

193. Discovering Naturally Occurring Allelic Variants Associated with Biomass Recalcitrance in *Populus trichocarpa*

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Project Goals: The BioEnergy Science Center (BESC) is focused on the fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves 1) designing plant cell walls for rapid deconstruction and 2) developing multitolerant microbes for converting plant biomass into biofuels in a single step (consolidated bioprocessing). BESC biomass formation and modification research involves working directly with two potential bioenergy crops (switchgrass and *Populus*) to develop varieties that are easier to break down into fermentable sugars. We are using both testing and generation of large numbers of natural and modified plant samples as well as developing genomics tools for detailed studies into poorly understood cell wall biosynthesis pathways.

Adaptive variation in natural populations is a rich source of genetic variation that can be mined for accelerated domestication of biofuel crops. 1034 unique native genotypes from 22 river systems/ 5 subpopulations have been established in 4 common gardens and have been fully resequenced using Illumina HiSeq platform. Approximately, 40 million SNP with more than 50% of loci having a minor allele frequency < 1%; $\pi = 0.007$ and a match rate to reference genotype of 97.7%. Measuring a wide variety of traits in clonally-replicated common gardens over multiple years and environmental conditions we were able to associate vegetative phenology, height and diameter growth, cell wall chemistry and sugar release phenotypes to specific SNP markers. For the adaptive phenotypes these genes include a UDP-Galactose transporters and glycosyl hydrolases. Elite genotypes carrying these beneficial alleles have been identified and display a 905 improvement in growth relative to the wild-type clones. For cell wall phenotypes these genes include an EPS synthase, a copper-related ATOX-1, a calcium transporting ATPase, an amino acid transporter, a MYB transcription factor and a protein kinase. Allele specific variants at each of these loci result in sugar release analysis that show up to 1.4X lower lignin, 2.7X increase in sugar release, and up to 2.4X higher ethanol yield compared to wildtype genotypes. The top performing genotypes consistently show high sugar yields in low severity hydrothermal pretreatment conditions and at low enzyme loadings. Several favorable alleles produce similar favorable phenotypes in transgenic *Arabidopsis*.

The ultimate goal of these efforts is to identify candidate genes that can be commercially deployed in superior biomass feedstock lines that express reduced recalcitrance, increased sugar yield and long term economic and environmental sustainability.

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