Poplar GWAS and eQTL Analyses Reveal a Key Transferase Gene, HCT2, as a Missing Link in Plant Growth-Defense Tradeoffs

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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 will address 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 and C6 esters) using CBP at high rates, titers and yield in combination with cotreatment or pretreatment. And CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Poplar is a fast-growing woody perennial with robust genetic tools and data. However, challenges remain in gaining functional information on its many genes and pathways. Secondary metabolite biosynthesis is a complex and precise process that is catalyzed by numerous enzymes that are under the control of complex transcriptional regulatory networks. The identification of key regulators in secondary metabolite biosynthesis remains restricted by low throughput techniques. We integrated genome-wide associated studies (GWAS) and expression-based quantitative trait loci (eQTL) studies in *Populus trichocarpa* to identify genetic elements controlling the abundance of *cis*- and *trans*-3-*O*-caffeoylquinic acid, key intermediates in lignin biosynthesis and important compounds with numerous therapeutic roles including antioxidant and antimicrobial activity. We found that the abundances of these metabolites were not only significantly associated with single nucleotide polymorphisms (SNPs) in a hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase gene (*PtHCT2*), but were also correlated with the expression levels of the same gene. eQTL mapping revealed that *PtHCT2* expression was regulated by putative cis-acting elements, which coincided with GWAS SNP associations and were located in a W-box element, a binding site for WRKY transcription factors. Further analyses in co-expression networks, transcriptional response to infection by the fungal pathogen *Sphaerulina musiva*, and *in vitro* validation of transcriptional regulation suggested that *PtHCT2*

is involved in both caffeoylquinic acid biosynthesis and defense response, providing one example of the long sought after a mechanistic link in growth-defense tradeoffs.

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