High-throughput functional characterization of *Populus trichocarpa* UDP-glycosyltransferases

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI addresses strategic barriers to the current bioeconomy in the areas of 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to specialty biofuels (C4 alcohols, C6 esters and hydrocarbons) using CBP at high rates, titers and yield in combination with cotreatment, pretreatment or catalytic upgrading. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Poplar is a fast-growing woody crop currently used for paper, pulp, and plywood with potential as a domestic feedstock for bioenergy and bioproducts. In order to breed or engineer poplar genotypes that are high-yielding, robust, and can be converted into value-added chemicals, we need to understand poplar secondary metabolism and its genetic basis. Secondary metabolism is important in plant response to stressors and influences resource allocation to and away from the cell wall. For example, lignin shares phenylpropanoid precursors with poplar phenolic glycosides involved in defense against insect herbivores, pathogens, UV radiation, and drought.[1, 2] UDP-dependent glycosyltransferases (UGTs) are responsible for catalyzing sugar transfer to phenolics and other metabolites, which influences their localization within cells and tissues, physiological role, and metabolic fate. UGTs are a large multi-functional enzyme class, so determining which ones have roles in secondary metabolism and which substrates they act upon from sequence homology alone is not currently possible.

We used a *P. trichocarpa* metabolite-genome wide association study to identify UGT encoding genes with single nucleotide polymorphisms (SNPs) associated with increased and decreased levels of metabolites.[3-5] Next, we profiled the activities of the enzymes against a substrate panel of phenolics and precursors; we identified enzymes with activities unique from those previously observed in *A. thaliana*.[6] Several of these enzymes with unique activity profiles are undergoing *in vivo* untargeted validation. This work will advance understanding of poplar secondary metabolism and aid in developing a robust, high-yielding feedstock.

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