Field Experiments of Nine Switchgrass TOP Lines

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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* L.) is a perennial warm-season C4 grass that has been identified as a candidate lignocellulosic bioenergy crop because of its rapid growth rate, nutrient use efficiency, and widespread adaptation throughout eastern North America. Cell wall recalcitrance in switchgrass and other lignocellulosic feedstocks is a major economic barrier for enabling efficient enzymatic, microbial, or chemical breakdown of cell wall carbohydrates into fermentable sugars. Recent research has focused on developing switchgrass lines that are more amenable to the fermentation process, either by genetic engineering or by selection of low-recalcitrant lines through association analyses.

Greenhouse studies have identified several potentially successful transgenic routes for reducing cell wall recalcitrance and/or improving growth in switchgrass. An important validation step, especially for genetically engineered plants, is to perform multi-year field studies, which is a vital goal of BESC. It is well known that the greenhouse is not always predictive of crop performance in the field. Herein we present data from nine BESC "TOP Lines" and appropriate controls from agronomically-relevant University of Tennessee (Knoxville) field studies, in which plants were grown under the USDA APHIS BRS release into the environment permits for two or three field seasons. Data includes: (1) agronomic performance (morphology and end-of-season biomass), (2) lignin content and composition by high-throughput py-MBMS, (3) sugar release by high-throughput enzymatic assays, (4) ethanol yield by separate hydrolysis and fermentation assays, and (5) incidence of switchgrass rust, caused by the pathogen *(Puccinia emaculata)*.

COMT (caffeic acid 0-methyltransferase) down-regulated switchgrass lines grown in the field for three years (2011-2013) had consistently lower lignin levels, reduced S/G ratios, and improved sugar release across all three years. By the end of year three, both transgenic lines produced 36--41% more ethanol than controls and produced equivalent biomass as controls with no difference in switchgrass rust incidence. The MYB4 over-expressing plants grown for three years (2012-2014) had decreased lignin, improved sugar release, and improved ethanol yields of up to 50%, with one line also producing 63% more biomass than the control in year two. The MYB4 transgenic lines were similar to the control in rust susceptibility with the exception of line L1, which did not exhibit any rust symptoms for the duration of the experiments. Some miRNAl56 overexpressing lines, which were grown for three years (2013-2015), had decreased lignin content, reduced syringyl/guaiacyl (S/G) ratios, and improved sugar release. One line produced 25-56% more biomass relative to the control across all three years. Rust susceptibility varied significantly among the different lines and among years. *Galacturonosyltransferase4* (GAUT4) down-regulated lines grown for three years (2013-2015) had altered S/G ratios and improved sugar release. All three transgenic lines produced significantly more biomass than the control, and one line also had a 23% increase in ethanol yield in year one. Although some GAUT4 lines showed increased rust susceptibility relative to the control in year two, no differences among transgenic lines and the control were observed in year three. One *folylpolyglutamate synthase 1* (FPGSl) down- regulated line had 27% higher biomass relative to the control, whereas a second line produced 7% more ethanol with no change in biomass production in year one. No changes in rust susceptibility were observed between FPGS1 transgenic lines and the control in year two. Several natural variant lines grown for two years (2014-2015) had reduced lignin, increased sugar release, and improved biomass production compared with the control, with one line also showing an increase in ethanol yield in year two. One GAUTl down-regulated line produced 35% more biomass than the control at the end of the first growing season (2015), whereas another line had improved sugar release. One ERF/SHN over-expressing line produced 41% more biomass than the control at the end of the first growing season (2015). Data are currently being collected and analyzed for switchgrass lines with over-expression of GA2-ox and down-regulation of CWG, both of which were planted in late spring of 2016 and have just completed their first growing season.

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