113. Modulating plant cell wall for biofuel at GLBRC

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Projects goals : Great Lakes Bioenergy Research Center (GLBRC) is focused on sustainable production of crops with desirable biofuel traits and efficient conversion of biomass into fuels and chemicals. To achieve the goals, GLBRC has four research areas – Plants, Sustanability, Deconstruction and Conversion. The ultimate goal of plants area is to increase both the amount and quality of biomass by modulating cell wall digestibility through lignin modification and the metabolic and genetic system that control accumulation of oils and other easily digestible, energy-rich compounds in plant tissues.

To increase easily digestible hemicellulose (mixed-linkage glucan; MLG) in plant stem parenchyma cells, a Plants team in GLBRC is focused on understanding the factors required to accumulate large quantities of MLG including genetic regulation of MLG synthesis, identification of tissue specific promoters and characterization and engineering of MLG synthases.

To achieve the goals, we are using Brachypodium distachyon as a model grass species. MLG is one of the major components of cereal grains, and occupies up to 80% of cell walls of the Brachypodium endosperm. The MLG biosynthesis depends on the biochemical activity of membrane spanning glucan synthases encoded by the CSLH and CSLF cellulose synthase-like gene families. However, relatively little is known about their topology with respect to the biosynthetic membranes and requirement for producing two different linkages in the MLG glucan backbone. As the first step on the project, we have demonstrated the topology of CSLF6 protein derived from Brachypodium (BdCSLF6) using heterologous expression systems. Using live cell imaging and immuno-electron microscopy analyses of tobacco epidermal cells expressing BdCSLF6, we demonstrate that a functional YFP fusion of BdCSLF6 is localized to the Golgi apparatus and that the Golgi localization of BdCSLF6 is sufficient for MLG biosynthesis. By implementing protease protection assays of BdCSLF6 expressed in the yeast Pichia pastoris, we also demonstrate that the catalytic domain, the N-terminus and the C- terminus of the protein are exposed in the cytosol. Furthermore, we found that BdCSLF6 is capable of producing MLG not only in tobacco cells but also in Pichia, which generally does not produce MLG. Together, these results support the conclusion that BdCSLF6 can produce both of the linkages present in the (1,3; 1,4)-β-D-glucan chain of MLG and that the product is channeled at the Golgi into the secretory pathway for deposition into the cell wall.

This initial approach allowed us to set up the condition for analyzing MLG synthases. To accomplish our goals, we are currently engineering BdCSLF6 to improve its ability to produce large quantity of MLG.

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