

Control of lignin and general phenylpropanoid biosynthesis in maize

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Grasses, including maize, are a major source of agricultural biomass, offering significant opportunities for increasing renewable fuel production. Efficient biofuel production is influenced by lignin content which can significantly reduce extractable sugar content. To explore the possibility of altering lignin, we are investigating regulators of the maize phenylpropanoid pathway.

The maize R2R3-MYB P1 controls accumulation of phlobaphenes in seed coats and biosynthesis of insecticidal *C*-glycosyl flavones in silks. Few steps of these pathways are known, and only the *A1* gene encoding dihydroflavonol reductase is confirmed as immediate target of P1. To establish the overall regulatory function of *P1*, we performed a genome-wide analysis combining RNA-Seq and ChIP-Seq using two maize varieties varying highly in their *P1* expression. Computational analysis of the data revealed that *P1* particularly plays a role in flavonoid biosynthesis, and to a lesser extent in other branches of the phenylpropanoid pathway.

Based on the mechanisms by which P1 controls gene expression, we hypothesized that closely related R2R3-MYBs control other phenylpropanoid pathway branches. *ZmMYB40* and *ZmMYB95* are two R2R3-MYBs expressed in most plant tissues, and both have been identified as positive transcriptional regulators, with *ZmMYB40* binding one P1-binding site in the *A1* promoter. Distinct from P1, however, *ZmMYB40* overexpression in maize cells induces the accumulation of phenylpropanoids, but not flavonoids, suggesting a function different from P1. In order to identify direct targets for *ZmMYB40* and *ZmMYB95*, transgenic lines harboring RNAi constructs to silence the respective genes were generated. Lines showing low expression levels are used for cell wall composition and target gene expression analysis. Characterization of these R2R3-MYBs in combination with two negative regulators of lignin biosynthesis (*ZmMYB31* and *ZmMYB42*) will help explain the intricate regulation of lignin and lignin precursors. These studies will also help identify approaches for genetic improvement of other important grass species for biofuel production.

This project was funded by grants DOE DE-FG02-07ER15881 and NSF DBI-0701405.

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