Asparagine Cycling Alleles Strongly Impact Grain Protein Concentration

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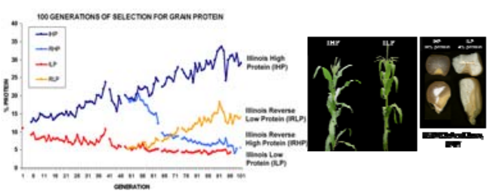
Abstract

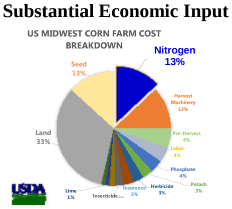
The Illinois Long Term Selection Experiment (ILTSE) for grain protein concentration began in 1896 and has generated phenotypic extremes for grain protein and nitrogen use efficiency traits. The high protein lines also distinctly hyperaccumulate asparagine. Asparagine is an amino acid that functions in storage and transport of nitrogen in maize. Asparagine concentrations are modulated by two asparagine cycling genes: asparagine synthetase and asparaginase. High and low protein lines from the ILTSE have lesions in the promoters of these genes that appear to change gene expression and have been divergently driven to fixation in the ILTSE populations. To test the role of these asparagine cycling alleles, near isogenic lines (NILs) were generated where high protein line alleles were introgressed into the low protein line and vice versa. The NILs differ for grain protein as measured by near infrared reflectance and an *fl2zein*-RFP transgene where red kernel color reports expression of the maize storage protein, zein. The introgression of high protein alleles into the low protein background increased grain protein, whereas low protein alleles decreased grain protein. The degree of change in protein mediated by asparagine cycling variants exceeds the variation observed for diverse maize inbred lines for the US Corn Belt.

Introduction

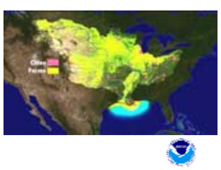


- The experiment began in 1896 from Burr's White.
- Cyril Hopkins established a set of experiments to try to change the grain protein concentration, both to higher protein and lower protein.
- After over 115 cycles of selection, the two lines are very different
- Grain protein in IHP is ~30%, low protein has bottomed out at ~4%, which may represent a biological minimum
- IHP accumulates an N storage molecule called asparagine
- The two also differ in their N use traits

Substantial Economic Input



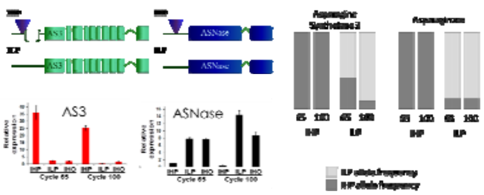
Ecological Footprint



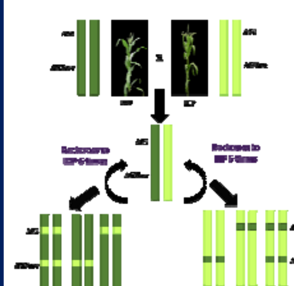
- The world population is expected to reach 9 billion by 2050, resulting in a need for 70-100% more food production over the next 40 years.
- Increased use of fertilizer nitrogen increased plant yields, but had negative impacts due to high input costs and environmental damages.
- Going forward, an important goal for agriculture will be to increase crop yields with fewer inputs from nitrogen fertilizer. Sustainable intensification of maize production can be aided by improvements in nitrogen use efficiency.

Asparagine Cycling Variation

- Asparagine cycling switches N between storage and growth form
- AS3 and ASNase have lesions in the promoter that correlate with changes in expression
- The alleles associated with the promoter lesions have been driven to fixation in the selection lines over the course of the experiment.

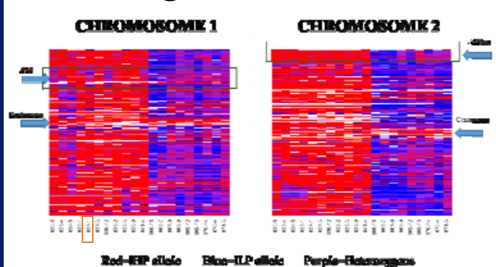


Building NILs



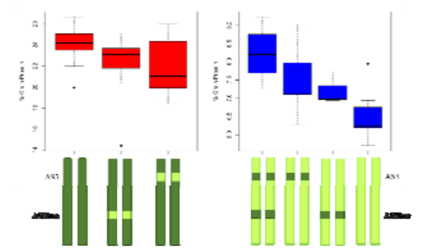
- Asparagine cycling alleles were introgressed from ILP into IHP, and vice versa
- BC2 populations were generated without the use of forward markers
- BC5 and BC6 populations were generated from 2011-2014, and selfed to get homozygous lines
- Introgression of both ILP alleles into IHP was not achieved

Background Markers



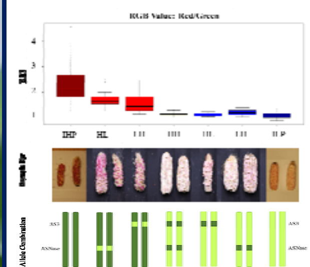
- Background markers were generated in summer 2013 using genotyping by sequencing.
- The ILP1 near-isogenic lines were BC1 populations, and the IHP1 populations were BC4.
- DNA was sequenced at the Institute for Genomic Diversity at Cornell University
- 955,690 SNPs were generated.
- Introgression size was approximated by binning SNPs in 100kb windows, and the area surrounding the AS3 and ASNase genes was assessed.
- The IHP background (predominantly red columns) has vast differences in the introgression size around AS3, but appears to have very small introgressions around ASNase.
- The ILP background (blue and purple columns) show the limited number of cross-overs expected for BC1 populations.

Grain Protein Results



- Introgressing the ILP allele for AS3 into IHP decreased grain protein from a mean of 24.1% to 21.8%, while ASNase decreased protein to 22.3%.
- Introgression of the IHP allele for AS3 increased grain protein from a mean of 6.5% to 7.2%, and ASNase increased protein to 7.7%. Introgressing both alleles from IHP into ILP increased grain protein to 8.2%.

Red Fluorescent Protein Marker System



- *fl2zein*-RFP transgene generated to track expression of zeins in the endosperm
- Redness quantified using an RGB color picker in ImageJ, and the red to green ratio was used as the phenotype, since it best correlates with grain protein.
- Trend for grain protein is not as clear using this marker system as with NIR, particularly for the ILP background.
- RFP specifically tracks zein concentration; NIR measures amine bonds. NIR measurements could be higher due to free asparagine rather than true grain protein.

Going Forward: 2016 Field Season

- Leaf tissue from the penultimate leaf was taken at noon and midnight for all allele classes from inbred NILs in 2015 and 2016 at anthesis. Amino acids were extracted, and amino acid concentrations will be analyzed using HPLC.
- All inbred introgression lines were grown again in 2016, with 6-8 plots per allele class. Ears were harvested on September 22nd, and phenotypic data is forthcoming
- Ten diverse maize inbreds were profiled for their AS3 and ASNase allele combinations. Plants were crossed to each of the introgression lines in 2015 to generate all possible allele combinations. Hybrid plants were grown in 2016 and also harvested September 22nd. Ear and plant biomass, N, and kernel traits will be analyzed.
- Genotyping by sequencing data for 2015 grown plants was sequenced by the Institute for Genomic Diversity at Cornell University and introgression size for the BC5 and BC6 plants will be determined.

Acknowledgements

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