

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) iso-sequencing 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 cell-like 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.

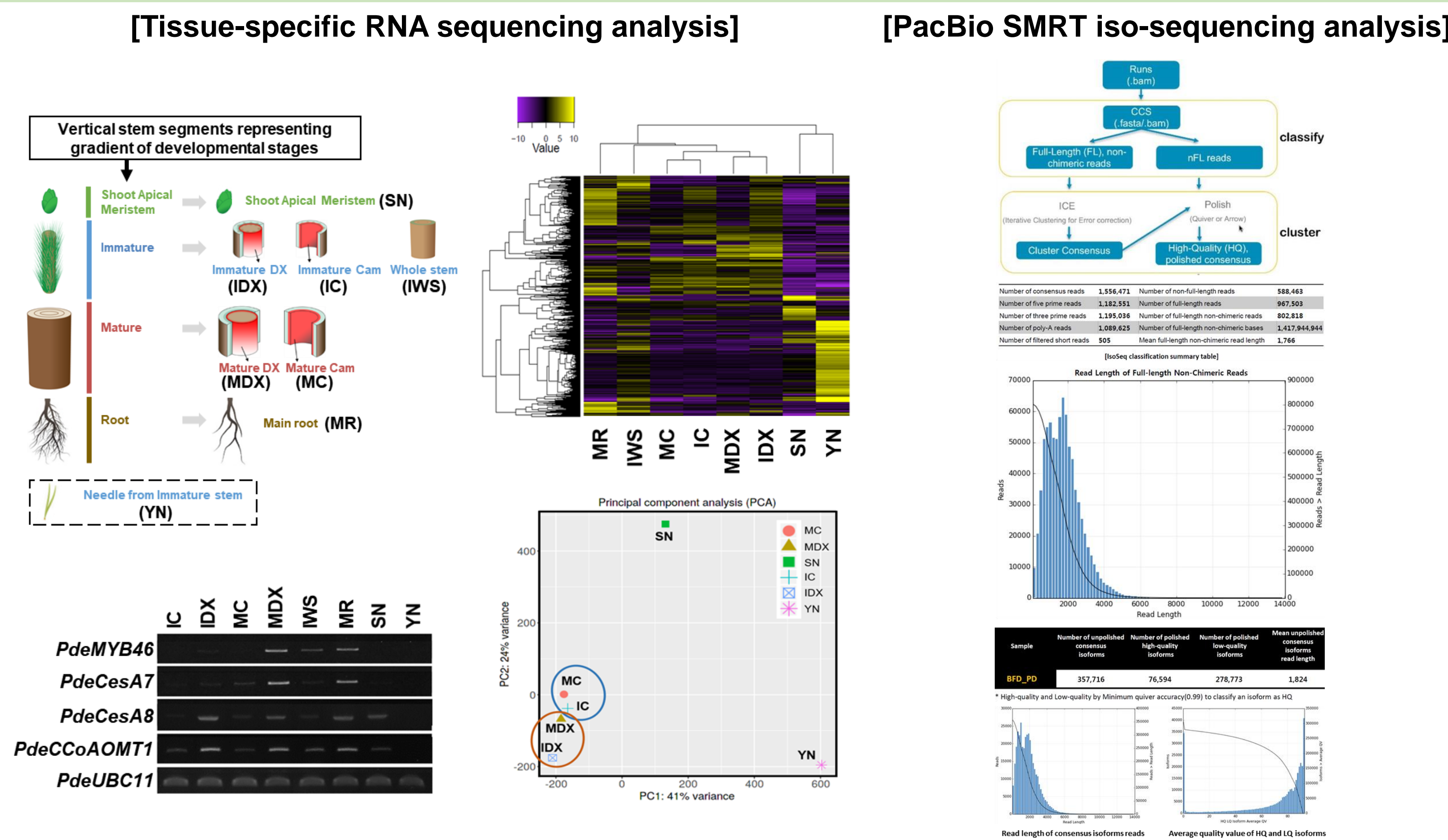
Introduction

- **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.

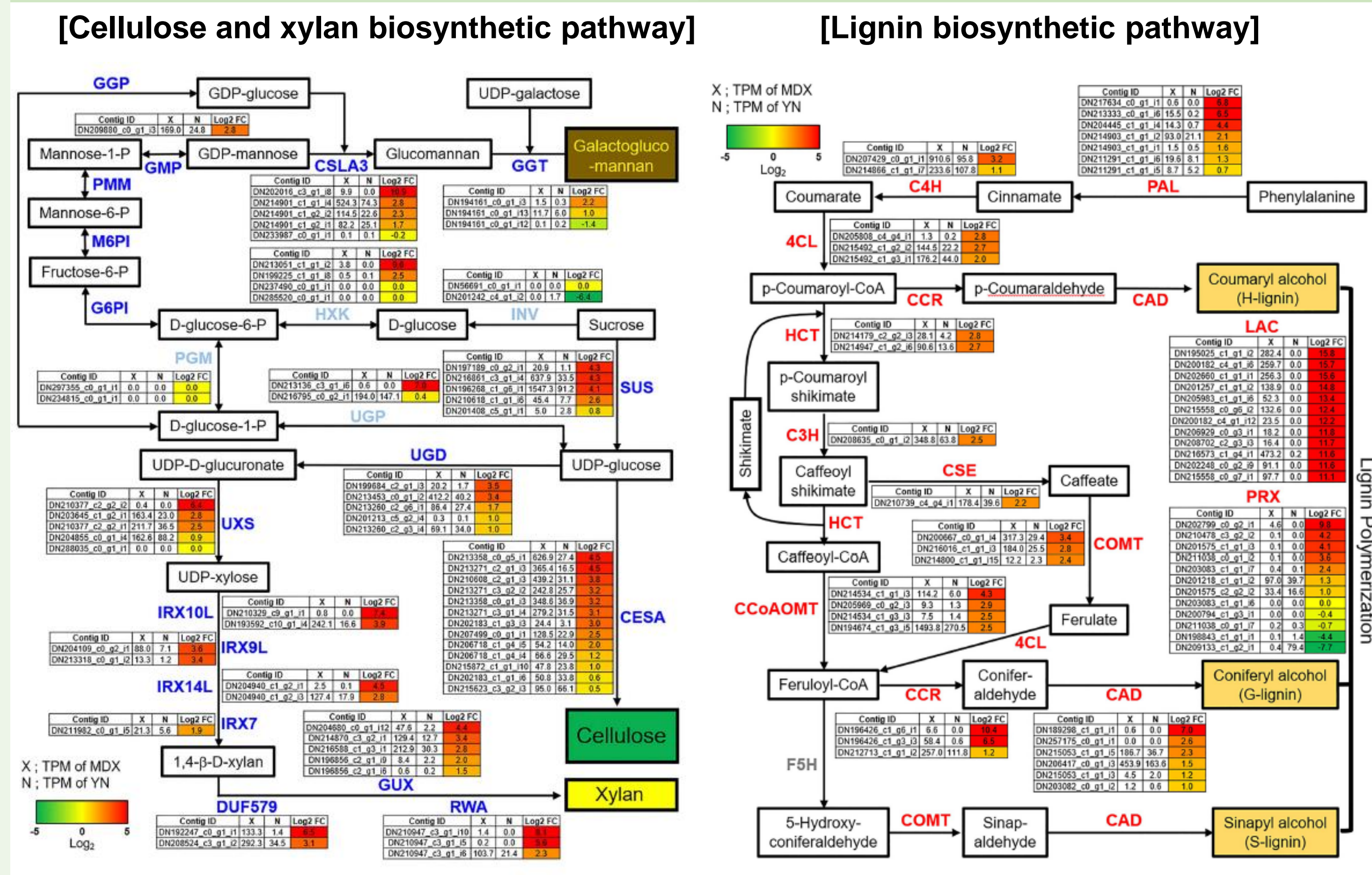


Results

Tissue-specific transcriptome analysis of *P. densiflora*



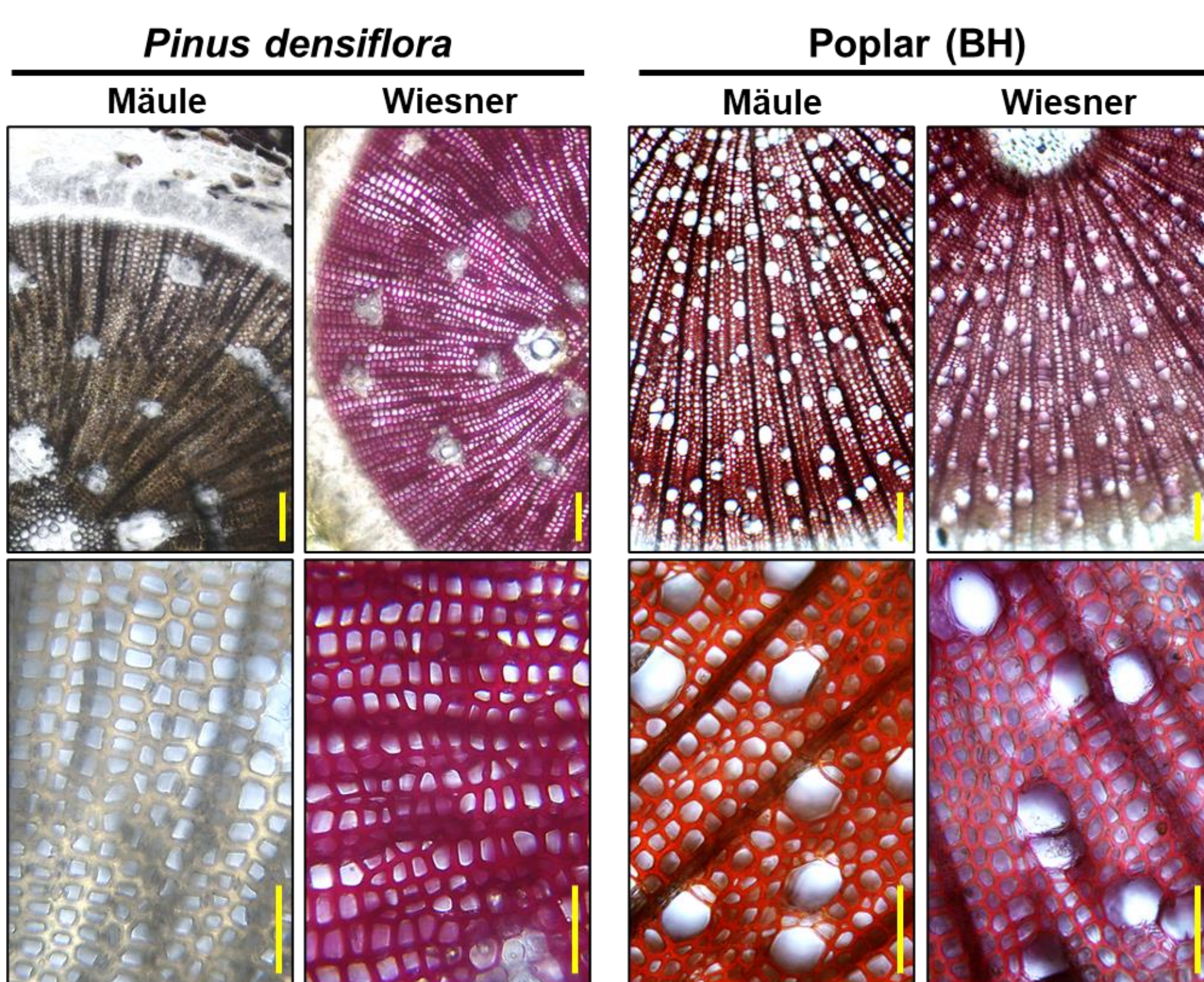
Identification of major players in the secondary wall formation in *P. densiflora*



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

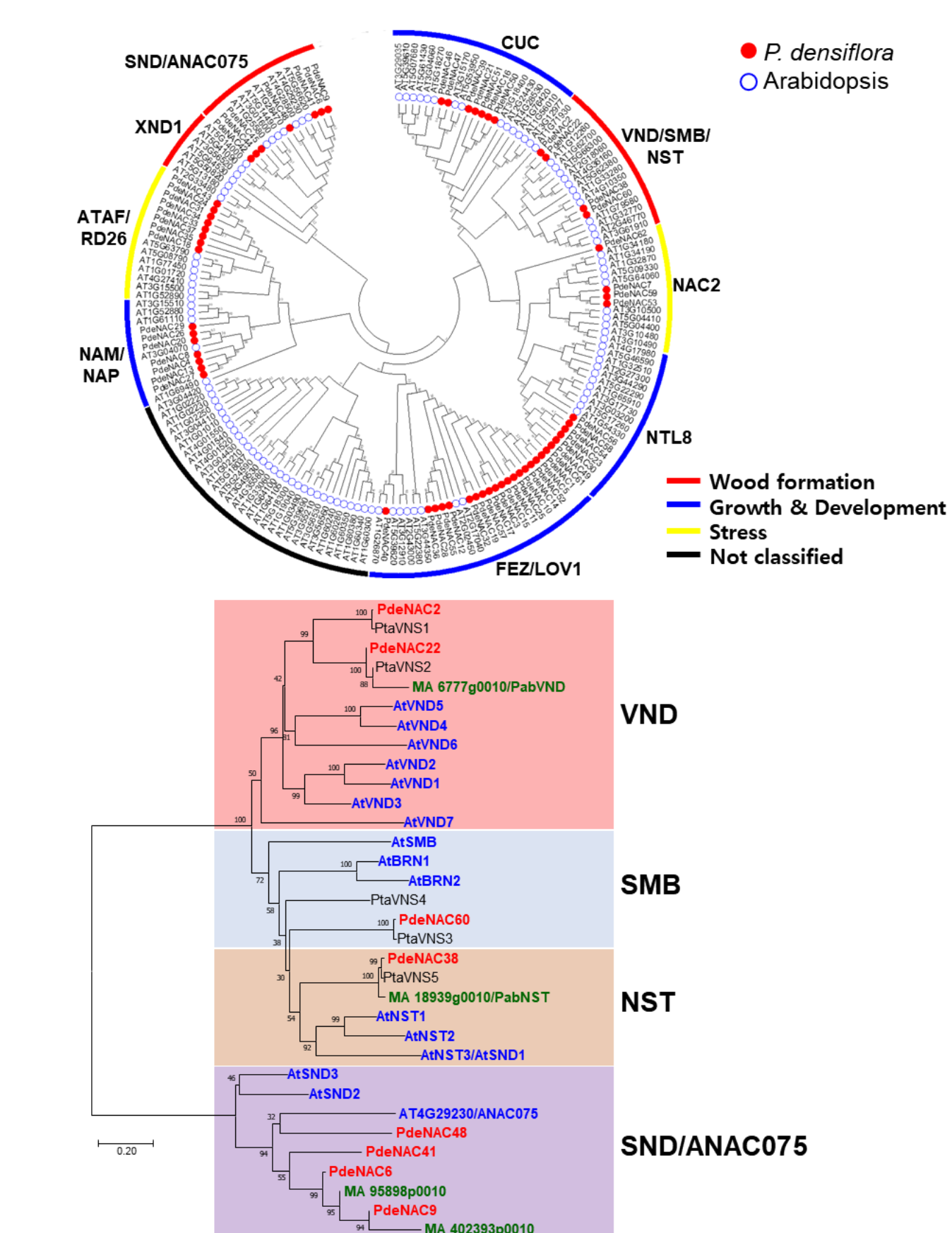
[Stem cross section of pine tree and hybrid poplar]

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



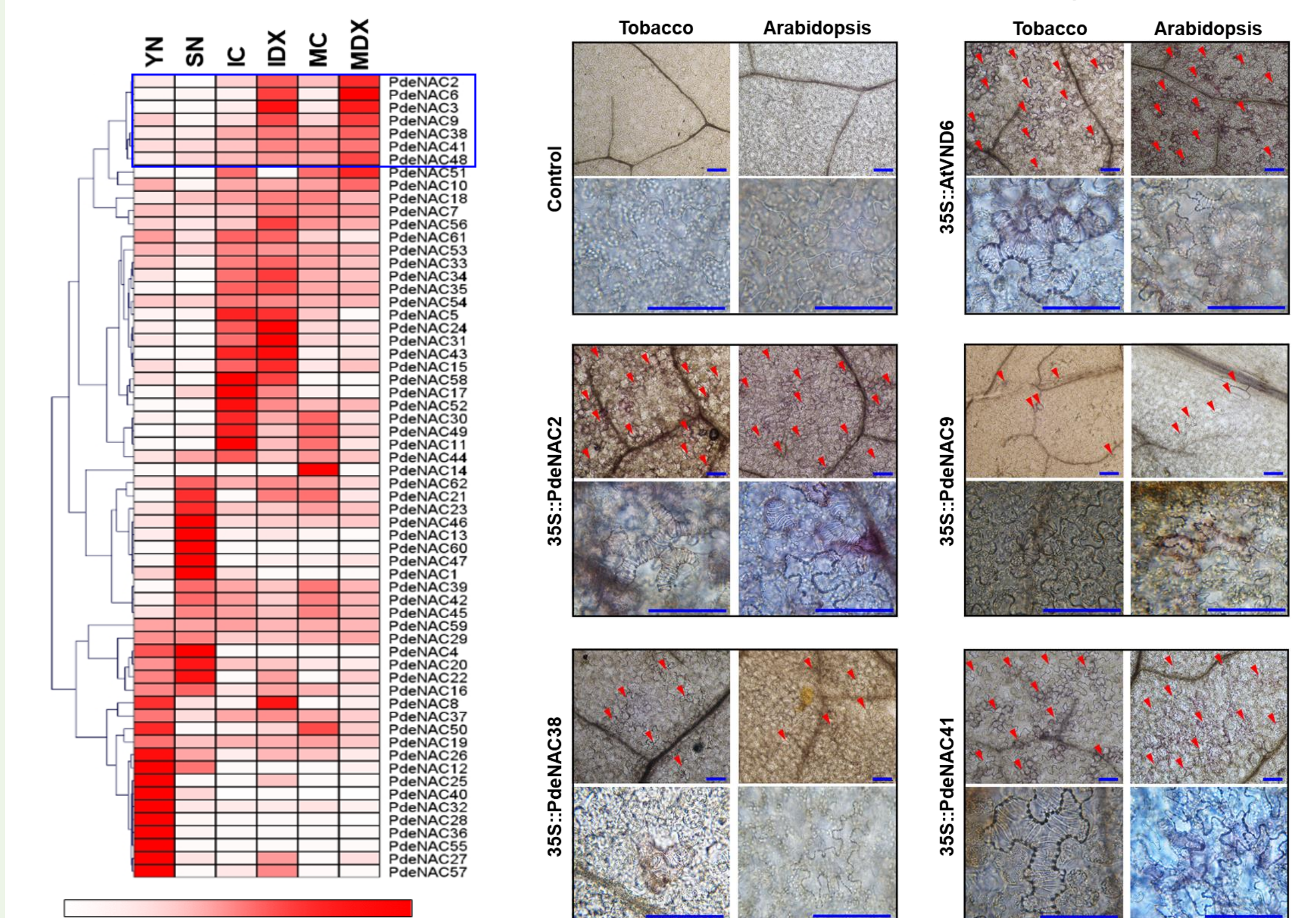
Identification of NAC transcription factor family in *P. densiflora*

[Phylogenetic analysis of PdeNAC family]



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]



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 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.