

Protein-protein interactions between members of the poplar biomass gene set

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As one of the fastest-growing and most productive trees in North America, poplar is a model biomass crop for producing biofuels, biopower and biomaterials. Poplar is also a valuable genomics research model with a completely sequenced genome and well-established methods for creating transgenic plants. Together, these attributes make poplar a logical subject for experiments aimed at a more detailed understanding of the molecular biology and genomics of biomass production, with the long-term goal being the development of a more efficient bioenergy feedstock. The development of microarrays that enable the measurement of gene expression across entire genomes has recently facilitated the identification of hundreds of new wood-associated genes in poplar and other models for wood formation. For example, we recently identified approximately 400 poplar genes that are highly upregulated in wood-forming tissue of *Populus trichocarpa*. The proteins encoded by these biomass-associated genes must interact with other proteins to function properly, suggesting that a more detailed understanding of protein-protein interactions occurring in cells of wood-forming tissues would contribute to advances in feedstock improvement. Because studying interactions involving so many proteins is not practical using plant cells, we are using the yeast two-hybrid system to test all possible pairwise combinations involving the 400 biomass-associated proteins. Furthermore, selected proteins will be tested by yeast two-hybrid for interactions with proteins from a wood cDNA library. Finally, selected genes are being expressed in poplar plants as affinity-tagged proteins and tested for their abilities to form complexes with other proteins extracted from woody tissue and identified using mass spectrometry. All of the interactions revealed by these three methods will form the beginning of a biomass protein-protein interaction map that can guide strategies for feedstock improvement. To date, we have successfully cloned more than 250 open reading frames (ORFs) that encode members of the poplar biomass gene set (<http://xylome.vbi.vt.edu/>), tested 11 transgenic poplar lines to confirm the expression of affinity-tagged proteins, and used yeast two-hybrid screens to identify novel interactions impacting lignification, cell expansion and calcium-dependent signal transduction.

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