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Wood Transcriptome Profiling Identifies Critical Pathway Genes of Secondary Wall Biosynthesis and Novel Regulators for Vascular Cambium Development in *Populus*

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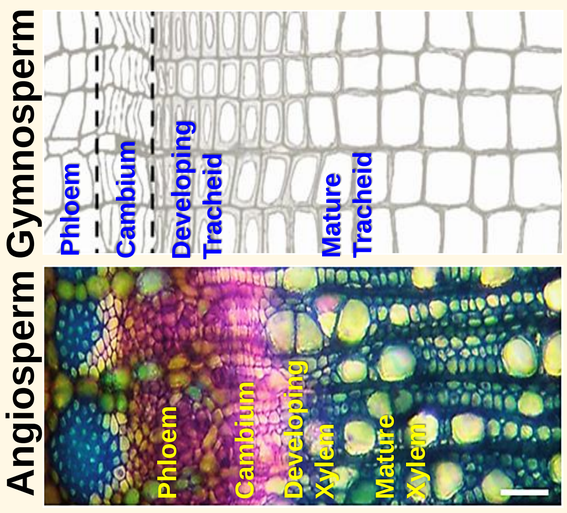
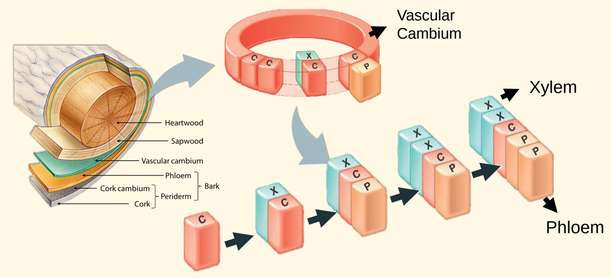
Abstract

Wood, the most abundant biomass on Earth, is composed of secondary xylem differentiated from vascular cambium. However, the underlying molecular mechanisms of wood formation remain largely unclear. To gain insight into wood formation, we performed a series of wood-forming tissue-specific transcriptome analyses from a hybrid poplar (*Populus alba* × *P. glandulosa*, clone BH) using RNA-seq. Together with shoot apex and leaf tissue, cambium and xylem tissues were isolated from vertical stem segments representing a gradient of secondary growth developmental stages (i.e., immature, intermediate, and mature stem). In a comparative transcriptome analysis of the 'developing xylem' and 'leaf' tissue, we could identify critical players catalyzing each biosynthetic step of secondary wall components (e.g., cellulose, xylan, and lignin). Several candidate genes involved in the initiation of vascular cambium formation were found via a co-expression network analysis using abundantly expressed genes in the 'intermediate stem-derived cambium' tissue. We found that transgenic Arabidopsis plants overexpressing the *PtrHAM4-1*, a GRAS family transcription factor, resulted in a significant increase of vascular cambium development. This phenotype was successfully reproduced in the transgenic poplars overexpressing the *PtrHAM4-1*. Taken together, our results may serve as a springboard for further research to unravel the molecular mechanism of wood formation, one of the most important biological processes on this planet.

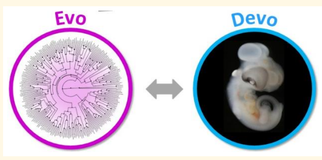
Introduction

Mechanism of Wood Formation





- **How cambium cells were initiated and maintained?**
- **How xylem cell fate is specified ?**

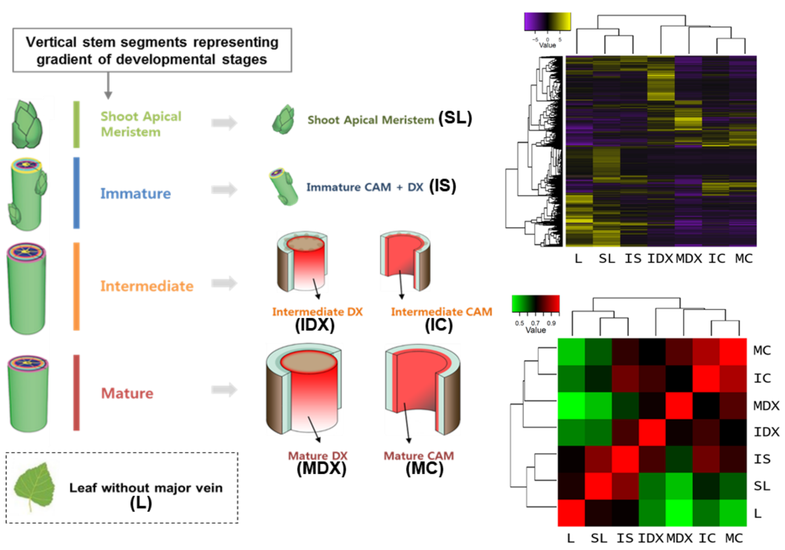


'*Evo-Devo*' genomics approach

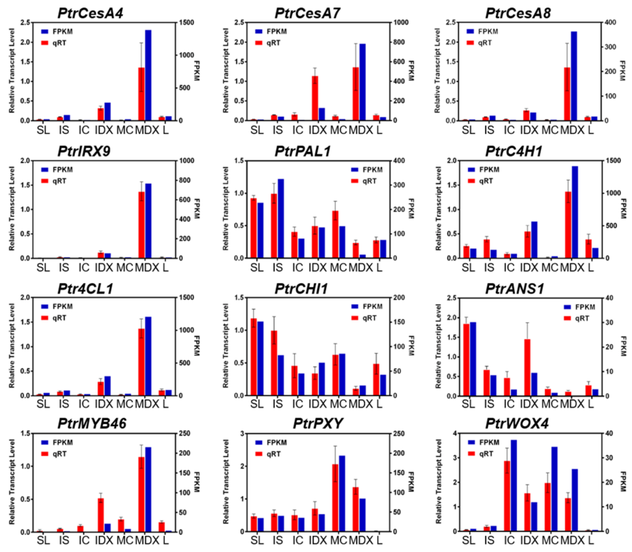
Results

Wood forming tissue-specific transcriptome analysis of hybrid poplar

[Tissue-specific RNA sequencing analysis]

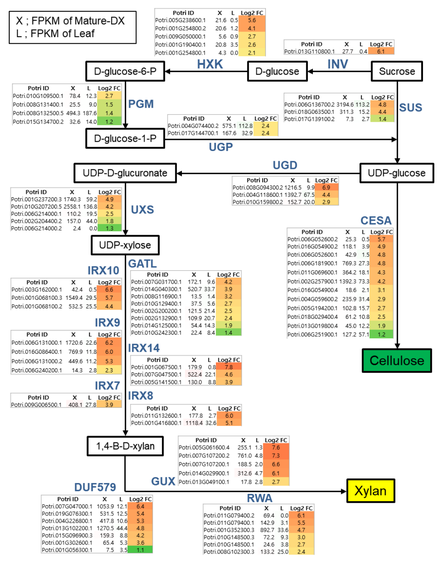


[Confirmation of RNA-seq results by independent qRT-PCR analysis]

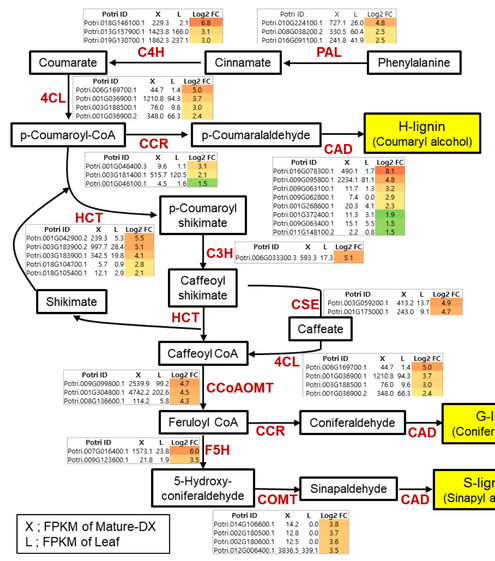


Identification of critical pathway genes for secondary wall biosynthesis in *Populus*

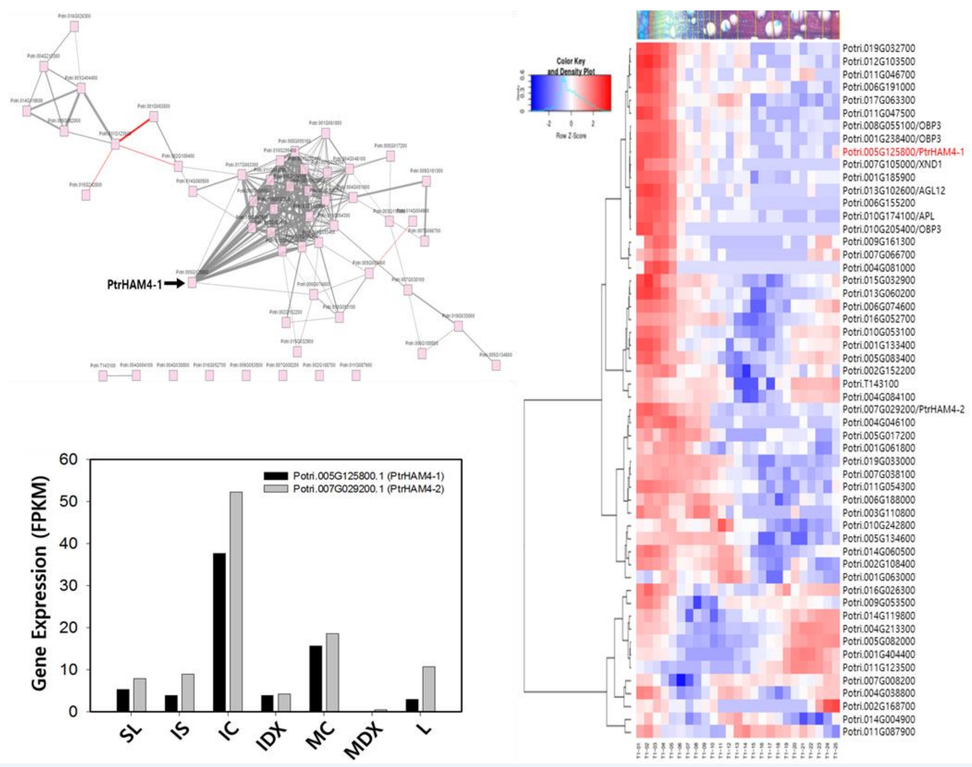
[Cellulose and xylan biosynthetic pathway]



[Lignin biosynthetic pathway]

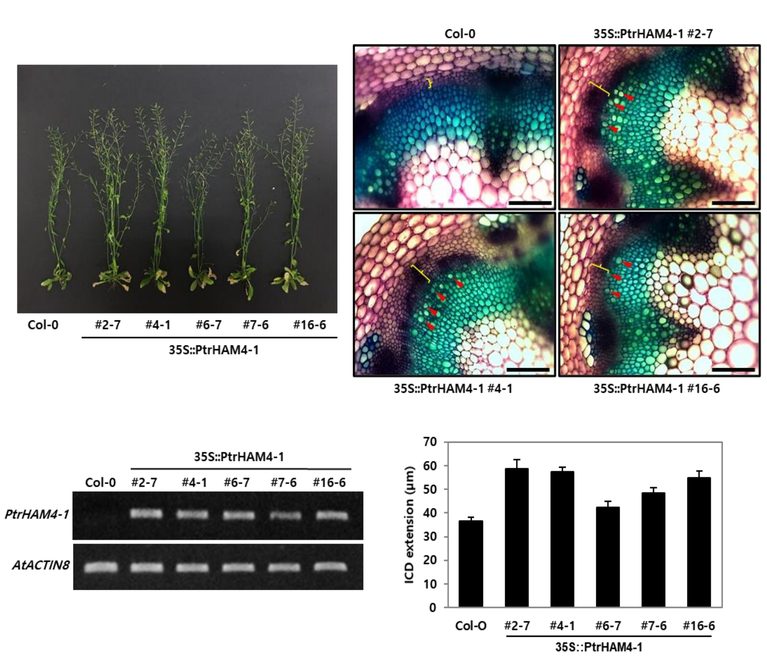


Identification of *PtrHAM4-1*, preferentially expressed in cambium tissue

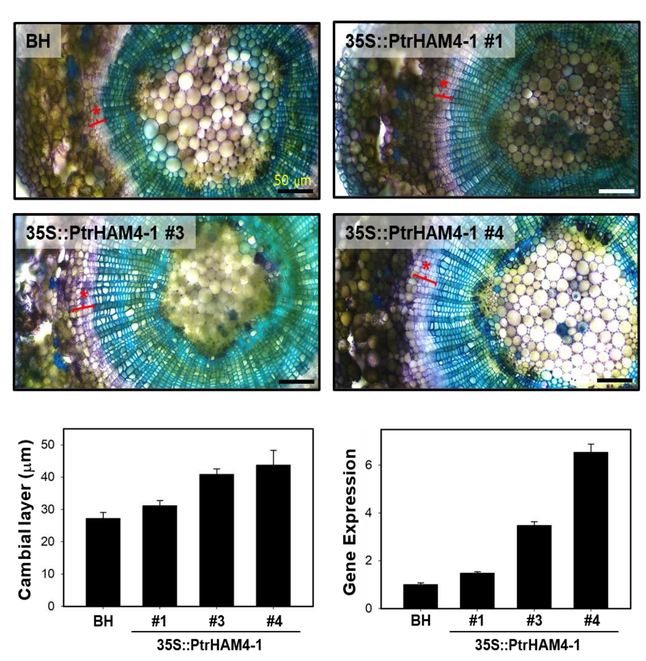


Overexpression of *PtrHAM4-1* enhanced vascular cambium development in both transgenic Arabidopsis and poplar plants

[Overexpression of *PtrHAM4-1* in transgenic Arabidopsis increased cambium development]



[Transgenic poplar overexpressing *PtrHAM4-1* resulted in an increased cambium development]



Conclusion & Discussion

- In summary, a comprehensive **wood-forming tissue-specific transcriptome analysis** from a **hybrid poplar** successfully pinpointed many essential genes involved in the **biosynthetic pathways of secondary wall components**.
- These genes could be focal points for the **biotechnological improvement of wood properties** within the production of **biomaterials and/or biofuels**.
- Furthermore, the transcriptional regulators involved in **vascular cambium development** were isolated and demonstrated their validity via functional characterization of ***PtrHAM4-1*** using a heterologous expression.
- Thus, our results may offer insights for disentangling the **complex mechanisms of wood formation**, one of the **most important biological processes** on this planet.

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