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GENOME-WIDE ASSOCIATION AND IDENTIFICATION OF *ZEA MAYS* CHITINASE GENE FAMILY

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Abstract *Maize (Zea mays L.) is one of the most important crops globally, whose yield and quality are threatened by various fungal pathogens such as Fusarium verticillioides, Aspergillus flavus, and Exserohilum turcicum. Chitinases, which belong to the glycosyl hydrolase (GH) enzyme family, specifically GH18, GH19, and GH20, play important roles in the plant's immune response against these fungal pathogens by breaking down the β-1,4-glycosidic bonds present within their cell walls' chitin. By performing Genome-Wide Association Studies (GWAS) combined with the identification of the gene families present within the genome, researchers can map the single nucleotide polymorphisms (SNPs) responsible for the disease resistance of these plants to specific chitinase loci. This study aims to provide a synthesis of the genomic analysis of the maize chitinase (ZmChi) genes, including their structural characteristics, their phylogenetic relationships, their chromosomal locations, their expression patterns, and their associations with resistance to these fungal pathogens.*

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Introduction

Maize (Zea mays L.) production is experiencing increasing pressure from both environmental and fungal disease factors. These diseases are caused by fungi that can break down the tissues of the maize plant, whereas the maize plants themselves produce defense proteins known as pathogenesis-related proteins, mainly chitinase and β-1,3-glucanase enzymes (Dos Santos and Franco, 2023; Linthorst and Van Loon, 1991). Plant chitinases randomly cleave internal glycosidic linkages in unbranched N-acetylglucosamine polymers (chitin), effectively disrupting fungal hyphal tip elongation and releasing pathogen-associated molecular patterns (PAMPs) that trigger systemic acquired resistance (SAR). The recent release of high-resolution maize reference genomes (such as B73 RefGen_v5) has made it possible to identify full gene families (Cheng et al., 2023; Lin et al., 2021). Recent bioinformatics surveys have identified between 39 and 43 distinct ZmChi genes in the maize genome. These genes have been linked to natural variations within the maize genome that are associated with resistance to destructive fungal pathogens that cause diseases like ear rot and leaf blight (Lin et al., 2021; Wang et al., 2025; Wang et al., 2024).

Genomic Organization and Structural Features of ZmChi Genes

The ZmChi gene family consists of three major glycosyl hydrolase families, GH18, GH19 and GH20, and can be found on all 10 maize chromosomes. Chitinases of classes I, II, IV, VI and VII are predominantly from the GH19 family (dominant in higher plants), whereas class III and V chitinases are from GH18 (ubiquitous in kingdoms) (Dong et al., 2015; Lu et al., 2020; Wang et al., 2024).

Key Structural & Chromosomal Characteristics

Chromosomal Architecture

Uneven distribution across chromosomes with significant gene clusters due to tandem duplication events on chromosomes 1, 2, 4 and 5 (Agulnik et al., 1996; Cannon et al., 2004).

Subcellular Targeting: Most of the predicted ZmChi proteins possess N-terminal signal peptides for protein sorting to the apoplast, central vacuole, or chloroplasts (Jarvis, 2008; Li and Chiu, 2010).

Conserved Motifs: The GH19 family contains conserved catalytic signatures ([LIVM]-G-F-C-Y

[GI]-x(2)-[SAG]), and the GH18 family has characteristic (β/α)8 barrel folds for substrate binding (Duan et al., 2025; Kahar et al., 2025)(Table 1).

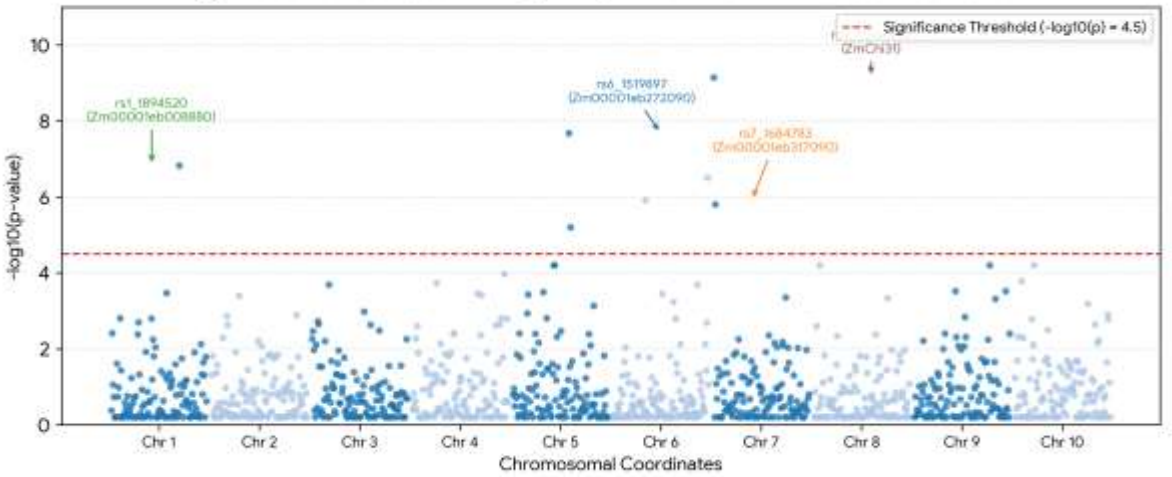
Table 1: Structural Classification and Features of Representative Maize Chitinase Genes

Gene ID (B73 v5)	GH Family	Class	Chr.	Exons	Isoelectric Pt (pI)	Subcellular Target	Primary Stress Induction
Zm00001eb317090	GH18	V	Chr 7	3	5.42	Apoplast	*F. verticillioides*, Drought
Zm00001eb272090	GH19	I	Chr 6	2	8.15	Vacuole	*F. verticillioides*, Salinity
Zm00001eb008880	GH20	Exo	Chr 1	8	6.08	Cell Wall	*F. verticillioides*, Cold
Zm00001eb167340	GH18	III	Chr 4	4	6.89	Apoplast	Wounding, Jasmonic Acid
Zm00001eb168350	GH18	III	Chr 4	4	7.12	Cytoplasm	Abscisic Acid (ABA)
ZmChi31	GH19	IV	Chr 8	2	8.65	Chloroplast	Multi-stress responsive (11 stresses)

Genome-Wide Association Mapping of ZmChi Loci

Genome-wide association studies (GWAS) use the historical recombination events in different inbred maize panels (e.g., the 282-line diversity panel or the Chinese inbred association groups) to map quantitative trait loci (QTLs) that control fungal disease resistance at single-nucleotide resolution (Cazares-Álvarez et al., 2024; Duan et al., 2025; Wang et al., 2024). Figure 1 shows a Genome-Wide Association Study (GWAS) with high resolution. Significant trait-associated SNP signals localized to particular ZmChi loci throughout maize chromosomes 1–10 are displayed in a Manhattan plot.

Figure 1: Genome-Wide Association Study (GWAS) Manhattan Plot for Maize Disease Resistance Traits



Identification of Major-Effect Trait-Associated SNPs

Fusarium & *Gibberella* Ear Rot Resistance: High-density SNP arrays (e.g., Maize 600K and GBS platforms) identified prominent candidate association signals located within 5–20 kb window regions flanking ZmChi loci (Table 2). Haplotype Fine-Mapping: Multi-allelic haplotype analysis of the lead candidate locus ZmChi31 revealed two major naturally occurring haplotypes (HapA and HapB) (Meena et al., 2025; Sarwar et al., 2026). Inbred lines harboring HapA exhibited a statistically significant reduction ($p < 0.001$) in kernel rot severity and lower internal fungal biomass compared to lines with HapB.

Table 2: Top GWAS Signals and Candidate Chitinase Loci for Disease Resistance Traits

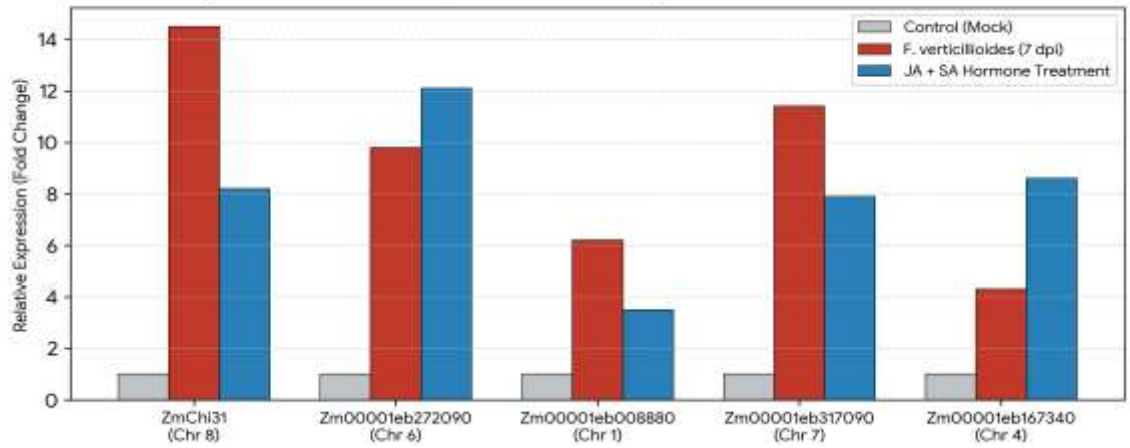
ssociated Trait	Marker SNP	Chr.	Position (bp)	-log10(p)	Lead Candidate Gene	Functional Defense Role
Fusarium Ear Rot (FER)	rs1_1894520	Chr 1	18,945,200	6.82	Zm00001eb008880	Exo-chitinase cleavage activity
Southern Corn Leaf Blight	rs6_1519897	Chr 6	151,989,702	7.67	Zm00001eb272090	GH19 Endochitinase hyphal degradation

Aspergillus flavus Resistance	rs8_12548901	Chr 8	125,489,010	9.14	ZmChi31	Chloroplastic/Apoplastic PR protein
Gray Leaf Spot (GLS)	rs7_1684783	Chr 7	16,847,839	5.91	Zm00001eb317090	Cell-wall structural defense network

Expression Profiling and Interaction Networks

Quantitative RT-PCR and transcriptome analyses demonstrate that specific chitinase genes display strong differential expression upon fungal pathogen attack. For example, following infection by Fusarium verticillioides, at least 10 core ZmChi genes exhibit notable upregulation by 7 days post-inoculation (dpi) (Lou et al., 2024; Zeng et al., 2024; Zhang et al., 2022). Figure 2 shows differential transcript abundance (fold change relative to control) of key ZmChi candidate genes under *Fusarium verticillioides* infection and defense hormone treatment of Japanic acid (JA) + salicylic Acid (SA).

Figure 2: Differential Transcriptional Activation of Representative ZmChi Genes Under Stress



Protein-Protein Interaction (STRING) Dynamics

Functional interaction network modeling demonstrates that ZmChi proteins physically and functionally interact with key cell-wall remodeling enzymes (cellulose synthases, pectin methylesterases, and β-hexosaminidases) as well as defense amplifiers (subtilisin-like proteases and PR-1). Notably, Zm00001eb317090 functions as a central hub node in the interaction network, connecting over 30 candidate functional partners during defense responses (Guerriero et al., 2015; Wang et al., 2024; Yang et al., 2025).

Marker-Assisted Selection and Breeding Implications

Determining the genetic variations that exist within the ZmChi gene family can help to develop diagnostic tools for the breeding of maize. KASP Marker Assay Development: Some of the important SNPs that were discovered in the GWAS study (e.g., rs8_12548901) can be converted into a KASP marker assay to enable selection of the desired background in the backcross breeding program. Pyramiding Resistance Alleles: Stack-combination of the GH19 (ZmChi31) and GH18 (Zm00001eb317090) loci can be achieved through genomic selection to create inbred lines with multiple resistances (Cheng et al., 2025; Hossain and Roslan, 2024). CRISPR/Cas9 Promoter Designing: The ZmChi gene contains MYB, WRKY, and ERE elements within its promoters, which can be targeted for CRISPR/Cas9 to allow for the creation of an inducible gene promoter with CRISPR/Cas9 without affecting the gene's yield (Cui et al., 2023; Qiu et al., 2025; Shi et al., 2021).

Conclusion

The critical function of chitinases in broad-range fungal disease resistance in maize has been discovered through the identification of 39-43 members of the ZmChi gene family, followed by a GWAS map of maize with respect to these genes. Some of the important functional loci within the ZmChi gene family include ZmChi31 and Zm00001eb317090, both of which have been identified as high-priority candidate genes for functional gene editing and marker-assisted breeding for resistance against fungal diseases in maize.

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Statements and Declarations

Data Availability statement

All relevant data are within the manuscript file.

Author's Contribution Statement

QA, and QH collected data and wrote manuscript equally. RA, and SK make final editing. QA supervised the whole research work. All authors have read the final manuscript and approve its submission.

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Not applicable

Conflict of interest

The investigation was undertaken without any financial conflicts of interest or any other commercial relationships that could be seen as such by any of the authors.