

## Discovery and Modification of Photomorphogenic Regulation in Maize

*Submitted by Matthew E. Hudson, University of Illinois*

Hudson: mhudson@uiuc.edu

### **IMBA Project 2007-3**

Project period: 10/1/06-9/30/08

**Progress report** (covers period of 10/1/06-6/30/07)

#### **Non-technical 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.

The principle behind the project is that there are responses, shared by all plants, that cause them to become elongated or “leggy” in response to certain light environments. The responses occur when plants grown under conditions where light quality is not optimal (for example, in a densely planted cornfield). These responses limit yield when planting densities are high. In order to control these responses, we intend to identify the responsible genes and alter them, by using genetic approaches such as TILLinG (a way to artificially knock out genes in maize). So far, our project has identified five genes which may be useful for this process, and has begun the TILLinG process on four of them.

Three specific objectives were proposed, to be accomplished during the funding period. Our Year 1 progress towards these goals is below:

#### **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. We have proceeded to perform candidate gene analysis using quantitative PCR techniques for the genes identified in Objective 2.

#### **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.

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

We have developed functional qPCR primers for the 15 candidate genes identified as part of Objective 2. Using quantitative PCR, we have determined for the 15 candidate genes if they are transcriptionally regulated in response to shade avoidance and other light signals. Both homeodomain genes are strongly down-regulated by de-etiolating light signals and strongly up-regulated by a shade-avoidance signal. For the 13 bHLH genes, a more complex picture has emerged, with an apparent distinction between members of the family in terms of response. The results for bHLH expression are presented in Table 1.

**Table 1:** The fold-change in mRNA levels in response to several light treatments for the 13 bHLH genes (blank cells in the table represent experiments that have yet to be completed). \*statistically significant by ANOVA at  $P \leq 0.05$

	A	B	C	D	E	F	G	H	I	J	K	L	M
red 1 hr	1.75*	0.88	0.72	0.85	0.26*	0.70				1.01			0.47
red 12 hr	1.71*	1.57	0.95	1.42	0.38*	1.22				1.11			0.97
far-red 1 hr	1.83*	1.31	0.95	1.12	0.23*	1.10				1.01			1.25
far-red 12 hr	1.42	1.02	0.40	1.02	0.15*	0.85				1.47			1.68
end of day far red 1 hr	1.20	1.21	0.78	0.71	1.13	1.39	1.05	0.91	0.71	1.56	2.16*	2.14*	1.11

Note that two of the bHLH genes respond strongly to de-etiolating light stimuli, and two to the “planting density” stimulus of EODFR. These four genes are our targets for genetic modification, along with one homeodomain gene.

We have also developed primers and ordered TILLinG mutants for four genes (three bHLH and one homeodomain), we have obtained mutants in one of these genes and have homozygous lines now. We used a number of techniques to obtain the full genic sequence of these four genes in both B73 and W22.