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. The efficiency of biofuel production is influenced by lignin content which can significantly reduce the amount of extractable sugars. To explore the possibility of altering lignin content we are investigating regulators of the phenylpropanoid pathway.

The maize pericarp is the outside layer of the seed coat, and it acts as the first barrier to insects and pathogens. It is also important for the quality of canned corn and popcorn as a moisture barrier. In maize, C1 and P1 are both members of the R2R3-MYB class and control different branches of the phenylpropanoid pathway (biosynthesis of anthocyanins, and various phenylpropanoids and phlobaphenes, respectively). In maize seed coats or pericarps, P1 controls the accumulation of phlobaphene pigments, in addition to the biosynthesis of insecticidal *C*-glycosyl flavones in silks. To date, only few early steps of these pathways are known, and only the *A1* gene encoding dihydroflavonol reductase (DFR) has been confirmed as immediate target of P1. In order to establish the overall regulatory function of *P1*, we performed a genome-wide analysis combining RNA-Seq and ChIP-Seq in P1-rr, specifying red pericarp and red cob glume color and P1-ww, which harbors a null P1 allele lacking phlobaphene pigments. We successfully obtained short read sequence tags from mRNA and ChIP DNA extracted from pericarp cells of P1-rr and P1-ww at two different developmental stages, 15 and 25 DAP. The computational analysis of RNA-Seq and ChIP-Seq data revealed that *P1* particularly plays a role in the flavonoid pathway, and to a lesser extent in the other branches of the phenylpropanoid pathway.

C1 controls, together with its cofactor, the bHLH R, the accumulation of anthocyanins, another branch of the phenylpropanoid pathway. Based on the mechanisms by which P1 and C1 control gene expression and supported by mutations that switch the activity of one to the other, we hypothesized that it was very possible that other R2R3 MYB^{PtoA} clade members would control other branches of the biosynthesis of phenolics. *ZmMYB40* and *ZmMYB95* are two R2R3-MYBs expressed in most plant tissues, and *in situ* mRNA hybridization showed their expression strongly associated with vascular bundles. Both of these MYBs have been identified as positive transcriptional regulators and ZmMYB40 binds the high-affinity 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, maize transgenic lines harboring RNAi constructs to silence the respective genes have been generated. We identified lines showing decreased *ZmMYB40* or *ZmMYB95* expression that will be used for cell wall composition analyses in addition to target gene expression analysis. Characterization of these MYBs in combination with

investigation of two confirmed negative regulators of lignin biosynthesis (ZmMYB31 and ZmMYB42) will help explain the intricate regulation of lignin and lignin precursors in maize. These studies will also help identify approaches for genetic improvement of maize and other important grass species as feedstocks for biofuel production.

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