# Altered Transcript Abundances of Aspergillus Flavus and Transgenic Maize Overexpressing a Bacterial Glutamate Dehydrogenase (GDH) Reduces Aflatoxin Contamination of Grain

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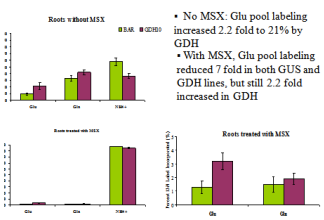
*Aspergillus flavus* is a filamentous fungus that causes an ear and kernel rot in maize (*Zea mays* L.). Ear rots are often associated with the accumulation of aflatoxin in the infected plant. The ability of *A. flavus* to produce aflatoxin depends on the nature and the composition of the substrate. Transgenic maize lines expressing a bacterial NADPH dependent *gdhA* (glutamate dehydrogenase) gene showed increased nitrogen metabolism with an overall increase in some amino acids in the ear. Previous studies showed that *A. flavus* exhibits decreased conidiation and aflatoxin accumulation on GDH$^+$ maize lines. The aim here was to use microarray studies to assess changes in the expression patterns of *A. flavus* genes on GDH$^+$ maize. Used was artificial inoculation in the field of dough stage maize kernels of inbred 'H99' transgenic or non transgenic with *gdhA*. The mRNA was isolated after harvest. Increased by more than 2 fold on GDH$^+$ maize were 747 *A. flavus* transcripts, only 27 decreased. Hydrolases (EC 3.-), kinases (EC 7.-) and ABC transporters (non-enymatic) were the major protein families increased. No effect on toxin pathway genes was detected. No maize transcripts were increased by GDH$^+$, though 95 were reduced significantly by two fold or more. Reduced by GDH were transcripts encoding trehalase, branched chain amino acids, primary carbon metabolism and polyphenol synthesis. Metabolites increased by the reduced transcripts in GDH$^+$ maize included cellulose to lignin ratios; non-branched side chain amino acids; and some toxic products of special nitrogen metabolism. Therefore, the *gdhA* gene was inferred to confer partial resistance to *A. flavus* by repressing pathways in a way that lead to altered compositions, stronger cell walls and increased defense metabolite concentrations, pathways different from endogenous resistance mechanisms discovered to date. See patents 5,998,700; 6,329,573; and pending.

## GDH Improved Yield and NUE

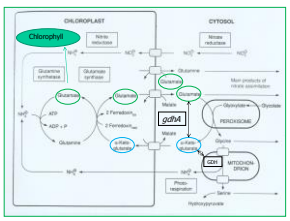
| Irrigation methods | Lateral Lengths m | Applied water m3/ha | Yield kg/ha GDH+ | Yield kg/ha GDH- | Yield Boost kg/ha | WUE (kg/m$^3$) GDH+ | WUE (kg/m$^3$) GDH- | WUE boost (kg/m$^3$) |
|---|---|---|---|---|---|---|---|---|
| | 40 | 7725.16 | 12885.27 | 11131.35 | 1753.92 | 1.67 | 1.44 | 0.23 |
| CM2DIS | 60 | 10338.91 | 12235.62 | 9392.12 | 2843.50 | 1.18 | 0.91 | 0.27 |
| | 80 | 13757.42 | 12023.18 | 8659.01 | 3364.17 | 0.87 | 0.63 | 0.24 |
| | 40 | 7638.29 | 12623.69 | 9903.82 | 2719.87 | 1.65 | 1.30 | 0.35 |
| CM1DIS | 60 | 10382.71 | 12015.51 | 8155.87 | 3859.64 | 1.16 | 0.79 | 0.37 |
| | 80 | 13782.14 | 11871.72 | 8945.58 | 2926.14 | 0.86 | 0.65 | 0.21 |
| | 40 | 8932.25 | 12029.28 | 8793.02 | 3236.26 | 1.35 | 0.98 | 0.37 |
| TDIS | 60 | 10652.88 | 11034.12 | 8052.40 | 2981.72 | 1.04 | 0.76 | 0.28 |
| | 80 | 15212.7 | 11429.77 | 6852.14 | 4577.63 | 0.75 | 0.45 | 0.30 |

## N Assimilation Fluxes in Roots

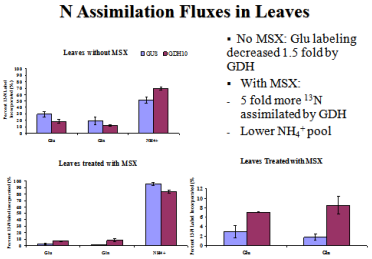



- No MSX: Glu pool labeling increased 2.2 fold to 21% by GDH
- With MSX, Glu pool labeling reduced 7 fold in both GUS and GDH lines, but still 2.2 fold increased in GDH

## Reactions in Glutamate Homeostasis



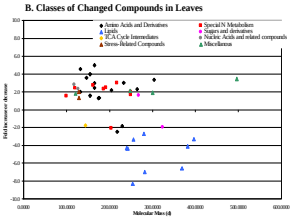

## N Assimilation Fluxes in Leaves




- No MSX: Glu labeling decreased 1.5 fold by GDH
- With MSX:
  - 5 fold more $^{13}N$ assimilated by GDH
  - Lower $NH_4^+$ pool

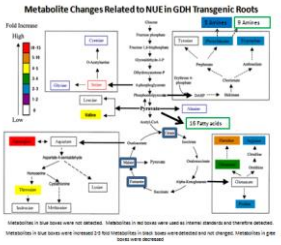
A. Water Deficit Resistance in GDH Tobacco

B. Classes of Changed Compounds in Leaves

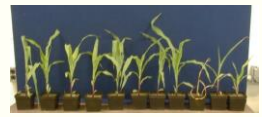



91 empirical formulas determined of 98 changed metabolites
47 of 91 leaf metabolites assigned to classes

Metabolite Changes Related to NUE in GDH Transgenic Roots




D.



E.

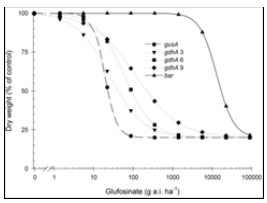
Yield increased – lignin decreased major insoluble N store

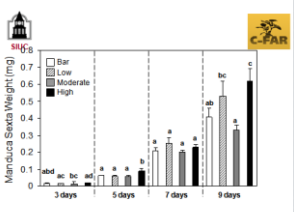
Table 2: Effect of the gdhA gene on the field performance hybrid maize B73xLL3-272 during dry years.

| Line | Yield Ears (lha$^{-1}$) | Barren (lha$^{-1}$) | Height Plant (cm) | Ear (cm) | Total Leaf N (g kg$^{-1}$) | Soluble Leaf N (g kg$^{-1}$) | Yield Weights (g kg$^{-1}$) | Moisture (kg m$^{-3}$) | Adjusted Yield (Mg ha-1) |
|---|---|---|---|---|---|---|---|---|---|
| GDH-LL3 | 24112 | 21524 | 160 | 81 | 34.9 | 3.3 | 220 | 672 | 1.83 |
| GUS-DL3 | 22542 | 16880 | 156 | 74 | 28.5 | 2.1 | 196 | 690 | 1.57 |
| LSD | 1274 | 3099 | 4.9 | 1.7 | 2.8 | 0.3 | 4.0 | 6.7 | 0.46 |

a amino acids and ammonium were not detected, nd soluble proteins that were unchanged
b Yield was adjusted to remove the effect of different moisture contents

**Herbivory Experiment.** Five-week-old *Nicotiana tabacum* with varying amounts of GDH (glutamate dehydrogenase expression; exposed to *M. sexta* L. (Lepidoptera: Sphingidae) herbivory.



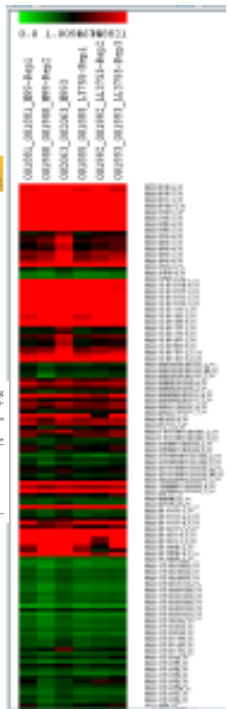

## Potential eQTL Associated with *A. flavus* resistance

| Fold Increase | EST-TC | Encoded Proteins |
|---|---|---|
| 2.82 | TC559384 | Probable histone H2A.5; |
| 3.08 | TC482386 | Putative enoyl-CoA hydratase; |
| 3.14 | TC462902 | Putative uncharacterized protein; |
| 3.15 | TC508624 | Isoflavone reductase |
| 3.17 | TC465779 | NADP-specific isocitrate dehydrogenase |
| 3.30 | TC504480 | Peroxin Pex14; |
| 3.43 | TC505842 | Invertase; |
| 3.56 | TC474624 | Farnesylated protein 3; |
| 3.86 | TC499349 | Beta-glucosidase aggregating factor 1; |
| 4.13 | TC523175 | Secretory carrier-associated membrane pr |
| 4.33 | TC460003 | Putative phosphoglycerate dehydrogenase |
| 4.70 | TC481822 | Proline-rich protein family-like; |
| 5.06 | TC483082 | Trehalase; n=1; |
| 6.09 | TC466654 | Dicer-like protein; |
| 18.48 | TC479627 | Branched-chain amino acid aminotransfera |

Table 4. Average daily gain of pigs fed a diet containing a genetically modified (*gdhA+*) corn or a similar non-transgenic (*gdhA-*) corn

| Item | Corn *gdhA+* | Corn *gdhA-* | Pooled SEM | *P*-value |
|---|---|---|---|---|
| Period 1 | | | | |
| Initial wt, kg | 46.1 | 48.8 | 1.84 | 0.33 |
| Final wt, kg | 49.9 | 52.1 | 1.94 | 0.45 |
| ADG, kg | 0.35 | 0.22 | 0.12 | 0.84 |
| Period 2 | | | | |
| Initial wt, kg | 52.1 | 49.9 | 1.93 | 0.45 |
| Final wt, kg | 58.1 | 58.5 | 2.57 | 0.92 |
| ADG, kg | 0.40 | 0.37 | 0.08 | 0.17 |

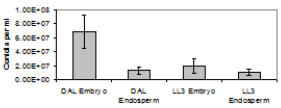
GDH grain was slightly superior in piglet growth



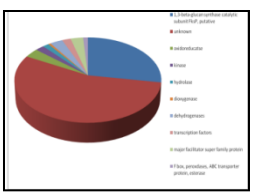
## eQTL associated with *A. flavus* resistance by associationmapping

| P value for association | Number SNPs tested for assoc. | Fold decrease | Transcript | Annotation | Location |
|---|---|---|---|---|---|
| 0.006 | 6 | 3.14 | TC462902 | uncharacterized protein | 9:151,968,248-151,972,265 |
| 0.009 | 11 | 4.13 | TC523175 | Secretory carrier-associated membrane protein 2 | 3: 191,058,631-191,062,929 |
| 0.009 | 3 | 5.05 | TC483082 | Trehalase | 1: 79,993,854-79,998,232 |
| 0.01 | 8 | 3.29 | TC504480 | Peroxin Pex14 | 6:166,050,667-166,054,905 |
| 0.02 | 3 | 4.33 | TC460003 | phosphoglycerate dehydrogenase | 2: 171,064,523-171,068,554 |
| 0.06 | 3 | 3.07 | TC482386 | enoyl-CoA hydratase | 5:191,095,225-191,095,775 |
| 0.07 | 2 | 3.55 | TC474624 | Farnesylated protein 3 -C | 4:195,752,798-195,757,168 |
| 0.1 | 3 | 3.16 | TC465779 | NADP-specific isocitrate dehydrogenase | 3:213,296,534-213,300,701 |
| 0.17 | 2 | 3.14 | TC508624 | Isoflavone reductase | 3:135,103,166-135,107,483 |

## Conidiation on GDH$^+$ corn kernels




The production of conidia by *A. flavus* on the embryos of LL3 corn kernels (*gdhA+*) is reduced by 73% compared to the conidiation on DAL corn kernels (*gdhA-*)



**Summary: P**lant metabolism was altered in ways that improve NUE, WUE, nutritional value, disease resistance, biofuel characteristics and alternate uses of plants by products. Understanding the molecular basis will help unravel the control of N metabolism and N signaling.