194. First Year Field Results of *PvMYB4*-overexpressing Transgenic Switchgrass and Other Multiple 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 multitalented microbes for 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 generation 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.

High biomass production and wide adaptation has made switchgrass (*Panicum virgatum* L.) a leading dedicated lignocellulosic feedstock candidate in the United States. One of the major limitations is the recalcitrance of complex carbohydrates to hydrolysis for conversion of lignocellulosic biomass into biofuel. Lignin is a primary contributor to recalcitrance as it creates a physical and chemical barrier to enzymatic access of cell wall polysaccharides. Therefore, genetic manipulation of the lignin biosynthetic pathway in an effort to reduce lignin content is a promising approach for overcoming this inherent cell wall recalcitrance.

Transgenic switchgrass plants with reduced lignin content were produced by over-expressing MYB4 transcription factor, an R2R3 type MYB repressor of the lignin biosynthetic pathway. Resulting MYB transgenic plants had reduced height, increased tillering, and reduced lignin under greenhouse conditions. Sugar release efficiency of some lines increased by 300%, which yielded up to 2.7 times more ethanol compared to control plants.

These analyses were performed on greenhouse-grown plants using live, green plant tissue and produced results that could be of great interest to the biofuel industry. However, results from using senesced and brown plant tissue harvested at the end of the growing season from the field experiments and productivity and performance of field-grown transgenics could potentially differ from greenhouse-grown transgenics because of exposure of plants to environmental stresses not present in a greenhouse setting. Therefore, it is essential to determine whether the improved traits observed in greenhouse experiments will be maintained in the field in order to further assess the relevance of these transgenics to the biofuel industry.

Field experiments of MYB4 transgenic switchgrass have been underway at the UT Plant Sciences Unit in Knoxville, Tennessee for two field seasons. In this project, plants are being assessed for 1) agronomic performance including biomass metrics (tiller height, plant width, tiller number) and biomass yield (dry weight), 2) cell wall chemical characterization including lignin content and lignin composition prior to and after senescence, 3) biorefinery performance including pretreatment response, sugar release efficiency, and ethanol yield, and 4) disease susceptibility of transgenic plants compared to the controls including the rust disease.

Several lines of MYB4-overexpressing plants did not survive the first year of field experiments with even more mortality over the first field winter. Out of 4 transgenic lines survived in the field, 2 lines had increased biomass yield and two lines had decreased biomass yield compared to the controls. Biomass produced appeared to be inversely associated with the expression level of the MYB4 gene. Cell wall recalcitrance analyses indicated lower lignin content in 2 transgenic lines compared to the controls and no significant difference in the S/G ratio between transgenics and the controls. The 2 transgenic lines with lower lignin content also had increase in sugar release compared to the controls. Three transgenic lines had an increase of 36%, 29% and 16% in ethanol yield compared to the controls. The most prevalent diseases observed were rust and leaf spot caused by a *Bipolaris* species on both transgenic and the controls. Additional data are forthcoming from the second field season.

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