Identification of Grass Stem Specific Promoters for Improvement of Biofuel Crops by Metabolic Engineering

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Project Goals: The major goal of our project is to manipulate the levels and composition of hemicellulose in the cell walls of biofuel crops. We therefore seek to understand the regulation of hemicellulose biosynthesis in sufficient detail so we can manipulate these processes in the plant to optimize their use as biofuel feedstocks. Specifically we are studying the grass specific hemicellulose mixed-linkage glucan (MLG), which can accumulate to very high level in tissues such as the endosperm. We wish to replicate the very large accumulation of MLG seen in endosperms cell walls in stem pith parenchyma cells in order to increase the sink capacity of these cells and to provide a more digestible wall with a high C6 to C5 ratio.

Plants store a variety of compounds as reserves of energy and carbon skeletons for future needs. Many of these compounds have other uses in cells such as the structural role that hemicelluloses play in cell walls or the use of lipids to construct membranes. In some cases these compounds are allocated in dedicated storage tissues, some of which are derived form the stem. One such example is potato tubers, their developmental origin being evident from typical stem cell type arrangement. Another example is the stems of grasses, such as sugar cane and sorghum, which store large amounts sucrose and sometimes starch. In the later case, carbon accumulation often occurs during periods of slow growth to use when more favorable conditions resume. These naturally occurring mechanisms suggest that it should be possible to engineer bioenergy crops to store polysaccharides in the cell wall in vegetative tissues, particularly stem pith parenchyma, to be easily broken down into sugars for conversion to biofuels or other high-value compounds.

Our primary goal is to gain sufficient knowledge of the control of hemicellulose deposition to manipulate the accumulation of large amounts of mixed-linkage glucan (MLG) in the stem parenchyma tissue of grasses. We chose this tissue because grasses use this location to store a large variety of compounds, such as starch and sucrose as mentioned above, without detrimental affects on plant growth, and because it represents a very large storage compartment, approximately a third of the aerial part of the plant for the larger grasses such as maize and sorghum. We chose MLG because it is a polysaccharide exclusively composed of glucose, is easily extracted from the wall and enzymatically digested, and because it accumulates to large

extent in a number of grass tissues. The most pronounced example of such a tissue is the Brachypodium seed endosperm where MLG constitutes 40% of the total dry weight, giving rise to very thick cell walls almost exclusively consisting of MLG polysaccharide.

To accomplish this goal, we will require promoters that are specific for the stem parenchyma tissue and that are active at this location at the correct developmental stage. Additionally, we will need to understand how tissues such as the endosperm accumulate large quantities of MLG in the absence of cellulose accumulation.

We will present our progress on the isolation of stem pith parenchyma specific promoters. Using transcriptional profiling of developing internodes and stem pith enriched tissue samples from Brachypodium we have identified a series of candidate genes exhibiting pith parenchyma specific expression. We have cloned the upstream promoter regions of these candidate genes and are in the process of evaluating these constructs as promoter-GUS fusions by stable transformation of Brachypodium. We will present the analysis of the first four of these candidate promoters and detailed analysis of one that exhibiting high expression in the pith parenchyma cell type throughout the stem. We also present our work on using transient transformation of stem pith cells using a gene gun, which will accelerate our ability to define the promoter region required for tissue specificity.

In parallel with evaluating our promoter candidates by GUS expression characterization, we are producing a collection of transgenic plant materials of Brachypodium and maize ectopically expressing the MLG synthase gene *BdCSLF06* using our stem pith promoter candidates. These plants will server to further evaluate the promoters and provide crucial insights into the requirements for accumulation of MLG in the cell wall.

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