**Gene Discovery for Maize Responses to Nitrogen**

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**The NitroGenes Team**

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

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**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.

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**Project Progress**

1. **Gene Discovery via RNA Expression Profiling**

   - Near 10,000 genes were expressed in maize tissues collected from B73 x Mo17 plants grown with or without supplemental N.
   - The primary physiological response to N stress is upregulated 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 ears was isolated from these tissues, which was hybridized to microarrays produced by the Maize Oligonucleotide Microarray Project at the University of Arizona.

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 Protein 1 (IHP1) recombinant 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 0 or 200 kg ha⁻¹ of supplemental N. In adjacent plots within a replicated field design selected for QTL are being conducted for the following traits:
     - NUE, N uptake, N utilization by developing grain
     - N and grain biomass at anthesis, harvest
     - N and grain N concentration at anthesis, harvest
     - N metabolites (nitrate, amino acids) in leaves and ears
     - 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.

3. **Validation of Candidate Genes**

   - Leaf amino acid profiles 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 pathway for asparagine accumulation.

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**Table 1. Classification of eQTL responsive genes in maize embryos and kernels per category.**

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N uptake</td>
<td>135</td>
</tr>
<tr>
<td>N utilization</td>
<td>100</td>
</tr>
<tr>
<td>Grain yield</td>
<td>90</td>
</tr>
<tr>
<td>Biomass</td>
<td>75</td>
</tr>
<tr>
<td>RNA expression</td>
<td>50</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td>20</td>
</tr>
<tr>
<td>Stress response</td>
<td>10</td>
</tr>
<tr>
<td>Membrane trafficking</td>
<td>5</td>
</tr>
<tr>
<td>Cell cycle</td>
<td>3</td>
</tr>
</tbody>
</table>

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Maize IBG 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.

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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.