Increasing biomass in grasses by identifying genes involved in mixed-linkage glucan biosynthesis

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Project Goals: The major goal of this project is to increase the quality and quantity of grass biomass. Specifically we aim to increase the amount of the easily digestible cell wall polysaccharide mixed-linkage glucan in grass cell walls. To achieve this goal, we have identified a transcription factor likely involved in mixed-linkage glucan biosynthesis that should allow us to better understand mixed-linkage glucan biosynthesis.

Mixed-linkage glucan (MLG) is a polysaccharide that is highly abundant in grass endosperm cell walls and at lower amounts in other tissues. We know that the enzymes produced by the genes *CSLF* and *CSLH*, members of the cellulose synthase-like gene families, are able to synthesize MLG but it is unknown if other genes participate in the production of MLG. We generated a large set of Brachypodium transcriptional profiling data that allowed us to identified a trihelix family transcription factor (*BdTHX1*) that is highly co-expressed with *BdCSLF6*. Using published data from other groups we were able to show that *THX1* and *CSLF6* are also co-expressed in wheat and maize [1, 2]. These co-expression data suggests that *THX1* is involved in the regulation of MLG biosynthesis.

Determining the genes regulated by this transcription factor could reveal more genes involved in MLG accumulation. To find such genes we conducted chromatin immunoprecipitation (ChIP)-seq experiments using immature Brachypodium seeds and an anti-BdTHX1 polyclonal antibody we produced. The ChIP-seq experiment identified the second intron of *BdCSLF6* as one of the most enriched sequences. The binding of BdTHX1 to the *BdCSLF6* intron was confirmed using electrophoretic mobility shift assays (EMSA). A gene encoding a grass specific glycoside hydrolase family 16 (GGH16-1) endotransglucosylase/hydrolase was also discovered in the ChIP-seq data and the binding was confirmed by EMSA. Such enzymes have been implicated in the incorporation of hemicelluloses in to the wall and as such could be involved in MLG accumulation. We have expressed GGH16-1 in *Pichia* and we are in the process of testing its activity on MLG. Motif analysis of the DNA regions shown to bind BdTHX1 by ChIP-seq and EMSA revealed that BdTHX1 binds to previously described elements bound by trihelix transcription factors termed GT-elements. GT-elements were also found in the introns of *CSLF6* gene in rice and maize.

Our work provides information on genes likely involved in MLG accumulation and

further characterization of these genes should allow for the improvement of biomass for biofuel production.

References

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