Expression variation associated with artificial selection for grain protein concentration in maize
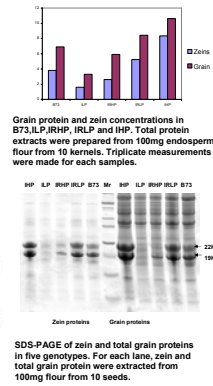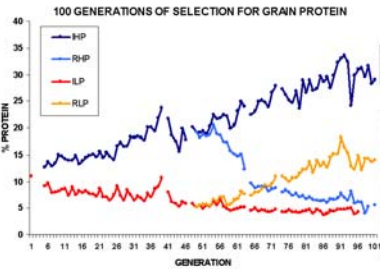
Han Zhao, Martha Schneerman, Ellen Taylor, and Steve Moose*
Crop Sciences, University of Illinois at Urbana-Champaign
***Contact: smoose@uiuc.edu**

Abstract

Evolutionary changes in mRNA expression are important contributors to phenotypic differences between species. Thus, documenting expression variation in response to artificial selection is likely to reveal genomic targets of directed evolution within species. This study tests the hypothesis that genes which show altered RNA expression among the Illinois Protein Long Term Selection Strains are also likely to be functional targets of phenotypic selection. Microarray experiments comparing leaf and seed RNA expression profiles from the Illinois High Protein (IHP), Illinois Low Protein (ILP), Reverse Illinois High Protein (IRHP) and Reverse Illinois Low Protein (IRLP) identified a set of features for which expression patterns are associated with grain protein concentration. These genes include those encoding the 19kDa and 22kDa α-zeins (endosperm storage proteins), protease inhibitor, genes involved in nitrogen assimilation within leaves, and genes that participate in the remobilization of nitrogen from leaves to seeds. DNA sequencing and RT-PCR assays validated RNA expression differences. Our results suggest that artificial selection has altered multiple physiological pathways and the mRNA expression of their associated genes.

Illinois long term Selection for grain protein in maize

More than 100 generations of selection in the open-pollinated variety Burr's White has generated four populations that span the known extremes for grain protein concentration.

Grain protein and zein concentrations in B73,ILP,IRHP, IRLP and IHP. Total protein extracts were prepared from 100mg endosperm flour from 10 kernels. Triplicate measurements were made for each samples.

SDS-PAGE of zein and total grain proteins in five genotypes. For each lane, zein and total grain protein were extracted from 100mg flour from 10 kernels.

mRNA expression profiling among the protein strains

36515 features

IHP vs ILP 10598 | IHP vs IRHP 3468
717(365)
IRLP vs ILP 3565

31489 features

IHP vs ILP 5651 | IHP vs IRHP 3182
501(323)
5185 IRLP vs ILP

Venn diagrams illustrating relationships among differentially expressed features (t-test, *p* < 0.005, FDR < 0.05) for each of the three comparisons of the IHP, ILP, IRHP and ILP inbred lines.

(A) V14 leaf tissue.
(B) Seeds harvested at 16 days after pollination (DAP).

The group of genes highlighted in red represents genes differentially expressed in all three comparisons, the number in parentheses indicating those whose changes in expression are also correlated with grain protein concentration.

Leaves | **Seeds**

We used the Arizona long-oligo arrays to conduct transcriptional profiling. Data were analyzed by Genespring and R. Hierarchical clustering of the differentially expressed genes from leaves and seeds (2-fold, FDR<0.05). Rows represent individual gene; columns represent individual M value.

Summary of differentially expressed features correlated to grain protein concentration

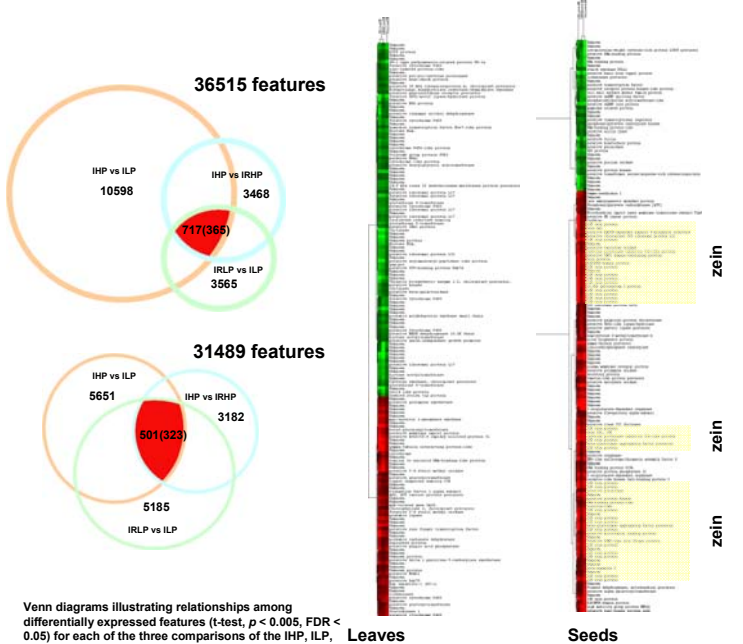|	Features	gDNA sequences IHP	gDNA sequences ILP	RT-PCR	Indel
Leaf					
IHP>ILP,IHP>IRHP,IRLP>ILP	151	4	4	4(1)	2
IHP<ILP,IHP<IRHP,IRLP<ILP	214	4	4	8(1)	1
Seed					
IHP>ILP,IHP>IRHP,IRLP>ILP	181	18	10	8(1)	6
IHP<ILP,IHP<IRHP,IRLP<ILP	142	5	4	6(0)	3
Leaf and Seed					
IHP>ILP,IHP>IRHP,IRLP>ILP	2	0	0	0	NA
IHP<ILP,IHP<IRHP,IRLP<ILP	10	2	2	2(0)	0
Other (IHP>ILP or IHP<ILP)	81	77	31	44(5)	12

The number in parentheses indicating those whose changes in expression are not consistent with microarray results.

The elongation factor 1 alpha subunit gene sequence alignment between IHP and ILP. This gene expression variations in leaves are correlated with grain protein concentration.

The protease inhibitor gene sequence alignment between IHP and ILP. This gene expression variations in seeds are correlated with grain protein concentration.

Selection for grain protein concentration has dramatically altered the expression of α-zein genes

22K Zeins | **15K Zein** | **50K Zein** | **Protease Inhibitor**

PBF | **O2**
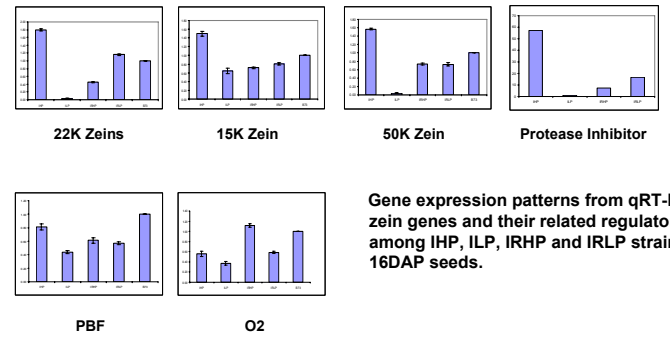
Gene expression patterns from qRT-PCR of zein genes and their related regulatory genes among IHP, ILP, IRHP and IRLP strains at 16DAP seeds.

Summary

❖Gene expression variations of leaves and seeds were observed suggesting that long term selection for high and low protein has resulted in the modification of multiple biological processes.

❖Functional annotation is under way and genes showing the most consistent differences in expression are being validated by qRT-PCR.

Future work

➤ Distinguish genetic drift and genetic hitchhiking from artificial selection effect on gene expression variation using high-resolution segregating population derived from the cross of IHP x ILP.

➤ Reveal and compare some promising candidate genes' DNA and expression variations among different cycles of the selection experiment.

➤ Determine trans- or cis-elements responsible for protein concentration difference.

➤ 4. Explore the genomic responses to artificial selection in maize.