Genome-Scale Discovery of Cell Wall Biosynthesis Genes in *Populus*

Steve P. DiFazio[1,2]* (spdifazio@mail.wvu.edu), Wellington Muchero,[2] G.T. Slavov,[1] J. Martin,[3] W. Schackwitz,[3] E. Rodgers-Melnick,[1] C. P. Pennacchio,[3] U. Hellsten,[3] L. Pennacchio,[3] L. E. Gunter,[2] P. Ranjan,[2,4] D. Rokhsar,[3] **G. A. Tuskan**,[2,4] and **Paul Gilna (BESC PI)**[2]

[1]West Virginia University, Morgantown, West Virginia; [2]BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee; [3]Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, California

http://bioenergycenter.org

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 multitalented 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 number of natural and modified plant samples as well as developing genomic tools for detailed studies into poorly understood cell wall biosynthesis pathways.

The primary goal of the *Populus* Activity in BESC has been the identification of genes controlling cell wall formation which ultimately positively impact sugar release, i.e., overcoming recalcitrance. The quantitative genomics portion of the project has focused on Quantitative Trait Locus (QTL) analysis in two large interspecific families, and association genetics to mine natural variation in *Populus trichocarpa*. Specifically, we established two QTL populations, one in West Virginia and one in eastern Oregon and have created a saturated genetic map containing >6000 genetic markers. This map was used to identify regions of the *Populus* genome that control sugar release. Six such regions were found and, in combination with the transcript profiling and association genetics results, six genes within these regions have been verified as improving sugar release. In addition, in the association mapping study we collected 1,100 genotypes from across the native range of *Populus trichocarpa*, established clonal replicates of each genotype in three common gardens in the Pacific Northwest and subjected two-year-old samples from the Corvallis, OR site to the high through-put compositional and recalcitrance phenotyping pipeline established at NREL. Simultaneously, we created a 36,000 single nucleotide polymorphisms (SNP) genotyping Infinium chip based on resequencing data generated by JGI from 15 alternate *P. trichocarpa* genotypes. This SNP array was used to interrogate all 1,100 genotypes found in the common gardens. Association genetics statistical approaches were used to identify specific SNP within specific genetic loci that controlled sugar release and other relevant cell wall phenotypes. From this analysis we identified 46 genes and their amino acid substitutions that are controlling the phenotypes measured in this population. The average increase in sugar yield associated with each SNP is approximately 26% above the wild type control. These genes have been initiated within the *Populus* transformation pipeline managed by ArborGen. We are continuing to phenotype the QTL and association populations for wood chemistry and sugar release, as well as a wide array of traits that will impact productivity in addition to recalcitrance, thereby paving the way for follow-on studies and commercialization during the next phases of the project.

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