Ongoing Results From a Candidate Gene Pipeline for Testing Effect of DNA Sequences On Aflatoxin Accumulation and *A. Flavus* Resistance in Maize

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Introduction

Many projects have identified candidate genes for resistance to aflatoxin accumulation or *Aspergillus flavus* in maize using genetic mapping, genomics, or proteomics studies. However, only a small percentage of these candidates have been validated under field conditions and their relative contribution to resistance, if any, is unknown. Here, we present the ongoing results of a candidate gene testing pipeline that consists of steps for identifying, testing, and verifying the association of any maize gene sequence with resistance. The pipeline includes four QTL mapping populations and one association mapping panel, all of which have been fully phenotyped over multiple years and locations for aflatoxin accumulation resistance and associated phenotypes. The QTL populations have been genotyped with markers at low density, and the association panel via Genotyping By Sequencing. More than two dozen genes identified as possible resistance candidates in the literature or in our CFRAS database of candidate gene information (http://www.agbase.msstate.edu/) have been put into the candidate gene testing pipeline. SNPs and InDel polymorphisms within each gene that map to the correct genomic location were tested for phenotypic effect on aflatoxin accumulation resistance, and results are presented here.

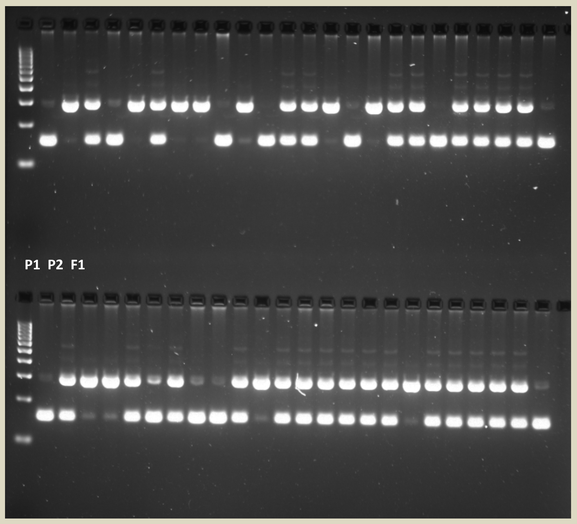
Results

Polymorphisms were found in the sequences of the seventeen candidate genes, and were used to map the genes in the 1 - 4 QTL mapping populations. Size assays (for P450, PER1, chloroplast precursor and the three chitinase genes) were amplified and separated on agarose gels for mapping; SNP assays (for all other genes) were visualized on the with the KASPAR SNP detection system (Kbiosciences) on a Fluorostar plate reader (BMG Labtech) (Fig. 1).

Polymorphisms for all genes were run in all mapping populations for which they were polymorphic, using JoinMap 4. All genes mapped to the expected locations in all cases (Fig. 3; Table 1). The phenotypic effect of the polymorphisms was estimated in the same mapping populations via composite interval mapping in QTL cartographer 2.5 (Table 1). Polymorphisms in or near 7 genes were associated with a QTL of LOD > 2.4, often in more than one population (Table 1). Eleven were associated with aflatoxin in one or more environments via the GLM in TASSEL, although only 8 of these occurred in more than one environment, or with more than one linked SNP, which is a better indication that the association was not a false positive result. In only three cases were QTL results also found in association analyses, but these were the strongest results in both analyses in most cases (Table 1, highlight).

Table 1. Information on the candidate genes tested via the pipeline to date, including QTL effects. An asterisk indicates that a linked SSR was mapped in the QTL mapping population, rather than the gene itself.

MaizeSequence ID	EST or Uniprot ID	Gene Name	Chromosomal Location	Reference	Polymorphic QTL population (description below; box)	LOD of significant QTL effect	Significance in Assoc. Panel (p)
GRMZM2G085661	Q43257	Cytochrome P450	4: 3260685	Frey et al., 1997	MpT, MpVa	MpVa = 2.5	none
GRMZM2G129761	A2SZW8	1-Cys peroxiredoxin PER1	7: 168,785,597	Chen et al., 2007	MpT, MpVa, MpNC	none	0.009 (3x)
GRMZM2G016561	CF038389	Hypothetical protein	3:140291029	CFRAS-DB query	MpNC	none	1.18E-04 (1x)
GRMZM2G152470	TC221535	Unk. homocysteine S-methyltransferase	3:117292752	CFRAS-DB query	MpT	none	0.001 (2x)
GRMZM2G057262	TC230106	Hypothetical protein	4:155846482	CFRAS-DB query	MpB	MpB = 7.0	none
GRMZM2G114702	TC237439	Hypothetical protein	4:85917427	CFRAS-DB query	MpNC, MpT	MpNC =2.5	none
GRMZM2G181192	AY241545.1	Glyoxalase I	10:4444027	Chen et al., 2004	MpVa	none	0.001503 (1x)
GRMZM2G015419	DQ335768.1	Lipoxygenase 10	4:239236528	Chen et al., 2007	MpB, MpNc, MpVa	none	0.001761 (4x)
GRMZM2G304548	X54064	Trypsin Inhibitor (TI-10)	2:157316072	Chen et al., 1998	MpB*, MpNc*, MpVa, MpT*	MpB = 8.0, MpNc = none, MpVa = 5.0, MpT = 2.5	pending
GRMZM2G007555		Heat Shock Protein (22kd)	1:167255241	Walbot et al., 1999	MpVa	none	0.006547 (1x)
GRMZM2G47716	AW424439	chloroplast precursor	4:27096658	Walbot et al., 1999	MpT, MpVa, MpB	MpB = 2.5, MpVa = 2.4, MpB = 2.4	2.23E-04 (3x)
GRMZM2G161414	TC2316745	NUP85-like	5:168420220	Kelley et al., 2012	pending	pending	0.005132 (2x)
GRMZM2G352855	TC2388325	Lechithin Cholesterol acyltransferase (LCAT)	2:166760989	Kelley et al., 2012	MpT, MpVa, MpB	pending	none
GRMZM2G119689	Q9SBI2	Incw-2: Invertase 2 *(mn1)*	2:56806376	Chourey et al., 2006	MpVa	none	none
GRMZM2G051943	P29022	Chitinase A	2:33533501	Shoresh & Harmann, 2008	MpB, MpNC, MpVa, MpT	MpVa = 5.5	3.49×10^{-10} (12x)
GRMZM2G453805	P93518	Chitinase 1 = Prm3	3:176569695	Shoresh & Harmann, 2008	MpVa*, MpT*, MpB	MpB = 2.4	9.00×10^{-7} (11x)
GRMZM2G064360	B6TFQ3	Basic endochitinase 1	5:63229609	Shoresh & Harmann, 2008	MpNC*, MpB*, MpVa*, MpT*	MpT = 9.0	1.15×10^{-4} (6x)



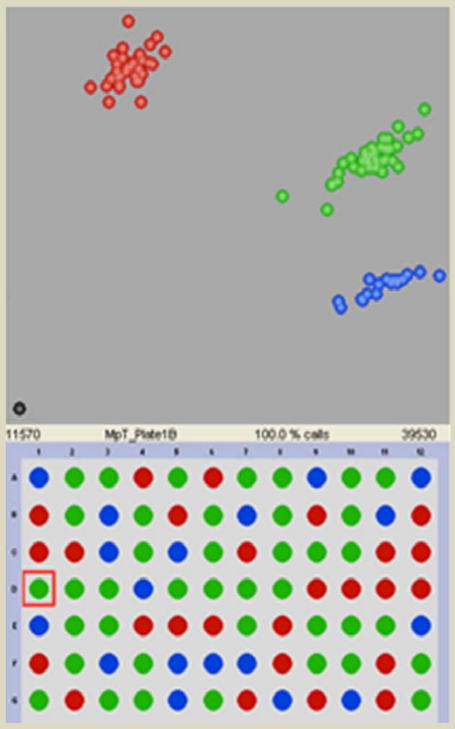


Figure 1. SSR (left) and SNP (right) genotyping of 96 individuals in a linkage mapping population, including parents and F1 (P1, P2, and F1 of the gel; well A1, B1 and A2 of the SNP image), showing the expected 1:2:1 segregation.

Description of populations: QTL populations were F2:3 mapping populations created from the following crosses: MpB = Mp313E x B73; MpVa = Mp313E x Va35; MpT = Mp715 x T173; MpNC = Mp717 x NC300. The association mapping panel consisted of 300 diverse inbred lines testcrossed onto Va35.

Discussion

Four genes (Trypsin Inhibitor and three Chitinase genes) have shown significant linkage or association with aflatoxin accumulation resistance in several cases (Table 1, highlight). Polymorphisms explaining a significant level of the phenotypic variation will make excellent markers for marker assisted improvement of this aflatoxin accumulation resistance, pending validation via transgenics or NILs. Candidates may aid in seeking pathways or networks that will lead to a greater understanding of the interactions between maize, fungus, and the production of aflatoxin. Even when polymorphisms show no significant effect via QTL or association mapping, this does not prove the gene(s) do not influence the trait. Other polymorphisms (alleles) not present in any of the parents of our QTL mapping populations may still affect the trait. This is less likely to occur in the association panel of 300 diverse individuals (thus allowing the testing of many more possible polymorphisms at a time), however, the genes may have shown a higher association in other environments not tested in this study. Therefore, lack of association does not prove lack of effect, although it reduced the chances of an effect. Please contact M. Warburton to submit candidate gene sequences to the pipeline.

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