



# Wood transcriptome analysis of *Pinus densiflora* identifies genes critical for secondary cell wall formation and NAC transcription factors involved in tracheid formation

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### **Abstract**

Although conifers have significant ecological and economic value, information on transcriptional regulation of wood formation in conifers is still limited. Here, to gain insight into secondary cell wall (SCW) biosynthesis and tracheid formation in conifers, we performed wood tissue-specific transcriptome analyses of *Pinus densiflora* (Korean red pine) using RNA sequencing. In addition, to obtain full-length transcriptome information, PacBio single molecule real-time (SMRT) isosequencing was carried out using RNAs from 28 tissues of *P. densiflora*. Subsequent comparative tissue-specific transcriptome analysis successfully pinpointed critical genes encoding key proteins involved in biosynthesis of the major secondary wall components (cellulose, galactoglucomannan, xylan, and lignin). Furthermore, we predicted a total of 62 NAC (NAM, ATAF1/2 and CUC2) family transcription factor members and identified seven PdeNAC genes preferentially expressed in developing xylem tissues in *P. densiflora*. Protoplast-based transcriptional activation analysis found that four PdeNAC genes, homologous to VND, NST and SND/ANAC075, upregulated GUS activity driven by an SCW-specific cellulose synthase promoter. Consistently, transient overexpression of the four PdeNACs induced xylem vessel celllike SCW deposition in both tobacco (Nicotiana benthamiana) and Arabidopsis leaves. Taken together, our data provide a foundation for further research to unravel transcriptional regulation of wood formation in conifers, especially SCW formation and tracheid differentiation.

## Introduction

- **Trees** are perennial growth and have winter dormancy and spring regrowth.
- **Secondary growth** (i.e., wood formation) from a vascular cambium.
- **Conifers** dominate in boreal forests of the northern hemisphere despite the small number of species, playing a crucial role in ecosystem function, and important producers of raw material for paper, solid fuels, liquid biofuels and biomaterials.
- Korean Red Pine (*Pinus densiflora*) is one of the most important tree species in our country because of their distribution, ecological contribution and economic values.







#### **Results**

Identification of major players in the **Tissue-specific transcriptome analysis of** P. densiflora secondary wall formation in *P. densiflora* [PacBio SMRT iso-sequencing analysis] [Cellulose and xylan biosynthetic pathway] [Lignin biosynthetic pathway] [Tissue-specific RNA sequencing analysis] X ; TPM of MD> JDP-galactose N ; TPM of YN Contig ID X N Log2 F DN209880\_c0\_g1\_i3 159.0 24.8 28 Vertical stem segments representing gradient of developmental stages GDP-mannose PAL C4H Phenylalanine oot Apical Meristem (SN) annose-6 Fructose-6 b-Coumaraldehyde (IWS) D-glucose-6-P 197355\_c0\_g1\_i1\_0.0\_0.0 14815\_c0\_g1\_i1\_0.0\_0.0 shikimate D-glucose-1-F Contig ID X N Log2 X N S N MR WS MC JDP-D-glucuronat UDP-glucose (YN) 🔺 MDX N193592\_c10\_g1\_i4 242.1 16.6 N214534\_c1\_g3\_i3 7.5 N194674\_c1\_g3\_i5 1493.8 2 MR SN YN PdeMYB46 Conifer onifervl alcoho Feruloyl-CoA aldehyde (G-lignin) PdeCesA7 Contig ID X N Log2 F DN211982\_c0\_g1\_i5 21.3 5.6 1.9 PdeCesA8 1,4-β-D-xylan X ; TPM of MDX PdeCCoAOMT1 N; TPM of YN GUX **DUF579** RWA PdeUBC11 5-Hydroxy-Sinapyl alcoho Sinap-PC1: 41% variance coniferaldehyde aldehyde (S-lignin)



#### Identification of *PdeNACs* involved in tracheid formation in *P. densiflora*

[Ectopic xylem vessel-like cell formation in both tobacco and Arabidopsis leaves by transient overexpression of four PdeNAC genes]





**Confirmation of absence of S-lignin** in *P. densiflora* wood

[Stem cross section of pine tree and hybrid poplar]

- Mäule staining; S-lignin staining
- Wiesner staining; lignified cell staining

Pinus densiflora		Poplar (BH)	
Mäule	Wiesner	Mäule	Wiesner
SILL COM			
		AP PAN	
A SEC			100000
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[Phylogenetic analysis of PdeNAC family]





## **Conclusion & Discussion**



- In summary, by comprehensive transcriptome analysis of wood-forming tissues of P. densiflora, we successfully identified many essential genes involved in biosynthesis of secondary wall components.
- These genes could be focal points for biotechnological improvement of wood properties to produce biomaterials and/or biofuels, including galactoglucomannan biosynthesis to utilize hemicellulosic 6-carbon sugars or use of laccases for lignin polymerization.
- Furthermore, we identified NAC transcriptional regulators involved in tracheid differentiation and confirmed our findings by functional characterization using a heterologous expression system.
- Our study findings provide insights that may prove useful for disentangling the complex mechanisms of wood formation, one of the most important **biological processes** on this planet.



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