Genomic Responses to Artificial Selection in Maize

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Abstract

Prior studies have detected evidence for selection among maize genes associated with domestication and crop improvement (1,2). However, it is more difficult to demonstrate selection upon individual genes or alleles associated with quantitative traits in recent breeding populations. The Illinois Protein Strains represent four related populations (Illinois High Protein, Illinois Low Protein, Illinois Reverse High Protein, Illinois Reverse Protein) that have been subjected to 105 cycles of artificial divergent recurrent selection for grain protein concentration. The application of functional genomics approaches to the unique genetic resource of the Illinois Protein Strains promises to reveal mechanistic insights into the responses of the complex maize genome to artificial selection. Our results to date have identified a number of genes whose RNA expression appears to be responsive to selection for grain protein concentration, including the zein seed storage protein genes and a number of genes predicted to function in pathways associated with nitrogen accumulation in developing maize seeds.

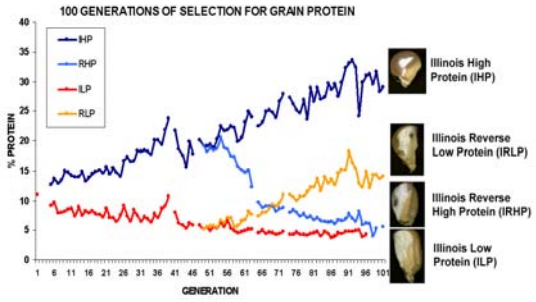


Figure 1. Selection responses in the Illinois Protein Strains. IHP and ILP are the product of 105 cycles of recurrent selection (20% index). Beginning at cycle 48, reversing the direction of selection within IHP and ILP created the IRLP and IRHP strains.

Experimental Methods

- Developing seeds of self-pollinated field-grown plants of inbred lines derived from the Illinois Protein Strains were sampled at 16 days after pollination. Five ears were sampled from each inbred.
- mRNA was extracted from whole seeds of these ears, reverse-transcribed to cDNA, and hybridized to long-oligo arrays produced by the Maize Oligonucleotide Array Project. Hybridized cDNAs were post-labeled using the Genisphere amplification system and then scanned using an Axon GenePix scanner. Dye swaps were nested within the 5 replicate labelings.
- After spot-finding, raw data was normalized using programs within Bioconductor, then imported into GeneSpring v. 7.2 for further analysis.

Comparison	# Significant Features[a] (56,370 total)	# Differentially Expressed Features[b]
IHP vs. ILP	42,896 (76%)	930 (2.2%)
IHP vs. IRHP	40,555 (72%)	651 (1.6%)
IHP vs. ILP & IRHP	39,993 (71%)	97 (0.2%)

Table 1. Microarray results.

[a] Features that passed both a spot quality check and showed signal above background on each of the five replicate slide sets.

[b] Features that showed significance difference by t-test, $p < 0.05$, following Bonferonni correction for multiple tests.

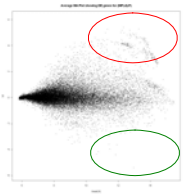


Figure 2. MA scatter plot of IHP vs. ILP comparison.
Log values of signal intensity versus ratio. The two circles indicate genes showing 5-fold expression differences between the two samples.

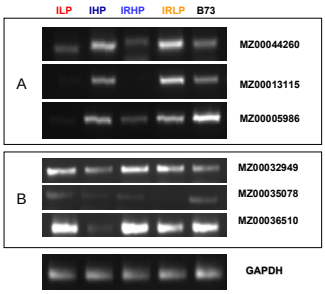


Figure 3. RT-PCR analysis of six candidate gene transcripts.
RT-PCR amplifications are from 5 genotypes with 33 cycles. GAPDH, as a house-keeping gene, was amplified with 30 cycles.

A. mRNA expression levels are positively related to protein concentrations among ILP, IHP, IRHP and IRLP.

B. mRNA expression levels are negatively related to protein concentrations among ILP, IHP, IRHP and IRLP.

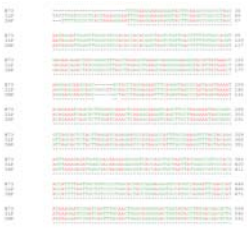


Figure 4. DNA sequence alignment (CLUSTAL W) among B73, IHP and ILP.

	Z22-4,14 (9)	Z22-5 (5)	Z22-7 (3)	Z22-22, D87 (2)	Z19A (39)	Z19B (33)	Z19D (5)	Z27 (10)	O2 (10)	PBF (1)	BiP (1)
IHP vs. ILP	7.8	6.3	24.6	15.2	5.0	3.6	17.6	0.9 *	1.4 *	4.7	1.1 *
IHP vs. IRHP	1.2 *	1.4 *	1.4 *	1.3 *	1.1 *	0.9 *	3.1	0.7 *	0.8 *	2.0 *	1.2 *

Table 2. Microarray results for zeins and zein regulators.

The relative expression of microarray features specific to zein family classes or subclasses, the O2 and PBF transcription factors, and the BiP ER protein chaperone are shown. The number of features present on the array for each gene class is indicated below each gene name in parentheses. * not significantly different expression level, by t-test at $p>0.05$

The coordinated directional changes in different a-zein gene subfamilies indicates that a-zein responses to selection in IHP and ILP are mediated by a trans-acting global transcriptional mechanism.

Only the Z19D genes showed significant expression differences in IRHP compared to IHP, despite large reductions in a-zein accumulation. This result suggests that the decrease in a-zein accumulation in RHP may be the result of post-transcriptional mechanisms. The lack of expression response for BiP suggests protein body formation is normal.

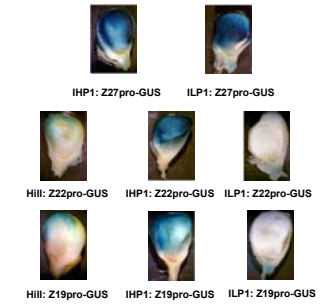
ID	Gene Description	Ratios (IHP/ILP)
MZ00035078	Exonuclease-like protein	0.05
MZ00032949	Unknown protein	0.08
MZ00036558	Putative dehydratase/deaminase {Oryza sativa (japonica cultivar-group);}	0.09
MZ00029853	Putative basic blue copper protein {Oryza sativa (japonica cultivar-group);}	0.12
MZ00036510	Unknown protein	0.13
MZ00036510	Exonuclease-like {Oryza sativa (japonica cultivar-group);}	0.13
MZ00041459	Putative histone H2A {Oryza sativa (japonica cultivar-group);}	0.13
MZ00024395	Putative outer envelope protein {Oryza sativa (japonica cultivar-group);}	0.13
MZ00013641	Putative histone H2A protein {Oryza sativa (japonica cultivar-group);}	0.14
MZ00010087	Putative copper chaperone {Oryza sativa (japonica cultivar-group);}	0.14
MZ00026755	Putative GDSL-motif lipase/hydrolase protein {Oryza sativa (japonica cultivar-group);}	0.15
MZ00031066	DUR3 {Oryza sativa (japonica cultivar-group);}	0.15
MZ00035899	Putative alliinase {Oryza sativa (japonica cultivar-group);}	10.05
MZ00031275	Putative brassinosteroid-insensitive 1 {Hordeum vulgare subsp. vulgare;}	10.24
MZ00037663	Mitochondrial carrier protein {Ribes nigrum;}	10.92
MZ00044260	Response regulator Cip1 [imported] - maize {Zea mays;}	11.05
MZ00046960	Putative amino acid permease {Oryza sativa (japonica cultivar-group);}	11.47
MZ00013115	Putative membrane protein {Oryza sativa (japonica cultivar-group);}	11.72
MZ00005986	Putative sugar transporter (alternative splicing product) {Oryza sativa (japonica cultivar-group);}	11.75
MZ00037998	Unknown protein {Oryza sativa (japonica cultivar-group);}	11.82
MZ00045319	Putative gag protein {Zea mays;}	12.19
MZ00031314	Putative CTV.22 {Oryza sativa (japonica cultivar-group);}	12.23
MZ00019419	Putative oligouridylate binding protein {Zea mays;}	13.08
MZ00029809	Putative trehalose-6-phosphate phosphatase {Oryza sativa (japonica cultivar-group);}	13.47
MZ00031708	Probable GCN4-complementing protein F23C21.2 [imported] - Arabidopsis thaliana {Arabidopsis thaliana;}	14.82
MZ00024383	Protein transport protein SEC61 gamma subunit. {Oryza sativa;}	14.82
MZ00007434	Peroxidase-like protein {Arabidopsis thaliana;}	15.17
MZ00044195	Secretory acid phosphatase precursor {Oryza sativa;}	15.55
MZ00056319	Photosystem I P700 chlorophyll A apoprotein A2 (PsaB) (PSI-B). {Zea mays;}	17.58
MZ00044351	Putative MAP kinase {Hordeum vulgare subsp. vulgare;}	17.89
MZ00007530	SWP1 protein-like {Oryza sativa (japonica cultivar-group);}	18.15
MZ00030874	Receptor-like kinase {Zea mays;}	18.24
MZ00050994	Cytochrome P450 {Vigna radiata;}	19.21
MZ00040854	Putative acetyltransferase {Oryza sativa (japonica cultivar-group);}	20.03

Table 3. Additional non-zein candidate genes whose expression changes greater than 5-fold between IHP and ILP.

Figure 5. Zein promoter-GUS transgene activity in the IHP1 versus ILP1 backgrounds.

The microarray results indicate that the protein selection experiment has acted on a global regulatory system for a-zein gene expression. To test if a-zein promoter sequences are sufficient to respond to this system, a-zein promoter-GUS transgenes obtained from Pioneer Hi-Bred were introgressed into the IHP1 and ILP1 inbred backgrounds.

- The Z22 and Z19 α-zein promoters direct weak expression around the periphery of the endosperm at 16 DAP in the Hill transformation background, which is consistent with previous reports of endogenous α-zein gene expression.
- For both the Z22 and Z19 promoters, expression was increased in IHP and reduced in ILP relative to Hill.



Next steps:

1. Distinguish genetic drift and genetic hitchhiking from artificial selection effect using high-resolution linkage mapping population derived from the cross of IHP x ILP.
2. Reveal and compare DNA and expression variations among different cycles of the selection experiment.
3. Determine trans- or cis-elements responsible for protein concentration difference.
4. Explore the genomic responses to artificial selection in maize.

References:

1. Wright, S. I., I. V. Bi, et al. (2005). The effects of artificial selection of the maize genome. Science 308(5726): 1310-1314.

2. Yamasaki, M., M. I. Tenaillon, et al. (2005). A large-scale screen for artificial selection in maize identifies candidate agronomic loci for domestication and crop improvement. Plant Cell 17(11): 2859-2872.

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