Genetic Control of the Carbon-Nitrogen Balance in Leaves of the Illinois Protein Strains

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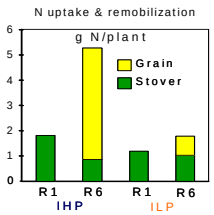
Abstract

Carbon catabolism and nitrogen assimilation are two fundamentally crucial and often competitive processes taking place within maize vegetative tissues. Previous studies have shown regulation of carbon fixation by nitrate and ammonium, as well as regulation of nitrogen assimilation by sugars, yet little is known about the interface between carbon and nitrogen pathways and the gene families controlling these junctions. We utilized the Illinois High Protein (IHP) and Illinois Low Protein (ILP) lines of maize, two products of divergent selection that differ widely in their respective abilities to sequester and utilize nitrogen, as a system for exposing altered control points for C and N metabolism. Preliminary global gene expression profiles were analyzed to determine possible patterns of metabolic coordination in response to environmental factors (light/N) and among genotypes. Hypotheses formulated from the expression profiles will be presented in conjunction with data from real-time qRT-PCR assays on candidate genes and putative housekeeping genes. Progress made towards characterizing differences in candidate gene structure and family densities among genotypes will also be reported in terms of the development of fosmid genomic libraries from IHP and ILP. Insights gained from these studies will be discussed in the context of future experiments that will examine carbon exchange, metabolite abundance and protein accumulation.

Introduction

The IHP and ILP lines of maize represent an ideal system for exploring the coordinated regulation of carbon (C) and nitrogen (N) pathways in crop plants. As the products of over 100 cycles of selection for grain protein concentration, the IHP and ILP strains differ widely in their ability to sequester and utilize N, especially during grain filling, wherein N is translocated from vegetative source tissues to developing kernels (Rizzi *et al*., 1996, *Maydica* 41: 325-332; Below, 2002, *Handbook of Plant and Crop Phys*.). Although the processes responsible for establishing an optimal balance of C and N within source tissues are critical determinates of plant growth and yield, little is known about the connections between the C and N pathways.

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



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

Figure 1: Differences in N metabolism between IHP and ILP.

(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.

Other studies have shown that the content of asparagine is about 30 times higher in the pedicel-placenta-chalazal tissues and endosperm of IHP than in ILP and constitutes up to 40% of the total amino acid pool in both tissues of IHP (Dembinski *et al*., 1991, *Plant Physiol. Biochem*, 29:549). The dramatic increase in asparagine at the expense of glutamine and aspartate in the transported amino acids of IHP compared to ILP may reflect differences in asparagine synthetase (AS) activity. Increased differences in asparagine in IHP are consistent with the role of asparagine as a preferred N storage and transport amino acid because of its high nitrogen-to-carbon ratio.

Methods and Molecular Tools

In order to study how carbon and nitrogen pathways are controlled at the genetic level, molecular tools are being developed to characterize the structure, function, and expression characteristics of the genes involved. Studies at the level of RNA expression are particularly relevant for genes like AS, which is difficult to study as an enzyme because of purification and instability issues. Global and specific RNA expression assays are crucial to our understanding of C and N metabolism, yet even these studies are confounded by the presence of multiple gene family members. Roughly one-third of the maize genes sequenced thus far appear to exist in gene families (Messing et al, 2004, PNAS, 101:40).

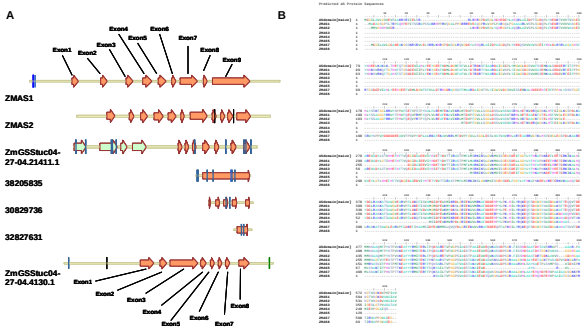


Figure 2: Family of Putative AS DNA and Protein Sequences

[a]Alignments of putative AS genes identified from publicly-available genomic contigs (www.plantgdb and www.ncbi.nlm.nih.gov). Coding regions (orange arrows) for all genes except ZMAS1 were predicted using FGENESH gene prediction software for monocots (http://www.softberry.com/berry.phtml).

[b]Alignments of predicted proteins (created using FGENESH) to protein sequence of ZMAS1.

Until more genomic and transcriptomic sequence becomes available, assays to monitor gene expression will be biased against poorly characterized gene family members and divergent, but functional alleles. To avoid this bias and to further characterize the genetic and regulatory structure of important genes, strategies such as RACE or library screening must be employed. We are utilizing the EpiCentre Biotechnologies *CopyControl™* fosmid library production kit to create a liquid-propagated genomic DNA library of the Illinois Selection Strains.

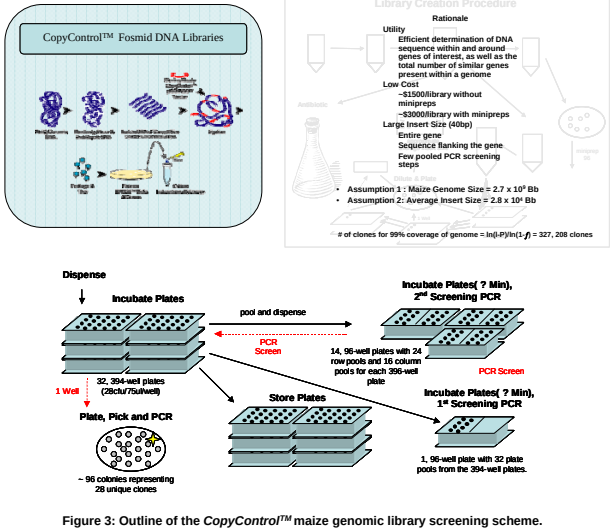


Figure 3: Outline of the *CopyControl™* maize genomic library screening scheme.

Current and Future Progress

Determining Internal Controls for Quantitative Plant Gene Expression Studies

Table 1: Summary of statistics measuring gene expression stability from real-tme qRT-PCR assays

GENE	MEAN[a]	F[b]	MSE-ANOVA[c]	CV[d]	SLOPE[e]	INTERCEPT	R^2	MS-REG[f]	STABILITY INDEX[g]
Ubi-Conj	33.0	8.79**	1.15	3.25	-0.26	35.44	0.38	0.36	-0.90
MZEACT	35.2	4.94*	1.42	3.38	0.16	21.12	0.14	0.14	0.54
MZE19	24.3	27.52**	1.14	4.39	0.37	17.37	0.79	0.75	1.63
Ubiquitin	18.6	22.99**	0.90	5.10	0.37	19.77	0.62	0.58	1.90
Sal1	22.4	7.91**	0.65	3.60	0.53	14.83	0.91	0.86	1.91

Experiment and data analysis based on the methodology of Brunner *et al*., 2004, *BMC Plant Biology*, 4:14. [a]Data are based on analysis of C_T values of five putative control genes assayed in six different tissues: ILP-night; B73-night; IHP-night; ILP-day, H99-day, IHP-day. Putative control genes are ordered, top to bottom, from those tending to show the highest stability to those showing the lowest. [b]Approximate F-tests (conducted with SAS) of variance among tissue samples tested. *, P<0.05; **, P<.01. [c]MSE-ANOVA represents variance among experiments and RT-PCR reactions within experiments. [d]Coefficient of variation. [e]Slope of regression of gene means against overall means for the different samples. Intercepts and coefficient of determination (R^2) for the estimated regression lines. [f]Mean square of deviation of means from estimated regression line (MS-REG), which estimates the degree to which genes deviate from the linear model in their level of mean expression for a particular tissue sample. [g]Stability index is the product of CV and slope (multiplication of columns 4 and 5). Genes whose expression shows the lowest random variation within tissue samples due to variation among experiments of PCR reactions (MSE-ANOVA), and whose expression depends least in a predictable way on tissue sample (slope), are preferred as controls.

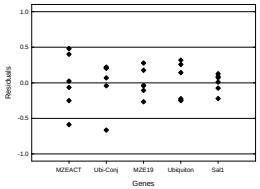


Figure 4: Scatterplot of residuals after regression of tissue means from each experiment on overall mean for all genes. Genes were ordered based on absolute value of mean (decreasing from left to right).

T Grouping	Mean C_T	N	Tissue
A	23.45	3	ILPn
A	22.99	3	B73n
A	22.83	3	IHPn
B	21.90	3	ILPd
B	21.64	3	H99d
B	21.63	3	IHPd

Figure 5: Fisher's LSD t test for C_T for Sal1 amplification among four genotypes sampled either during the day (d) or at night (n). Tissues with same T grouping do not exhibit significant variation in Sal1 gene expression.

Carbon Fixation and Nitrogen Assimilation in the Leaves of IHP and B73 Grown with Varying N in Hydroponics

Current RNA expression studies are focusing on the C4 photosynthesis reactions (Wingler *et al*., *Plant Physiol*: 120, 539-546), and their possible interface with N assimilation (Fig. 6), as indicated by studies showing regulation of C fixation by nitrate and ammonium (Sugiharto and Sugiyama, 1996, *Plant Physiol*: 98, 1403-1408), as well as regulation of N assimilation by sugars (Chevalier *et al*., 1992, *The Plant Journal* 98: 1403-1408). These studies are utilizing B73 plants and IHP plants, which have been shown to contain high total N yield, high N uptake after silking and high N remobilization from the leaves and stalk after anthesis (Rizzi *et al*., 1996, *Maydica* 41: 325-332). These experiments (Fig. 7) are being conducted in a hydroponic system according to the methodology of Below and Gentry (1987, *Journal of Fertilizer Issues*, 4 (3), 79-85).

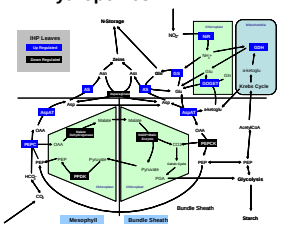


Figure 6: Possible interactions between C fixation and N assimilation.

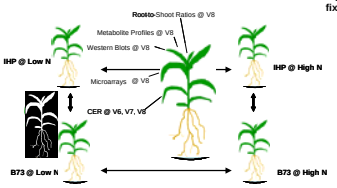


Figure 7: Hydroponic Experiments.

Acknowledgements

Funding is from the NSF