205. Matrix Cell Wall Polysaccharide Synthesis Gene 1 Knockdown in Switchgrass – a BESC TOP Line

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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.

Lignocellulosic biomass such as woody *Populus* and perennial switchgrass is composed of the complex structures of lignin, cellulose, matrix polysaccharides (pectin and hemicellulose) and cell wall proteins. Due to its complex structure, lignocellulosic feedstock is highly recalcitrant to bioconversion into ethanol by microbes. Here we modified Matrix Cell wall Polysaccharide Synthesis gene 1 (*mcps1*) expression in switchgrass, *Populus*, rice and foxtail millet using an RNAi and Virus-Induced Gene Silencing (VIGS) approach to determine the effects of reduced expression of the encoded protein on recalcitrance and ethanol yield. The *MCPS1* gene was selected as a target due to its high expression in tissues enriched in secondary walls, since secondary wall-containing tissues are the main source of biomass in these feedstocks. The knockdown expression of MCPS1 in switchgrass (*Pvmcps1-KD*) resulted in increased tiller numbers and increased biomass yield. Down regulation of the switchgrass *MCPS1* gene yielded a significant 15% increase in glucose and total sugar/gm biomass and ethanol yields up to 36% (mg/g cellulose) and 51% (mg/g dry biomass), respectively, greater than controls. Similarly, 36% more glucose and 31% more total sugar release was obtained in foxtail millet *Simcps1-KD* VIGS knockdown lines. Down-regulation of MCPS1 homologs in *Populus* (*Pvmcps1*-KD) resulted in 6-44% greater plant height and 7-27% increased stem diameter compared to WT as well as 3-7% greater glucose release. Rice transgenic knockdown (*Osmcps1-KD*) lines had greater plant height, tiller numbers and dry biomass. The generation of biomass with reduced recalcitrance and increased biomass can lower biofuel production costs and increase biomass yield/acre, thereby positively affecting the biofuel industry. Efforts are underway to determine the mechanism by which reduced expression of MCPS1 leads to increased sugar release and enhanced growth.

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