jbei
Joint BioEnergy Institute

Selection, Cloning and Functional Characterization of Rice-Diverged, Cell Wall-Related Glycosyltransferases

Miguel Vega-Sánchez* (mevegasanchez@ucdavis.edu), Laura Bartley, Peijian Cao and Pamela Ronald (pcronald@ucdavis.edu) Department of Plant Pathology, University of California, Davis, CA 95616 and Joint BioEnergy Institute, Feedstocks Division, Emeryville, CA 94710

Understanding plant cell wall biosynthesis is crucial for the development of the next generation of biofuels derived from lignocellulosic material. Current limitations in the harvest of fermentable sugars from cellulose derive from the inherent recalcitrance of plant cell walls. Basic knowledge of how the structure and composition of the cell wall can be modified to obtain biomass suitable for efficient and economically viable biofuel production is needed. Glycosyltransferases (GTs) are a large, multifamily class of enzymes that form glycosidic bonds between donor nucleotide sugars and acceptor substrates. Among the GTs are the enzymes responsible for the synthesis of important cell wall polysaccharides, including cellulose, hemicellulose and callose. However, the function, substrate specificity and biochemical activity of the majority of GTs are unknown. Many aspects of grass and other commelinoid monocot cell walls are distinct from that of better-studied dicots. As many of the preferred feedstocks for future bioenergy initiatives include grasses such as switchgrass and Miscanthus, the model species of choice to advance our understanding of monocot-specific aspects of the cell wall is rice (Oryza sativa). Rice was the first grass species to have its full genome sequenced and abundant functional genomic resources have accumulated in recent years. To identify potential targets involved in cell wall synthesis, we mined the recently created and publicly available rice GT phylogenomic database (http://ricephylogenomics.ucdavis.edu/cellwalls/gt/) to select a list of 33 rice-diverged GTs with high expression in above-ground, vegetative tissues. Cloning of these genes is underway, and will be followed by the creation of a variety of expression constructs for functional analysis in both transgenic plants and heterologous systems for protein expression, biochemical activity determination and protein-protein interaction network generation. We have ordered insertion and activation-tagged lines from worldwide repositories for cell wall composition/modification analyses as well as phenotypic characterization. Among this group of genes, we will study in detail a subset within GT family 2, the grass-specific Cellulose synthase like (Csl)-F and H sub-families, by generation of gene family knockdowns using artificial miRNAs. We will characterize other putative GTs from unknown families as well. We anticipate that by focusing on grass-specific GTs involved in cell wall synthesis and modification in plant tissues important for the production of lignocellulosic-derived biofuels, we can facilitate the improvement of genetic traits in second-generation bioenergy crops such as switchgrass.

U.S. DEPARTMENT OF ENERGY | Office of Science