Identification of QTL and eQTL for Nitrogen Use Efficiency in the IBMRI x IHP1 Population

Devin Nichols, Fred Below, and Stephen Moose
Department of Crop Sciences, University of Illinois, Urbana, IL 61801

Abstract and Introduction

Nitrogen (N) is often a yield-limiting nutrient in the production of maize. The identification of genes involved in nitrogen use efficiency (NUE) and the use of such genes to develop hybrids with greater NUE could have both economic and environmental benefits. The objective of this study was to identify QTL controlling NUE and its component traits. The mapping population used was a set of hybrids developed by crossing lines from the IBMRI population to the Illinois High Protein (IHP1) inbred. This population takes advantage of the high mapping resolution of the IBMRIs and the high N uptake phenotype of IHP1. A set of 243 hybrids was grown with and without supplemental N in an N-responsive environment. Measurements of plot yield, stover fresh weight, stover dry weight, stover N concentration, and grain protein, oil, and starch concentration were made and used to calculate NUE and its component traits. Additionally, mRNA expression levels of several N-responsive genes was measured in the population using quantitative PCR for use in expression QTL analysis. Results from the QTL analysis of these traits will be presented here.

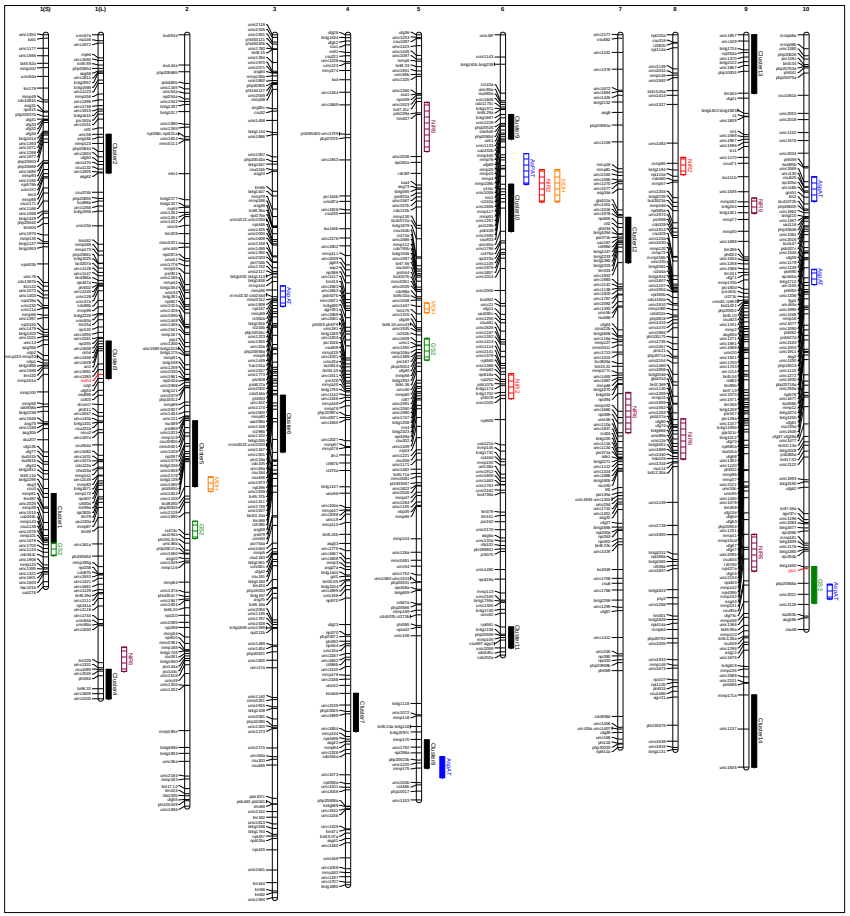

Figure 1 (above): Representation of the 10 maize chromosomes showing the position of 14 QTL clusters (black bars) where QTL for 5 or more NUE-related phenotypic traits were coincident. The positions of eQTL identified for Glutamine Synthetase (GS2, green bars), Nitrite Reductase (NiR2, red bars; NiR6, maroon bars), Malate Dehydrogenase (MDH, orange bars), and Aspartate Aminotransferase (AspAT, blue bars) are also displayed. Cis-eQTL are shown as solid bars while trans-eQTL are shown as hollow bars with horizontal markings. The physical location of each of the genes used for eQTL mapping is indicated in red. QTL mapping for phenotypic and eQTL was performed using Composite Interval Mapping (CIM) in Windows QTL Cartographer. Significance thresholds were determined using permutation tests.

Materials and Methods

- **IBMRIs (Lee et al., 2002. *Plant Mol. Biol*. 48:453) whose genotypes were confirmed at a sample of 10 marker loci were crossed as females by the IHP1 (Uribelarrea et al., *Crop Science* 44: 1593) tester.**
- **IHP1 is derived from the High Protein Strain of the Illinois Long Term Selection Experiment and represents the known extreme for seed protein concentration (30%) and N uptake (Uribelarrea et al. *Field Crops Research* 100: 82)**
- **The field site for these experiments was a nitrogen responsive nursery which has been managed for >15 years for nitrogen fertility.**
- **In 2006 and 2007, a set of 243 hybrids was grown in 3 replications of single-row plots at each of two N rates: 0 and 252 kg N ha^{-1}.**
- **Biomass and N accumulation in the stover and grain fractions was determined from four plants per plot sampled at physiological maturity (R6 growth stage). N assimilation in leaves at the V12 growth stage was measured by assaying nitrate concentration in leaf midrib tissue. Reproductive success was measured as the proportion of plants producing an ear. Kernel number and kernel weight were determined from R6 grain samples, which were also used to estimate grain protein, oil, and starch concentrations using NIR. These data were used to calculate plot grain yield, stover N content, grain N content, total plant N content, NUE, N uptake efficiency (NupE), N utilization efficiency (NutE), and yield response to N (ΔN).**
- **The following formulas were used to calculate NUE, NupE, and NutE:**

$$NUE = \frac{Yield_{+N} - Yield_{-N}}{N\ rate} \qquad NupE = \frac{Plant\ N_{+N} - Plant\ N_{-N}}{N\ rate} \qquad NutE = \frac{Yield_{+N} - Yield_{-N}}{Plant\ N_{+N} - Plant\ N_{-N}}$$

- **Leaf tissue was collected at VT for expression QTL (eQTL) analysis in 2007. The expression of several N-responsive genes was measured using qRT-PCR assays.**
- **Composite Interval Mapping (CIM) with 1334 markers was used to map QTL and eQTL for all measured and calculated traits using Windows QTL Cartographer software (Version 2.5). Permutation tests were run to determine the LOD thresholds for each trait.**

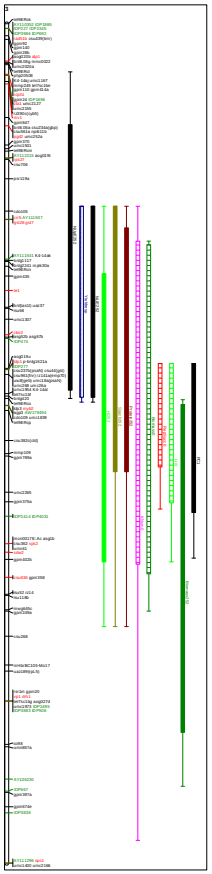

Figure 2 (Left): Close-up view of QTL Cluster 6 on maize chromosome 3 (shown on Figure 1 at left) showing the individual traits having QTL in the region. The Box and Whisker Plots represent confidence intervals for the QTL with the box representing the peak LOD-1 interval and the whisker representing the peak LOD-2 interval. Genes known to be located in the region are shown in red and gene-based markers in the region are shown in green. Information about genes and gene-based markers was obtained from maizegdb.org.

Results and Conclusions

- **Multiple QTL were identified for each of the 30 NUE-related phenotypic traits analyzed in 2006.**
  - **Over 250 QTL were identified in total.**
  - **Due to the large number of QTL, we chose 14 regions where 5 or more QTL clustered for in depth analysis (Figure 1).**
- **eQTL were identified for each gene tested.**
  - **A cis-eQTL was identified for GS2 on chromosome 10 and AspAT on chromosome 5 (Figure 1).**
  - **Between 3 and 5 trans-eQTL were identified for each gene.**
  - **Coincident trans-eQTL for NiR2, MDH, and AspAT on chromosome 6 (Figure 1) suggest co-regulation of the genes by a factor in the region.**
  - **A trans-eQTL for AspAT which co-localized with the cis-eQTL for GS2 on chromosome 10 (Figure 1) suggests that AspAT expression may be regulated by GS2 or glutamine concentration in the plant.**
- **Analysis of QTL clusters, such as the one on chromosome 3 (Figure 2), has identified regulatory genes which are considered candidate genes controlling NUE.**
  - **Some interesting genes in the chromosome 3 region include cko2, crr5, ldp1, and myb2.**

Planned Research

- **Phenotypic data from 2007 season will be analyzed to determine which QTL are stable across years.**
- **eQTL analysis will be performed on additional N-responsive genes including Asparagine Synthetase and Asparaginase to determine if there are any factors which co-regulate these N-responsive genes.**
- **QTL analysis will be performed on earshoot amino acid data to determine if QTL for amino acid concentrations or ratios are coincident with phenotypic QTL or eQTL .**
- **Regions containing QTL clusters will be further analyzed with developing bioinformatic resources to identify candidate genes. We will focus on regions which are shown to be stable across years.**
- **Putative candidate genes will be tested using techniques such as quantitative PCR to evaluate whether they could be the casual gene of the QTL effect.**

Acknowledgements

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