

Transcriptional Responses to a Century of Phenotypic Selection in Maize

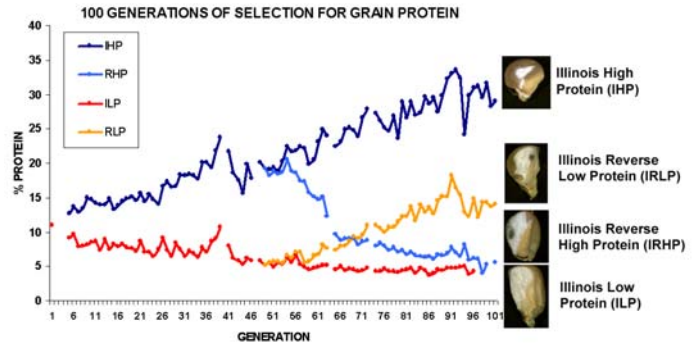
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ABSTRACT

The Illinois Protein Strains are the result of the longest continuous genetic selection experiment in higher plants. Initiated in 1896, 105 cycles of divergent recurrent selection for grain protein concentration, and 57 cycles of reverse selection, have produced four populations that span the known phenotypic extremes in maize for grain protein concentration and a number of correlated traits. To gain insights into gene expression responses to long-term selection, we have conducted mRNA expression profiling of the Illinois Protein Strains, with an initial focus on developing seeds. Microarray comparisons of inbreds derived from IHP and ILP reveal that approximately 2% of the genes expressed in developing seeds show significant differences, with the seed storage protein genes exhibiting the most dramatic changes. Similar comparisons of IHP and RHP aid in identifying the subset of gene expression responses to phenotypic selection instead of random genetic drift. Further analyses of the structure and expression of seed storage protein genes do not find major changes in the alleles present among the selected populations, suggesting trans-acting mechanisms contribute to the observed transcriptional responses. Continued characterization of mRNA expression profiles within this unique genetic resource will provide additional insights into how the mRNA expression of genes and pathways responds to breeding and selection.

105 cycles of both forward and reverse selection (20% index, 60 individuals) in the open-pollinated variety Burr's White has generated four populations that span the known extremes for grain protein concentration within maize.



Microarray Comparisons of Seed Gene Expression in the Illinois Protein Strains

Experiment Design

- Inbred lines with grain protein concentration representative of the population mean were produced from each population by self-pollination.
- Plants from each inbred were grown in the 2004 field nursery in four replicated plots.
- Plants were self-pollinated and one ear from each plot was harvested at 16 days after pollination, representing the onset of the linear phase of grain-filling.
- mRNA was extracted from whole seeds of these ears, reverse-transcribed to cDNA, and then labeled with either Cy3 or Cy5.
- cDNAs were hybridized (5 slide-set replicates per comparison) to the maize long-oligo arrays purchased from the University of Arizona 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						
		Total	2X up in IHP	2X down in IHP	5X up in IHP	5X down in IHP	10X up in IHP	10X down in IHP
IHP vs. ILP	42,896 (76%)	930 (2.2%)	318	370	178	70	120	24
IHP vs. IRHP	40,555 (72%)	651 (1.6%)	252	336	19	7	3	0
IHP vs. ILP & IRHP	39,993 (71%)	97 (0.2%)	70	27	1	2	0	0

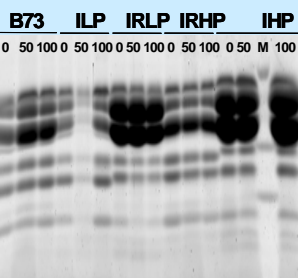
^a Features that passed both a spot quality check and showed signal above background on each of the five replicate slide sets.

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

- A higher proportion of genes were found to be differentially expressed in the IHP vs. ILP comparison relative to the IHP vs. IRHP comparison, which is consistent with the number of generations of divergent selection separating IHP from ILP (105) versus IRHP (57).
- Only 97 features were found to be differentially expressed in both the IHP vs. ILP and IHP vs. IRHP comparisons. These represent genes whose differential expression is likely to be the result of selection rather than genetic drift.

Changes in Zein Gene Expression and Structure among the Illinois Protein Strains

Zein protein accumulation



SDS-PAGE analysis of alcohol-soluble proteins from mature seeds. The Illinois Protein Strains exhibit large differences in the accumulation of α -zeins (Z22 & Z19), but not other zein classes. The B73 inbred is included for reference.

Zein pathway mRNA expression data from microarray analysis

	Z22-4,14 (9)	Z22-5 (5)	Z22-7 (3)	Z22-22, D87 (2)	Z19A (39)	Z19B (33)	Z19D (5)	Z50 (10)	Z27 (10)	Z16 (21)	Z10 (4)	O2 (10)	PBF (1)	BIP (1)
IHP vs. ILP	7.8	6.3	24.6	15.2	5.0	3.6	17.6	4.6	0.9*	1.2*	1.2*	1.4*	4.7	1.1*
IHP vs. IRHP	1.2*	1.4*	1.4*	1.3*	1.1*	0.9*	3.1	0.9*	0.7*	0.9*	0.8*	0.8*	2.0*	1.2*

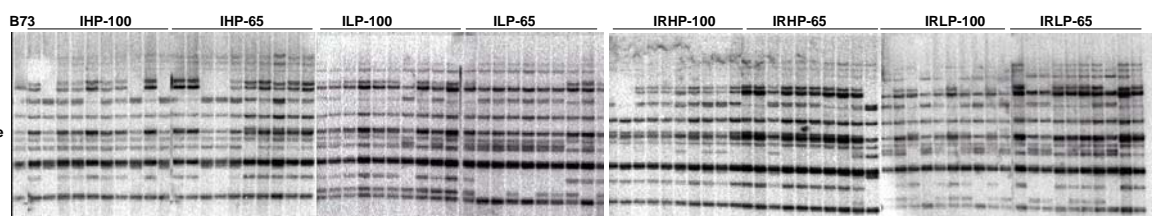
The relative expression of microarray features specific to zein family classes or subclasses, the O2 and PBF transcription factors, and the BiP ER protein chaperone are shown. The number of features present on the array for each gene class is indicated below each gene name in parentheses.

* not significantly different expression level, by t-test at $p > 0.05$

- The specificity of features permits estimates of gene expression differences among distinct zein genes
- The largest mRNA expression differences between IHP and ILP are observed for the Z22 and Z19 genes, which is consistent with the observed changes in the accumulation of α -zeins, but not other zein classes.
- The coordinated directional changes in different α -zein gene subclasses indicates that α -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 α -zein accumulation. This result suggests that the decrease in α -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.

DNA gel blot analysis of 22-kDa genes among 10 individuals from the indicated selection cycles and Illinois Protein Strain populations.

Allelic variation among 22-kDa α -zein genes in the Illinois Protein Strains



Allelic diversity exists, but observed patterns are not predictive of grain protein concentration. Similar results were observed for the 19-kDa zein genes. These observations further support the hypothesis that differences in 22-kDa zein accumulation are most likely regulated by trans-acting factors.