

Isolation and characterization of homeodomain leucine zipper class-III transcription factor gene in *Eucalyptus tereticornis*

V Dharanishanthi and Modhumita Ghosh Dasgupta*

Division of Plant Biotechnology, Institute of Forest Genetics and Tree Breeding, Coimbatore 641002, India.

Received 19 September 2014; revised 16 October 2015; accepted 26 November 2015

Wood is the most abundant raw material and is used as industrial product and renewable bioenergy resource. Genomic studies in woody perennials have revealed the involvement of several wood associated transcription factors regulating the coordinated process of wood formation. The homeodomain-leucine zipper (HD-ZIP) is one of the plant-specific transcription factor known to play key role in plant development. It is expressed in provascular cells and cambial meristem where it has been proposed to regulate vascular development. In this study, the homeodomain-leucine zipper class III transcription factor gene (*EtHB2*) was isolated from leaf tissue of *Eucalyptus tereticornis*. The complete coding domain sequence was 2535 base pairs (encodes for 844 amino acids) in length and harbored the signature domains including homeodomain leucine zipper, START and MEKHLA. Tissue specific expression profiling of *EtHB2* revealed its predominant expression in developing xylem tissues.

Keywords: *Eucalyptus*, HD-ZIP, plant development, transcription factor, xylem.

Introduction

Wood formation stipulates the coordinated expression of a vast number of functional and regulatory genes. Several reports on genes involved in the biosynthesis of polysaccharides, lignins, cell wall proteins and transcription factor in forest trees using classical biochemical and molecular analysis are documented¹⁻⁹. The importance of transcriptional regulation of secondary growth has been emphasized in recent studies¹⁰⁻¹⁴. Several members of NAC (VND, WND, SND), MYB, zinc finger, LIM domain, MADS-box, WRKY, ARF-Aux/IAA and homeodomain–transcription factors are reported to regulate secondary wall biosynthesis¹⁵⁻¹⁹.

Homeodomain-leucine zipper proteins are plant-specific transcriptional factors known to play key role in plant development including meristem regulation²⁰, organ and vascular development²¹, hormones mediated response²² and response to environmental conditions²³. Based on sequence conservation, gene structure and function, HD-ZIP genes are classified into four subfamilies named HD-ZIP class I, II, III, and IV²⁴⁻²⁶. The HD-ZIP III class of transcription are reported to be specifically involved in embryogenesis²⁷, meristem regulation²⁸⁻³¹, lateral organs initiation²⁸, leaf

polarity^{27,31,32}, vascular system development^{29,32,33} and auxin transport^{34,35}. This class of transcription factor is characterized by presence of four signature domains including homeodomain (HD) for DNA binding; leucine zipper (LZ) involved in dimerization; START (STeroidogenic Acute Regulatory protein related lipid Transfer) with sterol and lipid binding properties and MEKHLA positioned in the C-terminal end is involved in response to light, oxygen and redox potential sensing³⁶⁻³⁸.

Recently, HD-ZIP III was reported to play a vital role in vascular development in *Arabidopsis thaliana*, *Zinnia elegans*, *Populus trichocarpa* and *Eucalyptus nitens*^{18,19,39,40}. However, the exact mechanism of HD-ZIP III genes controlling secondary vascular differentiation remains unclear. In *Arabidopsis*, five members of HD-ZIP III including *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*), *REVOLUTA/INTERFASCICULAR FIBERLESS1* (*REV/IFL1*), *ATHB8*, and *ATHB15/ CORONA (CNA)/ INCURVATA4 (ICU4)* was documented³². Several studies in *Arabidopsis* mutants revealed that *PHB*, *PHV* and *REV* predominantly expressed in the vascular bundles, adaxial region of lateral organs and apical meristem, while the expression of *ATHB8* and *CNA* was documented in vascular bundles and procambial region⁴¹⁻⁴³. HD-ZIP class III transcription factor from *P. trichocarpa* (*PtrHox 5*, 7, 16, 23, 31, 39, 44, and

*Author for correspondence

Tel: +91- 422-2484123; Fax: +91- 422-2430549
modhumitaghosh@hotmail.com, ghoshm@icfre.org

61) were highly expressed in secondary xylem²⁶. In *E. nitens*, SNPs present in *Eni-HB1* were associated with multiple wood property traits including microfibril angle, pulp yield and total lignin⁴⁴. The present study was aimed at isolation, characterization and expression profiling of HD-ZIP-III transcription factor gene (*EtHB2*) from *Eucalyptus tereticornis*, a tropical eucalyptus species.

Materials and Methods

Plant Materials

Leaf tissues were harvested from nineteen-year-old *E. tereticornis* (Kupiano Provenance, seed lot no. 13398, Papua New Guinea) maintained in the seed orchard at Karunya Research Plot, Coimbatore, India. Fresh tissues were harvested and immediately frozen in liquid nitrogen and stored at -80°C until RNA isolation.

RNA Isolation and cDNA Synthesis

Total RNA was extracted from leaf tissues using plant RNA isolation kit (Chromous Biotech, Bangalore, India). The quality of RNA was checked on a 1% agarose gel and concentration was determined using NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, USA). Subsequently, total RNA was treated with RNase free DNase I, (Fermentas, Pittsburgh, USA) as per manufacturers' procedure and first strand cDNA was synthesized from 1 µg total RNA using the first strand cDNA synthesis kit (Fermentas, Pittsburgh, USA).

Amplification and Characterization of *EtHB2* Gene

Five primer pairs targeting the HD-ZIP class III TF from *E. grandis* was synthesized³⁹ (Table 1) and amplified in 20 µL PCR reaction containing 200 ng of cDNA, 20 nM forward and reverse gene specific primers, 2 µL of 10 × *Taq* buffer, 2 unit of Prime *Taq* polymerase (Genetbio, Nonsan, Korea) and 0.8 µL of 2.5 mM dNTP mix (Sigma Aldrich, St Louis, USA). PCR was performed in Veriti 96 Well Thermal Cycler (Applied Biosystems, Foster City, USA) using the following programme: initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 60°C for 30 sec, 72°C for 2 min and final extension at 72°C for 10 min. The amplified fragments were separated on a 0.8% agarose gel. One hundred base pair DNA ladder (Chromous Biotech, Bangalore, India) was used to determine the size of the amplicons. The PCR fragments were cloned using InsTAclone™ PCR Cloning Kit (Fermentas, Pittsburgh, USA) using the

manufacturers' procedure and sequenced. Further, two primer pairs were designed and synthesized from *E. tereticornis* sequence to span the complete gene. All the primer pairs were designed using the online software Primer3 (<http://fokker.wi.mit.edu/primer3/>) and synthesized at IDT gene technologies (Canada).

Phylogenetic Analysis

Sequence analysis was performed using the Basic Alignment Search Tools (BLASTn and BLASTx) from National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov). The full length conserve domain sequence (CDS) was derived from overlapping fragments using CAP3 program (<http://pbil.univ-lyon1.fr/cap3.php/>) and analyzed with ORF (Open Reading Frame) finder program (www.ncbi.nlm.nih.gov/projects/gorf/). Conserved domains were identified using online CD-search tool (www.ncbi.nlm.nih.gov/structure/cdd/wrpsb.cgi). The deduced amino acid sequence was aligned and compared with HD-ZIP III proteins from *E. grandis* (*EgrHB*), *P. trichocarpa* (*PtrHOX16*) and *A. thaliana* (*AtHB15/CNA*) and the conserved domains were viewed using CLC sequence viewer 6.9.1 (www.clcbio.com). The phylogenetic relationship with other major class III HD-ZIPs was determined using MEGA 5.1⁴⁵ using neighbour joining model with 1000 bootstrap replications.

Expression Analysis of *EtHB2* in Different Tissues of *E. tereticornis*

Plant Material, Quantitative Real-time PCR (qRT-PCR)

The tissue-specific expression pattern of *EtHB2* was conducted using qRT-PCR in leaf, internode and developing xylem tissues. RNA extraction and cDNA synthesis was performed as per protocol described before. Semi quantitative real time PCR primer pairs were designed using Beacon Designer Software (PREMIER Biosoft International, USA) and primer pairs QRT *EtHB2* FP: 5'-AGAACCTGTGGCA TCACTCAGA-3' and QRT *EtHB2* RP: 5'-AGATC CCACCTTGAAG ACAAGC-3' were used for qRT-PCR analysis. qRT-PCR was performed using SYBR green chemistry using the ABI PRISM 7500 step one plus Sequence Detection System (Applied Biosystems, USA). The reaction mixtures included: 200 ng of cDNA, 10 µL of 2x SYBR Green PCR Master Mix, 100 nM of forward and reverse primer, and 7 µL of nuclease-free water in a 20 µL of final volume reaction mix. The cycling conditions for amplification included polymerase activation (10 min

at 95°C), followed by 40 cycles of 95°C for 15 sec, 60°C for 1 min. A positive control, negative control, no template control was also included. The melt curve analysis was performed for the primer pair to confirm product specificity. *EtAct* and *EtSAND* were used as reference genes for normalization of data⁴⁶. The relative expression was calculated using the delta-Ct method⁴⁷.

Results and Discussion

Isolation and Characterization of *ETHB2* Gene

The seven primer pairs targeting the HD-ZIP class III transcription factor from *E. grandis* and *E. tereticornis* amplified at approximately 500 to 1200 bp (Table 1). The sequences were assembled to generate a single contigs of 2535 bp. The complete CDS was deposited in the GenBank with accession number KF472162.1. The nucleotide sequence of *EtHB2* showed 99% similarity to HB1-like protein (*EcxBH1*) from *Eucalyptus cladocalyx*, 96% similarity to class III HD-ZIP (*CNA2*) from *Medicago truncatula* and 93% similarity to the putative class III HD-ZIP gene from *Hevea brasiliensis*.

The translated protein encoded by *EtHB2* has 844 amino acid residues with a predicted molecular mass of 92912 kDa and a deduced isoelectric point of 6.23. The major component of deduced amino acids consisted of 10.9% of leucine, 9.2% of serine and 8.5% of alanine. The translated product showed 99% similarity to HB-1 like protein from *E. cladocalyx*, 92% similarity to DNA binding protein from *Ricinus communis*, 91% similarity to homeobox-leucine zipper family protein/ lipid-binding START domain-containing protein from *Theobroma cacao* and 86% identity to ATHB-15 from *A. thaliana*.

The conserved domain search documented that the *ETHB2* contained HD, LZ, MEKHLA and START domains which are also reported from their orthologs from *A. thaliana*, *Medicago truncatula*, *Oryza sativa*, *Physcomitrella patens*, *P. trichocarpa*, *Ricinus communis*, *Selaginella moellendorffii*, *Vitis vinifera* and *Zea mays*^{18, 21, 38, 48}. One of the significant features of HD-ZIP III proteins is the presence of a MEKHLA domain in the C-terminus, which contains highly conserved amino acids including Met (M), Glu (E), Lys (K), His (H), Leu (L), Ala (A)²⁵. This domain shares significant similarity with PAS domain, which has a role in light, oxygen and redox potential sensing³⁸. The amino acid sequence of *EtHB2* was compared with *EgrHB*, *PtrHox16* and *ATHB-15* and all the four signature domains were shown to be highly conserved across the orthologs (Fig. 1).

Phylogenetic Analysis

The phylogenetic relationship of *EtHB2* with its orthologs from other plant species is shown in Fig. 2. This analysis revealed that *EtHB2* is closely related to *EgrHB*, *VvHB3*, *CsHB4*, *CpHB2*, *PCN*, *RcHB3*, *MeHB-5/6*, *MtCNA-1/2*, *GmHB-7/8/9*, *AtHB15/CNA*, *AlHB15/CNA* and *MgHB-5/6* forming a single clade, which is in agreement with previous report¹⁸. Five HD-ZIP III gene family members of *A. thaliana* were included in the phylogeny (*AtHB8*, *AtHB15/CNA*, *AtREV*, *AtPHB* and *AtPHV*), formed two major clades: *AtHB8*, *AtHB15/CNA* clade and *AtREV*, *AtPHB* and *AtPHV* clade, with *AtPHB* and *AtPHV* as a sister pair. The predominant expression of the *EtHB2* orthologs from *Populus*

Table 1 — Details of primer pairs targeting *EtHB2*

Primers ID	Sequence(5'-3')	Amplicon Size (bp)	Similarity
EgrHB1-1 F	GTATTGCTTTTCTCCATTTTGC	1145	<i>Eucalyptus cladocalyx</i> , HB1 (99%)
EgrHB1-2R	TCAATAACTTGTTTCATCGCAGTG		
EgrHB1-3 F	TGAATATTTCTGGAGATGGCAAC	1077	
EgrHB1-4 R	ATTGTGGAGGACTCATACAATGG		
EgrHB1-5 F	AATACACAAAATGGTCCAAGCAT	876	<i>Medicago truncatula</i> CNA2 (96%)
EgrHB1-6 R	ATACGAAACCCAGAAGGAAGAAG		
EgrHB1-7 F	GTTAAGCAGGGGATTTAATGAGG	984	
EgrHB1-8 R	CCACAGGTTCTTGAGAATCAACT		<i>Hevea brasiliensis</i> classIII HD-ZIP gene (93%)
EgrHB1-9 F	ACTATCGCATTTGAGTTTGCATT	775	
EgrHB1-10 R	CACTACACAGACATGGCAAAAAGA		
EtHB1-11 F	AAGCAAATCAAGGTCTGGTTC	1268	
EtHB1-12 R	AGTATGCATCAATACTGTTGTCAGC		
EtHB1-13F	GTTTCTCAGCTGGGTGTATGAGAATG	523	
EtHB1-14 R	ACTCCCATCTTCCAGAACAGAAGTAT		

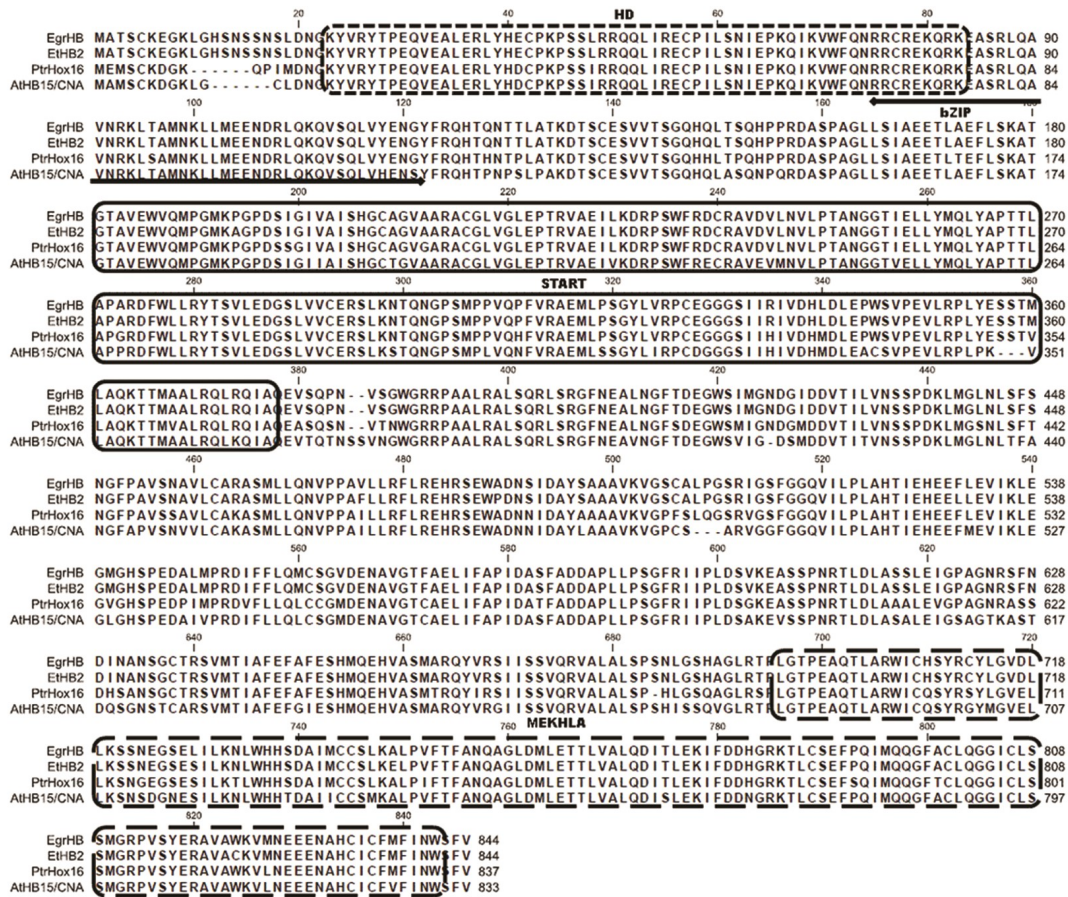


Fig. 1 — Alignment of the deduced amino acid sequences of EtHB2 with HD-ZIP III proteins from *E. grandis* (EgrHB), *P. trichocarpa* (PtrHox16) and *A. thaliana* (AtHB15/CNA). The alignment was produced using CLC sequence viewer 6.9.1. The conserved domains are shown in box and bZIP domain is underlined. Abbreviation: HD=Homeodomain, bZIP=basic leucine zipper domain, START= Steroidogenic Acute Regulatory protein related lipid Transfer domain and MEKHLA domain.

(PCN) and *Arabidopsis* (*AtHB15/CNA*) in the cambial and xylem tissues is reported^{18,42,43}. The present study grouped these orthologs together suggesting their synteny during evolution.

Tissue-Specific Expression Pattern of *EtHB2*

The relative expression analysis of *EtHB2* was performed in leaf, internode and developing xylem using qRT-PCR (Fig. 3). The expression of *EtHB2* was 4 and 2 fold higher in developing xylem and internode tissue, respectively when compared to leaf tissue. This result was comparable with earlier studies from *P. trichocarpa* where the expression of *PCN*, *PtrHox31* and *PtrHB7* was found to be mainly in the cambial zone and xylem^{18,19,26}. Similarly, *CNA* (*Arabidopsis*) and *ZeHB-13* (*Zinnia*) showed ectopic expression in procambium and promote xylem differentiation⁴².

The tissue-specific expression patterns of *EtHB2* and its orthologs reported from other plants suggest that this transcription factor can play a vital role in secondary xylem development and may have a significant effect on the wood properties. The strong genetic association ($P < 0.01$) was found between SNPs in *Eni-HB1* and multiple wood property traits including microfibril angle, pulp yield and total lignin⁴⁴ in *E. nitens* suggesting that *Eni-HB1* influenced the lignin and cellulose biosynthesis pathways leading to cell wall development. The predominant expression of *EtHB2* in the developing xylem tissues also suggests its significant role in regulation of secondary cell wall development in *E. tereticornis*.

The isolation of the full length gene of *EtHB2* can provide a new resource to *Eucalyptus* breeders to use this potential candidate gene in association mapping and genetic engineering studies for modifications of lignocellulosic biomass.

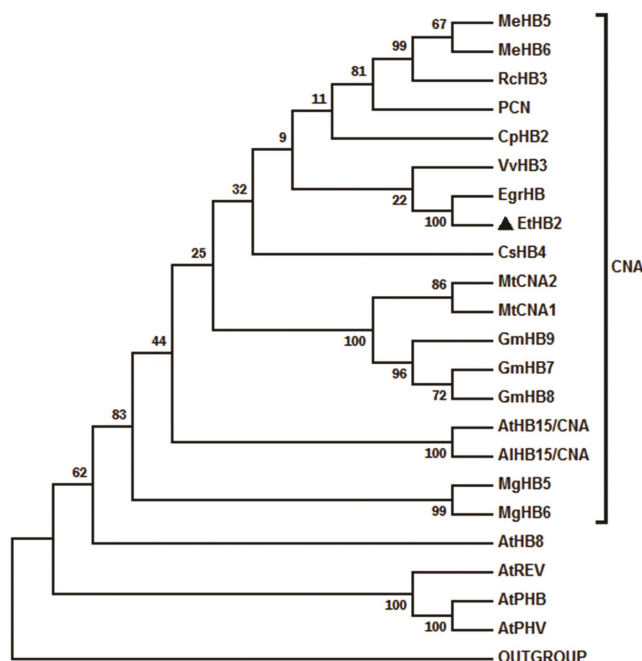


Fig. 2 — Neighbour joining tree of *EtHB2* (represented by black triangle) with other homologues constructed using MEGA 5. 1. Abbreviations: Me, *Manihot esculenta*; Rc, *Ricinus communis*; P, *Populus trichocarpa*; Cp, *Carica papaya*; Vv, *Vitis vinifera*; Egr, *Eucalyptus grandis*; Et, *Eucalyptus tereticornis*; Cs, *Cucumis sativus*; Mt, *Medicago truncatula*; Gm, *Glycine max*; At, *Arabidopsis thaliana*; Al, *Arabidopsis lyrata*; Mg, *Mimulus guttatus*; CNA, CORONA; PHB, PHABULOSA; PHV, PHAVOLUTA; REV, REVOLUTA; PCN, POPCORONA and Out group- *PpHB1* from *Physcomitrella patens*.

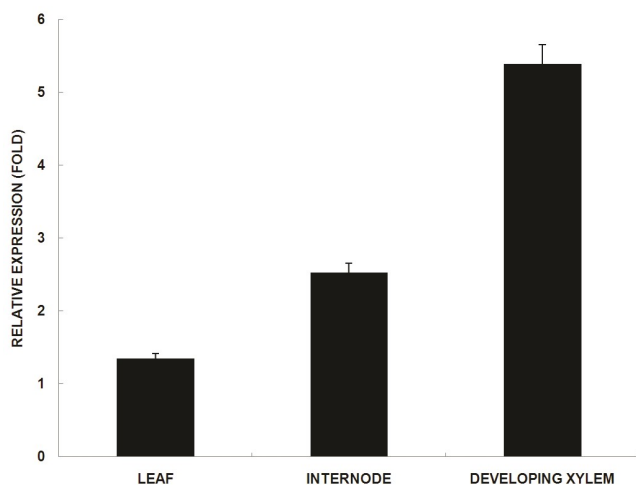


Fig. 3 — Tissue specific expression of *EtHB2*. The relative expression (Mean $\pm$ SE, $n=3$) was calculated using delta-Ct method.

Acknowledgements

The authors acknowledge the research grant received from Department of Biotechnology, Government of India. The author also acknowledges Dr. S. Viswanathan, Head, Division of Plant pathology, Sugarcane Breeding Institute, Coimbatore for providing qRT-PCR facility.

References

- Allona I, Quinn M, Shoop E, Swope K, Cyr S *et al*, Analysis of xylem formation in pine by cDNA sequencing, *Proc Natl Acad Sci USA*, 95 (1998) 9693-9698.
- Whetten R W, Mackay J J & Sederoff R R, Recent advances in understanding lignin biosynthesis, *Ann Rev Pl Physiol P Mol Biol*, 49 (1998) 585-609.
- Zhang Y, Sederoff R R & Allona I, Differential expression of genes encoding cell wall proteins in vascular tissues from vertical and bent loblolly pine trees, *Tree Physiol*, 20 (2000) 457-466.
- Plomion C, Leprovost G & Stokes A, Wood formation in trees, *Plant Physiol*, 127 (2001) 1513-1523.
- Boerjan W, Ralph J & Baucher M, Lignin biosynthesis, *Annu Rev Plant Biol*, 54 (2003) 519-549.
- Peter G & Neale D, Molecular basis for the evolution of xylem lignifications, *Curr Opin Plant Biol*, 7 (2004) 737-742.
- Mellerowicz, E J & Sundberg B, Wood cell walls: biosynthesis, developmental dynamics and their implications for wood properties, *Curr Opin Plant Biol*, 11 (2008) 293-300.
- Dejardin A, Laurans F, Arnaud D, Breton C, Pilate G *et al*, Wood formation in Angiosperms, *C R Biologies*, 333 (2010) 325-334.
- Schuetz M, Smith R & Ellis B, Xylem tissue speciation, patterning and differentiation mechanisms, *J Exp Bot*, 64 (2013) 11-31.
- Patzlaff A, Newman L J, Dubos C, Whetten R W, Smith C *et al*, Characterization of PtMYB1, an R2R3-MYB from pine xylem, *Plant Mol Biol*, 53 (2003) 597-608.
- Prassinis C, Ko J H, Yang J & Han K H, Transcriptome profiling of vertical stem segments provides insights into the genetic regulation of secondary growth in hybrid aspen trees, *Plant Cell Physiol*, 46 (2005) 1213-1225.
- Andersson-Gunneras S, Mellerowicz E J, Love J, Segerman B, Ohmiya Y *et al*, 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 (2006) 144-165.
- Bomal C, Bedon F, Caron S, Mansfield S D, Levasseur C *et al*, Involvement of *Pinus taeda* MYB1 and MYB8 in phenylpropanoid metabolism and secondary cell wall biogenesis: a comparative in planta analysis, *J Exp Bot*, 59 (2008) 3925-3939.
- Wilkins O, Nahal H, Foong J, Provart N J and Campbell M M, Expansion and diversification of the *Populus* R2R3-MYB family of transcription factors, *Plant Physiol*, 149 (2009) 981-993.

- 15 Groover A & Robischon M, Developmental mechanisms regulating secondary growth in woody plants, *Curr Opin Plant Biol*, 9 (2006) 55-58.
- 16 Zhong R & Ye Z H, Regulation of cell wall biosynthesis, *Curr Opin Plant Biol*, 10 (2007) 564-572.
- 17 Zhong R, McCarthy R L, Lee C & Ye Z H, Dissection of the transcriptional program regulating secondary wall biosynthesis during wood formation in poplar, *Plant Physiol*, 157 (2011) 1452-1468.
- 18 Du J, Miura E, Robischon M, Martinez C, Groover A *et al*, The *Populus* Class III HD ZIP Transcription Factor POPCORONA affects cell differentiation during secondary growth of woody stems, *PLoS One*, 6 (2011) 210-214.
- 19 Zhu Y, Song D, Sun J, Wang X & Li L, PtrHB7, a class III HD-Zip gene, plays a critical role in regulation of vascular cambium differentiation in *Populus*, *Mol Plant*, 6 (2013) 1331-1343.
- 20 Sessa G, Carabelli M, Sassi M, Ciolfi A, Possenti M *et al*, A dynamic balance between gene activation and repression regulates the shade avoidance response in *Arabidopsis*, *Genes Dev*, 19 (2005) 2811-2815.
- 21 Prigge M J & Clark S E, Evolution of the class III HD-Zip gene family in land plants, *Evol Dev*, 8 (2006) 350-361.
- 22 Nakamura M, Katsumata H & Abe M, Characterization of the class IV homeodomain-leucine zipper gene family in *Arabidopsis*, *Plant Physiol*, 141 (2006) 1363-1375.
- 23 Henriksson E, Olsson A S B, Johannesson H, Johansson H, Hanson J *et al*, Homeodomain leucine zipper class I genes in *Arabidopsis* Expression patterns and phylogenetic relationships, *Plant Physiol*, 139 (2005) 509-518.
- 24 Sessa G, Carabelli M, Ruberti I, Lucchetti S, Baima S *et al*, Identification of Distinct Families of HD-ZIP Proteins in *Arabidopsis thaliana*, *Plant Mol Biol*, 81 (1994) 411-426.
- 25 Ariel F D, Manavella P A, Dezar C A & Chan R L, The true story of the HD-Zip family, *Trends Plant Sci*, 12 (2007) 419-426.
- 26 Hu R, Chi X, Chai G, Kong Y, He G *et al*, Genome-wide identification, evolutionary expansion, and expression profile of homeodomain-leucine zipper gene family in poplar (*Populus trichocarpa*), *PLoS One*, 7 (2012) e31149.
- 27 Prigge M J, Otsuga D, Alonso J M, Ecker J R, Drews G N *et al*, Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in *Arabidopsis* development, *Plant Cell*, 17 (2005) 61-76.
- 28 Otsuga D, DeGuzman B, Prigge M J, Drews G N & Clark S E, REVOLUTA regulates meristem initiation at lateral positions, *Plant J*, 25 (2001) 223-236.
- 29 Baima S, Possenti M, Matteucci A, Wisman E, Altamura M *et al*, The *Arabidopsis* ATHB-8 HD-zip protein acts as a differentiation-promoting transcription factor of the vascular meristems, *Plant Physiol*, 126 (2001) 643-655.
- 30 Williams L, Grigg S P, Xie M, Christensen S & Fletcher J C, Regulation of *Arabidopsis* shoot apical meristem and lateral organ formation by microRNA miR166g and its AtHD-ZIP target genes, *Development*, 132 (2005) 3657-3668.
- 31 McConnell J R, Emery J, Eshed Y, Bao N, Bowman J *et al*, Role of PHABULOSA and PHAVOLUTA in determining radial patterning in shoots, *Nature*, 411 (2001) 709-713.
- 32 Emery J F, Floyd S K, Alvarez J, Eshed Y, Hawker N P *et al*, Radial patterning of *Arabidopsis* shoots by class III HD-ZIP and KANADI genes, *Curr Biol*, 13 (2003) 1768-1774.
- 33 Kim J, Jung J H, Reyes J L, Kim Y S, Kim S Y *et al*, microRNA-directed cleavage of ATHB15 mRNA regulates vascular development in *Arabidopsis* inflorescence stems, *Plant J*, 42 (2005) 84-94.
- 34 Mattsson J, Ckurshumova W & Berleth T, Auxin signaling in *Arabidopsis* leaves vascular development, *Plant Physiol*, 131 (2003) 1327-1339.
- 35 Morelli G & Ruberti I, Shade avoidance responses driving auxin along lateral routes, *Plant Physiol*, 122 (2000) 621-626.
- 36 Johannesson H, Wang Y & Engstrom P, DNA-binding and dimerization preferences of *Arabidopsis* homeodomain-leucine zipper transcription factors in vitro, *Plant Mol Biol*, 45 (2001) 63-73.
- 37 Schrick K, Nguyen D, Karlowski W M & Mayer K F, START lipid/sterol-binding domains are amplified in plants and are predominantly associated with homeodomain transcription factors, *Genome Biol*, 5 (2004) R41.
- 38 Mukherjee K & Bürglin T R, MEKHLA, a novel domain with similarity to PAS domains, is fused to plant homeodomain-leucine zipper III proteins, *Plant Physiol*, 140 (2006) 1142-1150.
- 39 Bhuiyan N, *Identification of genes influencing wood fibre properties in Eucalyptus nitens*. PhD Thesis, The University of Melbourne, Melbourne, Australia, 2008.
- 40 Cote C L, Boileau F, Roy V, Ouellet M & Levasseur C, Gene family structure, expression and functional analysis of HD-Zip III genes in angiosperm and gymnosperm forest trees, *BMC Plant Biol*, 10 (2010) 273.
- 41 Baima S, Nobili F, Sessa G, Lucchetti S, Ruberti I *et al*, The expression of the ATHB-8 homeobox gene is restricted to provascular cells in *Arabidopsis thaliana*, *Development*, 121 (1995) 4171-4182.
- 42 Ohashi-Ito K & Fukuda H, HD-Zip III homeobox genes that include a novel member, ZeHB-13 (*Zinnia*) /ATHB-15 (*Arabidopsis*), are involved in procambium and xylem cell differentiation, *Plant Cell Physiol*, 44 (2003) 1350-1358.
- 43 Green K A, Prigge M J, Katzman R B & Clark S E, CORONA, a member of the class III homeodomain leucine zipper gene family in *Arabidopsis*, regulates stem cell specification and organogenesis, *Plant Cell*, 17 (2005) 691-704.
- 44 Southerton S G, MacMillan C P, Bell J C, Bhuiyan N, Downes G *et al*, Association of allelic variation in xylem genes with wood properties in *Eucalyptus nitens*, *Australian Forestry*, 73 (2010) 259-264.
- 45 Tamura K, Peterson D, Peterson N, Stecher G, Nei M *et al*, MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, *Mol Biol and Evol*, 28 (2011) 2731-2739.
- 46 Karpaga Raja Sundari B & Dasgupta MG, Selection and validation of reference genes for real-time qRT-PCR normalization in different tissues of *Eucalyptus tereticornis*, *Silvae Genetica*, 61 (2012) 221-300.
- 47 Yuan J S, Reed A, Chen F & Stewart C N, Statistical analysis of real-time PCR data, *BMC Bioinformatics*, 7 (2006) 85.
- 48 Sessa G, Steindler C, Morelli G & Ruberti I, The *Arabidopsis* ATHB-8, -9 and -14 genes are members of a small gene family coding for highly related HD-Zip proteins, *Plant Mol Biol*, 38 (1998) 609-622.