

QTL mapping and candidate gene discovery of metabolomic signatures in switchgrass

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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.

Switchgrass (*Panicum virgatum* L.) is an emerging bioenergy crop undergoing rapid domestication efforts, and thus there is significant interest in understanding its genomic and metabolic pathways regulating important agronomic traits. Metabolomics reveal the chemical footprint of organisms, providing insight into the pathways that govern physiology, stress response, digestibility and sustainability. Here, we combine high-throughput metabolic analysis with genetic and trait mapping to identify the genomic regions, and ultimately genes, that underlie variation in crucial traits.

We developed a F2 switchgrass population, constructed a high-density genetic map, and generated metabolic profiles for a subset of 152 switchgrass F2 progeny using gas chromatography-mass spectrometry (GCMS). Quantitative Trait Loci (QTL) analysis associated these metabolomic signatures with recombination patterns in our genetic map, with 134 significant QTL for 101 metabolomic compounds, including compounds involved in lignin and stress response pathways. In particular, we found 9 lignan compounds with overlapping peaks on Chromosome 2N, explaining up to 77% of metabolite concentration, and identified 4 monolignol diversification candidate genes. Additionally, 4-O-coumaroyl- and 4-O-caffeoyl quinic acids shared a significant peak on Chromosome 3N. A candidate gene underlying this QTL varies with ecotype, with upland genotypes having low expression and numerous mutations and deletions. *In vitro* profiling of its biochemical activity confirmed that quinic acid is the preferred substrate. Similar analyses, combining genomic analysis, RNASeq, sequencing, CRISPR-Cas9 knockouts, and gene substrate affinity analysis, are being carried out to identify candidate genes underlying metabolomic variation for other mQTL. Understanding the genomic regulation of diverse primary and secondary metabolites provide insight into switchgrass recalcitrance, herbivore and disease resistance, drought tolerance, and other emergent sustainability and biomass traits.

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