Functional genomic and cross-species studies uncover novel regulators of phenylpropanoid biosynthesis

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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.

The phenylpropanoid pathway is responsible for the synthesis of a wide variety of bioactive chemicals, including lignin, p-coumaric acid, and flavonoids. However, genetic mechanisms regulating the carbon flux towards desirable molecules remain largely unknown. In order to maximize valuable bioproducts yield in biomass feedstocks, we leveraged the genomic and genetic resources of *Populus trichocarpa* genome-wide association studies (GWAS) population to identify and characterize genes and specific alleles underlying the regulation of phenylpropanoid biosynthesis. Furthermore, we extended *Populus* discoveries to other bioenergy feedstocks through cross-species studies.

By taking a GWAS approach using ~1,000 *P. trichocarpa* natural variants, we have linked one *Populus* 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase gene (named *PtrEPSP-TF*) to lignin biosynthesis. Subsequently, our Omics analyses of *Populus* transgenic lines overexpressing *PtrEPSP-TF* and biochemical characterization of PtrEPSP-TF protein revealed that PtrEPSP-TF carries a DNA-binding helix-turn-helix motif and functions as a transcriptional regulator of the phenylpropanoid pathway upstream of MYB46 (Xie et al., 2018). Meanwhile, our GWAS analysis led to the identification of high-impact single nucleotide polymorphisms (SNPs) in the *PtrEPSP-TF* gene. Biochemical and transactivation analyses demonstrated that one of these high-impact SNPs resulted in the substitution of 142nd amino acid and dramatically impairs the DNA-binding and transcriptional activity of PtrEPSP-TF (Xie et al., 2020). These discoveries provide molecular targets for genetic engineering and genome-editing to regulate phenylpropanoid biosynthesis to improve biomass

feedstocks characteristics. We continue to utilize our discoveries to identify new regulators of the phenylpropanoid pathway and to gain mechanistic understanding of their regulatory actions.

References

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