

141. BdMYB48 Regulates Biofuel Feedstock Attributes in the Model Grass *Brachypodium distachyon*

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Project Goals: (1) Identify regulators of grass cell wall biosynthesis and biomass accumulation. (2) Analyze key transcription factor function and genomic binding sites. (3) Determine the effects of regulator perturbation on growth and amenability to deconstruction and cell wall properties.

Plant biomass offers a sustainable low cost alternative to fossil fuels and grasses such as miscanthus, sorghum, and switchgrass can provide ample biofuel feedstocks. While there is a need to better understand the molecular switches governing grass biomass accumulation, few have been characterized. Therefore, we conducted a screen for *Brachypodium distachyon* transcription factor proteins that interact with the regulatory regions of genes that encode cell wall biosynthetic enzymes. *B. distachyon* serves as a suitable model for energy crop research due to its close phylogeny to species such as switchgrass and miscanthus. In addition, it has many attributes characteristic of an ideal model organism, including a completely sequenced genome, self-compatible rapid life cycle, mutant collections, and genetic transformation. Seven of the 14 transcription factor proteins we observed that had a significant affinity with cell wall promoters were MYBs. Based on gene expression and amino acid homology, *BdMYB48* was selected for further characterization. The *BdMYB48* transcript is abundant in *B. distachyon* stem tissue, which accounts for a majority of above ground biomass. In order to functionally characterize *BdMYB48*, gain-of-function mutants (*Ubi::BdMYB48*) were generated by constitutively over expressing the full length coding region under the maize ubiquitin promoter. Similarly, dominant repressor mutants (*Ubi::BdMYB48:CRES*) were generated by over expressing the full-length coding region fused to a dominant repressor. Despite both types of transgenics having no significant changes in flowering time, gain-of-function lines had greater aboveground biomass accumulation while *Ubi::MYB48:CRES* plant biomass was reduced.

Interestingly, cellulose and lignin biosynthesis genes were significantly down regulated with a striking reduction in sclerenchyma fiber cell lignification in the *Ubi::BdMYB48:CRES* plants. Moreover, acetyl bromide soluble lignin content was significantly reduced in *BdMYB48:CRES* plants and modestly increased in *Ubi::BdMYB48* plants. Considering lignin is inversely correlated with bioconversion efficiency phenotypes, ethanol yield was measured after culturing stems with *Clostridium phytofermentans*. As expected, a decrease in ethanol yield was observed for *Ubi::BdMYB48* and a significant increase for *Ubi::BdMYB48:CRES* samples. Overall, these data suggest a cell type specific role for *BdMYB48* in *B. distachyon* secondary wall synthesis.

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