

# 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*-acetylglucosamine (GlcNAc) units linked via  $\beta$ -1,4-glycosidic bonds. Plant chitinolytic enzymes hydrolyze chitin, and acting alone or in combination with  $\beta$ -1,3-glucanase 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 MaizeGDB ([www.maizegdb.org](http://www.maizegdb.org)), [www.maizesequence.org](http://www.maizesequence.org), Uniprot ([www.uniprot.org](http://www.uniprot.org)) and Interpro (<http://www.ebi.ac.uk/interpro/>) MaizeCyc, ([maizecyc.maizegdb.org](http://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 Willcox 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.

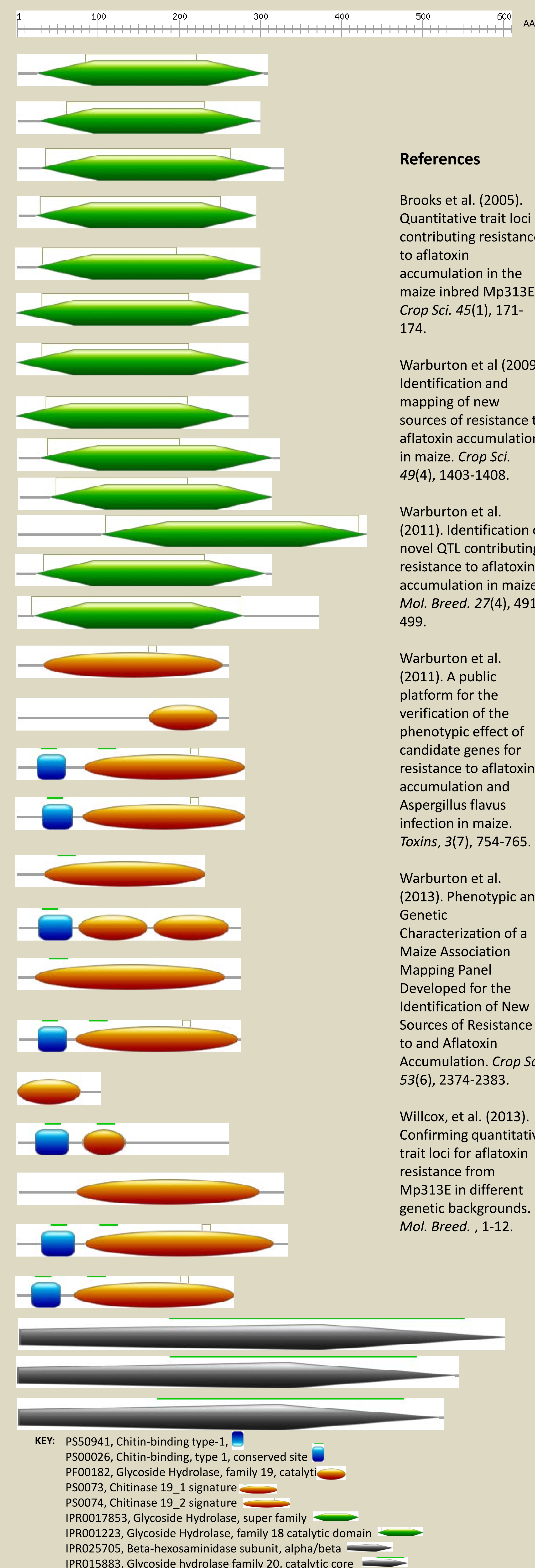
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**Table 1.** ID and descriptive characteristics of the 29 chitinase enzymes studied in up to four QTL mapping and one association mapping population for aflatoxin accumulation resistance. Multiple associations and significant QTL in one or more test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

Gene	Glycoside Hydrolase Family	Description	Bin	Chrom	Start Position	GO Annotation	# SNPs <sup>1</sup>	# associations	QTL pops <sup>3</sup>	LOD <sup>4</sup>
GRMZM2G162505	GH-18	Putative uncharacterized protein	1.08	1	240,766,113	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	8	0 (1)	MpVa MpB MpT MpNC	2.6 0.8 0.5 1.2
GRMZM2G403475	GH-18		3.04	3	118,806,302	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	7	1 (3)	MpVa MpT MpNC	0.8 1.2 0.6
GRMZM5G837822	GH-18	Hevamine-A	3.06	3	176,570,295	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	12	2 (9)	MpVa MpB MpT	1.2 1.8 3.4
GRMZM2G453805	GH-18	Putative uncharacterized protein, chn1	3.08	3	212,009,199	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	11	2 (4)	MpVa MpB MpT	1.2 0.4 1.2
GRMZM2G133781	GH-18	Putative uncharacterized protein	4.02	4	6,624,888	GO:0005975 - carbohydrate metabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	7	0 (1)	MpVa MpB	1.2 1
GRMZM2G358153	GH-18	Chitinase 1 Putative uncharacterized protein	4.03	4	12,082,697	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	5*	3 (6)	MpVa MpB MpNC	0.4 2.4 1.2
GRMZM2G057766	GH-18	Chitinase 1	5.06	5	197,564,845	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	8*	0 (0)	MpVa	0.6
GRMZM2G141456	GH-18	Chitinase Putative uncharacterized protein	5.08	5	215,173,302	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	4	0 (4)	MpNC	0.6
GRMZM2G447795	GH-18	Xylanase inhibitor protein 1	6.05	6	129,086,004	GO:0005975 - carbohydrate metabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	3*	0 (0)	MpT	0.4
GRMZM2G162359	GH-18	Putative uncharacterized protein	7.01	7	10,651,271	GO:0005975 - carbohydrate metabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds	6	1 (4)	MpVa MpB MpT	0.6 0.6 0.8
GRMZM2G037694	GH-18	Chitinase Putative uncharacterized protein	8.03	8	144,588,215	GO:0006032 - chitin catabolic process GO:0004568 - chitinase activity	4	1 (5)	MpVa MpNC	1.4 0.8
GRMZM2G400999	GH-18	Xylanase inhibitor protein 1	10.00	10	1,980,938	GO:0005975 - carbohydrate metabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	13*	3 (7)	MpVa MpB MpT	2 0.8 10
GRMZM2G090441	GH-18	Chitinase Putative uncharacterized protein	10.04	10	107,900,408	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004568 - chitinase activity	10	0 (2)	MpVa MpB MpT	1.2 0.4 1.8
GRMZM2G099454	GH-19	Putative uncharacterized protein	1.01	1	7,403,531	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0016998 - cell wall macromolecule catabolic process GO:0004568 - chitinase activity	11	0 (3)	MpVa MpB MpT	3.8 1.8 1.8
GRMZM2G103668 GRMZM2G454531	GH-19	Hypothetical protein LOC100275812 Pseudogene	1.05	1	85,545,979 85,545,422	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0016998 - cell wall macromolecule catabolic process GO:0004568 - chitinase activity	7	0 (2)	MpVa MpB MpT	2 0.8 10
GRMZM2G051943	GH-19	Endochitinase A precursor/ chitinase cta1, chiA, chita, chitinase A1	2.04	2	33,534,101	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004568 - chitinase activity GO:0008061 - chitin binding	5	1 (6)	MpVa MpB MpT MpNC	5 5.8 0.8 1
Possibly same as GRMZM2G052175 GRMZM2G051921 AC186025.4 FGD08	GH-19	Putative uncharacterized protein	2.04	2	33,506,460	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004568 - chitinase activity GO:0008061 - chitin binding	6	1 (2)	MpVa MpB MpT MpNC	5 5.8 0.8 1
GRMZM2G052175 Possibly same as GRMZM2G051943	GH-19	Endochitinase A umc1783	2.04	2	33,537,139	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0016998 - cell wall macromolecule catabolic process GO:0004568 - chitinase activity	5	1 (6)	MpVa MpB MpT MpNC	5 5.8 0.8 1
GRMZM2G064360	GH-19	Basic endochitinase 1	5.03	5	63,229,136	GO:0006032 - chitin catabolic process GO:0016998 - cell wall macromolecule catabolic process GO:0004568 - chitinase activity GO:0008061 - chitin binding	3	0 (1)	MpVa MpB MpT MpNC	1.4 3.2 0.9 1.6
GRMZM2G389582 GRMZM2G389557	GH-19	Chitinase chn3 Clone 304975 mRNA sequence	5.03	5	63,277,951 63,277,675	GO:0006032 - chitin catabolic process GO:0016998 - cell wall macromolecule catabolic process GO:0004568 - chitinase activity	6	0 (2)	MpVa MpB MpT MpNC	1.4 3.2 0.9 1.6
GRMZM2G129189	GH-19	Endochitinase PR4 Putative uncharacterized protein	5.05	5	182,518,442	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0016998 - cell wall macromolecule catabolic process GO:0004568 - chitinase activity GO:0008061 - chitin binding	6	1 (2)	MpVa MpB MpT MpNC	1.2 3.2 0.6 1.6
GRMZM2G145518 GRMZM2G447967	GH-19	Putative uncharacterized protein	6.01	6	82,813,531 82,813,748	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004568 - chitinase activity GO:0008061 - chitin binding	5	1 (2)	MpVa MpT MpNC	1.6 0.5 1.6
GRMZM2G145461	GH-19	chitinase candidateL00973, chn*-L00973, pCh2, uiu5(chn), MZECHITC	6.01	6	82,862,563	GO:0006032 - chitin catabolic process GO:0016998 - cell wall macromolecule catabolic process GO:0004568 - chitinase activity GO:0008061 - chitin binding	0 none found	na	MpVa MpT MpNC	1.6 0.5 1.6
GRMZM2G168364	GH-19	Endochitinase A2 Putative uncharacterized protein	7.03	7	134,135,706	GO:0006032 - chitin catabolic process GO:0016998 - cell wall macromolecule catabolic process GO:0004568 - chitinase activity	15	0 (5)	MpVa MpB MpT MpNC	1.2 1.6 2.2 1.6
GRMZM2G062974	GH-19	Basic Endochitinase A	8.03	8	88,812,713	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004568 - chitinase activity GO:0008061 - chitin binding	8	1 (3)	MpVa MpB MpNC	1.4 1.2 0.8
GRMZM2G005633	GH-19	Endochitinase B Precursor; Fragment	10.04	10	127,370,239	GO:0005975 - carbohydrate metabolic process GO:0006032 - chitin catabolic process GO:0004568 - chitinase activity GO:0008061 - chitin binding	7	0 (8)	MpVa MpB MpT	1.2 0.4 1.8
GRMZM2G134251	GH-20	Beta-hexosaminidase beta chain uncharacterized	1.02	1	27,303,824	GO:0005975 - carbohydrate metabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004563 - beta-N-acetylhexosaminidase activity	6*	0 (1)	MpB MpT	1.8 0.8
GRMZM2G034598	GH-20	Beta-hexosaminidase beta chain	6.01	6	34,442,195	GO:0005975 - carbohydrate metabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004563 - beta-N-acetylhexosaminidase activity GO:0045735 - nutrient reservoir activity	7	0 (1)	MpVa MpT MpNC	1.6 0.5 1.6
GRMZM2G117405	GH-20	Beta-hexosaminidase	8.03	8	164,558,359	GO:0005975 - carbohydrate metabolic process GO:0004553 - hydrolase activity, hydrolyzing O-glycosyl compounds GO:0004563 - beta-N-acetylhexosaminidase activity	13	4 (14)	MpVa MpB MpNC	1.4 1.2 0.8

<sup>1</sup> The number of SNPs tested in the association mapping panel of 288 lines, for which there was a polymorphism with a Minor Allele Frequency greater than 3.5%, within a +/- 10KB window of the candidate gene (usually within +/- 1KB).  
<sup>2</sup> The number of SNP/trait associations at  $p < 10^{-3}$ , (and  $p < 10^{-2}$  in parentheses) counting each environment and the average over environments as traits.  
<sup>3</sup> The QTL mapping populations in which the SNP segregated and was mapped. MpV = Mp313E x Va35; MpB = Mp313E x B73; MpT = Mp715 x T173; MpNC = Mp717 x NC300.  
<sup>4</sup> The LOD score at which the peak QTL was seen (if any).  
 \* no nearby SNPs were found, and these tested were downstream of the gene 25K

**Figure 1.** Protein structure and conserved motifs of the 29 chitinase enzymes studied; enzymes are listed in the same order as Table 1.



## References

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