Characterization of *Populus* Transgenic Plants Overexpressing PtDUF266A (OXPtDUF266A) and Biofuel Production

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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.

Determining the function of proteins containing Domains-of-Unknown Function (DUF) is a challenge once their phenotypic value is identified. In this study, one member of *Populus* DUF266 proteins, PtDUF266A, was investigated as a potential candidate for improved biofeedstocks for biofuel production. DUF266-containing proteins have been considered as 'not classified glycosyltransferases (GTnc)' because they are phylogenetically distinct from the GTs. In specific *Populus* transgenic plants overexpressing DUF266-containing proteins (*OXPtDUF266A)* were characterized by diverse analysis approaches including (1) wet chemical bulk analysis for chemical composition, (2) anthrone assay for cellulose content, (3) gel permeation chromatography (GPC) analysis for molecular weights of lignin and cellulose, (4) gene expression analysis for effects on biomass biosynthesis gene regulation, and (5) nuclear magnetic resonance (NMR) analysis for structural information of lignin.

The results showed that the *OXPtDUF266A* were larger than WV94 under the same growing conditions. In the *OXPtDUF266A,* the glucose and cellulose contents notably increased. In addition, degree of polymerization of cellulose in the OXPtDUF266A transgenic plants also increased. However, its cellulose crystallinity index was not changed. Based on gene expression analysis, cellulose biosynthesis-related genes such as *CESA* and *SUSY* were upregulated in the *OXPtDUF266A* transgenic plants. The gene expression analysis results indicate that overexpression of PtDUF266A induced more expression of cellulose biosynthesis genes. Besides the cellulose, physicochemical

properties of lignin in the *OXPtDUF266A* transgenic plants were also analyzed by NMR and GPC analyses. The lignin molecular weights and lignin S/G ratio in the transgenic plants were not notably changed.

In addition, to evaluate the *OXPtDUF266A* transgenic plants as a suitable feedstock for biofuel production, sugar release of the transgenic plants was tested. Compared to the wild-type plants, the *OXPtDUF266A* transgenic plants showed more sugar release (38% higher). Increase of total cellulose content in the *OXPtDUF266A* transgenic plants resulted in an increase of sugar release. Overall, the overexpression of *PtDUF266A* showed great potential as a feedstock for biomass utilization.

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