

Modified Expression of HD-ZIP III and NAC Transcription Factors Impact Biomass Formation and Sugar Release Efficiency in *Populus*

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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.

We are undertaking a multipronged, genetics- and genomics-based, research strategy to examine regulation of cell wall and biomass properties in *Populus*. Here, we present results from studies of genes belonging to the Homeodomain-leucine zipper class III (HD-ZIP III) and NAC domain transcription factor families. HD-ZIP III and NAC domain transcription factor family members have been previously known to regulate a diverse array of plant pathways ranging from embryo, shoot, leaf and flower development to biotic and abiotic stress response and hormone signaling. We examined the roles of selected gene family members, with enhanced expression in secondary wall-enriched xylem tissue, in determining biomass properties including sugar release efficiency of *Populus*. Transgenic *Populus deltoides* plants were generated via overexpression or down-regulation under the control of either a vascular-specific 4-coumarate-CoA ligase (*4CL*) promoter or that of a ubiquitous promoter. Morpho-anatomical, metabolic, physiological, cell wall chemistry studies revealed the extent of impact on biomass properties. Such modifications in gene expression resulted in a range of phenotypes; from changes in cell wall chemistry with no apparent compromise on plant growth and performance to pleiotropic effects to favorable phenotypic combination of enhanced growth and reduced recalcitrance. We further performed SNP (single nucleotide polymorphism) network analyses to extract genotypic and phenotypic correlations significant to the targeted genes. Using *PdWND1B* (NAC) and *PdHB3* (HD-ZIP III) as examples, we discuss alternate modes of action of these transcription factor genes in impacting biomass formation and sugar release efficiency in *Populus*.

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