Gene Discovery for Maize Responses to Nitrogen

PI Stephen Moose
University of Illinois
Functional genomics

co-PI Fred Below
University of Illinois
Physiology and field evaluations of maize N response

co-PI Ed Buckler IV
USDA-ARS/Cornell Univ.
Genetic mapping and statistical analysis

Collaborator Matt Hudson
University of Illinois
Bioinformatics

Project Summary

Nearly 10 million tons of N fertilizer are applied annually to the maize crop worldwide to increase grain yield. Improving maize nitrogen use efficiency (NUE) will reduce input costs and the energy requirements for maize production, as well as reduce concerns about the environmental impacts of excess N fertilizer. This project is applying the latest genomics resources to discover maize genes that are associated with nitrogen use efficiency (NUE), defined as grain yield per unit of available N. The information learned will provide new opportunities for improving NUE in maize and other crops through breeding or biotechnology approaches. Findings will also be applied to the improvement of NUE in tropical maize germplasm adapted to low soil fertility environments, through collaboration with the International Institute for Tropical Agriculture (IITA) in Nigeria.

Project Objectives

1. Discover N-responsive genes in root, shoot and developing ear tissues of maize.
2. Obtain comprehensive amino acid profiles for maize plants grown with different rates of supplemental N.
3. Identify QTL controlling nitrogen use efficiency and its component traits.
4. Map genetic loci that control N-responsive mRNA expression (eQTL).
5. Validate candidate genes through genetic approaches.
6. Build a relational database and web interface for public access to project data.

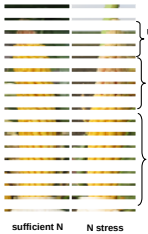
The NitroGenes Team

Participants on the NitroGenes project from both Cornell and Illinois, during the Cornell group's visit to Illinois in July.

Project Progress

1. Gene Discovery via RNA Expression Profiling

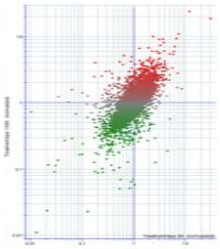
Comparison of earshoots harvested at anthesis from B73 x Mo17 plants grown either with or without supplemental N.



The primary physiological response to N stress that is most highly correlated with grain yield and NUE is a reduction in the number of fully developed kernels, which is most pronounced at the tip of the ear.

The tip portion (25%) of developing earshoots was sampled from B73 x Mo17 hybrid plants grown in the field either with or without supplemental N.

mRNA isolated from these tissues was hybridized to microarrays produced by the Maize Oligonucleotide Microarray Project at the University of Arizona.



~ 9,000 features passed quality check for both N treatments, where signal intensities for both dyes was above background on all eight replicate slides.

326 features show statistically significant expression differences in response to N

- 122 genes were upregulated by N
- 204 were downregulated by N.

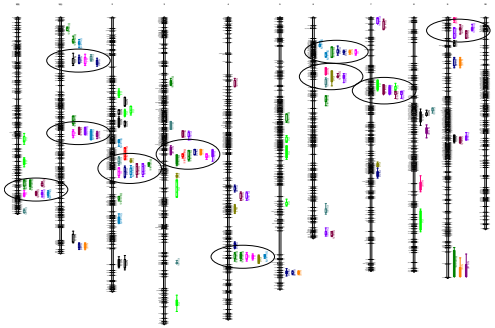
Table 1: Classification of N-responsive genes in maize earshoots (number of genes per category, total: ↑N, ↓N)

amino acid metabolism/transport (7: 2, 5)	energy/ATP production (2: 1, 1)	regulatory proteins (3: 0: 3)
biochemical modification (3: 1, 2)	growth regulators (3: 2: 1)	RNA processing (4: 1, 3)
central carbon metabolism (9: 2, 7)	lipid metabolism (4: 2, 2)	signal transduction (14: 3, 11)
cell cycle (3: 0, 3)	membrane trafficking (6: 1, 5)	stress-responsive (9: 8, 1)
cellular differentiation (2: 2, 0)	no match (67: 34, 33)	transcription factors (17: 3, 14)
cell wall synthesis/degradation (6: 3, 3)	phosphate assimilation/transport (2: 2, 0)	translation (16: 14: 2)
chromatin factors (1: 0, 1)	photosynthesis (8: 7, 1)	transporters (10: 4, 6)
cytoskeleton (5: 1, 4)	protein regulation (8: 4, 4)	unknown (105: 28, 77)

2. High-Resolution Genetic Mapping of Genes Controlling N-Responsive Traits

The intermated B73 x Mo17 recombinant inbred lines (IBMRIs) are the highest resolution mapping population available in maize. The Illinois High Protein1 (IHP1) inbred line exhibits the greatest capacity for N uptake measured in maize. Hybrids between 250 of the IBMRIs crossed to IHP1 were grown in 2006 and 2007, with each hybrid receiving either no or 250 kg/ha of supplemental N in adjacent plots within a replicated field design Searches for QTL are being conducted for the following traits:

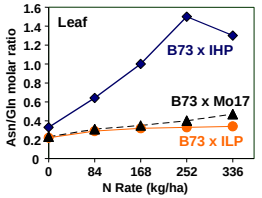
- NUE, N uptake, N utilization by developing grain
- stover and grain biomass at anthesis, harvest
- stover and grain N concentration at anthesis, harvest.
- N metabolites (nitrate, amino acids) in leaves and earshoots
- grain yield and its components, kernel number and weight
- RNA expression for key N metabolism genes (eQTL)
- Activities of enzymes for N assimilation and C/N balance.



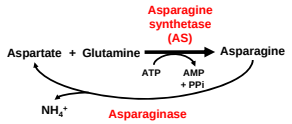
Maize IBM genetic map showing locations of QTL for N-responsive traits as colored bars to right of each virtual chromosome. Solid bars represent QTL identified at high N, horizontally lined bars represent QTL identified at low N, and vertically lined bars represent QTL for N response. Some chromosomal regions harbor QTL for multiple traits.

3. Validation of Candidate Genes

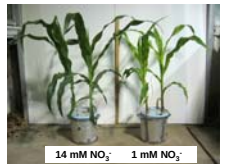
Leaf amino acid profile changes in response to N among B73 x IHP1, B73 x ILP1, and B73 x Mo17 hybrids. The transport and storage amino acid asparagine (Asn) increases relative to metabolically active glutamine (Gln) in response to N and the IHP genotype.



The metabolic pahtway for asparagine accumulation.



B73 and IHP1 plants were grown in hydroponics under either N-deficient (1 mM NO_3^-) or N-sufficient (14 mM NO_3^-) conditions. Leaves were sampled from these plants during both the day and night. RNA was isolated and the relative expression of genes encoding an asparagine synthetase (AS2) or asparaginase isoform was assayed by qRT-PCR.



AS2 RNA expression increases in response to N and is also elevated in IHP1 relative to B73, particularly during the day. Conversely, asparaginase RNA expression is repressed by N, and is greatly reduced in IHP1 compared to B73.