanthi and Ghosh Dasgupta 2016; Mizrachi et al. 2017). However, only few TFs regulating wood formation have been characterized in *Eucalyptus* sp. till date (Goicoechea et al. 2005; Legay et al. 2010; Hussey et al. 2011; Negishi et al. 2011; Yu et al. 2015; Dharanishanthi and Ghosh Dasgupta 2016; Soler et al. 2017). Further, the interaction of wood-related TFs in naturally occurring phenotypes with distinct wood properties in relation to holocellulose and lignin content is not reported in this genus. Hence, a co-expression network specific to TFs was constructed in the present study to identify key TF regulators governing natural variation in wood property in *E. tereticornis*.

Materials and methods

TFs were mined from the expression datasets of 18,987 transcripts (GEO Accession number GSE73030) (Dharanishanthi and Ghosh Dasgupta 2016) using Wood-Formation Related Genes database (WFRGdb, http://me.lzu.edu.cn/woodformation/). Further, *Eucalyptus* nucleotide sequences were used to search the complete protein sequences of *Arabidopsis* using BlastX with (*e* value cutoff of 1^{e-5}) in the non-redundant database of NCBI and TAIR (v10) and the best hits (lowest *e* value) were selected as *Arabidopsis* orthologs.

Expression profiles of TFs in four *E. tereticornis* genotypes (SWMG-6 and CW-8 with low lignin/high cellulose content; KUP-14 and NKR-49 with low cellulose and high lignin content) were sourced from the earlier study (Dharanishanthi and Ghosh Dasgupta 2016). The fold expression data were filtered for significantly regulated (up- and down-regulated) transcripts across all genotypes and transcripts exhibiting ± 0.8-fold difference in expression with a statistical significance of $p < 0.05$ were considered as differentially regulated. Hierarchical clustering was conducted with the CIMminer (http://discover.nci.nih.gov/cimminer/home.do) software for the two distinct groups to document gene expression clusters specific to the phenotypes.

The array used in the present study had 1190 TFs representing all major families. For construction of the co-expression network, the expression profiles of all TFs represented in the array was considered. A correlation matrix of all transcripts was made by calculating pair-wise Pearson correlation coefficient using normalized expression value across all genotypes using Co-Express 1.5 software (http://www.bioinformatics.lu/CoExpress/) with threshold of > 0.97. The number of significantly co-expressed TFs was 697. The TFs were functionally annotated and their position in chromosome, protein domains, and gene ontology were defined based on the recent genome assembly of *E. grandis* using Phytozome v12.1. GO enrichment was done using EUCANEXT (http://bioinfo03.ibi.unicamp.br/eucalyptusdb/) and pathway analysis was conducted in Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa and Goto 2000; Kanehisa et al. 2012).

The transcription factor network was constructed with 697 nodes and 14,017 edges using Cytoscape software (http://www.cytoscape.org) using default parameters (Shannon et al. 2003). Each node represented a transcript and the edge represented the strength of co-expression. Duplicate edges and self loops were manually removed from the network. The degree was calculated for each node based on the number of edges connected to a node. Highly connected nodes (Hub TFs) were identified from the network. Subsequently, guided gene networks were constructed for major secondary cell wall biogenesis-related TFs including *EtERF2, EtVND7, EtSND2* and *EtMYB103*.

Results

The functional annotation of the selected TFs revealed that they spanned all 11 chromosomes with maximum representation of 95 TFs in chromosome 6 and minimum representation of 41 in chromosome 4 (Supplementary table 1). GO mapping of transcripts categorized 373 TFs under biological process, 817 under molecular functions and cellular components included 156 TFs (Supplementary Fig. 1). Pathway analysis revealed that maximum number of TFs represented the plant hormone signal transduction pathways.

The major families of TFs represented in the array belonged to MYB, NAC, WRKY, VND, SND1, LIM, HD-ZIP, Znf and bZIP. The major TFs and their representation in the co-expression network are presented in Table 1.

Differential transcript expression revealed that the fold expression of TFs ranged from −5.40 to 6.36 and maximum expression was documented in *bHLH* (5.59), *LRR* (4.71), *Znf-C3HC4* (6.36) and *TDR* (3.68), while *TDR* (−4.23), *MYB91* (−3.11), *WRKY40* (−2.99) and *WRKY23* (−5.40) documented minimum expression across SWMG-6, CW-8, KUP-14 and NKR-49, respectively. Comparison of the expression data

Table 1 Details of major transcription factor families used in co-expression network construction

Transcription factor family	Functional domain	No. of transcripts
AP2-EREBP	AP2	84
ARF	Auxin_resp	15
AUX-IAA	AUX_IAA	35
bHLH	HLH	57
bZIP	bZIP_1 bZIP_2 bZIP_Maf	35
GRAS	GRAS	41
HSF	HSF_DNA-bind	28
MYB/MYB related	Myb_DNA binding	105
NAC	NAM	50
PHD	PHD	30
HB	Homeobox/ HD-ZIP	46
SBP	SBP	21
WRKY	WRKY	36

across the two phenotypic groups revealed similar expression patterns (Fig. 1). In SWMG-6 and CW-8, the fold expression ranged from (−4.23) (*TDR*) to 5.59 (*bHLH*), while in KUP-14 and NKR-49 the fold expression ranged from −5.40 (*WRKY23*) to 6.36 (*Znf-C3HC4*). No distinct expression pattern across the two phenotypic groups was documented.

The TF co-expression network was constructed with 697 nodes and 14,017 edges. The degree of the network ranged from 5 to 69. The major TFs with high degree of interactions included *EtERF2, EtVND7, EtWRKY70, EtSND2, EtMYB103* and*Et HD-ZIP1*. *EtERF2* was identified as top hub gene with 69° (Fig. 2). The details of the TFs interacting with the top ten hub transcripts are shown in Table 2.

Guided network of few major secondary cell wall biogenesis-related TFs was constructed. *EtERF2* which co-expressed with 69 other TFs interacted with known cell wall biogenesis-related TFs such as *EtSND2, EtVND1, EtVND4, EtVND6, EtMYB70, EtGRAS* and *EtSCL8* (Fig. 3a). Another xylogenesis-specific TF, *EtVND7* (NAC family TF), identified as the second major hub transcript, co-expressed with 68 transcripts including *EtARF16, EtMYB20, EtMYB36, EtMYB70, EtNAC075* and *EtWRKY 21* (Fig. 3b). The known xylogenesis master regulator *EtSND2* interacted with 64 TFs including *EtMYB70, EtVND1, EtVND4, EtVND6, EtWRKY41, EtbZIP* and *EtbHLH92* (Fig. 3c). *EtMYB103* interacted with its paralogs such as *EtMYB4R, EtMYB5, EtMYB61* and *EtMYB70* (Fig. 3d).

Discussion

In tree species, the composition of secondary cell wall determines its industrial utility and hence its biogenesis involving physiological, biochemical and molecular processes has been a topic of active research in tree science (Andersson-Gunnerås et al. 2006; Du and Groover 2010; Zhong et al. 2010; Wang and Dixon 2012; Hussey et al. 2013; Zhong and Ye 2014; Hefer et al. 2015; Shinya et al. 2016; Jokipii-Lukkari et al. 2018). However, secondary growth leading to wood formation is an exceptionally complex developmental event controlled by numerous gene families involved in the biosynthesis of polysaccharides, lignin and cell wall proteins. Hence, integrating multiple level of trait information using system model enables characterization of individual genes, pathways and gene networks operational during secondary development (Mizrachi and Myburg 2016; Mizrachi et al. 2017; Jokipii-Lukkari et al. 2018).

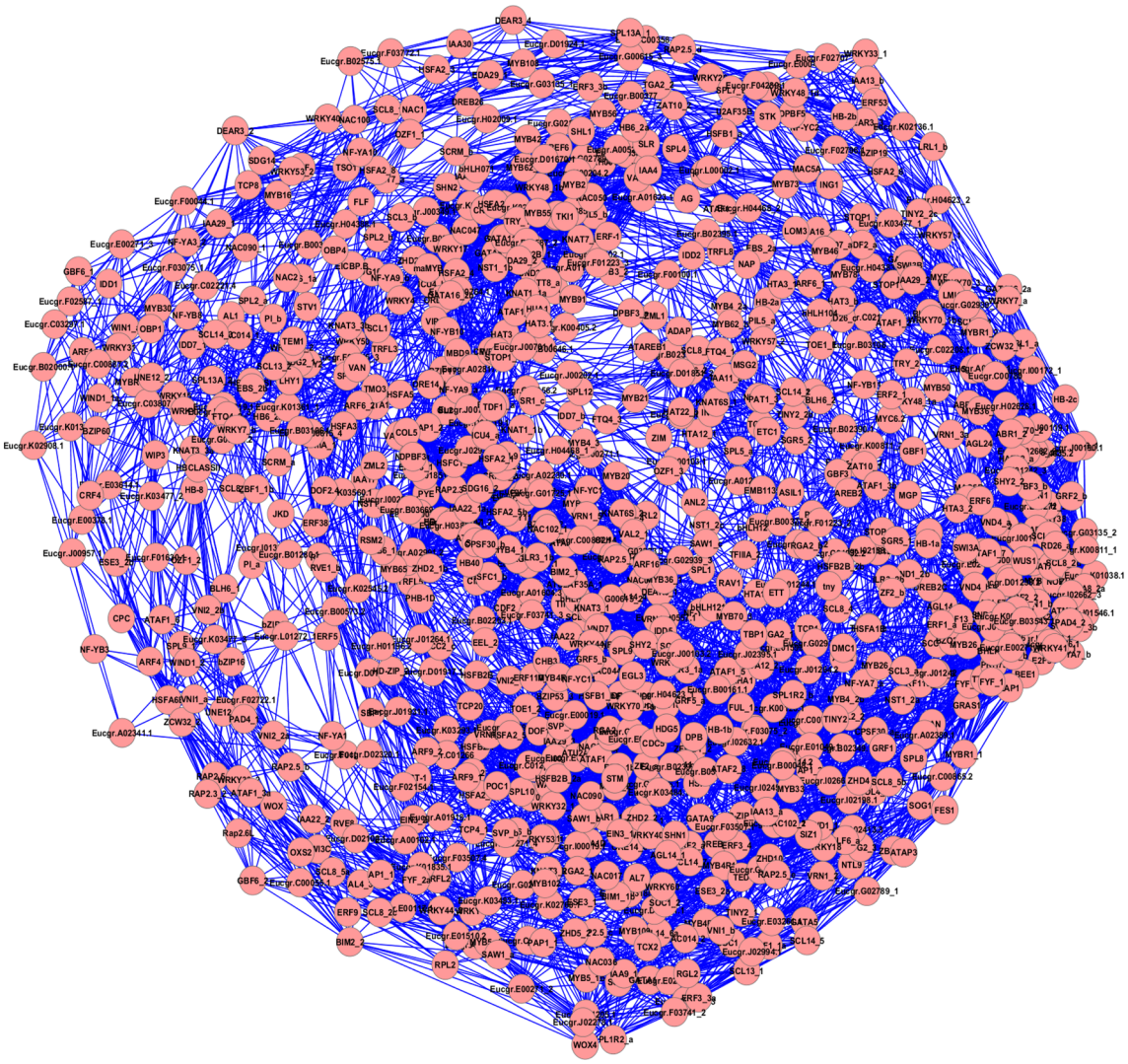
Transcriptional regulation of wood formation

Wood formation is governed by multi-tier regulation at spatial and temporal levels (Zhang et al. 2014). The anatomical variation in wood is primarily governed by integration of environmental and developmental cues to the molecular process of transcriptional regulation (Du and Groover 2010). Hierarchical control of wood formation by TF networks has been documented (Li et al. 2012; Zhong and Ye 2014). This network is a complex multi-leveled feed-forward loop regulatory system (Zhong and Ye 2014) and several families of TFs interact to regulate secondary cell wall development (Cassan-Wang et al. 2013; Xu et al. 2015; McCarthy et al. 2009; Sundell et al. 2017; Sakamoto et al. 2016; Shi et al. 2017; Jokipii-Lukkari et al. 2018). This hierarchical-tier system involves the secondary cell wall-related NAC TFs (*SND1, NST1/2* and *VND6/7*) as top level master regulators, while *SND3, XND* and *MYBs* are reported to act as second-level switches interacting with downstream TFs such as *BES1, SND2, C3H14, KNAT7* and cell wall-related structural genes involved in biosynthesis of secondary cell wall components (Cassan-Wang et al. 2013; Hussey et al. 2013; Zhong and Ye 2014; Ye and Zhong 2015; Lamara et al. 2016). In the present study, TF-specific co-expression network in *E. tereticornis* identified several hub TFs with high degree of interactions including *EtERF2, EtVND7, EtWRKY70, EtSND2, EtMYB103* and *Et HD-ZIP1*, which is in agreement with earlier studies.

Ethylene-mediated transcriptional regulation of secondary development

The role of phytohormones in regulating vascular cambium activity has been intensively studied (reviewed by

Fig. 1 Hierarchical clustering of transcription factors in *E. tereticornis* genotypes



Nodes represented in brown color
Edges depicted in blue color

Fig. 2 Co-expression network of transcription factors in *E. tereticornis*

Ye and Zhong 2015). Ethylene, a multifunctional phytohormone is known to regulate secondary development in short-lived plants and wood formation in woody perennials. On exogenous application, it stimulates cambial growth and reaction wood formation (Du and Yamamoto 2007; Love et al. 2009; Felten et al. 2018). The role of ethylene as stimulator of cambial activity is reported from Arabidopsis (Etchells et al. 2012), poplar (Love et al. 2009; Andersson-Gunnerås et al. 2003; Vahala et al. 2013) and *Picea abies* (Ingemarsson 1995). Further, accumulation of ethylene during TW formation is also reported (Andersson-Gunnerås et al. 2003; Du and Yamamoto 2003).

Ethylene-mediated transcriptional regulation is reported to occur in all developmental stages of secondary growth including secondary cell wall formation, cambium–xylem expansion phase, xylem maturation, lignin and xylem biosynthesis (Seyfferth et al. 2018). The APETALA2/Ethylene-Responsive element-binding Factor (AP2/ERF) is a plant-specific TF superfamily and their regulatory function in plant development is well established (Phukan et al. 2017). Heterologous expression of *ERFs* in transgenic systems including poplars (Felten et al. 2011, 2018; Vahala et al.

Table 2 Details of top listed hub transcription factors identified in the co-expression network

TF ID	Description	TF family	Domain	Degree
ERF2	Ethylene-responsive element-binding factor 2	AP2-EREBP	AP2	69
VND7	NAC 007	NAC	NAM	68
AP2L	AGAMOUS-like 8	MADS	SRF-TF	68
VND6	NAC 007	NAC	NAM	67
PAD4	alpha/beta-Hydrolases superfamily protein	C2H2	zf-C2H2	66
Eucgr.D01238.2	Zinc finger (CCCH-type/C3HC4-type RING finger) family protein	C3H	zf-CCCH	66
SHR	GRAS family transcription factor	GRAS	GRAS	66
WRKY70	WRKY DNA-binding protein 70	WRKY	WRKY	65
NF-YA7	Nuclear factor Y, subunit A7	CCAAT	CBFB_NFYA CBFD_NFYB_HMF CCAAT-Dr1 NF-YB NF-YC	65
Eucgr.E02477	Transducin/WD40 repeat-like superfamily protein	C3H	zf-CCCH	64
WRKY38	WRKY DNA-binding protein 38	WRKY	WRKY	64
BEE1	BR-enhanced expression 1	bHLH	HLH	63
HD-ZIP1	Homeobox-leucine zipper family protein/lipid-binding START domain-containing protein	HB	Homeobox KNOX1 KNOX2	63
SND2	NAC domain-containing protein 73	NAC	NAM	63
WRKY41	WRKY family transcription factor	WRKY	WRKY	63

2013; Felten and Sundberg 2013), *Isatis indigotica* (Ma et al. 2017); rice (Ambavaram et al. 2011) and tobacco (Liu et al. 2017) had resulted in significant changes in cell wall composition. Similarly, over-expression of an ACC oxidase, *PttACO1* increased wood formation in poplar (Love et al. 2009). In *Eucalyptus* hybrid (*Eucalyptus urophylla* × *Eucalyptus grandis)*, two AP2/ERF transcripts were strongly up-regulated in genotypes with high lignin and low cellulose content (Shinya et al. 2016).

The only study to understand the role of ethylene-related genes using co-expression network was reported in poplar (Seyfferth et al. 2018). The network identified *EIN3D* and 11 ERFs as hub transcripts. Additionally, 221 TFs belonging to *NAC, MYB, C2H2, bZIP, bHLH, ERF*, and *TALE* TF families were also documented in the network. A similar co-expression pattern was documented in the present study and *EtERF2* was identified as the top hub transcript which interacted with 69 other TFs such as *EtSND2, EtVND1, EtVND4, EtVND6* and *EtMYB70*. The occurrence of known secondary cell wall-related TFs such as SND2 in the poplar TF network is in agreement with the present study in *E. tereticornis*. These studies support the hypothesis that transcriptional mechanisms governing wood formation in angiosperms is conserved across lineages but are modulated by environmental, experimental or genetic perturbations (Zinkgraf et al. 2017).

Conclusion

Documenting molecular phenotypes generated due to transcript expression patterns enable better understanding of trait variation (Porth et al. 2013; Du et al. 2016; Thavamanikumar et al. 2014). Hence, integration of systems genetics approaches with marker identification has been advocated by several research groups (Feltus 2014; Baute et al. 2016; De Maeyer et al. 2016; Basnet et al. 2016). Co-expression networks have enabled identification of candidates for marker assays (Verbeke et al. 2013) and increased the reliability of eQTL association analysis in humans (Jia and Zhao 2014). In a recent study, network-based association analysis was reported in *E. grandis* × *E. urophylla* and expression patterns of transcripts governing secondary cell wall biosynthesis was correlated with structure and processability of wood (Mizrachi et al. 2017). In the present study, several candidate TFs with distinct expression variation across *E. tereticornis* phenotypes were identified in the co-expression network which could presumably affect the wood composition in the species. The study has identified major hub TFs which can act as candidates for marker discovery and accelerate *Eucalyptus* breeding for productivity improvement through selection of industrially suitable phenotypes by marker-assisted selection.

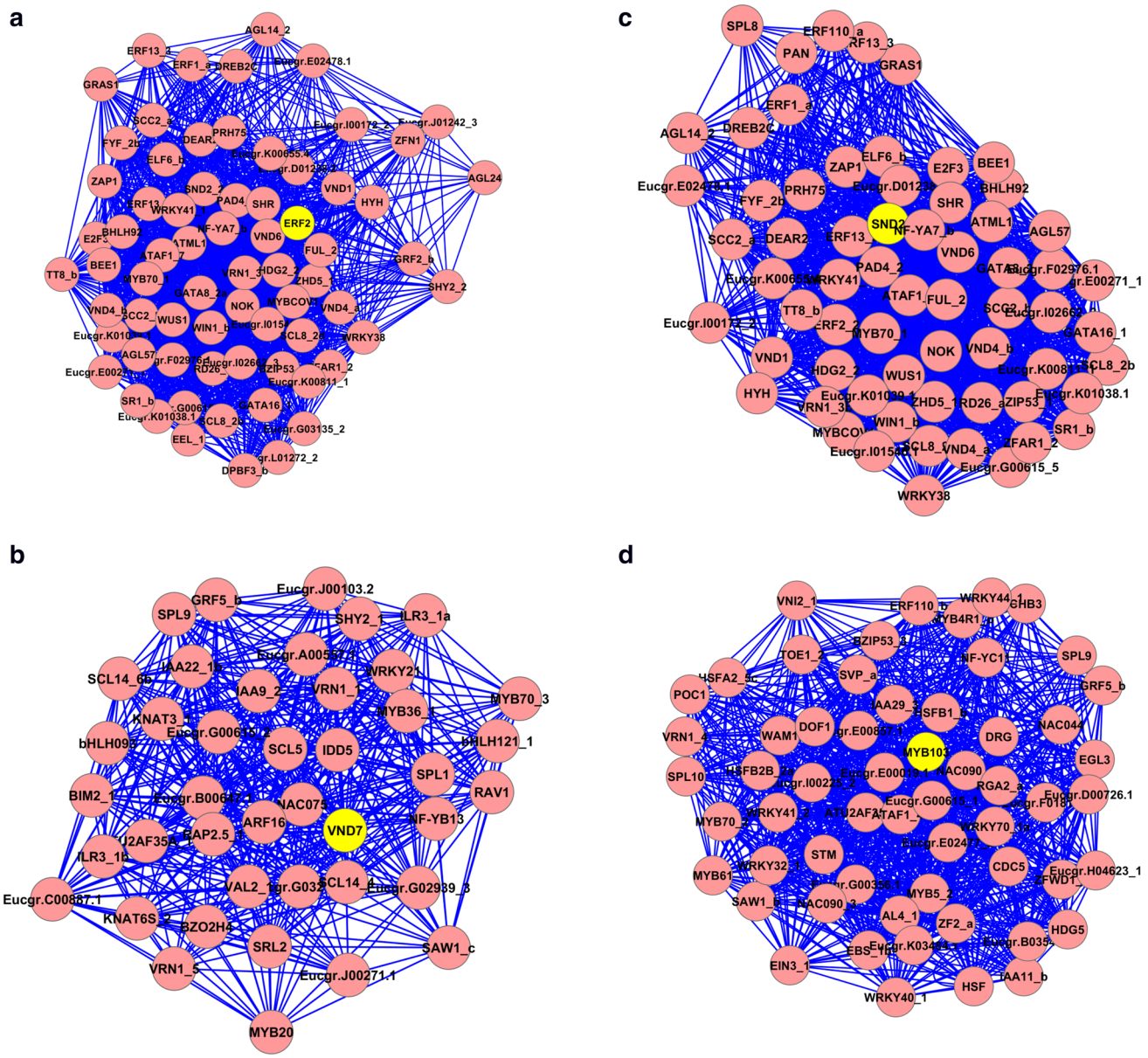


Fig. 3 **a** Guided network of *EtERF2*. **b** Guided network of *EtVND7*. **c** Guided network of *EtSND2*. **d** Guided network of *EtMYB103*

Acknowledgements We acknowledge the guidance given by Dr. Jennifer Dewoody, National Forest Genetics Laboratory, USDA Forest Service, Placerville, CA; Dr. Sucheta Tripathy, Structural Biology and Bio-informatics Division, Indian Institute of Chemical Biology, Kolkata, India; Dr. Saravanakumar Selvaraj, Centre for Pharmacology and Toxicology, Hannover Medical School, Hannover, Germany, and Dr. Gayatri Ramakrishnan, Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India, for developing gene regulatory networks. The funding for the research work was provided to Dr. Modhumita Ghosh Dasgupta by Department of Biotechnology, Government of India, under the research project with grant number BT/PR10055/PBD/16/772/2007. The funding support as research fellowship was provided to Dr. Veeramuthu Dharanishanthi by Department of Biotechnology, Government of India.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Ambavaram MM, Krishnan A, Trijatmiko KR, Pereira A (2011) Coordinated activation of cellulose and repression of lignin biosynthesis pathways in rice. Plant Physiol 155:916–931

Andersson-Gunnerås S, Hellgren JM, Björklund S, Regan S, Moritz T, Sundberg B (2003) Asymmetric expression of a poplar ACC

oxidase controls ethylene production during gravitational induction of tension wood. Plant J 34:339–349

Andersson-Gunnerås S, Mellerowicz EJ, Love J, Segerman B, Ohmiya Y, Coutinho PM, Nilsson P, Henrissat B, Moritz T, Sundberg B (2006) Biosynthesis of cellulose-enriched tension wood in *Populus*: global analysis of transcripts and metabolites identifies biochemical and developmental regulators in secondary wall biosynthesis. Plant J 45:144–165

Barros E, Staden CA, Lezar S (2009) A microarray-based method for the parallel analysis of genotypes and expression profiles of wood-forming tissues in *Eucalyptus grandis*. BMC Biotechnol 9(51):1472–6750

Basnet RK, Carpio DPD, Xiao D, Bucher J, Jin M, Boyle K, Fobert P, Visser RGF, Maliepaard C, Bonnema G (2016) A systems genetics approach identifies gene regulatory networks associated with fatty acid composition in *Brassica rapa* seed. Plant Physiol 170:568–585

Baute J, Herman D, Coppens F, De Block J, Slabbinck B, Dell'Acqua M, Pè ME, Maere S, Nelissen H, Inzé D (2016) Combined large-scale phenotyping and transcriptomics in maize reveals a robust growth regulatory network. Plant Physiol 170:1848–1867

Cassan-Wang H, Goue N, Saidi MN, Legay S, Sivadon P, Goffner D, Grima-Pettenati J (2013) Identification of novel transcription factors regulating secondary cell wall formation in *Arabidopsis*. Front Plant Sci 4:189

De Maeyer D, Weytjens B, De Raedt L, Marchal K (2016) Network-based analysis of eQTL data to prioritize driver mutations. Genome Biol Evol 8:481–494

Dharanishanthi V, Ghosh Dasgupta M (2016) Construction of co-expression network based on natural expression variation of xylogenesis-related transcripts in *Eucalyptus tereticornis*. Mol Biol Rep 43:1129–1146

Du J, Groover A (2010) Transcriptional regulation of secondary growth and wood formation. J Integr Plant Biol 52:17–27

Du S, Yamamoto F (2003) Ethylene evolution changes in the stems of *Metasequoia glyptostroboides* and *Aesculus turbinata* seedlings in relation to gravity-induced reaction wood formation. Trees Struct Funct 17:.522–528

Du S, Yamamoto F (2007) An overview of the biology of reaction wood formation. J Integr Plant Biol 49:131–143

Du Q, Gong C, Wang Q, Zhou D, Yang H, Pan W, Li B, Zhang D (2016) Genetic architecture of growth traits in *Populus* revealed by integrated quantitative trait locus (QTL) analysis and association studies. New Phytol 209:1067–1082

Etchells JP, Provost CM, Turner SR (2012) Plant vascular cell division is maintained by an interaction between PXY and ethylene signalling. PLoS Genet 8:e1002997

Felten J, Sundberg B (2013) Biology, chemistry and ultrastructure of tension wood. In: Fromm J (ed) Cellular aspects of wood formation. Springer, Berlin, pp 203–224

Felten J, Vahala J, Love J, Gorzsás A, Gerber L, Kumar M, Kangasjärvi J, Sundberg B (2011) Ethylene signaling via ethylene response factors (ERFs) modifies wood development in hybrid aspen. BMC Proc 5:115

Felten J, Vahala J, Love J, Gorzsas A, Ruggeberg M, Delhomme N et al (2018) Ethylene signaling induces gelatinous layers with typical features of tension wood in hybrid aspen. New Phytol. https://doi.org/10.1111/nph.15078

Feltus FA (2014) Systems genetics: a paradigm to improve discovery of candidate genes and mechanisms underlying complex traits. Plant Sci 223:45–48

Goicoechea M, Lacombe E, Legay S, Mihaljevic S, Rech P, Jauneau A, Lapierre C, Pollet B, Verhaegen D, Chaubet-Gigot N, Grima-Pettenati J (2005) *EgMYB2*, a new transcriptional activator from *Eucalyptus* xylem, regulates secondary cell wall formation and lignin biosynthesis. Plant J 43:553–567

Groover AT, Nieminen K, Helariutta Y, Mansfield SD (2010) Wood formation in *Populus*. In: Jansson S, Bhalerao RP, Groover AT (eds) Genetics and genomics of *Populus*. Springer, New York, pp 201–224

Hefer CA, Mizrachi E, Myburg AA, Douglas CJ, Mansfield SD (2015) Comparative interrogation of the developing xylem transcriptomes of two wood-forming species: *Populus trichocarpa* and *Eucalyptus grandis*. New Phytol 206(4):1391–1405

Hussey SG, Mizrachi E, Spokevicius AV, Bossinger G, Berger DK, Myburg AA (2011) 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 11:173

Hussey SG, Mizrachi E, Creux NM, Myburg AA (2013) Navigating the transcriptional roadmap regulating plant secondary cell wall deposition. Front Plant Sci 4:1–21

Ingemarsson BSM (1995) Ethylene effects on peroxidases and cell growth patterns in *Picea abies* hypocotyl cuttings. Physiol Plant 94:211–218

Ingvarsson PK, Street NR (2011) Association genetics of complex traits in plants. New Phytol 189:909–922

Jia P, Zhao Z (2014) Network assisted analysis to prioritize GWAS results: principles, methods and perspectives. Hum Genet 133:125–138

Jokipii-Lukkari S, Delhomme N, Schiffthaler B, Mannapperuma C, Prestele J, Nilsson O, Street NR, Tuominen H (2018) Transcriptional roadmap to seasonal variation in wood formation of Norway Spruce. Plant Physiol 176:2851–2870

Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30

Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M (2012) KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Res 40(D1):D109–D114

Kirst M, Basten CJ, Myburg AA, Zeng ZB, Sederoff RR (2005) Genetic architecture of transcript-level variation in differentiating xylem of a *Eucalyptus* hybrid. Genetics 169:2295–2303

Lamara M, Raherison E, Lenz P, Beaulieu J, Bousquet J, MacKay J (2016) Genetic architecture of wood properties based on association analysis and co-expression networks in white spruce. New Phytol 210:240–255

Legay S, Sivadon P, Blervacq AS, Pavy N, Baghdady A, Tremblay L et al (2010) *EgMYB1*, an R2R3 MYB transcription factor from eucalyptus negatively regulates secondary cell wall formation in *Arabidopsis* and poplar. New Phytol 188:774–786

Li E, Bhargava A, Qiang W, Friedmann MC, Forneris N, Savidge RA, Johnson LA, Mansfield SD, Ellis BE, Douglas CJ (2012) The Class II KNOX gene KNAT7 negatively regulates secondary wall formation in *Arabidopsis* and is functionally conserved in *Populus*. New Phytol 194:102–115

Liu Y, Wei M, Hou C, Lu T, Liu L, Wei H et al (2017) Functional characterization of populus *PsnSHN2* in coordinated regulation of secondary wall components in tobacco. Sci Rep 7:42

Long TA, Benfey PN (2006) Transcription factors and hormones: new insights into plant cell differentiation. Curr Opin Cell Biol 18:710–714

Love J, Björklund S, Vahala J, Hertzberg M, Kangasjärvi J, Sundberg B (2009) Ethylene is an endogenous stimulator of cell division in the cambial meristem of *Populus*. Proc Natl Acad Sci USA 106:5984–5989

Ma R, Xiao Y, Lv Z, Tan H, Chen R, Li Q et al (2017) AP2/ERF Transcription Factor, Ii049, positively regulates lignan biosynthesis in *Isatis indigotica* through activating salicylic acid signaling and Lignan/Lignin pathway genes. Front Plant Sci 8:1361

McCarthy RL, Zhong R, Ye ZH (2009) MYB83 is a direct target of SND1 and acts redundantly with MYB46 in the regulation of

secondary cell wall biosynthesis in *Arabidopsis*. Plant Cell Physiol 50:1950–1964

Mizrachi E, Myburg AA (2016) Systems genetics of wood formation. Curr Opin Plant Biol 30:94–100

Mizrachi E, Verbeke L, Christie N, Fierro AC, Mansfield SD, Davis MF, Gjersing E, Tuskan GA, Montagu MV, de Peer YV, Marchal K, Myburg AA (2017) Network-based integration of systems genetics data reveals pathways associated with lignocellulosic biomass accumulation and processing. Proc Natl Acad Sci 114:1195–1200

Negishi N, Nanto K, Hayashi K, Onogi S, Kawaoka A (2011) Transcript abundances of LIM transcription factor, 4CL, CAld5H and CesAs affect wood properties in *Eucalyptus globulus*. Silvae Genet 60:288–296

Ohashi-Ito K, Fukuda H (2010) Transcriptional regulation of vascular cell fates. Curr Opin Plant Biol 13:670–676

Phukan UJ, Jeena GS, Tripathi V, Shukla RK (2017) Regulation of apetala2/ethylene response factors in plants. Front Plant Sci. https://doi.org/10.3389/fpls.2017.00150

Porth I, Klápště J, Skyba O, Friedmann MC, Hannemann J, Ehlting J, El-Kassaby YA, Mansfield SD, Douglas CJ (2013) Network analysis reveals the relationship among wood properties, gene expression levels and genotypes of natural *Populus trichocarpa* accessions. New Phytol 200:727–742

Sakamoto S, Takata N, Oshima Y, Yoshida K, Taniguchi T, Mitsuda N (2016) Wood reinforcement of poplar by rice NAC transcription factor. Sci Rep 6:19925

Salazar MM, Nascimento LC, Camargo EL, Gonçalves DC, Lepikson Neto J, Marques WL, Teixeira PJ, Mieczkowski P, Mondego JM, Carazzolle MF, Deckmann AC, Pereira GA (2013) Xylem transcription profiles indicate potential metabolic responses for economically relevant characteristics of *Eucalyptus* species. BMC Genom 14:201

Schuetz M, Smith R, Ellis B (2013) Xylem tissue speciation, patterning and differentiation mechanisms. J Exp Bot 64:11–31

Seyfferth C, Wessels B, Jokipii-Lukkari S, Sundberg B, Delhomme N, Felten J, Tuominen H (2018) Ethylene-related gene expression networks in wood formation. Front Plant Sci 9:272

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504

Shi R, Wang JP, Lin YC, Li Q, Sun YH, Chen H, Sederoff RR, Chiang VL (2017) Tissue and cell-type co-expression networks of transcription factors and wood component genes in *Populus trichocarpa*. Planta 245:927–938

Shinya T, Iwata E, Nakahama K, Fukuda Y, Hayashi K, Nanto K, Rosa AC, Kawaoka A (2016) Transcriptional profiles of hybrid *Eucalyptus* genotypes with contrasting lignin content reveal that monolignol biosynthesis-related genes regulate wood composition. Front Plant Sci 7:443

Soler M, Plasencia A, Larbat R, Pouzet C, Jauneau A, Rivas S, Pesquet E, Lapierre C, Truchet I, Grima-Pettenati J (2017) The *Eucalyptus* linker histone variant EgH1.3 cooperates with the transcription factor EgMYB1 to control lignin biosynthesis during wood formation. New Phytol 213:287–299

Sundell D, Street NR, Kumar M, Mellerowicz EJ, Kucukoglu M, Johnsson C et al (2017) AspWood: high-spatial-resolution transcriptome profiles reveal uncharacterized modularity of wood formation in *Populus tremula*. Plant Cell 29:1585–1604

Thavamanikumar S, Southerton S, Thumma B (2014) RNA-Seq using two populations reveals genes and alleles controlling wood traits and growth in *Eucalyptus nitens*. PLoS One 9:e101104

Vahala J, Felten J, Love J, Gorzsas A, Gerber L, Lamminmaki A, Kangasjarvi J, Sundberg B (2013) 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 200:511–522

Vanholme R, Storme V, Vanholme B, Sundin L, Christensen JH, Goeminne G, Halpin C, Rohde A, Morreel K, Boerjan W (2012) A systems biology view of responses to lignin biosynthesis perturbations in *Arabidopsis*. Plant Cell 24:3506–3529

Verbeke LP, Cloots L, Demeester P, Fostier J, Marchal K (2013) EPSILON: An eQTL prioritization framework using similarity measures derived from local networks. Bioinformatics 29:1308–1316

Wang HZ, Dixon RA (2012) On–off switches for secondary cell wall biosynthesis. Mol Plant 5:297–303

Xu T, Ma T, Hu Q, Liu J (2015) An integrated database of wood-formation related genes in plants. Sci Rep. https://doi.org/10.1038/srep11422

Ye ZH, Zhong R (2015) Molecular control of wood formation in trees. J Exp Bot 66:4119–4131

Yu H, Soler M, San Clemente H, Mila I, Paiva JA, Myburg AA, Bouzayen M, Grima-Pettenati J, Cassan-Wang H (2015) Comprehensive genome-wide analysis of the Aux/IAA gene family in *Eucalyptus*: evidence for the role of EgrIAA4 in wood formation. Plant Cell Physiol 56:700–714

Zhang X, Cal AJ, Borevitz JO (2011) Genetic architecture of regulatory variation in *Arabidopsis thaliana*. Genome Res 21:725–733

Zhang J, Nieminen K, Serra JAA, Helariutta Y (2014) The formation of wood and its control. Curr Opin Plant Biol 17:56–63

Zhong R, Ye ZH (2014) Transcriptional regulation of biosynthesis of cell wall components during Xylem differentiation. In: Fukuda H (ed) Plant cell wall patterning and cell shape. Wiley, Hoboken, pp 351–377

Zhong R, Lee C, Ye ZH (2010) Evolutionary conservation of the transcriptional network regulating secondary cell wall biosynthesis. Trends Plant Sci 15:625–632

Zinkgraf M, Gerttula S, Groover A (2017) Transcript profiling of a novel plant meristem, the monocot cambium. J Integr Plant Biol 59:436–449