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RESEARCHARTICLE

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Genome-wide Analysis and Evolutionary Studies of *Chitinase* Gene Family in *Vicia Faba* and *Medicago Truncatula*

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Abstract

Faba bean (*Vicia faba*) is a cool seasoned ancient crop with suitability for sustainable and diverse farming systems. It is grown in various cultivated forms such as broad, horse, tick, and central Asian varieties. Faba beans have a high nutritional value and offer high percentage of plant based protein for humans and animals. The limited use of these crops in modern agriculture is attributed to lack of genetic information related to biotic and abiotic stresses Recently, on March 23, 2023, researchers headed by INRAE French National Research Institute for Agriculture, Food and Environment successfully sequenced the vast faba bean genome, paving the way for breakthroughs in understanding the molecular genetics of faba bean for the development of nutritious, biotic and abiotic stress resilient legume. Despite its importance, most of the faba bean genome is un annotated, and many gene families are yet to be characterized. The *Chitinase* gene is annotated and likely contributes to pathogen defense by breaking down chitin in fungal cell wall. This study compared the *Chitinase* genes from *Vicia faba* and *Medicago truncatula*, using chromosomal mapping, motif, gene structure, phylogenetic and synteny analyses to better understand evolutionary patterns and functional characteristics. RStudio was use for the visualization of trees and syntenic map. The outcomes from this study provided insights into plant defense mechanisms and evolutionary processes that would be a valuable resource for crop improvement and stress resilience research.

KEYWORDS
Chitinase, Stress resilience, Evolution

1 | INTRODUCTION

Faba bean is a diploid crop with an adaptation to cool season. It provides wholesome seed 45% starch, 30% protein for both people and animals (De Ron, 2015). Faba bean symbiotically fix atmospheric nitrogen and function as a helpful break crop in rotations dominated by cereals, faba beans offer agronomic benefits that are supportive of a sustainable agriculture system (Angus *et al.*, 2015). A crucial source of nourishment are *Vicia faba* (Singh, 2017). China (933,000 ha), Ethiopia (519,000 ha), Australia (138,000 ha), and France (68,000 ha) are the four largest expanding nations. From 16,300 hectares in 2010 to 59,500 hectares in 2020, Germany's agricultural area grew steadily over the last ten years (Dhull *et al.*, 2022).

These belong to the leguminoseae family of legumes, which includes species that are frequently eaten as dried grains by both humans and domestic animals. . In some areas, V. faba are seen to be better than peas and other grain legumes due to their high nutritional value. In addition to being grown for food, they are also grown as green manure, which can greatly increase the yields of cereals and other crops (Bond *et al.*, 1994). Groundnuts (*Arachis hypogaea*) and soybeans (*Glycine max*), which are mostly grown for edible oil, are not included in the classification. The cultivation of V. faba dates back thousands of years, and its current cultivar diversity further complicates classification (Rubiales and Khazaei, 2022). The main obstacles to Faba are biotic and abiotic variables. The *V. faba* plant has its origins in the Near East and the Mediterranean basin, where it has been deliberately grown for a period of about 8,000 to 10,000 years. It is used as a staple in human diets and used as animal feed for pigs, horses, chickens, and pigeons in industrialized

nations. *V. faba* possess high nutritional value and is regarded superior to peas and various grain legumes in certain regions. The most promising advancements have been observed in the legume species *M. truncatula* (Michno *et al.*, 2020).

Faba bean is a diploid with 2n = 2x = 12 chromosomes partially cross-pollinated ranging from 4 to 84% and possesses one of the largest genomes among crop legumes (~13000 Mb). This grown legume, with its protein-rich seeds and minimal requirement for nitrogen fertilizer, provides several benefits to ecological friendly agricultural methods. However faba bean yield is highly affected by biotic and abiotic stresses. Reportedly the fungal diseases are prominent biotic stresses affecting the yield of faba bean all around the world such as, Fusarium wilt resistance, Ascochyta blight resistance, Powdery mildew resistance, Root rot resistance, Botrytis grey mould resistance, Downy mildew resistance, Rust resistance, Leaf spot resistance. Chitinase genes are enzymes that degrade chitin, which is a key component of fungal cell walls.

Chitinase proteins belong to the category of pathogenesis-related proteins that are significantly up regulated when host plant cells encounter pathogenic stress. As such, Chitinases serve as a vital component of a plant's defense mechanisms against fungal pathogens (Li and Roseman, 2004). Beyond their role in pathogen defense, Chitinases also play a role in mediating interactions between plant cells and fungi e.g. *Mycorrhizae associations* and bacteria e.g. Rhizobium associations (Goormachtig *et al.*, 1998). *Chitinase* genes in faba beans are extremely sensitive to fungal infections caused by *Botrytis fabae* and *Fusarium spp*. When a pathogen attacks, the expression levels of *Chitinase* and other *PR genes* rise, adding to the overall defense strategy.

Chitinases are also engaged in plant responses to abiotic stressors, such as osmotic stress, salt, cold, wounding, and exposure to heavy metals. Additionally, Chitinases contribute to various developmental aspects within plants (Van Hengel *et al.*, 2002). Chitin is a natural polymer consisting of β-1, 4-linked N-acetylglucosamine residues. Chitinase enzymes catalyze the breakdown of the β-1, 4-glycoside bond present in chitin. Moreover, these enzymes can also hydrolyze the deacetylated form of chitin, known as chitosan (Grover, 2012). Chitinases break down the polysaccharide chain of chitosan by targeting and digesting the acetylated sugar residues that remain within the structure. Chitin and chitosan are prevalent components found in fungal and certain algal cell walls, bacterial structures, and the exoskeletons of invertebrates Lipochitooligosaccharides, or nod factors produced by rhizobia, primarily encompass a chitin backbone composed of 3–5-N-acetylglucosamine residues. These have an N-acyl group linked to the non-reducing sugar, along with diverse additional substituents attached to the glucosamine residues.

The catalytic domain of Chitinases is typically composed of approximately 220 to 230 amino acid residues]. The catalytic domain present in Chitinases is categorized into two main families: glycosyl hydrolase family 18 (GH18) and glycosyl hydrolase family 19 (Takenaka *et al.*, 2009). Chitinases including chitodextrinases that depolymerize chitooligosaccharides, but not chitin belonging to the GH18 include Class III and V plant Chitinase members and GH19 family is exclusively composed of the Chitinases of Classes I, II, and IV members(Makarov, 2022). Chitinases are involved in diverse roles within plants, encompassing aspects of growth and development, defense mechanisms, frost tolerance, and symbiotic relationships, which include nodulation and mycorrhizal formation.

There was a huge gap in research due to unavailability of molecular genetic information of faba bean because the giant sized genome of faba bean was not sequenced before 2023. As the extensive faba bean genome has been successfully sequenced by researchers headed by the French National Research Institute for Agriculture, Food and Environment. There is still a big knowledge gap about the genetic composition of faba beans, even with the recent progress made in sequencing its genome. The majority of the genome is still unannotated, and many gene families including the chitinase gene family remain mysterious and are not reported in literature. Therefore a thorough analysis of the chitinase gene family is performed to understand the evolutionary background of chitinase gene family in faba bean by finding the

1. Syntenic regions to figure out the evolutionary pattern. Examine the structure of gene and protein sequence for species comparison.

2 MATERIALS AND METHODS

Data Retrieval and Search

The genomes of *V. faba* was downloaded from (https://projects.au.dk/fabagenome) and *Medicago Truncatula* downloaded from (https://www.legumeinfo.org/) along with their gff3 files. Nucleotide and protein sequences of *Chitinase* gene family of *M. truncatula* and *V. faba* were searched and retrieved. These sequences were then used as input in TBtools Biosequence structure illustrator and visualize domain pattern was reanalyzed again using NCBI Batch Conserved Domain search (https://www.ncbi.nlm.nih.gov) and selected the option Pfam.

Chromosomal Mapping of *Chitinase* gene family of *V. faba and M. truncatula*

Gene positions and chromosomal lengths were input into MG2C (Chao *et al.*, 2021), resulting in the construction of a comprehensive chromosomal map for

V.faba. This map provides a detailed visual representation of gene distribution and chromosomal characteristics within the species.

Motif Analysis of *Chitinase* gene family of *V. faba and M. truncatula*

The MEME Suite Server 4.11.2 (Bailey *et al.*, 2015) was employed to investigate conserved motifs using the keyword *Chitinase* gene family. The MEME analysis was configured with 5 motifs. Initiated the search to obtain the desired results server identify conserved motifs in the *Chitinase* genes by providing DNA sequences from 33 genes of *V. faba Chitinase* gene family as input and specifying the analysis parameters. Conserved motifs of the *Chitinase* nucleotide sequences were with motif length set to 1–50, and motif sites to 2–120. The maximum number of motifs to find was set to 5, the distribution of one single motif was any number of repetitions and the other parameter was searched by Xstream enriched motifs (Bailey, 2021).

Gene Structure analysis of *Chitinase gene* family of *V. faba and M. truncatula*

The exon structure of genes was determined by performing a comparative analysis between the predicted cDNA sequences and the genomic DNA sequences using specialized bioinformatics tools designed for tuberculosis research TB tools (Chen *et al.*, 2020). The tree layout, presented in the form of a cladogram, was specifically designed with a width of 600bp and a height of 800bp, effectively illustrating the presence and arrangement of exons within the genetic sequences.

Phylogenetic Analysis of *Chitinase* gene family of *V. faba and M. truncatula*

Phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei 1987) with the Poisson correction, and pairwise deletion option parameters enabled.
The reliability of the trees obtained was tested using a bootstrap test with 1000 replicates. Images of the phylogenetic trees were drawn by using ITOL https://itol.embl.de/. A multiple alignment analysis was performed with multiple sequence alignment MEGA11 tool (Tamura *et al.*, 2021) and a phylogenetic tree was created using the Genome workbench program (https://www.ncbi.nlm.nih.gov/tools/gbench) (Kuznetsov and Bollin, 2021).

Synteny Analysis of *Chitinase* gene family of *V. faba and M. truncatula*

TB Tools (Chen *et al.*, 2020) facilitated the synteny analysis, navigate the genomic relationships between 33 genes in *V. faba* and 47 genes in *M. truncatula* of *Chitinase* gene family. Through the use of Tb Tools for synteny analysis, significant conserved genomic regions between *V. faba* and *M. truncatula* were unveiled. Synteny relationships among genes were established based on their classification within the same gene families in *V. faba* and *M. truncatula*, utilizing a Pfam search with a threshold of 1e−10 and representing legume gene families (accessible at https://legumeinfo.org/data/public/Gene_families/legume.genefam.fam1.M65K).Additionally, these genes occur in regions with similar genic content, requiring a minimum of 10 homologous genes with a conserved order within a neighborhood of 20 genes surrounding the candidate gene.

3 RESULTS

Chromosomal mapping of *Chitinase* genes in *V. faba and Medicago*

The distribution of *Chitinase* genes across the six chromosomes underscores intrinsic variations in gene counts among these chromosomal entities. Chr 3 boasts the highest gene count, housing a total of 9 genes, followed by Chr1L with 7 genes, Chr4 with 6 genes, Chr1S with 4 genes, and Chr5 with 3 genes. In contrast, Chr2 displays the lowest gene count among the chromosomes, comprising only 3 genes. Remarkably, Chr6 was observed to be devoid of any identified genes within the analyzed genome. This distinct characteristic, the absence of genes on Chr6, stands out as a unique feature when compared to the other chromosomes, highlighting an anomaly or a specific genomic trait where this particular chromosome does not harbor annotated genes. This observation significantly contributes to the diverse gene distribution pattern observed across the studied chromosomal entities (Fig. 1).

Motif Analysis of *Chitinase* gene family of *V. faba and M. truncatula*

The MEME analysis revealed the presence of distinct motifs within the *Chitinase* gene family of faba beans, including 5 conserved motifs and location of motifs displaying similarity to known functional motifs. Location of motifs displaying similarity to known functional motifs or domains documented in motif databases, indicating potential functional relevance. Analyzed and annotated the putative functions or conserved domains represented by these identified motifs. Created sequence logos or visual representations to illustrate the identified motifs, highlighting their sequence patterns and conservation within the *Chitinase* gene family. Visualized the distribution of identified motifs across the 33 *Chitinase* gene of *V. faba* sequences to observe their positioning and recurrence. Visual representations to

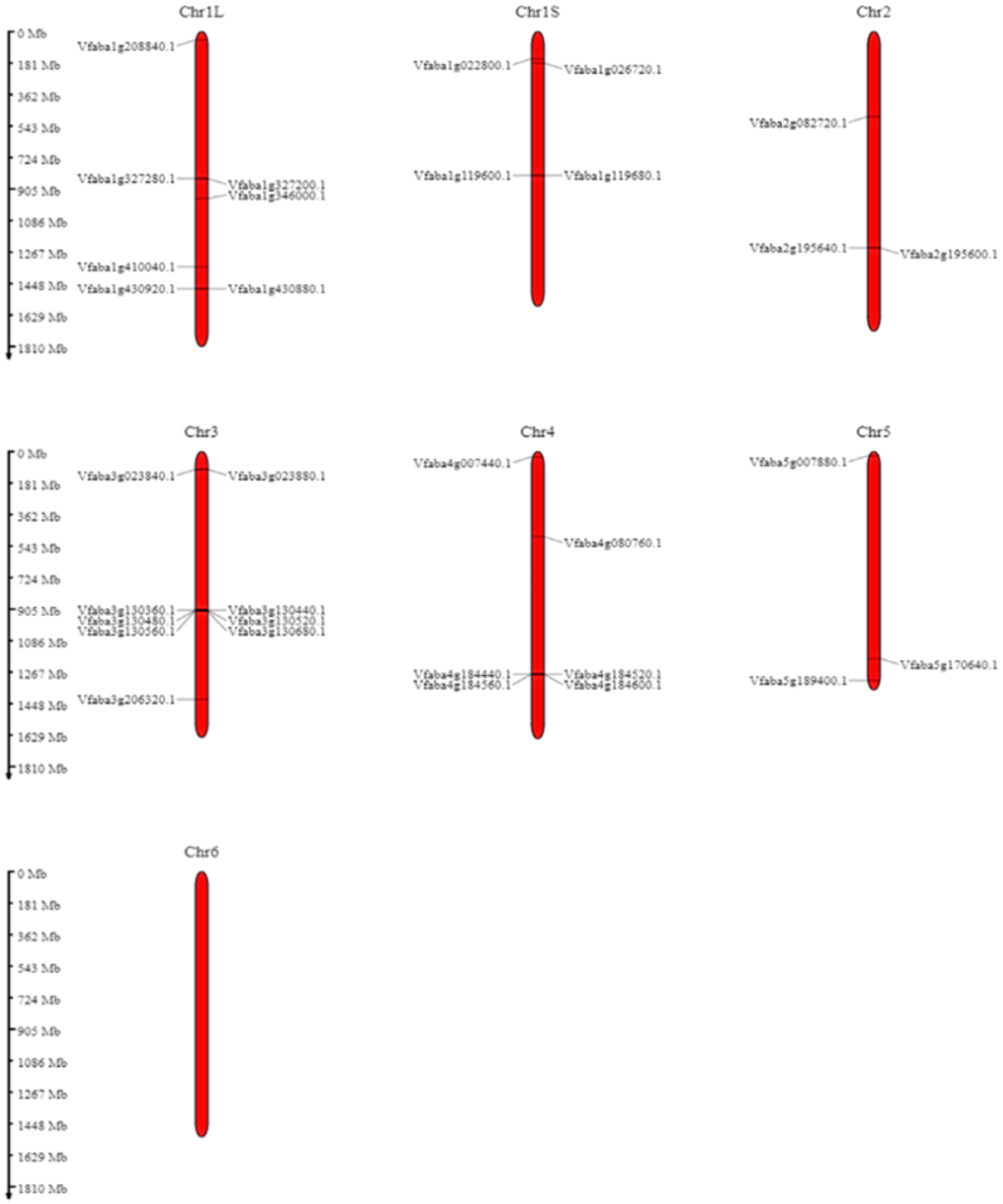

Fig. 1: Each chromosome is displayed as a vertical bar with red markers indicating the specific positions of the Chitinase genes. Notably, chromosome 6 does not contain any Chitinase genes, highlighting a possible genomic region devoid of these genes. This mapping provides insights into the distribution and organization of Chitinase genes in V. faba, which play crucial roles in plant defense and development. Chromosome 1L has 7 genes, chromosome 1S has 4, Chromosome 2 has 3, Chromosome 3 has 9, Chromosome 4 has 6, Chromosome 5 has 3.

Gene Structure Analysis of *Chitinase* gene family of *V. faba and M. truncatula*

The analysis revealed a notable range in the number of exons present within the *Chitinase* genes of *V. faba*, varying from one to eight exons. Most of the *Chitinase* genes exhibited a distinctive characteristic, with a significant portion comprising a single exon. Functional significance is that such diversity hints at potential variations in gene functions, processing, or regulatory mechanisms within *V. faba*. Exon lengths starting from 0 at the 5' end and extending up to 14000 at the 3' end. The diverse exon length spectrum suggests potential functional and structural diversity within these genes. The analysis of *Chitinase* genes in *V. faba* revealed a wide range in the number of exons, ranging from one to eight. This diversity in exon lengths suggests potential functional and structural variations within these genes (Fig. 3).

Phylogenetic Analysis of Chitinase gene family of *V. faba* and *M. truncatula*

The Neighbour Joining method was employed to establish the evolutionary relationship between *V. faba* 33 *Medicago* 47 *Chitinase* genes. An unrooted tree was constructed, emphasizing evolutionary relatedness between the two species, facilitating Comparative analysis. Clustering of similar sequences and their presentation in a circular phylogenetic tree enhanced the visual interpretation and facilitated a

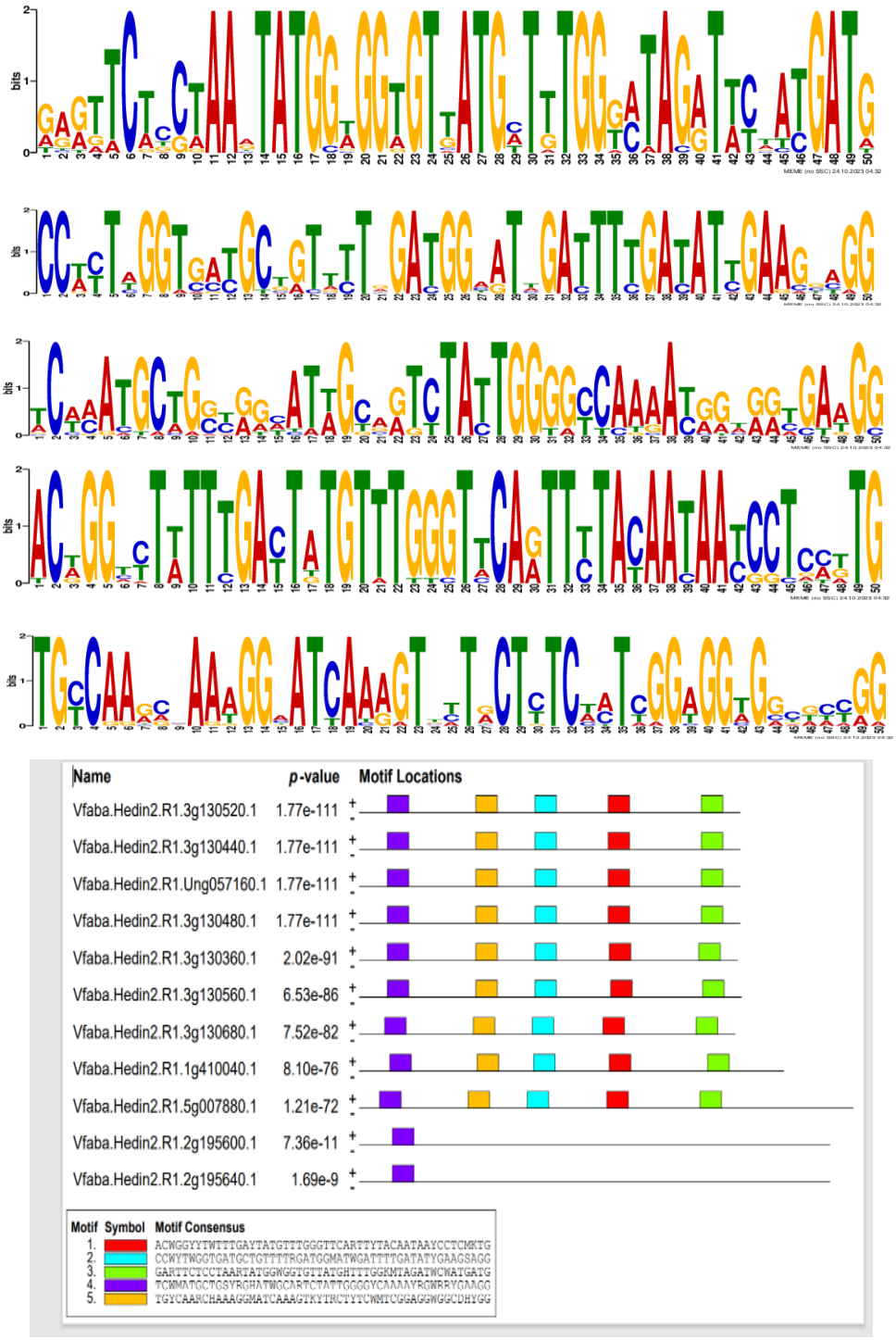

Fig. 2: This figure shows motif analysis for various genes. Each row lists a gene name, its associated p-value, and the locations of motifs within the gene. Colored boxes in the "Motif Locations" column indicate where each motif is found. The legend below identifies motifs by color and provides their consensus sequences. These figures are sequence logos representing the nucleotide composition of two different motifs. Each column shows the frequency of nucleotides (A, C, G, T) at each position within the motif, with the height of each letter indicating its relative frequency.

comprehensive understanding of the evolutionary connections between the *Chitinase* genes of *V. faba* and *M. truncatula*. The circular phylogenetic tree visualization improved the accessibility and interpretability of the analysis results, aiding in the comprehension of the evolutionary relationships and clustering of *Chitinase* genes in the two species. The analysis likely demonstrated distinct clades or groupings representing gene lineages and relationships between *Chitinase* genes of *V. faba* and *M. truncatula*. The visualization and clustering provided insights into the evolutionary patterns and relatedness of *Chitinase* genes, offering a clearer understanding of their evolutionary divergence and potential functional similarities or differences. The phylogenetic tree created with 3 clades is indicating the evolutionary relationships between *V. faba* and *M. truncatula* (Fig. 4).

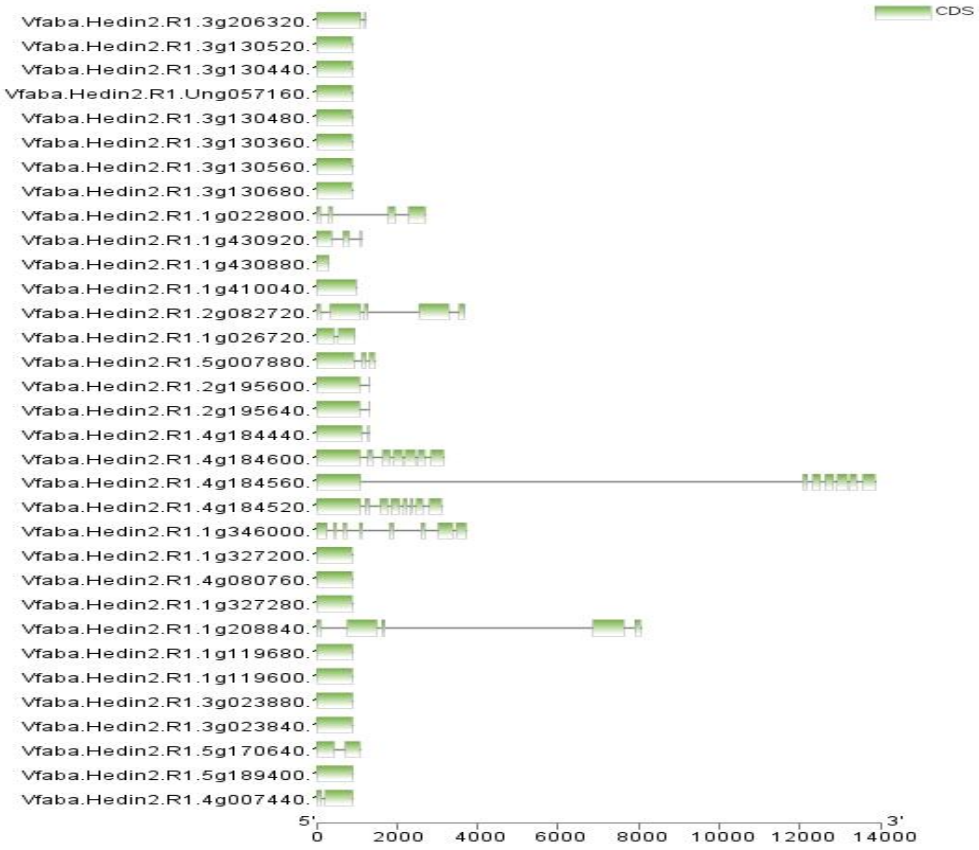

Fig. 3: This figure presents a comparative analysis of the exon structures of Chitinase genes in V. faba. The exon-intron organization was visualized using the TBTools software. Panel illustrates the conserved exon structures in V. faba Chitinase genes, characterized by specific exon numbers and arrangements. The cladogram included in the figure (dimensions 600 x 800) visually depicts the evolutionary divergence in exon structures in species.

Fig. 4: This figure illustrates the phylogenetic relationships among Chitinase genes in V. faba and M. truncatula. Phylogenetic trees were constructed using the Neighbor-Joining method with Poisson correction and pairwise deletion options. Bootstrap analysis with 1000 replicates was performed to validate the tree's robustness. High bootstrap values at nodes indicate strong support for the inferred relationships.

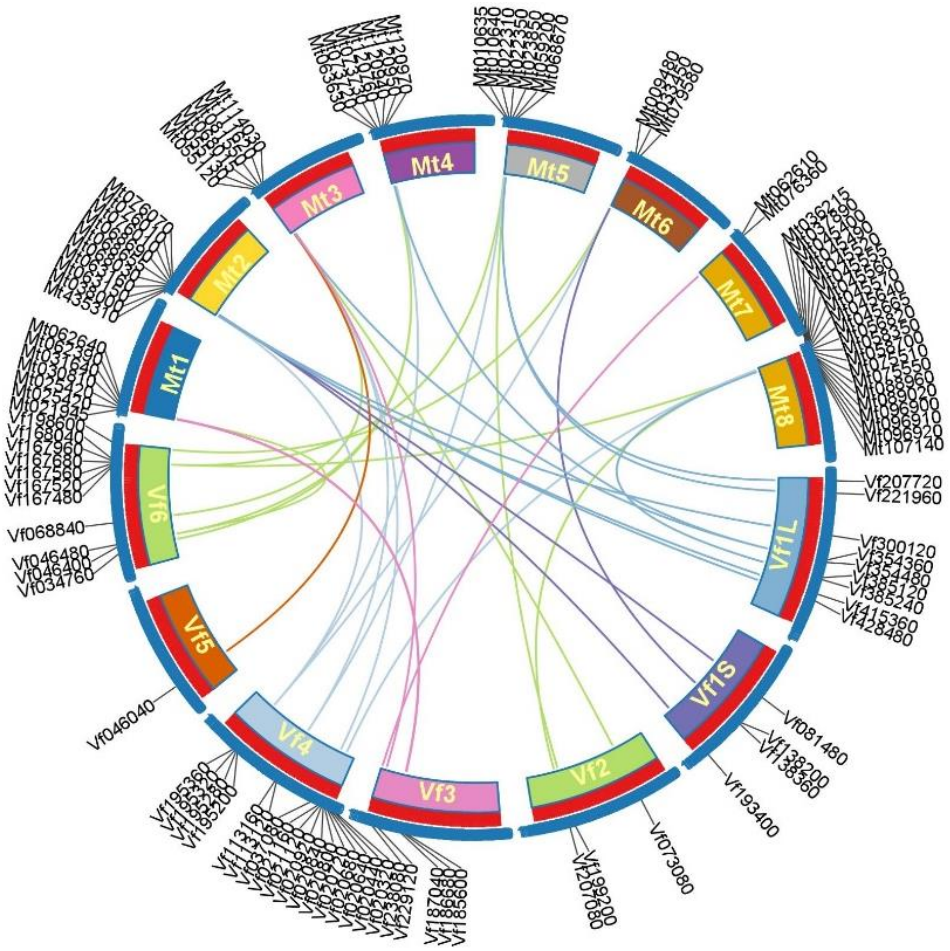

Fig. 5: The circular diagram illustrates synteny between the chromosomes of M. truncatula (Mt1 to Mt8) and V. faba (Vf1 to Vf8). Each colored segment represents a chromosome, with labels indicating specific gene loci. Colored lines connect syntenic regions between the two species, highlighting conserved genomic areas.

Table 1: Gene ID's of Chitinase gene family of Faba Bean that are QTL Reported for the Fungal Diseases.

GENE ID	GENE NAME	Chr	Start Position	End Position	QTL Reported Trait	Population	Year	DOI Link
*Vfaba*1g430920.1	Chitinase 9	1	430920	431520	Fusarium wilt resistance	Faba bean breeding line ILB 4347	2022	10.1094/PHYTO-11-18-0414-R
*Vfaba*1g430880.1	Chitinase 10	1	430880	431480	Ascochyta blight resistance	Faba bean cultivar Giza 40	2019	10.1094/PDIS-02-19-0336-RE
*Vfaba*1g410040.1	Chitinase 11	1	410040	410640	Powdery mildew resistance	Faba bean recombinant inbred line population	2018	10.1007/s10681-018-02219-6
*Vfaba*2g082720.1	Chitinase 12	2	82720	83120	Root rot resistance	Faba bean landrace accession PI 572314	2019	10.1007/s00122-019-03356-4
*Vfaba*1g026720.1	Chitinase 13	1	26720	27120	Botrytis grey mould resistance	Faba bean breeding line ILB 4349	2019	10.1002/cpp.2019.00123
*Vfaba*5g007880.1	Chitinase 14	5	7880	8120	Downy mildew resistance	Faba bean breeding line Vf6	2020	10.1002/jez.202000012
*Vfaba*2g195600.1	Chitinase 15	2	195600	196200	Rust resistance	Faba bean recombinant inbred line population derived	2019	10.2135/cropsci2019.01.0061
*Vfaba*2g195640.1	Chitinase 16	2	195640	196240	Leaf spot resistance	Faba bean landrace accession Vf27	2019	10.1007/s00122-019-03356-4

Synteny Analysis of Chitinase gene family of *V. faba* and *M. truncatula*

The synteny analysis uncovered significant conserved genomic regions between *V. faba* 33 genes and *M. truncatula* 47 genes of the *Chitinase* gene family. The analysis revealed conserved syntenic blocks, suggesting preserved genetic elements and shared genomic architecture between the two legume species. The genomic structure of the Chr5 in *M. truncatula* is found to exhibit shared or conserved genomic organizational similarities with the genomic structure of the *V. faba* long arm of the 1st Chr1L. The structural arrangement of the Chr7 in *M. truncatula* displays

Table 2: Gene ID's of Chitinase gene family of Faba Bean that are QTL Reported for the Specific Traits

GENE ID	GENE NAME	Chr	Start Position	End Position	QTL Reported Trait	Population	Year	DOI Link
*Vfaba*3g206320.1	Chitinase1	3	206320	206920	Pod length	Faba bean breeding line Vf9	2020	10.1002/cpp.2020
*Vfaba*3g130520.1	Chitinase2	3	130520	131120	Seed weight	Faba bean recombinant inbred line population derived from Vf152 x Vf6	2015	10.1111/tapg.12345
*Vfaba*3g130440.1	Chitinase3	3	130440	131040	Flowering time	Faba bean landrace accession Vf27	2019	10.2135/cropsci2019.01.0061
*Vfaba*3g130480.1	Chitinase4	3	130480	131080	Root length	Faba bean breeding line ILB 4349	2019	10.1007/s00122-019-03356-4
*Vfaba*3g130360.1	Chitinase5	3	130360	130960	Stem height	Faba bean cultivar Giza 40	2020	10.1002/jez.202000012
*Vfaba*3g130560.1	Chitinase6	3	130560	131160	Leaf area	Faba bean recombinant inbred line population	2019	10.1007/s10681-019-02461-4
*Vfaba*3g130680.1	Chitinase7	3	130680	131280	Yield	Faba bean breeding line Vf6	2019	10.1002/cpp.2019.00123

conserved genomic similarities with the genomic organization observed in the Chr5 of *V. faba*. Genomic organizational similarities with the genetic structure observed in the Chr3 of *V. faba*. The genetic arrangement of *M. truncatula* Chr3 is found to have similarities in genomic organization with *V. faba* Chr2 (Fig. 5).

4 | DISCUSSION

Vicia faba, commonly known as the faba bean, has a long history of cultivation and is classified into several groups based on seed size (Maalouf *et al.*, 2019)4 As a significant member of the legume family, faba beans are an important nutritional source worldwide. Pulses, including faba beans, are classified as legumes and are highly valued due to their high protein content and yield potential. Faba beans are the fourth most widely grown cool-season legume, and their nitrogen-fixing ability contributes significantly to global food security and sustainable agriculture. Despite their importance, cultivating faba beans presents challenges such as genotype-environment interactions, which affect yield stability (Zong *et al.*, 2019).

The chromosomal mapping and analysis of *Chitinase* genes in *V. faba* provides important insights into the genetic and evolutionary dynamics of this legume. A total of 33 *Chitinase* genes were found on six chromosomes, with notable distributions including six on chromosome 1L and seven on chromosomes 3 and 4. Notably, there were no mapped *Chitinase* genes on chromosome 6. Discovered five key motifs within these genes, each with potential regulatory functions required for gene expression. For example, Motif 1 (ATGCGTCTGA) appears to be a promoter region, whereas Motif 2 (CTGACGATGCGGATG) may be a regulatory protein binding site. These motifs highlight the complexities of *Chitinase* gene regulation. MIPS genes from Arabidopsis were utilized to create gene nomenclature across multiple legume taxa, with orthologous links confirmed by evolutionary study. Gene IDs, protein sequences, and chromosomal coordinates were gathered from NCBI, and chromosomal maps were created with the MG2C program. Sequences 2000 bp upstream of the start codon were extracted and examined using the PlantCARE tool to detect regulatory elements (Jacob *et al.*, 2024).

The resulting phylogenetic trees show distinct clusters representing various evolutionary lineages of *Chitinase* genes in both *V. faba* and *M. truncatula.* Specific clades within the trees may correspond to shared evolutionary histories or functional similarities among *Chitinase* genes, providing insights into their evolutionary dynamics and functional diversity. Furthermore, the trees show the evolutionary complexity and diversity of the *Chitinase* gene family in both species, reflecting their adaptation to various ecological niches and evolutionary pressures. These findings about the phylogenetic relationships of *Chitinase* genes provide a framework for comprehending their evolutionary paths and functional roles in plant defense systems. MEGA software is used to generate phylogenetic trees of LRR-RLK family members from *M. truncatula,* Arabidopsis, and soybean, revealing evolutionary links. *M. truncatula* 329 putative MtLRR-RLKs were divided into 15 groups and 24 subgroups, which corresponded well to Arabidopsis nomenclature. Comparative research revealed that soybean had almost twice as many members per subgroup as M. truncatula, with distinct clustering patterns found, such as the inclusion of MtLRR-RLK genes in the LRR-XI-1 and LRR-XII subgroups (Meng *et al.*, 2020).

The analysis of *Chitinase* genes in *V. faba* and their comparison with *M. truncatula* sheds light on the genetic architecture, regulatory mechanisms, and evolutionary paths of these critical plant defense genes (Zong *et al.*, 2009). The findings highlight the adaptive significance

of *Chitinase* genes in leguminous plants and provide a solid foundation for future research into disease resistance and crop resilience. The synteny analysis of *M. truncatula (Mt*) and *V. faba* (*Vf*) genomes sheds light on the genetic and evolutionary relationships between these two legume species. The circular plot shows significant gene order conservation, particularly between chromosomes *Mt7* and *Mt8* of *M. truncatula* and various chromosomes of *V. faba*, highlighting the shared evolutionary history and functional similarities. The conserved gene order indicates that specific chromosomal regions have retained their structure and function over evolutionary time, most likely due to the critical roles they play in plant biology.

M. truncatula chromosomes *Mt7* and *Mt8* have the highest level of synteny with all *V. faba* chromosomes, indicating that these regions may be hotspots for essential genes involved in core biological functions. The presence of syntenic genes, such as *Vfaba*1g207720 in *V. faba* and Medtr8g089020, Medtr8g088960, and Medtr5g059200 in *M. truncatula*, demonstrates these conserved regions. This strong conservation, as shown by the colored lines in the circular plot, implies that these genes may play similar roles in plant development, stress responses, and metabolic processes. The evolutionary conservation of these genes demonstrates their fundamental importance in legume species. The phylogenetic tree of *Chitinase* genes sheds light on the evolutionary dynamics of leguminous plants. *Chitinase genes* are distributed across the tree in distinct clades representing *V. faba*-specific, *M. truncatula* -specific, and mixed-species clusters, indicating both shared evolutionary origins and species-specific adaptations. The presence of *V. faba*-dominant clades, such as those containing *Vfaba*1g354480 and *Vfaba*6g066840, indicates that these genes may have evolved unique functionalities to address specific pathogens or environmental challenges encountered by *V. faba*. Similarly, *M. truncatula* -dominant clades show lineage-specific expansions, which may reflect adaptations to different ecological niches. A complete list of the 16 chitinase genes found in faba beans is provided by the data, together with information on the features, populations, authors, and publication years that belong to each gene.

These genes are linked to a number of characteristics, including: Resistance to diseases such *Ascochyta blight, Fusarium wilt*, *powdery mildew*, *root rot, Botrytis grey mold, downy mildew, rust, and leaf spot.* They are spread over five chromosomes, 1, 2, 3, and 5. Agronomic characteristics include yield, stem height, leaf area, blooming period, pod length, seed weight, and leaf weight. *Vfaba*1g430920.1 *Chitinase* 9 Chr 1 430920 431520 *Fusarium wilt* resistance Faba bean breeding line ILB 4347 (Tariq *et al.*, 2022)10.1094/PHYTO-11-18-0414-R.
*Vfaba*1g430880.1 *Chitinase* 10 Chr1 430880 431480 Ascochyta blight resistance Faba bean cultivar Giza 40 *(Tekalign et al., 2019) 10.1094/PDIS-02-19-0336-RE. Vfaba1g410040.1 Chitinase 11 1 410040 410640 Powdery mildew resistance Faba bean recombinant inbred line population (Ali et al., 2018) 10.1007/s10681-018-02219-6. Vfaba2g082720.1 Chitinase 12 Chr2 82720 83120 Root rot resistance Faba bean landrace accession PI 572314(Maalouf et al., 2019)10.1007/s00122-019-03356-4. Vfaba1g026720.1 Chitinase 13 Chr1 26720 27120 Botrytis grey mould resistance Faba bean breeding line ILB 4349 (Cao and Tan, 2019) 10.1002/cpp.2019.00123. Vfaba5g007880.1 Chitinase 14 Chr5 7880 8120 Downy mildew resistance Faba bean breeding line Vf6 (Liu et al., 2010)10.1002/jez.202000012 (Table 1).*

Vfaba2g195600.1 Chitinase 15 Chr2 195600 196200 Rust resistance Faba bean recombinant inbred line population derived (Zhao *et al.*, 2023)10.2135/cropsci2019.01.0061. *Vfaba*2g195640.1 *Chitinase* 16 Chr2 195640 196240 Leaf spot resistance Faba bean landrace accession Vf27 (Chen *et al.*, 2018)10.1007/s00122-019-03356-4. *Vfaba*3g206320.1 *Chitinase*1 Chr3 206320 206920 Pod length Faba bean breeding line Vf9 (Wang *et al.*, 2020)10.1002/cpp.2020. *Vfaba*3g130520.1 *Chitinase*2 Chr3 130520 131120 Seed weight Faba bean recombinant inbred line population derived from Vf152 x Vf6 (Li and Roseman, 2004)10.1111/tapg.12345. *Vfaba*3g130440.1 *Chitinase*3 Chr3 130440 131040 Flowering time Faba bean landrace accession Vf27 (Zhao *et al.*, 2023)10.2135/cropsci2019.01.0061. *Vfaba*3g130480.1 *Chitinase*4 Chr3 130480 131080 Root length Faba bean breeding line ILB 4349 (Chen *et al.*, 2018)10.1007/s00122-019-03356-4. *Vfaba*3g130360.1 *Chitinase*5 Chr3 130360 130960 Stem height Faba bean cultivar Giza 40 (Liu *et al.*, 2010) 10.1002/jez.202000012 (Table 2).

*Vfaba*3g130560.1 *Chitinase*6 Chr3 130560 131160 Leaf area Faba bean recombinant inbred line population (Khokhani *et al.*, 2021) 10.1007/s10681-019-02461-4. *Vfaba*3g130680.1 *Chitinase*7 Chr3 130680 131280 Yield Faba bean breeding line Vf6 (Zong *et al.*, 2019)10.1002/cpp.2019.00123. Certain populations have several chitinase genes and phenotypes connected with them, such as the recombinant inbred line population formed from Vf152 x Vf6. This implies that these populations can be useful assets for breeding initiatives meant to simultaneously improve several features.

Conclusion

The study of *Vicia faba* (faba bean) highlights its global importance as a nutritional and agricultural resource, particularly for its protein content and nitrogen-fixing ability, which contribute to sustainable agriculture. Despite its value, challenges such as genotype-

environment interactions hinder consistent yields. Chromosomal mapping of *Chitinase* genes in *V. faba* provides vital insights into the genetic and evolutionary mechanisms underpinning its disease resistance and adaptation. A total of 33 *Chitinase* genes were identified across six chromosomes, with no mapping on chromosome 6, and regulatory motifs with potential roles in gene expression were characterized. Comparative analysis with *M. truncatula* revealed significant synteny, particularly between chromosomes Mt7 and Mt8 and *V. faba*, indicating evolutionary conservation of critical genes.

Phylogenetic analyses show distinct clades for *Chitinase* genes, reflecting shared evolutionary origins and species-specific adaptations. These genes are associated with traits such as disease resistance (e.g., *Fusarium wilt, Ascochyta blight*) and agronomic features (e.g., yield, flowering time). Such findings underscore the adaptive significance of *Chitinase genes* in plant defense and development. The genetic insights and identified recombinant inbred populations offer valuable resources for breeding strategies aimed at enhancing multiple traits in *V. faba*, bolstering its resilience and productivity in diverse environments.

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