

Discovery and Modification of Photomorphogenic Regulation in Maize

Submitted by: Matthew Hudson, University of Illinois

Hudson: mhudson@uiuc.edu

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Progress Report (covers period of 1/1/08-12/31/08)

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A. Executive summary

This project sets out to discover genes to allow the next generation of maize varieties to grow at higher densities (more plants per acre). We are aiming for plants that grow at higher density than previous generations of inbred and hybrid cultivars, without compromising yield. Thus, plants containing these genes should produce more grain per acre than current varieties. By our corporate collaborators incorporating these genes into new maize varieties, the trait of increased planting density can be passed into the commercial domain and become available to farmers. Since the last update, we have identified one gene that encodes a protein that interacts directly with phytochrome B and may participate directly in the signaling pathway for shade avoidance. We have identified a gene related to the gene family of interest together with Dr. Edward Buckler of USDA ARS (Cornell U) which we are characterizing. We have also cleared the final legal barriers to the collaborative experiments with Syngenta which should be complete by Summer 2009.

B. Research Activities and Progress for Past Year

Objective 1: Conduct expression profiling of maize seedlings given End of Day Far Red (EODFR) treatments.

We have validated the controlled environment conditions, performed the EODFR experiments and produced 10-fold replicated RNA samples for the transcriptional profiling experiment. Due to a delay in the execution of a legal agreement between the University and Syngenta, the corporate partner on this project, the samples could not be transferred to Syngenta until January 2009. In the meantime we selected 15 genes by sequence similarity to genes known in Arabidopsis to be involved in this process (Objective 2) and one gene using Nested Association Mapping in collaboration with Dr. Ed Buckler (ARS, Cornell University). We have progressed with genetic and molecular characterization of these genes.

Objective 2: Identify and characterize phytochrome signaling pathway components using genome bioinformatics and comparative genomics with Arabidopsis and rice

We have selected 15 candidate genes from the maize EST database and emerging maize genome sequence based on genome bioinformatics and comparative genomics. These

genes are composed of 13 basic helix-loop-helix transcription factors and 2 homeodomain-bzip transcription factors. Last year we developed quantitative PCR assays for all these transcripts and identified four that are strongly transcriptionally regulated in response to shade avoidance and other light signals. These four are our targets for genetic modification (Objective 3).

Objective 3: Perform detailed transcriptional, transgenic and genetic analysis of prioritized genes

As a result of the delay in the Syngenta agreement we have not yet been able to commence the generation of transgenic maize plants, and have used methods other than the microarray experiment to select candidate genes. We have developed specific gene-targeting primers and used the TILLinG service at Purdue University to produce directed mutants in four genes (three bHLH and one homeodomain). To accomplish this we needed to first obtain the full genic sequence of these four genes in both B73 and W22, which was completed this year, discovering extensive polymorphism in the case of two genes and some polymorphisms in the other two. In the case of one gene, a bHLH we term zPIF for Zea Phytochrome Interacting Factor, have obtained two missense mutant alleles and now have field-grown plants carrying these mutations back-crossed, genotyped and homozygous. Detailed characterization of the phenotypes of the homozygotes is ongoing. While it is clear that no strong field phenotype is seen, we expect to be able to determine subtle effects in shade avoidance responses through field trials at different densities and using growth chamber experiments.

We have also taken this gene, cloned it into an *in vitro* expression system, and produced the protein. We have in addition cloned full-length protein-coding genes for phytochrome A, B and C, for the first time, and produce the full-length proteins *in vitro* and also synthetically add the bilin chromophore necessary for the proteins to be active light receptors. The protein products of these genes likely control shade responses. We have been able to show that these proteins interact, in a cell-free system, only when the system is subjected to red light (Figure 2).

Red light
control zPIF phyB

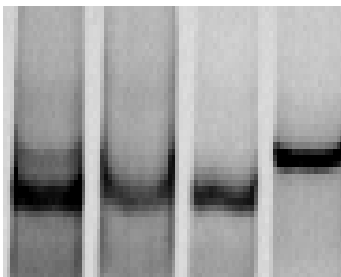


Figure 1: interaction of phytochrome B (phyB) with the bHLH protein zPIF. In vitro synthesized phyB and zPIF (right lanes) were mixed in a co-immunoprecipitation reaction. zPIF was then precipitated using an epitope tag. phyB co-precipitated under red light (left lane) but not under control conditions (far red light).

We have now cloned more bHLH transcripts into the protein expression system and we are currently investigating the degree to which they also have light-specific affinity for phytochrome proteins.

D. Work planned for coming year

Syngenta is now performing expression profiling on our RNA samples at their North Carolina site, and the results should be available shortly. In the coming year, data analysis and confirmation of the results will take place and publication of the microarray is anticipated.

Field trials for the bHLH TILLinG mutant and growth chamber studies will be completed this summer.

In the coming months we will determine *in vitro* affinities for all of the candidate genes related to zPIF with all of the maize phytochromes in the light-interconvertible Pr and Pfr forms.

E. Equipment purchases

None

F. Bibliography

Hudson, ME, Swaminathan, K, Kumar, I, Li, Y, Bellendir, S, Win, H, Fliege, C. 2007. Light Signal Transduction in Maize. 49th Annual Maize Genetics Conf., St. Charles, IL.

Kumar, I and Hudson, M,E. 2008. Phytochrome-interacting bHLH transcription factors and their role in maize photomorphogenesis. 50th Annual Maize Genetics Conf., Washington, DC.

Kumar, I, Swaminathan, K. and Hudson, M,E. 2009. Phytochrome-interacting bHLH transcription factors in maize and their role in photomorphogenesis. Manuscript in preparation.

We anticipate preparation of a further manuscript once the Syngenta microarray data is available.

G. Impact Items

Our work on the bHLH family in maize has allowed selection of candidate genes for association mapping in other laboratories.