Modification of GAUT12 Expression Affects Recalcitrance in the Woody Feedstock *Populus deltoides*

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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.

The deconstruction of feedstocks such as *Populus* for biofuel production is challenging due to the recalcitrance of woody biomass. *Populus* biomass is rich in cellulose, xylan and lignin with smaller amounts of pectin. The high degree of cross-linking and interactions between the wall polymers is a major barrier to the conversion of cellulose and other polysaccharides into ethanol without expensive chemical and/or enzymatic pretreatment. GAUT12 (GAlactUronosylTransferase12)/ IRX8 (IrRegular Xylem8) is a putative glycosyltransferase proposed to be involved in secondary cell wall glucuronoxylan and/or pectin biosynthesis.

We manipulated the expression of one of the two *Populus deltoides* GAUT12 homologs (*PtGAUT12.1* and *PtGAUT12.2)* via overexpression and RNAi approaches and determined the effects on biomass recalcitrance and growth. A 7–48% increase (*PdGAUT12.1*-OE) and 50–67% decrease (*PdGAUT12.1*-KD) in *Populus deltoides* GAUT12.1 transcript expression compared to controls yielded a 4–11% decrease and 4–8% increase, respectively, in glucose release following enzymatic saccharification of the biomass. The *GAUT12.1*-OE lines had reduced plant height and stem radial diameter of 6–54% and 8–41%, respectively, compared to controls, a phenotype opposite to the increased growth of *PdGAUT12.1*-KD lines with a 12–52% and 12–44% increase in plant height and radial stem diameter, respectively, compared to the controls (Biswal et al., *Biotechnology for Biofuels* **8**:41, 2015). The wood of *PdGAUT12.1*-OE lines had 12–17% increased galacturonic acid (GalA) and 14–20% increased xylose content. Conversely wood from *GAUT12.1*-KD lines had 25–47% reduced GalA and 17–30% reduced xylan content. There was no effect of *GAUT12.1* overexpression or downregulation on total lignin content. Taken together, the results suggest that GAUT12 is required for the synthesis of one or more pectin and xylancontaining structures in *Populus* and that there is an inverse relationship between the amounts of this structure in the wall and stem diameter, height and sugar release. We hypothesize that GAUT12 synthesizes a wall polymer that cross-links the wall and that increased amounts of this structure lead to a more cross-linked wall, greater recalcitrance and reduced growth. Reciprocally, we hypothesize that reduced amounts of the polymer(s) lead to loosened walls and hence decreased recalcitrance and increased growth. The results show that the directed down regulation of GAUT12 in *Populus* can lead to better woody feedstock for the biofuel industry.

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