

**Final Progress Report**  
**Engineering phenolics metabolism in the grasses using transcription factors**  
Grant No. DE-FG02-07ER15881

**Final Report:**

The project focused on two main aspects: 1) The generation and characterization of transgenic lines for ZmMYB40 and ZmMYB95 (encoding putative activators of the phenylpropanoid pathway), and 2) The characterization of ZmMYB31 and ZmMYB42 as two novel regulators of maize phenylpropanoid biosynthesis.

- 1) A large number of maize transgenic lines plants harboring dsRNA constructs for ZmMYB40 and ZmMYB95 were generated generated at the Iowa State University Plant Transformation Facility (ISU-PTF) and characterized molecularly (for the presence of the transgenes and the effect on the expression of ZmMYB40 and ZmMYB95. Lines with significant reduction (>80%) for one or both genes were found, but no phenotypic effect could be determined. Several cytological staining methods for lignin were applied, but no significant difference in lignin content with untransformed controls were detected. The characterization of these lines continues, considering the possibility that a) a stronger inhibition of mRNA accumulation for both genes is necessary to see an effect; b) that the levels of soluble phenolics (rather than lignin) is affected; and c) that these two regulators play a role other than controlling phenylpropanoid accumulation. Simultaneously, antibodies to these two genes were generated and chromatin immunoprecipitation (ChIP) experiments are under way to determine what the direct targets might be.
- 2) As part of a collaboration with Dr. David Caparros' group (Barcelona, Spain), we started the characterization of two other MYB transcription factors that had been suggested to function as repressors of phenylpropanoid gene expression. Antibodies to both of these proteins (ZmMYB31 and ZmMYB42) were generated, and used in ChIP experiments. As predicted, we demonstrated that these factors directly bind, and repress, phenylpropanoid genes in maize that include COMT (caffeic acid *O*-methyltransferase). These results were published (Fornale et al., 2010).

**List of Publications Resulting (and Acknowledging) from this Funding**

- Grotewold, E. (2008). Transcription factors for predictive plant metabolic engineering: are we there yet? *Curr Opin Biotechnol* **19**: 138-144.
- Grotewold, E and Gray, J. (2009) Maize transcription factors. In *The Maize Handbook*. J. Bennetzen and S. Hake (Eds). Pp: 693-713.
- Grotewold, E, and Springer, N. (2009) Decoding the transcriptional hardwiring of the plant genome. In *Annual Plant Reviews: Systems Biology*. Coruzzi, G. and Gutierrez, R. (eds). Blackwell Publishing. **35**: 196-227.
- Fornale, S., Shi, X., Chai, C., Encina, A., Irar, S., Capellades, M., Fuguet, E., Torres, J-L., Rovira, P., Puigdomenech, P., Rigau, J., Grotewold, E., Gray, J., and Caparros-Ruiz, D. (2010). ZmMYB31 directly represses maize lignin genes and redirects the phenylpropanoid metabolic flux. *Plant J.* **64**: 633-644.
- Feller, A., Machemer, K., Braun, E.L., and Grotewold, E. (2011) Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. *Plant J.* **66**: 94-116.