Optimizing MLG Production for Improved Biofuel Crop

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Project Goals:

The goal of this project is to improve biomass feedstock by increasing the levels of mixed-linkage glucan (MLG), a non-cellulosic polysaccharide, in biofuel crops. Since MLG is composed of only glucose, we expect to generate plants with an improved C6 to C5 ratio that would be more easily fermentable by yeast, consequently generating an enhanced biofuel feedstock. MLG is synthesized by CSLF and CSLH proteins. In previous studies increasing the levels of MLG in barley plants using a constitutive promoter led to growth penalties, demonstrating the choice of promoter and specific tissue location to overexpress the genes of interest in storage vegetative tissues is important to obtain healthy MLG-rich plants. To overcome the growth penalty caused by the accumulation of MLG we plan to optimize the expression of CSFL6 and modify stress response signaling pathways, allowing plants to adapt to MLG overproduction while maintaining healthy plant performance. This research is promising to advance significantly the digestibility of biofuel feedstock produced from grasses.

Abstract:

The focus of our work is to improve biofuel grasses with the fewest inputs resulting in desirable bioenergy feedstock at a low cost. For improved biofuels we aim to increase the amount of a cell wall component, mixed-linkage glucan (MLG). MLG is an important cell wall polysaccharide containing β-D-glucosyl residues with both (1,3) and (1,4) linkages making up 20% of cell wall

in grasses[5]. To do this we must understand how MLG is synthesized by CSLF and CSLH proteins[2,3]. Within the CSLF family, CSLF6 has been reported to be highly expressed in the model grass Brachypodium and is exclusively found in monocotyledons[3]. From our RNA seq data we also found that CSLF6 is highly expressed in early endosperm development of Brachypodium when MLG synthesis is most active[4]. Using Brachypodium, we have characterized a CSLF6 homologue, BdCSLF6, and established its functional requirements *in vitro* and *in vivo*[5]. Expressing our YFP tagged BdCSLF6 in tobacco, a species that does not produce MLG, we established that BdCSFL6 is localized to the Golgi membranes and is capable of producing MLG. We have also shown production of MLG when BdCSLF6 is expressed in Pichia, which supports that BdCSLF6 alone produces both β-(1:3, 1:4) linkages of MLG. In addition, we also have confirmed the protein topology of BdCSLF6 and proven that the catalytic domain of the enzyme is exposed to the cytosol, supporting that MLG is secreted into the Golgi compartment[5]. By analyzing our transgenic Brachypodium over-expressing YFP fused CSLF6 by a constitutive promoter, we also observed BdCSLF6 is localized to the Golgi but also to the endoplasmic reticulum (ER)[5]. These plants display an increased amount of MLG (~30%) when compared to WT (Bd21-3), yet have a stunted growth phenotype. To avoid growth penalties, we have identified several tissue and developmental specific promoters through RNA seq analysis from Brachypodium and confirmed by RNA *in situ* hybridization and GUS reporter system[1,6,7,8]. The tissue specific promoters selected will drive expression of BdCSLF6 in pith parenchyma cells that are functionally capable of holding increased amounts of MLG. In addition, we plan to modify stress response signaling pathway genes in these BdCSLF6 lines allowing the plants to cope better to the accumulation of MLG. We anticipate that engineering plants to contain increased amounts of MLG in tissue amenable to store MLG will allow for an easily digestible biofuel feedstock without compromising growth.

References

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