Comparison of Multiple Generations of TOP Line Transgenic Switchgrass with Reduced Recalcitrance for Enhanced Bioconversion to Fuels

Alexandru Dumitrache,[1] Jace Natzke,[1] Miguel Rodriguez Jr.,[1] Kelsey L. Yee,[1] Olivia A. Thompson,[1] Charleson R. Poovaiah,[1,4] Hui Shen,[1,5] Mitra Mazarei,[1,4] Holly L. Baxter,[1,4] Nancy L. Engle,[1] Angela Ziebell,[1,2] Robert W. Sykes,[1,2] Erica Gjersing,[1,2] Chunxiang Fu,[1,3] Zeng-Yu Wang,[1,3] Mark F. Davis,[1,3] Timothy J. Tschaplinski,[1] C. Neal Stewart, Jr.,[1,4] Richard A. Dixon,[1,5] Jonathan R. Mielenz,[1] Steven D. Brown[1]* (brownsd@ornl.gov), Brian H. Davison,[1] and **Paul Gilna**[1]

[1]BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, Tennessee; [2]National Renewable Energy Laboratory, Golden, Colorado; [3]The Samuel Roberts Noble Foundation, Ardmore, Oklahoma; [4]University of Tennessee, Knoxville; [5]University of North Texas, Denton

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 multi-talented microbes or 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 testing large numbers of natural variants and generating specific and modified plant samples as well as developing genomics tools for detailed studies into poorly understood cell wall biosynthesis pathways. BESC researchers provide enabling technologies in biomass characterization, 'omics, modeling and data management in order to (1) understand chemical and structural changes within biomass and (2) to provide insights into biomass formation and conversion mechanisms.

Switchgrass is a promising bioenergy feedstock, yet its recalcitrance is a key obstacle in large-scale bioconversion applications. Advancements in plant feedstocks, through transgenic and natural variant selection, has allowed for a variety of TOP Lines to emerge with improved phenotypic qualities. In this study, a yeast-based separate hydrolysis and fermentation (SHF) bioconversion assay was used to assess TOP Line switchgrass plants. Biomass included switchgrass lines with altered expression of genes involved in pectin and lignin biosynthesis and regulation as well as C1 metabolism. Multiple years of field-grown GAUT4, miRNA, MYB4, COMT, and FPGS altered lines were all analyzed for carbohydrate composition and ethanol yield and assessed using statistics and correlation analyses of various features of these TOP Lines. Overall, results revealed a generalized year-over-year increase in glucan and xylan plant content that is more pronounced for certain transgene targets, and an overall increase in ethanol yields for the modified crops, with highs of 36% more yield for overexpression of *PvMYB4* (MYB4 line) over parental controls. Statistical analysis revealed that selection of parental lines with improved properties and transformability had also one of the largest impacts on glucan content changes and ethanol yield increases. Future advances in natural parental line screening and a better understanding of reduced recalcitrance due to various genetic target modifications will aid efforts in developing industrially relevant switchgrass lines.

The BioEnergy Science Center 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.