Modification of Pectin Biosynthesis Leads to Higher Biomass Yield and Saccharification in Bioenergy Feedstock

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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.

Lignocellulosic biomass such as switchgrass is a feedstock for biofuel production. However, the critical barrier towards conversion of such biomass to biofuels is its inherent recalcitrance to deconstruction. Switchgrass biomass is rich in cellulose, xylan, and lignin with smaller amounts of pectin, a particularly complex cell wall polysaccharide whose degree of covalent and non-covalent cross-linking with itself and other wall polymers influences wall architecture. The pectin polysaccharides are the most structurally complex of the plant cell wall glycans, consisting of the polysaccharides homogalacturonan (HG) and rhamnogalacturonan I and II. Here we show that reduced expression of three HG-biosynthetic glycosyltransferases, the α-1,4-galacturonosyl-transferases GAUTl, GAUT4 and GAUT7, impacts both biomass yield and saccharification. All three GAUTs have been proposed to be involved in pectin biosynthesis. We manipulated the expression of *GAUT1, GAUT4* and *GAUT7* through an RNAi approach and determined the effects on biomass recalcitrance and growth. Glucose release per gram dry biomass was significantly increased by 24-35% in *PvGAUT1-KD* lines, 10-15% in *PvGAUT4-KD* lines and 12-22% in *PvGAUT7-KD* lines compared to controls. Total sugar release was increased by 17-31% in *PvGAUT1-KD* lines, 14-15% in *PvGAUT4-KD* lines and 9-14% in *PvGAUT7-KD* lines compared to controls. Silencing of these three genes leads to 7-43% increased plant height in *PvGAUT1-KD* lines, 12-17% in *PvGAUT4-KD* lines and

5-10% in *PvGAUT7-KD* lines, along with 110-230% more tillers compared to wild type. Both the increase in plant height and the greater number of tillers contributed to more aerial biomass in the transgenic lines than control plants. We hypothesize that specific GAUTs synthesize unique HG glycans that function as stand-alone polysaccharides and/or a glycans in wall glycoconjugates that are necessary for native cell wall integrity in grasses. We also hypothesize that reduced amounts of the pectic polymer(s) lead to loosened walls and hence decreased recalcitrance and increased growth in switchgrass.

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