Characterization of the Chitinase Gene Family for *A. Flavus* and Aflatoxin Accumulation Resistance in Maize

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Introduction

Chitin is a component of insect and fungal cell walls, and are made of N-acetylgluosamine (GlcNAc) units linked via β-1,4-glycosidic bonds. Plant chitinolytic enzymes hydrolyze chitin, and acting alone or in combination with β-1,3-glucanases, enzymes, defend against fungal pathogens. There are several chitinases, with different structures, substrate specificities, intracellular locations, and specific activities. Three main plant chitinase families do not share sequence similarity, 3D structures, or enzymatic mechanisms, and are thought to have evolved independently. Endochitinases cleave the chitin chain randomly, and include the Glycoside Hydrolase (GH) families 18 and 19. The exochitinases include GH-20 family members.

While some specific chitinase genes have been found to boost plant resistance to fungal infection and growth, the ability of all maize chitinase genes to reduce the growth of *Aspergillus flavus* and the subsequent production of aflatoxin by *A. flavus*, has not been well characterized. This study characterizes all maize chitinase genes in structure and function, including resistance to *A. flavus* infection and aflatoxin accumulation.

Materials and Methods

The literature, and databases at MaizeDB (www.maizegdb.org), www.maizesequence.org, Uniprot (www.uniprot.org) and Interpro (http://www.ebi.ac.uk/interpro/) MaizeCyc, (maizecyc.maizegdb.org) and Unigene (http://www.ncbi.nlm.nih.gov/unigene) were queried for any gene and/or protein with maize chitinolytic activity. Protein sequences were scanned for motifs at http://www.ebi.ac.uk/Tools/pfa/iprscan/ and domains built using http://prosite.expasy.org/cgi-bin/prosite/mydomains/. Candidate genes were confirmed to be associated with aflatoxin resistance according to Warburton et al., (2011). Genes were mapped in four QTL mapping populations, reported in Wilcox et al., (2013), Warburton et al. (2011); Warburton et al., (2009); and Brooks et al., (2005). Association mapping was done in a panel of 300 diverse inbred lines described in Warburton et al. (2013).

Population structure and a kinship matrix were calculated using 2000 SNPs, to correct for population substructure during association analysis using the Mixed Linear Models of TASSEL 3.0.

Conclusions

Although we have not been able to fully test every sequence yet, three GH-18 genes, three (or possibly four) GH-19 genes, and one GH-20 gene have strong, multiple lines of evidence for association with aflatoxin accumulation resistance in maize grain. (yellow highlighted lines, Table 1). These will be independently validated in future work. Because such different chitinases appear to be improving resistance, combining them into a single line may be useful for increasing resistance in maize.

Table 1. Id and descriptive characteristics of the 29 chitinase enzymes studied in up to four QTL mapping and one association mapping population for aflatoxin accumulation resistance. Multiple associations and significant QTL in one or more test locations are indicated in yellow highlight.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Chr</th>
<th>Chromosome Position</th>
<th>GO:0005975</th>
<th>GO:0006032</th>
<th>GO:0004553</th>
<th>GO:0004568</th>
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<tbody>
<tr>
<td>GRMZM2G090441</td>
<td>GH-18</td>
<td>1.05</td>
<td>347,788,419</td>
<td>chitin catabolic process</td>
<td>cell wall macromolecule catabolic process</td>
<td>hydrolase activity, hydrolyzing O-glycosyl compounds</td>
<td>carbohydrate metabolic process</td>
</tr>
<tr>
<td>GRMZM2G099454</td>
<td>GH-20</td>
<td>1.02</td>
<td>4,028,626</td>
<td>chitinase activity</td>
<td>chitin catabolic process</td>
<td>hydrolase activity, hydrolyzing O-glycosyl compounds</td>
<td>carbohydrate metabolic process</td>
</tr>
<tr>
<td>GRMZM2G040099</td>
<td>GH-19</td>
<td>1.07</td>
<td>34,099,658</td>
<td>chitinase activity</td>
<td>chitin catabolic process</td>
<td>hydrolase activity, hydrolyzing O-glycosyl compounds</td>
<td>carbohydrate metabolic process</td>
</tr>
</tbody>
</table>

References


Warburton et al. (2011) A public platform for the verification of the phenotypic effect of candidate genes for resistance to aflatoxin accumulation and *Aspergillus flavus* infection in maize. Toxins, 3(7), 754-765.
