

Gene discovery in lignification by gene expression and metabolic analyses

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As an alternative energy and carbon source to fossil resources, recently wood biomass has been attracted much attention. Wood biomass, mainly composed of cellulose, hemicellulose, and lignin, are synthesized actively in developing xylem of trees. The understanding of the synthetic mechanism of wood components would be greatly useful to modify wood biomass amenable to the future biorefinary.

Lignin is a phenolic polymer and the synthesis is called “lignification”. 4-Hydroxycinnamic alcohols (monolignols) formed via the cinnamate/monolignol pathway are the immediate precursors in the lignin biosynthesis. Several enzymes are responsible for the metabolism, and thus far, the genes encoding the enzymes were identified by biochemical characterization in various plant species. However, the transcriptional regulation of the genes is largely unknown.

In the present study, the author aimed to identify transcription factors involved in lignification. First, the author focused on MYB transcription factors of *Populus trichocarpa*. MYB gene family is the largest transcription factor family in plant. Previous studies revealed that several MYB transcription factors were involved in lignification as well as cellulose and hemicelluloses biosynthesis in pine, eucalypt, and *Arabidopsis thaliana*, but nothing was known about poplar MYB transcription factors. To narrow down the putative MYB genes involved in lignification, we searched MYB transcription factor genes in *P. trichocarpa* genomic database, and the gene expression among organs were evaluated by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). Three MYB genes are specifically expressed in developing xylem where lignification was actively occurred, suggesting that the genes were involved in lignification. To narrow down the target genes regulated by the MYB transcription factors, the expression of the genes which encode cinnamyl alcohol dehydrogenase (CAD), an enzyme that catalyzes the formation of monolignols, was evaluated using qRT-PCR. This analysis enabled us to predict a CAD gene putatively targeted by the xylem-specific MYB transcription factors. How these xylem-specific MYB transcription factor in *P. trichocarpa* regulate lignification remains to be elucidated.

Second, the author attempted to narrow down novel transcription factor genes involved in lignification in *A.thaliana*. Using co-expressed genes network analysis, we narrowed down several candidate transcription factor genes which had not been reported. Next, we selected knock-out and over-expression mutant lines of *A. thaliana*. The selected lines were cultivated and harvested to determine the quantities of lignin, intermediates, and related metabolites using GC-MS and LCMS-IT-TOF. As a result, several knock-out and over-expression lines revealed that decrease of the quantity of lignin and the intermediates of the cinnamate/monolignol pathway. These results suggest that the candidate transcription factor may be involved in lignification. Further studies are required to illustrate the relationship between lignin biosynthesis and the novel transcription factors, but the present study may be the first step to identify the novel transcription factor involved in lignification.