Genomic Responses to a Century of Divergent Selection in Maize

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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.

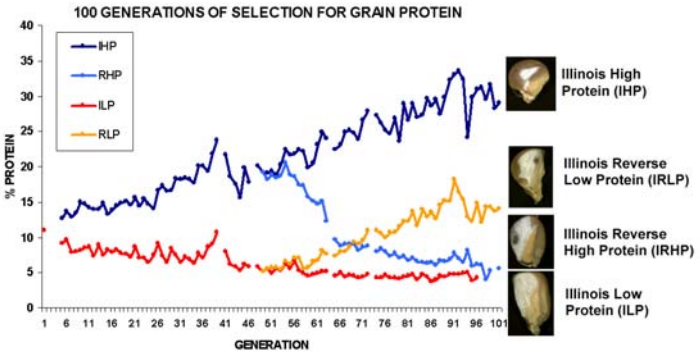


Fig. 1: Selection responses in the Illinois Protein Strains. IHP and ILP are the product of 103 cycles of recurrent selection (20% index). Beginning at cycle 48, reversing the direction of selection within IHP and ILP created the IRLP and IRHP strains. Gaps are years without data.

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

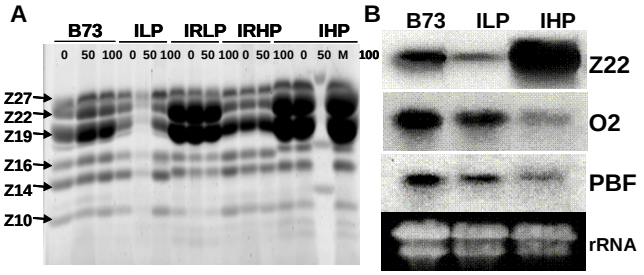


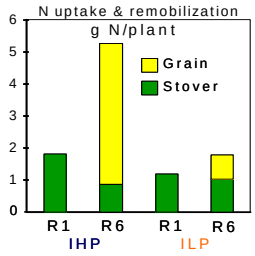
Fig. 2: Differences in expression of zein genes and regulatory factors in inbred lines derived from the Illinois Protein Strains.

(A) SDS-PAGE analysis of alcohol-soluble zeins in mature seeds from Illinois Protein Strain and B73 inbred plants grown at either 0, 50, or 150 lbs./acre supplemental N.

(B) RNA gel blot analysis of mRNA expression for 22-kDa zeins (Z22), the bZIP transcription factor OPAQUE2 (O2), and Dof-domain transcription factor PROLAMIN BOX BINDING FACTOR (PBF). Both O2 and PBF are known to regulate zein gene expression (Schmidt et al., 1992, *Plant Cell* 4: 689; Vicente-Carbajosa et al., 1997, *PNAS* 94: 7685; Marzabal et al., 1998, *Plant J.* 16: 41). EtBr-stained rRNAs are shown as a loading control.

Selection for grain protein concentration has changed correlated traits associated with whole plant carbon/nitrogen metabolism.

A



B

Amino Acid	B73	ILP	IHP
Total AAs[a]	17.8	9.9	68.1
Glutamine[b]	36	43	23
Aspartate[b]	13	22	6
Asparagine[b]	11	6	35
Arginine[b]	10	< 1	13
Serine[b]	7	4	2
Alanine[b]	6	4	1
Glutamate[b]	5	15	4

[a]Total amino acids in shank tissues reported as mg/g dry weight
[b]Individual amino acids reported as percentage of total

C

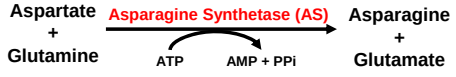


Fig. 3: IHP and ILP genotypes differ in N metabolism.

(A) N contents in stover and grain measured at the R1 (anthesis) and R6 (physiological maturity) growth stages for the IHP1 and ILP1 inbreds. Total N uptake is indicated by sum of the stover and grain fractions and N remobilization by the relative decrease in stover N at the R6 compared to R1 growth stage.

(B) Total amino acids (mg/g dry weight) measured in shank tissues (connecting ear to main stem) of B73, ILP1, and IHP1 inbreds at anthesis, which reflects amino acids being supplied to the developing seeds. The relative proportions of the major transported amino acids in shank tissues are also shown.

(C) The dramatic increase in asparagine at the expense of glutamine and aspartate in the transported amino acids of IHP compared to ILP probably reflects differences in asparagine synthetase (AS) activity. Increased differences in asparagine of IHP are consistent with the role of asparagine as a preferred N storage and transport of N amino acid as well as the and enhanced seed nitrogen content of Arabidopsis plants that overexpress AS (Lam et al., 2003, *Plant Physiol*. 132: 926.

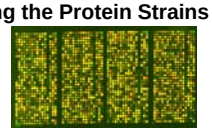
mRNA Expression Profiling among the Protein Strains

(A) Genotypes to compare:
IHP, ILP, IRHP, IRLP, B73 inbreds
IHP x B73 hybrid versus parental inbreds

(B) Tissues to profile:
- endosperm (8, 12, 16, 24, 32 DAP)
- ear leaf (16, 24, 32 DAP)
- roots & shoots (V5 stage in hydroponics)

(C) Plants grown with or without supplemental N (both in hydroponics and field)

(D) Traits to associate mRNA profiles:
- grain protein and starch concentration
- N uptake
- root architecture
- N remobilization from vegetative tissues
- transported amino acid profiles



We plan to use the long-oligo arrays being developed at Arizona to conduct transcriptional profiling. These experiments will not only identify candidate genes associated with grain composition and nitrogen metabolism traits, they will also characterize gene expression networks that have responded to selection. We are also collaborating with agricultural genomics companies to perform complementary mRNA profiling experiments with different platforms.

Candidate genes identified through mRNA profiling will be validated using combined physiological and genetic approaches:

1. Changes in allele frequencies in response to selection

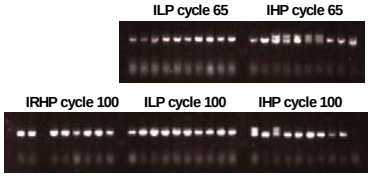


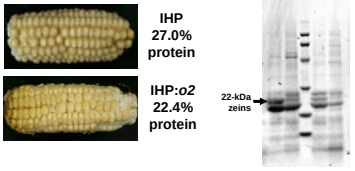
Fig. 4: Genetic variability at *o2* in Illinois Protein Strains.
10 individuals from ILP, IHP, and IRHP populations at cycles 65 and 100 were surveyed using SSR *umc1066* within the *o2* gene. A single allele was fixed in ILP at cycle 65 and 100, whereas two alleles were present in IHP in both cycles. Reverse selection from IHP led to fixation of same allele found in ILP. Thus, there appears to be an allele of *o2* associated with low protein. Alternatively, maintenance of variability at *o2* may permit continued response to selection for high protein.

2. Mutagenesis of candidate genes

Fig. 5: Phenotypes conditioned by *o2-R* null mutation in IHP background.

(A) The *o2* mutant causes an opaque mutant phenotype and reduces grain protein concentration.

(B) SDS-PAGE analysis of total mature seed protein from the IHP and B73 inbreds and their corresponding *o2* mutant near isogenic lines (6 backcrosses)



3. Association with QTL for grain composition and/or nitrogen metabolism traits.

Genotype	NUE	Yield at 0 N	Yield N response
B73 X CML52	110	2.4	4.7
B73 X CML247	102	3.2	3.6
B73 X Mo18W	99	1.9	5.2
B73 X CML69	88	2.8	3.6
B73 X B97	86	4.2	2.7
B73 X Oh7B	86	4.8	2.5
B73 X CML103	80	4.5	2.5
B73 X Ki11	74	1.2	6.2
B73 X Hp301	71	3.6	2.6
B73 X NC358	68	4.0	2.4
B73 X Ky21	67	2.0	3.8
B73 X CML228	63	2.5	3.1
B73 X Tzi8	63	3.5	2.5
B73 x M37W	62	4.3	2.2
B73 X Ki3	57	4.2	2.1
B73 x CML322	55	5.0	1.9
B73 X Il14H	55	3.9	8.4
B73 X NC350	52	5.2	1.8
B73 X Tx303	48	3.9	2.0
B73 X CML277	47	3.8	2.0
B73 X CML333	44	4.0	1.9
B73 X Mo17	44	5.5	1.7
B73 X P39	41	4.1	1.8
B73 X Oh43	38	4.7	1.7
B73 X MS71	28	5.9	1.4
FR1064 x LH185	54	5.9	1.8
FR1064 X ILP1	50	4.7	1.9
FR1064 x IRHP1	52	4.5	2.0
FR1064 x IRLP1	66	3.5	2.5
FR1064 X IHP1	70	2.4	3.4

Table 1: Variability for Nitrogen Use Efficiency (NUE) and Yield Responses in Diverse Hybrids.

In collaboration with Ed Buckler (Cornell Univ.), we evaluated hybrids where 25 inbreds selected to represent a wide range of maize allelic diversity were crossed to B73. Hybrids were grown in our nitrogen-responsive nursery during 2003 without supplemental N or with supplemental N at a rate of 168 kg/ha. Values for NUE (yield per unit of N supplied), yield without supplemental N (an indicator of N utilization), and yield response to N (an indicator of relative N uptake) are given for each hybrid. Also included in the evaluation were a current commercial hybrid (FR1064 x LH185) and hybrids where the four Illinois Protein Strain inbreds were crossed to FR1064.

A wide degree of variation for each trait is apparent in this set of hybrids, which can be associated with allelic variability in candidate genes for N response.

We will also use traditional linkage mapping approaches that take advantage of the high-resolution IBMRI mapping population as well as 500 lines derived from IHP x ILP followed by random mating for 8 generations (developed by John Dudley, Monsanto, and Renessen L.L.C.)

Acknowledgements

Funding from the Illinois Council for Food and Agricultural Research (C-FAR), Renessen L.L.C., Pioneer Hi-Bred, BASF Plant Sciences, and Syngenta.

John Dudley for seed of the Illinois Protein Strains

Ian Meyer, undergraduate student in the Moose lab