

Expression Quantitative Trait Nucleotide (eQTN) Mapping Reveals Transcriptional Regulatory Networks in *Populus trichocarpa*

Jin Zhang¹, Jay Chen^{1*} (chenj@ornl.gov), Meng Xie¹, Yongil Yang¹, Kaijie Zheng¹, Lee Gunter¹, Sara Jawdy¹, Timothy J. Tschaplinski¹, Nancy Engle¹, Erika Lindquist², Vasanth Singan², Kerrie Berry², Jeremy Schmutz^{2,3}, Wellington Muchero¹, and **Gerald A. Tuskan**¹

¹Center for Bioenergy Innovation, Oak Ridge National Laboratory, Oak Ridge, TN; ²U.S. Department of Energy Joint Genome Institute, Walnut Creek, CA; ³HudsonAlpha Institute for Biotechnology, Huntsville, AL

<https://cbi.ornl.gov>

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. CBI will maximize product value by *in planta* modifications and biological funneling of lignin to value-added chemicals.

Populus are being used for biomass production for a suite of industrial applications including biofuels conversion. Our understanding of biomass productivity and quality is limited by the fact that this complex trait requires the regulation and coordinated interactions of many genes. Identification of genetic networks regulating biomass productivity and quality remains largely unaccomplished and is urgently needed to inform genetic improvement of *Populus* feedstocks for biomass production and conversion.

To uncover the genetic regulatory landscape in the woody perennial bioenergy crop *Populus trichocarpa*, we performed an expression quantitative trait nucleotide (eQTN) mapping enabled by the whole-genome resequencing and RNA-seq analysis of *P. trichocarpa* natural variants. A panel of >8.2 million single nucleotide polymorphisms (SNPs) and nucleotide insertions and deletions (InDels) were obtained from whole-genome resequencing of 917 unrelated individuals of *P. trichocarpa*. Transcriptome data from 390 leaf and 444 xylem samples were analyzed and revealed that 16,030 and 15,496 genes, respectively, exhibited significant expression variation across the population. Through genetic mapping, *cis*- and *trans*-eQTNs were identified. Enriched transcription factor binding sites (TFBS) including *cis*-eQTN showed tissue-specific divergence. *trans*-eQTN analysis identified multiple hotspots that were significantly associated with expression of more than 100 putative target genes. Combined with genome-wide association studies (GWAS) of trait phenotypes, the upstream regulators of these phenotype-associated genes and their regulatory network were identified. These analyses have provided a comprehensive understanding of the genetic regulatory mechanisms underlying complex traits.

References

1. Zhang, J. et al. (2018). GWAS and eQTL analyses reveal roles of *HCT2* in caffeoylquinic acid biosynthesis and its regulation by defense-responsive transcription factors in *Populus*. *New Phytologist* 220: 502-516.

The Center for Bioenergy Innovation is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science.