1. BESC Switchgrass and Populus TOP Lines: Identifying and Analyzing the Best Candidate Lines for Enhanced Biofuel Production

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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 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 using both testing and generating 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.

Two goals of the BioEnergy Science Center (BESC) are to develop switchgrass and Populus lines with reduced recalcitrance and/or higher total cell wall sugar content and to understand the molecular and whole plant traits associated with this enhanced biofuel potential. BESC researchers have identified and vetted over 600 genes as targets to reduce recalcitrance and/or enhance total cell wall sugar content through transient or stable expression studies with switchgrass, Populus or model species. Switchgrass and Populus natural variants also were targeted for recalcitrance and cell wall sugar content evaluation. In total, thousands of transgenic and natural variant lines have been analyzed for enhanced biofuel potential and a process was developed to identify among these lines those with the greatest biofuel production potential (i.e., to identify TOP Lines). A TOP Line Selection Committee with experience in cell wall biochemistry, functional genomics, plant growth and development, and applied research evaluated the TOP Line candidates. A subset of lines with the best reduction in recalcitrance or enhanced cell wall sugar content while maintaining or enhancing growth traits were selected for detailed growth and wall analyses during large structured greenhouse and field trials. In addition to identifying high value biomass, the goal of this research is to identify a core set of biomass traits indicative of reduced recalcitrance (i.e., having increased biofuel potential). Teams of researchers were identified to grow, observe and harvest the lines and to evaluate TOP Line tissue using a variety of analytical approaches, with the goals of characterizing the growth morphology of each line under different conditions and determining the mechanisms of reduced recalcitrance. Specifically, a standardized protocol was developed for simultaneous growth of multiple TOP and comparator lines at the same location to enable meaningful comparisons between lines within the same growth experiment. The inclusion of internal reduced-recalcitrance control lines within each growth experiment allows normalization of the TOP Line findings between experiments. Comparisons across lines will allow researchers to identify, among the TOP Lines, those with the greatest potential to deliver improved biofuel production and to identify the number and

type of different "recalcitrance" mechanisms that impact production of biofuel from plant biomass. Data on TOP Line growth, biochemical and chemical characteristics are being incorporated into a Laboratory Information Management System (LIMS) to enable comparisons within and across lines. The entire process for the identification, growth and analysis of the BESC TOP lines is presented as a summary of the status of evaluation of some of the TOP Lines.

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