Modulating hemicellulose to improve bioenergy crops

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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, the CW constitutes the majority of the biomass destined for conversion. Therefore, we want to improve 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, characterizing and engineering MLG synthases are also required to produce MLG with high efficiency. To achieve our goals, we used *Brachypodium distachyon* as a model grass species. MLG is one of the major components of cereal grains, and MLG biosynthesis depends on the biochemical activity of membrane spanning glucan synthases encoded by the CSLH and CSLF cellulose synthase-like gene families. As the first step of the project, we demonstrated the topology of CSLF6 protein derived from Brachypodium (BdCSLF6) using heterologous expression systems. We reported 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 using its catalytic domain in the cytoplasm. The localization of BdCSLF6 was further confirmed in its native environment, and MLG was detected 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 in barley. To overcome the growth defect by over-accumulation of MLG, we generated plants with improved biomass by expressing the endoplasmic reticulm (ER) stress sensor IRE1 and crossed them with MLG over-accumulating Brachypodium plants to utilize the unfolded protein response, thereby alleviating stress caused by overexpression of BdCSLF6. We found that the transgenic lines for both IRE1OX and CSLF6OX maintained the increased biomass trait of the IRE1OX lines, supporting that IRE1 overexpression suppresses the growth penalty induced by BdCSLF6 overexpression. We also found an increased MLG level in the crossed line compared to wild type. To understand the tissue- and development-specific regulation of MLG synthesis and IRE1 expression, we have employed a new *in-situ* hybridization technique. Applying this technique in the Brachypodium seeds indicated that MLG synthesis is specifically activated in the endosperm with a tissue specific manner. With this

result, we expect to apply this technique in the other stem tissue to understand MLG synthesis, degradation and impact of plant growth.

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