

## **Disruption of *Brachypodium distachyon* Lichenase Alters Metabolism of Mixed-linkage Glucan and Starch**

Mingzhu Fan<sup>1\*</sup>(mzfan2012@gmail.com), Jacob K Jensen<sup>1,2</sup>, Sang-Jin Kim<sup>1</sup>, Jia-Yi Chan<sup>1</sup>, Starla Zemelis-Durfee<sup>1</sup>, Claudia Beaudry<sup>1</sup>, Federica Brandizzi<sup>1</sup>, **Curtis G Wilkerson<sup>1</sup>**

<sup>1</sup>Michigan State University, East Lansing; <sup>2</sup>University of Copenhagen, Copenhagen.

<https://www.glbrc.org/>

### **Project goals: Engineering bioenergy crops with increased accumulation of an easily fermentable cell wall polysaccharide MLG, without affecting plant growth.**

Biomass represents a significant energy resource and as such, it is important to improve the efficiency of its conversion to fuel by altering its chemical makeup. Mixed-linkage glucan (MLG), which is widely distributed in grasses, is an easily fermentable cell wall polysaccharide and ideal compound for the production of biofuels. Previous studies have shown that transgenic plants overexpressing MLG synthases accumulate more MLG but with detrimental growth defects and reduced biomass yields. In this study, we identify a gene encoding a lichenase we have named *Brachypodium distachyon* *LICHENASE 1* (*BdLCHI*), which is highly expressed in the endosperm of germinating seeds and elongating internodes. RNA in situ hybridization showed that it is also expressed in chlorenchyma cells of mature leaves and stems. *lch1* mutants generated using CRISPR-Cas 9 technology accumulated more MLG in the vegetative tissues examined and did not show significant growth defects. Compared with wild-type plants, disruption of *BdLCHI* resulted in an 8-fold increase in MLG content in senesced leaves. Immunolabeling with MLG monoclonal antibody showed that MLG in parenchyma cells of mature leaves and stems was not removed as was the case in wild-type plants. MLG stability during development was improved in *lch1* mutants.

Transcription of *BdLCHI* was induced by darkness in both wild-type and *lch1* mutants. While MLG content in wild-type plants decreased significantly after dark-incubation, *lch1* plants contained a similar amount of MLG as those grown under a regular light/dark cycle. Dark-incubated 8-week-old *lch1* plants also had a faster rate of starch breakdown than that in wild-type plants. Disruption of *BdLCHI* not only changed MLG accumulation but also altered starch degradation in darkness. These results indicate that engineering bioenergy crops by modifying the expression of lichenases is a promising strategy for biofuel production.

*This material is based upon work supported by the Great Lakes Bioenergy Research Center, U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018409.*