From Gene to Network: Switchgrass TOP Line RNA-seq Data Analysis Pipeline at BESC

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Project Goals: The BioEnergy Science Center (BESC) focuses on fundamental understanding and elimination of biomass recalcitrance. BESC's approach to improve accessibility to the sugars within biomass involves (1) improved plant cell walls for rapid deconstruction and (2) multi-talented microbes for converting plant biomass into biofuels in a single step [consolidated bioprocessing (CBP)]. Biomass research works with two potential bioenergy crops (switchgrass and *Populus)* to develop improved varieties and to understand cell wall biosynthesis pathways. We test large numbers of natural variants and generate specific modified plants samples. BESC's research in deconstruction and conversion targets CBP manipulating thermophilic anaerobes and their cellulolytic enzymes for improved conversion, yields, and titer. Enabling technologies in biomass characterization, 'omics, and modeling are used to understand chemical and structural changes within biomass and to provide insights into mechanisms.

Switchgrass (*Panicum virgatum*) has been used for more than a decade as a model crop for sustainable energy research. At BESC, a transformation pipeline was established to identify genes/pathways that improve bioenergy traits of switchgrass. In addition, evaluations of switchgrass natural variants for enhanced biofuel traits were conducted. Twelve transgenic or natural variant lines were identified as BESC switchgrass TOP Lines that had increased sugar release and normal or enhanced plant growth. Systematic analyses, including those of the transcriptome, were carried out for these plants in order to understand the underlying pathways controlling the improved traits.

RNA-seq, as well as other systematic analyses, was conducted on tissue from greenhouse-grown switchgrass TOP Line plants and their controls just entering reproductive stage 1 (R1). The RNA-seq and other TOP Line analysis data are stored in and retrieved from the BESC LIMS. From data quality control (QC) to gene expression analysis for differential expression, each RNA-seq dataset was processed through a compilation of publically available software. Using only reads mapped to one location of the genome, gene level assemblies were generated with HISAT2 and related programs originally with switchgrass genome assembly V1.1 and then later with genome assembly V3.1. To facilitate gene expression visualization at sample and gene levels, the RNA-seq data also are displayed in a BESC Jbrowse portal along with the switchgrass reference genome V1.1 and V3.1.

For each TOP Line, genes whose expression were different from the respective control levels were selected, using differential analysis software, DESeq, as well as an array-like method once an expression matrix was generated. In order to provide a richer biological context for results interpretation, differentially expressed genes were (via the identification of poplar orthologs) projected into our extensive poplar systems biology network models for further analysis. These networks help to identify changes contributing to the cell wall modification in the switchgrass RNAi TOP Lines, providing further genes to target to improve biofuel traits. Significantly modified pathways for cell wall biosynthesis also were identified using Mapman from differentially expressed gene list of the Top Lines. A selection of these genes is being further analyzed for their potential to reduce wall recalcitrance through additional network analyses and considered for functional studies in plants using transient and stable knockdown or overexpression strategies.

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