Multiple-approaches to engineer mixed-linkage glucan in sorghum

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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.

Mixed-linkage glucan (MLG), a β-(1,3;1,4)-glucose polymer, is one of the most abundant cell wall matrix components in grass species. Due to its simple structure and composition, which make MLG a highly digestible polymer, it has become the target of cell wall manipulation to improve bioenergy feedstocks for microbial conversion. Therefore, we are aiming to accumulate MLG in bioenergy sorghum, which exhibits high biomass yield and elevated content of soluble sugars. To increase the levels of MLG in this crop, we are focusing on MLG synthesis and degradation with the goal to overproduce MLG and prevent degradation of MLG in the stem. Overproduction of MLG hampers growth in some species, such as Brachypodium, rice and barley. Nevertheless, we established a technology to generate transgenic sorghum overexpressing a major MLG synthase (CSLF6) from either Brachypodium or sorghum (constructs named BdCSLF6 and SbCSLF6, respectively), which are known to generate a different frequency of β1-3 linkages in MLG, resulting in different types of fine structure of this polymer. The transgenic sorghum grown in a greenhouse showed higher MLG levels compared to wild type (WT) without any noticeable growth defects. In summer 2021, we conducted field trials (East Lansing, MI) with the T3 generation of CSLF6-sorghum transgenics and found no defects in growth and photosynthesis. MLG analyses from the field-grown sorghum were performed during development within single stem internodes. We observed that the transgenic lines have higher levels of MLG compared to WT, even in the fully elongated region of the internode where MLG degradation normally occurs. Furthermore, to modulate and reduce MLG degradation in sorghum stem and lead to higher levels of MLG, we identified and characterized three sorghum MLG endoglucanases (also called lichenases). We established that the three lichenases have similar enzymatic activity, with an optimal at pH5, between 25˚C and 45˚C. We also found that the three enzymes localize in the apoplast, where lichenase activity is expected to take place. In addition, we established the lichenases' expression pattern during development and light conditions, which led us to identify the lichenase that is likely responsible for MLG degradation in sorghum. We are now generating CRISPR-Cas9-mediated suppression of all three lichenases

in sorghum for crossing with the CSLF6-overexpressing lines to maximize the levels of MLG in the stem.

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