Modulating hemicellulose to improve bioenergy crop

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Project Goals: The goal of Bioenergy Plant Design team in Great Lakes Bioenergy Research Center (GLBRC) is to increase the quantity and quality of bioenergy crop biomass per hectare of land, which is crucial for the sustainable and economically viable production of lignocellulosic-derived bioproducts.

The plant cell wall (CW) is composed of polysaccharides and lignin, which have specific roles during plant's growth. Importantly, CW constitutes the majority of the biomass destined for conversion. Therefore, we want to achieve our goal by improving quantitative and qualitative CW traits such as elevating the production and accumulation of cell wall polysaccharides, including Mixed linkage (1,3;1,4)-β-glucan (MLG), a low-recalcitrance glucose polymer. Thus, characterization and engineering MLG synthases are also required to produce MLG with high efficiency.

To achieve the goals, we used *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 demonstrated 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.

To further investigate *in-vivo* MLG synthesis in its native environment, we have performed immuno-localization analyses with the MLG-specific antibody in Brachypodium and in barley. We found MLG present in the Golgi, post-Golgi structures and in the cell wall. Accordingly, analyses of a functional fluorescent protein fusion of CSLF6 stably expressed in Brachypodium demonstrated that the enzyme is localized in the Golgi as we have seen in tobacco. We also established that overproduction of MLG causes developmental and growth defects in Brachypodium as also occur in barley. Our results indicate that MLG production by BdCSLF6 occurs in the Golgi similarly to other cell wall matrix polysaccharides, and support the broadly applicable model in grasses that tight mechanisms control optimal MLG accumulation in the cell wall during development and growth.

References

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