Field Experiments of Seven Switchgrass TOP Lines

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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 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.

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. Downregulation of COMT (caffeic acid *O*-methyltransferase), a lignin biosynthetic gene, reduces lignin and the S/G ratio, thereby improving sugar release and ethanol yield. Overexpression of MYB4, a transcription factor that represses the expression of multiple lignin biosynthetic genes simultaneously, also reduces recalcitrance and improves sugar release efficiency and ethanol yield. Overexpression of miRNA156, a regulator of plant developmental processes, improves biomass yield and sugar release efficiency. Downregulation of CWG-1 (cell wall gene) improves biomass yield, sugar release efficiency, and ethanol yield. Downregulation of CWG-2 improves sugar release efficiency. Overexpression of ethylene response factor/shine (ERF/SHN) transcription factor improves biomass yield and sugar release in switchgrass. Downregulation of CWG-3 improves sugar release efficiency. In addition, natural accessions of switchgrass that produce high biomass with improved sugar release efficiency have been identified through association analysis.

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 seven BESC "TOP Lines" and appropriate controls from agronomically-relevant University of Tennessee (Knoxville) field studies, in which plants were grown under USDA APHIS BRS release into the environment permits for two or three field seasons. Data include: (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 SHF assays, and (5) incidence of switchgrass rust, caused by the pathogen (*Puccinia emaculata*).

COMT down-regulated switchgrass grown in the field for three growing seasons (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 tested 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 over three growing seasons (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 events were similar to the control in rust susceptibility with the exception of event L1, which did not exhibit any rust symptoms for the duration of the experiments. Some miRNA156 overexpressing plants had modest improvement in lignin content and S/G ratios, and ethanol yields in year one, with one line exhibited consistently higher biomass yields relative to the control for years one and two. Rust susceptibility varied significantly among the different lines and among years. Downregulated CWG-1 plants produced more biomass, but had unchanged recalcitrance metrics by year 2, however, one transgenic line had a 23% increase in ethanol yield in year one. Although some CWG-1 lines showed increased rust susceptibility relative to the control in year one, all lines (transgenic and control) were not different than controls by the end of year two. One line of downregulated CWG-2 switchgrass plants 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 CWG-2 transgenic lines and the control in year two. Several natural variant lines showed increased sugar release and improved biomass (up to 91% increase) in year one compared with the control, with one line also exhibiting a 9% increase in ethanol production. Data are currently being collected and analyzed for switchgrass plants with overexpression of the ERF/SHN transcription factor, and downregulation of CWG-3, both of which were planted in late spring of 2015 and have just completed their first growing season.

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