Both ChIP-SEQ and in planta gene modification indicate a function of PtaMYB221in lignin biosynthesis and secondary cell wall formation in poplar

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Genome-wide studies of multigene families involved in Eucalyptus phenylpropanoid metabolism and lignin branch pathway

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The biosynthesis of lignin monomers comprises ten enzymatic reactions catalyzed by enzymes encoded by members of multigene families. Lignin, one of the main components of plant secondary cell walls, is a major obstacle for improving industrial processes from several segments of the wood industry such as pulp and paper production. Eucalyptus grandis and E. globulus are major fiber sources for those industries and therefore have a substantial economic importance. The recent availability of the genome of Eucalyptus grandis provides research opportunities to understand the genomic architecture of those multigene families.

We performed a genome-wide survey of the ten E. grandis multigene families, and conducted comparative phylogenetic analyses by including bona fide lignin-biosynthetic genes from other species. We surveyed the expression profiles of all families' members through RNAseq in six E grandis tissues and, for a subset of putative bona fide lignin-biosynthetic genes, by RT-qPCR in a large panel of 16 E. globulus tissues.

All total, we identified 174 genes scattered all over the eleven E. grandis chromosomes. The four largest families (COMT, CAD, CCoAOMT and PAL) revealed to be extensively impacted by tandem gene duplication, which explains their significant expansion in comparison to other plant species. The combination of phylogeny and expression profiling analyses allowed us to define a Eucalyptus lignin toolbox featuring 29 members exhibiting strong, preferential expression in highly lignified tissues. All ten multigene families are represented in the toolbox.

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Both ChIP-SEQ and in planta gene modification indicate a function of PtaMYB221in lignin biosynthesis and secondary cell wall formation in poplar

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Poplar wood or secondary xylem is composed of vessels, fibers and parenchyma rays. Their secondary cell wall (SCW) consists of a number of layers sequentially deposited during cell differentiation. The formation of the SCW is controlled by a number of transcription factors (TF) that coordinate the expression of many genes involved in the biosynthesis, assembly, and deposition of the different SCW components. We investigated the function of one of them, PtaMYB221, a poplar ortholog of EgMYB1[1], known as a transcriptional repressor of the lignin biosynthetic pathway in Eucalyptus. Toward this end, we developed two complementary approaches, Chromatin Immuno-Precipitation followed by next-generation DNA sequencing (ChIP-SEQ) and *in planta* gene modification using genetic engineering. Using ChIP-SEQ with an antibody specific to PtaMYB221, we determined at the genome scale the set of PtaMYB221binding sites in differentiating secondary xylem of poplar bent trees: 488 putative targets of PtaMYB221 were identified including 15 genes potentially involved in lignin biosynthesis, 3 genes related to pectin degradation, 1 gene with a possible role in xylan biosynthesis, 1 gene involved in cellulose biosynthesis, 1 gene involved in xyloglucan biosynthesis and numerous TFs related to SCW development. Over-expression of native PtaMYB221 induced in transgenic poplars important sylleptic branching but had no effect on tension wood formation. On the reverse, dominant repression of PtaMYB221 resulted in poorly lignified fibers and parenchyma rays. We conclude that PtaMYB221 is important for lignin biosynthesis regulation during wood differentiation.

[1] S. Legay *et al.* (2007) *Plant Science,* **173**(5), 542-549.

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