CTU Journal of Innovation and Sustainable Development
ISSN 2588-1418 | e-ISSN 2815-6412

DOI:10.22144/ctujoisd.2026.035

Genome-wide analysis of pathogenesis-related 5 gene family involved in pathogenesis in sugar beet (*Beta vulgaris*)

Vu Xuan Duong[1], Le Thi Man[1], Le Thi Ngoc Quynh[2], Chu Duc Ha[3], Le Chi Toan[4], and Cao Phi Bang[1*]

[1]*Faculty of Natural Sciences, Hung Vuong University, Viet Nam*

[2]*Department of Biotechnology, Thuyloi University, Viet Nam*

[3]*Faculty of Agricultural Technology, VNU University of Engineering and Technology, Viet Nam*

[4]*Faculty of Biology, Hanoi Pedagogical University No. 2, Viet Nam*

**Corresponding author (phibang.cao@hvu.edu.vn)*

Article info.

Received 31 Jan 2026
Revised 11 May 2026
Accepted 21 Jun 2026

Keywords

Biotic stress, gene family, pathogenesis-related protein 5, sugar beet, thaumatin-like proteins

ABSTRACT

Thaumatin-like proteins (TLPs), classified as pathogenesis-related protein 5 (PR-5), constitute an important component of plant defense systems. However, systematic information on the TLP family in sugar beet (Beta vulgaris) remains limited. In this study, a genome-wide investigation of TLP genes was conducted to clarify their genomic features, evolutionary relationships, and expression behavior under biotic stress. A total of 21 TLPs were identified in the sugar beet genome. These genes showed uneven chromosomal distribution and displayed marked variation in gene length, exon-intron organization, and protein physicochemical properties. Phylogenetic analysis of Arabidopsis PR-5 proteins classified the sugar beet members into multiple conserved groups, with consistent gene structure patterns within each group. Expression profiling under Sclerotinia sclerotiorum infection revealed that BvTLP09, BvTLP10, BvTLP12, BvTLP04, and BvTLP01 were strongly induced, whereas other PR-5 genes showed weak, unchanged, or reduced transcriptional responses. Reanalysis of RNA-seq data from beet cyst nematode infection further demonstrated distinct expression patterns between resistant and susceptible varieties at early and late infection stages, indicating dynamic and genotype-dependent regulation of PR-5 genes. This study expands current knowledge of the PR-5 gene family in sugar beet and provides a basis for future functional studies on their roles in pathogen-responsive pathways.

1. INTRODUCTION

Sugar beet (*Beta vulgaris*) is a major sugar-producing crop that provides a significant proportion of the global sucrose supply and supports food, feed, and bio-based industries (Wolfgang et al., 2023). The crop is widely cultivated in temperate regions due to its high yield potential and adaptability to diverse agroecological conditions (Hoffmann & Kenter, 2018). However, sugar beet production faces persistent challenges from biotic stresses, including fungal, bacterial, and viral pathogens, as well as insect pests (Yu et al., 2020). Diseases such as leaf spot, root rot, and viral yellows cause severe yield reduction and deterioration of sugar quality. These constraints limit production

stability and increase management costs (Liu et al., 2025). A deeper understanding of the molecular mechanisms governing plant defense responses is essential for developing disease-resistant sugar beet cultivars and for reducing reliance on chemical control strategies.

Pathogenesis-related protein 5 (PR-5), also known as thaumatin-like protein, belongs to a well-characterized group of plant defense proteins that play important roles in resistance against a wide range of pathogens (Vaghela et al., 2022). PR-5 proteins are typically induced in response to fungal, bacterial, and viral infections and accumulate in the apoplast, where they exert antifungal activity by disrupting pathogen cell wall integrity and inhibiting fungal growth (de Jesus-Pires et al., 2020; Stec, 2006). Previous studies have shown that PR-5 proteins participate in plant innate immune responses and are associated with both basal defence and induced resistance (Almaghrabi et al., 2019; Dos Santos & Franco, 2023). Nowadays, PR-5 proteins have been identified in several plant species, such as dwarf *Ammopiptanthus* (*Ammopiptanthus nanus*) (Liu et al., 2023), qingke (*Hordeum vulgare*) (Wang et al., 2022), wheat (*Triticum aestivum*) (Ren et al., 2022), New Zealand spinach (*Tetragonia tetragonioides*) (Huang et al., 2024), and cotton (*Gossypium barbadense*) (Zhang et al., 2021). Despite their functional importance, comprehensive information on the PR-5 gene family in sugar beet remains limited. The number of *PR-5* genes, their genomic organization, evolutionary relationships, and potential functional diversification in sugar beet have not been systematically investigated.

The present study was designed to systematically investigate the PR-5 gene family in sugar beet at the genome-wide level. The objectives were to identify *PR-5* genes and characterize their genomic distribution, gene structures, and physicochemical properties, as well as to elucidate their evolutionary relationships through comparative phylogenetic analysis with *Arabidopsis*. In addition, this study aimed to examine transcriptional responses of *PR-5* genes under biotic stress conditions by integrating quantitative expression analysis during *Sclerotinia sclerotiorum* infection with reanalysis of RNA-seq data from beet cyst nematode infection in resistant and susceptible varieties. Through these analyses, the study seeks to provide a detailed framework for understanding *PR-5* gene diversity and regulation in sugar beet and to facilitate further investigation of their biological roles in plant-pathogen interactions.

2. MATERIALS AND METHODS

2.1. Identification of PR-5 members in sugar beet

PR-5 proteins in sugar beet were screened with *Arabidopsis* as the reference set, as previously described (Irigoyen et al., 2020). First, verified Arabidopsis PR-5 protein sequences (Sels et al., 2008) were retrieved from TAIR (Garcia-Hernandez et al., 2002) and used as query sequences. Second, BLASTP was performed against the sugar beet predicted proteome from the reference genome assembly (Dohm et al., 2014), with an E-value cutoff of $1e^{-5}$, a minimum query coverage of 50%, and a minimum sequence identity of 30%. Third, candidate hits were merged, and redundant isoforms were removed by retaining the longest protein per locus. Fourth, all non-redundant candidates were validated for the presence of the thionin domain by searching against the Pfam database (Mistry et al., 2021), with sequences lacking the characteristic thionin-like conserved cysteine pattern excluded.

2.2. Characterization of PR-5 members in sugar beet

The physicochemical characteristics of the identified PR-5 members in sugar beet were analyzed using the ExPASy ProtParam tool (Gasteiger et al., 2003). For each PR-5 protein sequence, the total number of amino acids, molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, and grand average of hydropathicity (GRAVY) were calculated to assess basic biochemical properties, predicted stability, thermostability, and hydrophobicity. The instability index was used to predict protein stability under physiological conditions, while the aliphatic index provided an estimate of relative thermostability, and GRAVY values were used to infer hydrophilic or hydrophobic character.

2.3. Phylogeny analysis of PR-5 members in sugar beet

Phylogenetic analysis was conducted to investigate the evolutionary relationships among PR-5 members in sugar beet and *Arabidopsis* (Sels et al., 2008), the latter serving as the reference species. Full-length amino acid sequences of all identified BvTLP proteins and verified PR-5 protein sequences from *Arabidopsis* (Sels et al., 2008), were aligned using ClustalX (Larkin et al., 2007) with default parameters. A phylogenetic tree was generated using the Maximum Likelihood method

in MEGA software (Kumar et al., 2024). The Jones-Taylor-Thornton amino acid substitution model was selected, with a uniform substitution rate among sites and complete deletion of gaps and missing data. The phylogenetic relationships were assessed through bootstrap analysis with 1,000 replicates.

2.4. Gene structure of PR-5 members in sugar beet

The gene structure of *PR-5* genes in sugar beet was analyzed using the Gene Structure Display Server (Hu et al., 2015). The full-length genomic DNA sequences and corresponding coding sequences of each identified *PR-5* gene were retrieved from the sugar beet genome database (Dohm et al., 2014). Genomic and coding DNA sequences were uploaded to the platform to determine the number of exons, exon lengths, intron lengths, and overall gene structure.

2.5. Expression analysis of *PR-5* genes under *Sclerotinia sclerotiorum* treatment

Expression patterns of *PR-5* genes in sugar beet under *S. sclerotiorum* infection were analyzed based on a previously published transcriptomic dataset (GEO accession number: GSE138039) (Sucher et al., 2020) available from the GEO NCBI. Sugar beet leaf samples included three uninfected controls and three infected samples. RNA libraries were prepared using standard Illumina protocols and sequenced on the Illumina HiSeq 2500 platform. According to the original GEO processing pipeline, raw reads were base-called using Illumina software, trimmed to remove adaptors, low-complexity, and low-quality sequences, and mapped to the sugar beet reference genome using the RNA-seq analysis function of CLC Genomics Workbench. Gene-level read counts were generated using the same CLC pipeline. A count table containing counts for all genes for all samples (Sucher et al., 2020) has been downloaded. In the present study, the sugar beet count matrix was imported into DESeq2, and read counts were normalized using the median-of-ratios method. *PR-5* gene expression changes were calculated as fold changes between infected and uninfected samples.

2.6. Expression analysis of *PR-5* genes under *Heterodera schachtii* inoculation

Reanalysis of RNA-seq data for *PR-5* expression under beet cyst nematode infection was performed using the public dataset deposited in the GEO NCBI under accession GSE135555 (Ghaemi et al., 2020). The experiment comprised a susceptible sugar beet line (7112*SB36) and a resistant cultivar (Nemakill), with root tissues collected at 4 days after inoculation (4 DAI and 10 DAI) with *Heterodera schachtii*, as well as mock-inoculated controls at the same time points. Two biological replicates were available for each genotype, treatment, and time point. RNA libraries were prepared using the QuantSeq 3′ mRNA-Seq Library Prep Kit and sequenced on the Illumina NextSeq 500 platform to generate single-end 76 bp reads. Raw read quality was assessed using FastQC, and low-quality reads were trimmed using Trimmomatic. Trimmed reads were aligned to the RefBeet1.1 sugar beet reference genome using STAR, and gene-level counts were obtained using the summarizeOverlaps function in the GenomicAlignments R package. A count table containing counts for all genes for all samples (Ghaemi et al., 2020) has been downloaded. In the present study, the count matrix was analyzed using DESeq2, and read counts were normalized using the median-of-ratios method. *PR-5* gene expression changes were calculated as $\log_2$ fold changes between infected and corresponding mock-inoculated controls for each genotype and time point.

3. RESULTS AND DISCUSSION

3.1. Identification and physicochemical properties of PR-5 members in sugar beet

A genome-wide analysis of the sugar beet genome identified 21 *PR-5* genes, which were designated *BvTLP01* to *BvTLP21* based on their chromosomal positions and locus order in the sugar beet genome annotation (Table 1). Each gene corresponded to a unique locus, ranging from Bevul.1G023900 to Bevul.9G138900. The *PR-5* genes were distributed across several chromosomes, with a notable concentration on chromosome 5, which contained six adjacent loci (*BvTLP09* to *BvTLP14*). Other *PR-5* genes occurred as single-copy loci on different chromosomes.

The lengths of *PR-5* genes varied substantially, ranging from 669 bp (*BvTLP09*) to 18,766 bp (*BvTLP19*). This variation exhibited differences in intron number and intron size rather than major changes in coding regions. The predicted protein lengths ranged from 210 amino acids (BvTLP13) to 679 amino acids (BvTLP19). Most PR-5 proteins fell within a typical size range of 220 to 350 amino acids, which is consistent with previously reported plant PR-5 proteins. BvTLP07 and BvTLP19 were larger proteins, suggesting the presence of extended domains that may support additional biological functions. Physicochemical analysis revealed that

the predicted molecular weights of PR-5 proteins ranged from 22.81 kDa to 74.23 kDa, in agreement with their protein lengths. The theoretical isoelectric point values ranged from 4.23 to 8.73, which indicates the presence of both acidic and basic proteins within the family. Several PR-5 members showed pI values below 6.0, while others exhibited neutral-to-alkaline pI values. The aliphatic index values of PR-5 proteins ranged from 53.65 to 88.03, which indicates moderate to high thermostability across the gene family. BvTLP19 showed the highest aliphatic index, which aligns with its large protein size and predicted structural stability. The GRAVY values ranged from -0.44 to 0.13, with most PR-5 proteins showing negative values (Table 1). This pattern demonstrated an overall hydrophilic character, which is consistent with the extracellular localization and antimicrobial function of thionin-like proteins. A small number of PR-5 proteins displayed slightly positive GRAVY values, which suggests limited hydrophobic regions that may contribute to interactions with pathogen membranes.

The number and characteristics of *PR-5* genes vary widely among plant species, which highlights lineage-specific expansion and functional diversification of this gene family. In the present study, 21 *PR-5* genes were identified in sugar beet, representing a moderate family size compared with other plants. This number is lower than that reported in polyploid or large-genome species such as wheat, which contains 129 *TaTLP* genes, largely due to whole-genome duplication and extensive segmental duplication events (Ren et al., 2022). In contrast, diploid or less duplicated genomes tend to harbor fewer PR-5 members, as observed in *T. tetragonoides* with 37 *TLP* genes (Huang et al., 2024), qingke with approximately 40 members (Wang et al., 2022), and cotton (Zhang et al., 2021), which contains an expanded TLP family associated with stress adaptation and disease resistance. Shrub and woody species adapted to harsh environments, such as *A. nanus*, also possess a relatively expanded PR-5 family, which has been linked to enhanced tolerance to cold and environmental stress (Liu et al., 2023). Despite differences in gene number, PR-5 proteins across plant species share conserved structural features, including the thaumatin domain, multiple conserved cysteine residues involved in disulfide bond formation, and predominantly hydrophilic properties, which support their antifungal and stress-related functions. Compared with species showing large PR-5 expansions, the moderate size of the sugar beet PR-5 family suggests a balance between conservation and diversification, which may suggest adaptation to its specific pathogen spectrum and environmental conditions rather than extensive genome duplication.

3.2. Classification of PR-5 members in sugar beet

Phylogenetic analysis based on the Maximum Likelihood method classified PR-5 members from sugar beet and *Arabidopsis* into eleven distinct groups (Groups I-XI), which were supported by moderate to high bootstrap values (Figure 1). Each group contained both PR-5 members from sugar beet and *Arabidopsis*, indicating conserved evolutionary relationships between sugar beet and *Arabidopsis* PR-5 proteins. This clustering pattern suggests that the major PR-5 lineages were established before the divergence of these two dicot species and were maintained during subsequent evolution.

Group I comprised AtTLP1, AtTLP5, and BvTLP08 and formed a well-supported clade. Group II consisted of BvTLP06, BvTLP14, and AtTLP22, which showed close evolutionary relationships between sugar beet and *Arabidopsis* members. Group III included BvTLP05, BvTLP18, AtTLP25, and AtTLP13 and showed high bootstrap support, suggesting functional conservation within this lineage. Group IV contained BvTLP19, AtTLP15, AtTLP24, and AtTLP4 and represented a conserved subgroup with limited gene expansion in sugar beet. Group V included BvTLP09, BvTLP10, BvTLP11, BvTLP12, BvTLP13, and AtTLP14, forming one of the largest sugar beet-expanded clades. Group VI comprised BvTLP01, AtTLP3, AtTLP9, AtTLP11, AtTLP19, and AtTLP21 and formed a distinct clade with strong support. Group VII included BvTLP02, BvTLP16, BvTLP20, AtTLP17, AtTLP18, and AtTLP20 and showed shared ancestry with limited lineage-specific expansion. Group VIII consisted of BvTLP07 and AtTLP23 and showed a clear one-to-one orthologous relationship. Group IX included AtTLP6, AtTLP7, AtTLP8, AtTLP15, and BvTLP15, forming a separate group with high bootstrap values. Group X contained BvTLP03 and AtTLP10 and formed a compact, well-supported clade. Group XI comprised BvTLP04 and BvTLP17, which formed a distinct lineage that may represent sugar beet-specific diversification.

Comparative analyses across plant species indicate that *PR-5* genes are consistently classified into

multiple phylogenetic groups that are largely conserved among angiosperms, despite substantial variation in gene family size. Studies in *Arabidopsis*, wheat, cotton, qingke, *T. tetragonoides*, and *A. nanus* have shown that PR-5 proteins cluster into a limited number of major groups, typically ranging from seven to ten clades, based on full-length protein sequences and conserved domains (Huang et al., 2024; Liu et al., 2023; Ren et al., 2022; Wang et al., 2022; Zhang et al., 2021). These groups often contain PR-5 members from both monocot and dicot species, which indicates that the main PR-5 lineages were established before the monocot-dicot divergence and have been maintained through evolution (Shatters et al., 2006). Functional studies in several plants further suggest that different phylogenetic groups may be preferentially associated with distinct biological roles, such as antifungal defense, abiotic stress tolerance, or developmental regulation (Mi et al., 2025; Zhu & Gao, 2025). The conserved multi-group classification of PR-5 proteins across plant species reveals a shared evolutionary origin, while lineage-specific expansion within individual groups provides a molecular basis for functional diversification and adaptive responses to diverse environmental and pathogenic pressures.

3.3. Exon/intron organization analysis of *PR-5* members in sugar beet

Exon-intron structure analysis of the 21 *PR-5* genes revealed clear structural patterns that aligned with their phylogenetic classification. The number of exons ranged from 1 to 10, indicating marked structural diversity within the PR-5 gene family (Figure 2). Genes within the same phylogenetic groups generally had similar exon numbers, supporting the evolutionary relationships inferred from the phylogenetic tree.

Particularly, six genes, *BvTLP09*, *BvTLP10*, *BvTLP11*, *BvTLP12*, *BvTLP13*, and *BvTLP18*, contained a single exon. These genes clustered mainly within the same phylogenetic group, which indicates strong structural conservation among closely related members. The compact structure of these genes suggests intron loss after gene duplication events within this lineage.

Five genes, *BvTLP02*, *BvTLP05*, *BvTLP06*, *BvTLP14*, and *BvTLP15*, contained two exons. These genes occupied adjacent positions in the phylogenetic tree and showed similar branch lengths, which indicates shared ancestry and conserved gene architecture. The presence of one intron in these genes suggests moderate structural complexity that remained stable during evolution.

Next, nine genes, *BvTLP01*, *BvTLP03*, *BvTLP04*, *BvTLP07*, *BvTLP08*, *BvTLP16*, *BvTLP17*, *BvTLP20*, and *BvTLP21*, consisted of three exons. These genes were distributed across several phylogenetic groups but formed small subclusters within each group. The consistent exon number among these members supports conservation of gene structure within each evolutionary lineage, despite variation in intron length and total gene size. Finally, *BvTLP19* showed a unique gene structure with ten exons and occupied a distinct position in the phylogenetic tree. This gene formed a separate branch from most other PR-5 members, which indicates early divergence and extensive structural expansion. The complex exon-intron organization of *BvTLP19* suggests multiple intron insertion events during its evolutionary history.

3.4. Expression patterns of *PR-5* genes under Sclerotinia *sclerotiorum* treatment

Expression analysis of *PR-5* genes in sugar beet under *S. sclerotiorum* infection revealed clear differences in transcriptional responses among family members (Table 2). Genes with fold-change values ≥ 2 were defined as up-regulated, whereas genes with fold-change values ≤ -2 were defined as down-regulated, as previously reported (Dalman et al., 2012).

Based on these criteria, several *PR-5* genes showed strong transcriptional induction in response to pathogen treatment. In particular, *BvTLP09* and *BvTLP10* were the most highly upregulated genes, with fold-change values of approximately 9,074.71-fold and 6,796.85-fold, respectively. *BvTLP12* also showed pronounced up-regulation with a fold change of *2,366.20*-fold, while *BvTLP04* exhibited significant induction with a fold change of 62.65-fold (Table 2). *BvTLP01* met the up-regulation threshold with a fold change of 3.65, which indicates activation in response to fungal infection. These genes represent major pathogen-responsive members of the PR-5 family. In contrast, *BvTLP03* and *BvTLP21* showed fold-change values of 0.46 and 0.53-fold, respectively. Among these, *BvTLP03* met the criterion for down-regulation, while *BvTLP21* showed a decreasing trend but did not reach the defined threshold. *BvTLP18* showed strong transcriptional repression, with a fold-change of 0.06, indicating marked down-regulation under *S. sclerotiorum* treatment. The remaining *PR-5* genes, including *BvTLP02*, *BvTLP05*, *BvTLP06*, *BvTLP07*,

BvTLP08, *BvTLP11*, *BvTLP13*, *BvTLP14*, *BvTLP15*, *BvTLP17*, *BvTLP19*, and *BvTLP20*, showed absolute values are below the threshold. These genes were classified as non-responsive under the tested conditions. Our study suggested that only a subset of *PR-5* genes shows strong transcriptional activation or repression during *S. sclerotiorum* infection. The highly induced genes, particularly *BvTLP09*, *BvTLP10*, *BvTLP12*, *BvTLP04*, and *BvTLP01*, are likely to play key roles in sugar beet defense responses, while the majority of *PR-5* members remain transcriptionally stable or weakly responsive.

PR-5 proteins are commonly associated with antifungal activity through effects on fungal membranes, β-1,3-glucans, or fungal enzymes, so these induced genes may contribute to local defense responses during fungal colonization. Since *S. sclerotiorum* is a necrotrophic pathogen, this induction should not be interpreted as direct evidence of resistance. These genes may instead participate in a wider stress response that includes antifungal protein accumulation, cell wall-associated defense, and regulation of tissue damage during infection. Additionally, the chromosome 5 cluster containing *BvTLP09* to *BvTLP14* is noteworthy, as it may represent a lineage-specific expansion of *PR-5* genes in sugar beet. Similar clustered distributions of *TLP* genes in cowpea and garlic have been linked to tandem duplication, gene family expansion, and possible functional redundancy or divergence among paralogs (Anisimova et al., 2021; de Jesús-Pires et al., 2024). In this study, *BvTLP09*, *BvTLP10*, *BvTLP11*, *BvTLP12*, and *BvTLP13* also belong to the same phylogenetic group and share compact single-exon structures, which supports a close evolutionary relationship among these clustered genes. The strong induction of *BvTLP09*, *BvTLP10*, and *BvTLP12*, and the weak responses of *BvTLP11*, *BvTLP13*, and *BvTLP14* suggest that this cluster may have undergone regulatory divergence after duplication. Some members may have retained pathogen-responsive functions, while others may have acquired different roles or may respond to other stresses, tissues, or infection stages. The chromosome 5 cluster should be considered a priority region for future promoter and duplication analyses, as well as functional assays, to clarify whether these genes act redundantly, cooperatively, or through sub-functionalized roles in sugar beet defense.

3.5. Expression patterns of *PR-5* genes under beet cyst nematode infection

Reanalysis of RNA-seq data revealed diverse transcriptional responses of *PR-5* genes in sugar beet roots following beet cyst nematode infection in susceptible and resistant varieties at 4 and 10 DAI (Figure 3).

At 4 DAI, transcriptional responses were limited in the susceptible variety. *BvTLP04* showed clear down-regulation with a fold change of -2.97, while *BvTLP10* also showed down-regulation (-2.63-fold). *BvTLP11* was the only gene that met the up-regulation threshold in the susceptible line at this stage, with a fold change of 2.95. In the resistant variety at 4 DAI, *BvTLP03* (-2.67-fold), *BvTLP14* (-2.08-fold), *BvTLP20* (-2.90-fold), and *BvTLP21* (-2.20-fold) were down-regulated, whereas *BvTLP06* was up-regulated with a fold change of 3.00. Other *PR-5* genes remained below the defined thresholds or showed no detectable expression.

At 10 DAI, stronger transcriptional responses were observed, particularly in the susceptible variety. *BvTLP06* showed pronounced down-regulation (-8.44-fold), while *BvTLP16* exhibited strong up-regulation (5.68-fold). *BvTLP11* also showed clear induction (3.16-fold) at this stage. In the resistant variety at 10 DAI, *BvTLP11* showed strong down-regulation (-4.03-fold), whereas *BvTLP15* (2.48-fold), *BvTLP16* (2.07-fold), and *BvTLP20* (2.25-fold) were up-regulated. The remaining *PR-5* genes showed fold change values within the non-responsive range or lacked detectable expression.

We found that *PR-5* gene expression under beet cyst nematode infection showed clear genotype- and time-dependent patterns. Early responses at 4 DAI were limited and primarily involved repression, whereas later responses at 10 DAI showed stronger, more divergent regulation between susceptible and resistant varieties. Genes such as *BvTLP06*, *BvTLP11*, *BvTLP15*, *BvTLP16*, and *BvTLP20* displayed consistent regulation that differed between genotypes or time points, which suggested potential roles in nematode-related defense responses and host resistance mechanisms.

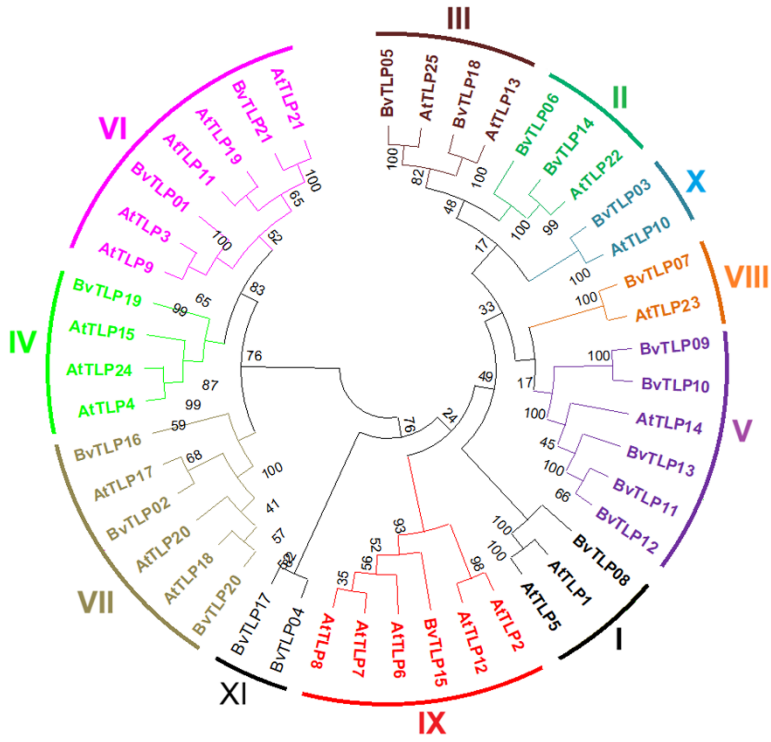

Figure 1. Classification of PR-5 proteins in *Arabidopsis* and sugar beet

The tree was constructed using full-length amino acid sequences with the Maximum Likelihood method. Sugar beet proteins are designated as BvTLPs, and Arabidopsis proteins were included as reference sequences. The proteins were classified into eleven groups (Groups I-XI), and bootstrap support values are indicated at the corresponding nodes.

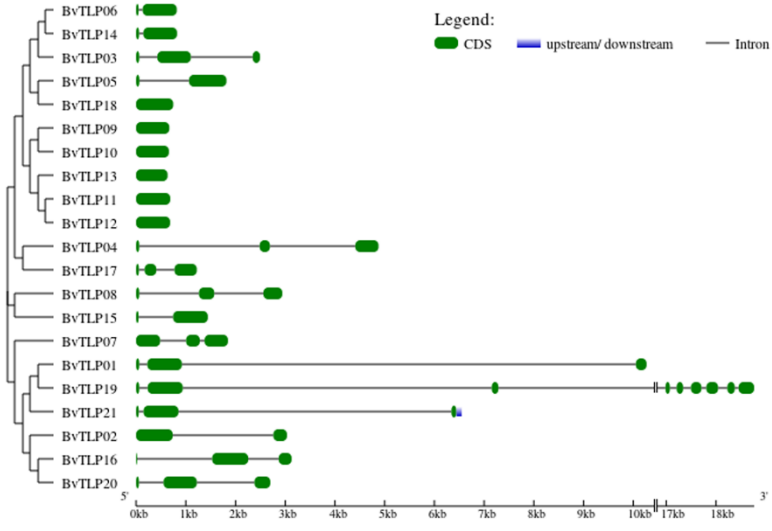

Figure 2. Gene structure of PR-5 members in sugar beet

Exons and introns are shown according to the genomic and coding sequences of each BvTLP gene. The figure illustrates variation in gene structure among members of the PR-5 family, including differences in exon number, intron number, and overall gene length.

Table 1. Characteristics of PR-5 proteins in sugar beet

Gene name	Locus name	Gene length	Protein length	Molecular weight	pI	Aliphatic index	GRAVY
BvTLP01	Bevul.1G023900	10267	323	33.75	4.43	68.61	-0.03
BvTLP02	Bevul.1G024200	3035	336	35.22	4.36	61.01	-0.07
BvTLP03	Bevul.1G098900	2493	294	31.87	8.21	74.97	0.03
BvTLP04	Bevul.1G143200	4876	245	26.09	4.51	64.49	-0.05
BvTLP05	Bevul.2G054100	1817	272	29.84	7.39	81.40	-0.09
BvTLP06	Bevul.2G228700	818	245	26.41	8.56	78.73	0.13
BvTLP07	Bevul.3G022000	1847	408	42.35	6.10	66.00	-0.02
BvTLP08	Bevul.3G185600	2940	249	26.36	8.73	72.57	0.09
BvTLP09	Bevul.5G032900	669	222	23.67	8.18	53.65	-0.44
BvTLP10	Bevul.5G033000	660	219	22.95	4.39	54.84	-0.24
BvTLP11	Bevul.5G033200	687	228	24.05	6.76	56.49	-0.08
BvTLP12	Bevul.5G033300	684	227	24.07	7.82	59.25	-0.10
BvTLP13	Bevul.5G033400	633	210	22.81	8.44	54.90	-0.24
BvTLP14	Bevul.5G137600	824	245	26.52	7.78	69.18	-0.17
BvTLP15	Bevul.5G160900	1443	249	25.77	4.23	59.92	-0.02
BvTLP16	Bevul.6G034400	3128	334	34.49	4.89	57.57	-0.11
BvTLP17	Bevul.7G046200	1221	246	26.52	8.09	65.45	-0.07
BvTLP18	Bevul.7G209100	747	248	26.82	8.33	76.33	0.03
BvTLP19	Bevul.8G070600	18766	679	74.23	5.91	88.03	-0.14
BvTLP20	Bevul.9G138800	2700	348	35.37	4.33	61.15	0.07
BvTLP21	Bevul.9G138900	6548	287	30.40	8.66	73.10	0.04

Table 2. Expression profiles of *PR-5* genes under *Sclerotinia sclerotiorum* treatment

Gene	Control		Sclerotinia sclerotiorum infected		Fold changes (*Sclerotinia sclerotiorum* infected/Control)
	Mean	SD	Mean	SD	
BvTLP01	1.981	0.543	7.223	1.920	3.65
BvTLP02	0.006	0.004	0.000	0.000	0.00
BvTLP03	0.026	0.022	0.012	0.017	0.46
BvTLP04	0.668	0.479	41.877	21.469	62.65
BvTLP05	0.002	0.003	0.000	0.000	0.00
BvTLP06	0.113	0.037	0.189	0.136	1.67
BvTLP07	0.024	0.015	0.024	0.017	1.01
BvTLP08	0.021	0.008	0.000	0.000	0.00
BvTLP09	0.126	0.064	1140.685	200.981	9074.71
BvTLP10	0.033	0.044	225.020	212.487	6796.85
BvTLP11	0.001	0.002	0.000	0.000	0.00
BvTLP12	0.005	0.006	12.969	5.140	2366.20
BvTLP13	0.007	0.003	0.000	0.000	0.00
BvTLP14	0.038	0.030	0.000	0.000	0.00
BvTLP15	0.002	0.002	0.000	0.000	0.00
BvTLP17	0.000	0.000	0.000	0.000	nd
BvTLP18	1.537	0.472	0.092	0.087	0.06
BvTLP19	0.666	0.417	0.844	0.093	1.27
BvTLP20	0.042	0.030	0.000	0.000	0.00
BvTLP21	0.463	0.141	0.245	0.017	0.53

Fold-change values represent the relative expression of BvTLP genes in infected samples compared with the corresponding control samples. The figure highlights differential transcriptional responses among PR-5 family members in response to fungal pathogen treatment.

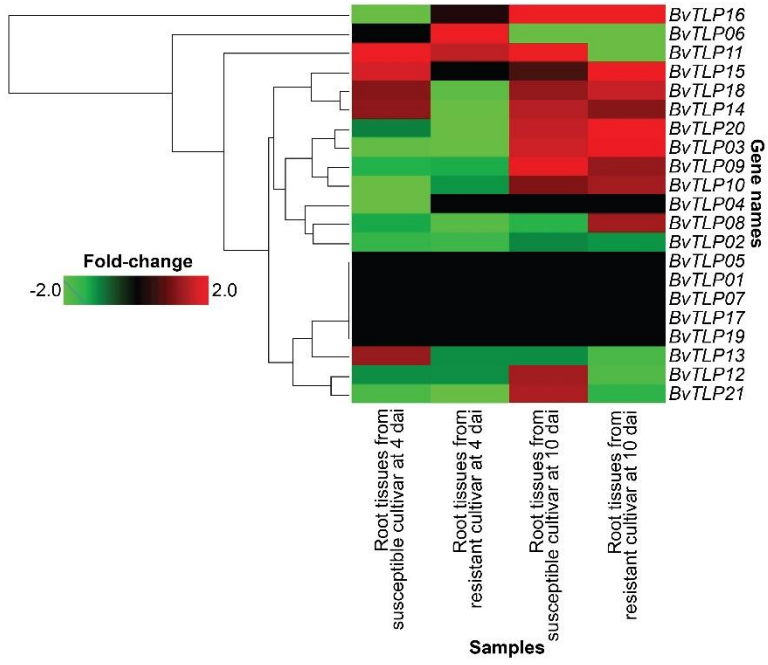

Figure 3. Expression profiles of *PR-5* genes under beet cyst nematode infection

Fold-change values show the relative expression of BvTLP genes in infected roots compared with mock-inoculated controls in susceptible and resistant sugar beet genotypes at 4 and 10 days after inoculation. The figure illustrates genotype- and time-dependent transcriptional responses of PR-5 genes during beet cyst nematode infection.

4. CONCLUSION

This study provides a comprehensive genome-wide characterization of the PR-5 gene family in sugar beet, including their identification, structural features, phylogenetic relationships, and expression profiles under biotic stress conditions. Twenty-one *PR-5* genes were identified and classified into distinct evolutionary groups that showed strong concordance between phylogenetic relationships and exon–intron organization, thereby supporting structural and evolutionary conservation within lineages. Physicochemical analysis revealed considerable diversity in protein size, isoelectric point, and hydrophobicity, suggesting functional differentiation among family members. Expression analyses demonstrated that *PR-5* genes exhibit highly divergent and stress-specific transcriptional responses. Several genes showed strong induction during *S. sclerotiorum* infection, while others remained unresponsive or were transcriptionally repressed. Reanalysis of RNA-seq data under beet cyst nematode infection further revealed genotype- and time-dependent expression patterns between resistant and susceptible varieties at early and late infection stages. Notably, five genes (including *BvTLP09*, *BvTLP10*, *BvTLP12*, *BvTLP04*, and *BvTLP01*, respectively) were strongly upregulated, whereas other remaining *BvPR-5* genes showed weak upregulation, unchanged, or downregulation under pathogen infection, which highlights their potential roles in sugar beet defense and resistance mechanisms. Taken together, these findings provide a useful basis for future functional studies aimed at clarifying the biological roles of *PR-5* genes in pathogen-responsive pathways. Although the identified *PR-5* genes may not be direct targets for resistance breeding, they provide informative molecular components for understanding defense regulation in sugar beet.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Almaghrabi, B., Ali, M. A., Zahoor, A., Shah, K. H., & Bohlmann, H. (2019). Arabidopsis thionin-like genes are involved in resistance against the beet-cyst nematode (*Heterodera schachtii*). *Plant Physiology and Biochemistry*, *140*(1), 55-67. https://doi.org/10.1016/j.plaphy.2019.05.005

Anisimova, O. K., Shchennikova, A. V., Kochieva, E. Z., & Filyushin, M. A. (2021) Pathogenesis-related genes of PR1, PR2, PR4, and PR5 families are involved in the Response to Fusarium Infection in garlic (*Allium sativum* L.). *International Journal of Molecular Sciences*, *22*(13), 6688. https://doi.org/10.3390/ijms22136688

Dalman, M. R., Deeter, A., Nimishakavi, G., & Duan, Z. H. (2012). Fold change and p-value cutoffs significantly alter microarray interpretations. *BMC Bioinformatics*, *13*(Suppl 2), S11. https://doi.org/10.1186/1471-2105-13-S2-S11

de Jesus-Pires, C., Ferreira-Neto, J. R. C., Pacifico Bezerra-Neto, J., Kido, E. A., de Oliveira Silva, R. L., Pandolfi, V., … & Benko-Iseppon, A. M. (2020). Plant thaumatin-like proteins: Function, evolution and biotechnological applications. *Current Protein & Peptide Science*, *21*(1), 36-51. https://doi.org/10.2174/1389203720666190318164905

de Jesús-Pires, C., Ferreira-Neto, J. R. C., Oliveira-Silva, R. L., Silva, J. B. D., Silva, M. D. D., Costa, A. F. D., & Benko-Iseppon, A. M. (2024) Genome-wide identification and stress responses of cowpea thaumatin-like proteins: A comprehensive analysis. *Plants (Basel)*, *13*(22), 3245. https://doi.org/10.3390/plants13223245.

Dohm, J. C., Minoche, A. E., Holtgrawe, D., Capella-Gutierrez, S., Zakrzewski, F., … & Himmelbauer, H. (2014). The genome of the recently domesticated crop plant sugar beet (*Beta vulgaris*). *Nature*, *505*(7484), 546-549. https://doi.org/10.1038/nature12817

Dos Santos, C., & Franco, O. L. (2023). Pathogenesis-related proteins (PRs) with enzyme activity activating plant defense responses. *Plants*, *12*(11), 2226. https://doi.org/10.3390/plants12112226

Garcia-Hernandez, M., Berardini, T. Z., Chen, G., Crist, D., Doyle, A., Huala, E., … & Zhang, P. (2002). TAIR: a resource for integrated *Arabidopsis* data. *Functional & Integrative Genomics*, *2*(6), 239-253. https://doi.org/10.1007/s10142-002-0077-z

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D., & Bairoch, A. (2003). ExPASy: The proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research*, *31*(13), 3784-3788. https://doi.org/10.1093/nar/gkg563

Ghaemi, R., Pourjam, E., Safaie, N., Verstraeten, B., Mahmoudi, S. B., Mehrabi, R., De Meyer, T., & Kyndt, T. (2020). Molecular insights into the compatible and incompatible interactions between sugar beet and the beet cyst nematode. *BMC Plant Biology*, *20*(1), 483. https://doi.org/10.1186/s12870-020-02706-8

Hoffmann, C. M., & Kenter, C. (2018). Yield potential of sugar beet - Have we hit the ceiling? *Frontiers in Plant Science*, *9*(1), 289. https://doi.org/10.3389/fpls.2018.00289

Hu, B., Jin, J., Guo, A. Y., Zhang, H., Luo, J., & Gao, G. (2015). GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics*, *31*(8), 1296-1297. https://doi.org/10.1093/bioinformatics/btu817

Huang, Z., Ding, Q., Wang, Z., Jian, S., & Zhang, M. (2024). Genome-wide identification and expression analyses of the thaumatin-like protein gene family in *Tetragonia tetragonoides* (Pall.) Kuntze reveal their functions in abiotic stress responses. *Plants*, *13*(17), 2355. https://doi.org/10.3390/plants13172355

Irigoyen, M. L., Garceau, D. C., Bohorquez-Chaux, A., Lopez-Lavalle, L. A. B., Perez-Fons, L., Fraser, P. D., & Walling, L. L. (2020). Genome-wide analyses of cassava Pathogenesis-related (PR) gene families reveal core transcriptome responses to whitefly infestation, salicylic acid and jasmonic acid. *BMC Genomics*, *21*(1), 93. https://doi.org/10.1186/s12864-019-6443-1

Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). MEGA12: Molecular Evolutionary Genetic Analysis version 12 for adaptive and green computing. *Molecular Biology and Evolution*, *41*(12), msae263. https://doi.org/10.1093/molbev/msae263

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., … & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, *23*(21), 2947-2948. https://doi.org/10.1093/bioinformatics/btm404

Liu, G., Tang, Y., Wang, H., Yu, S., Gao, H., Wang, Y., & Zhang, D. (2025). Advances in beet (*Beta vulgaris* L.) stress adaptation: Focus on transcription factors and major stress-related genes. *Plants*, *15*(1), 12. https://doi.org/10.3390/plants15010012

Liu, Q., Sui, X., Wang, Y., Zhu, M., Zhou, Y., & Gao, F. (2023). Genome-Wide Analyses of Thaumatin-like Protein Family Genes Reveal the Involvement in the Response to Low-Temperature Stress in *Ammopiptanthus nanus*. *International Journal of Molecular Sciences*, *24*(3), 2209. https://doi.org/10.3390/ijms24032209

Mi, X., Tang, M., Yang, C., Liang, S., Chen, Y., Qiao, D., & Chen, Z. (2025). Analysis of thaumatin-like protein (TLP) gene family in tea plant revealed that CsTLP8 contributes to tea blister blight resistance. *BMC Plant Biology*, *25*(1), 1014. https://doi.org/10.1186/s12870-025-06996-8

Mistry, J., Chuguransky, S., Williams, L., Qureshi, M., Salazar, G. A., Sonnhammer, E. L. L., … & Bateman, A. (2021). Pfam: The protein families database in 2021. *Nucleic Acids Research*, *49*(D1), D412-D419. https://doi.org/10.1093/nar/gkaa913

Ren, R., Zhou, X., Zhang, X., Li, X., Zhang, P., & He, Y. (2022). Genome-wide identification and characterization of thaumatin-like protein family genes in wheat and analysis of their responses to *Fusarium* head blight infection. *Food Production, Processing and Nutrition*, *4*(1), 24. https://doi.org/10.1186/s43014-022-00105-0

Sels, J., Mathys, J., De Coninck, B. M., Cammue, B. P., & De Bolle, M. F. (2008). Plant pathogenesis-related (PR) proteins: a focus on PR peptides. *Plant Physiology and Biochemistry*, *46*(11), 941-950. https://doi.org/10.1016/j.plaphy.2008.06.011

Shatters, R. G., Jr., Boykin, L. M., Lapointe, S. L., Hunter, W. B., & Weathersbee, A. A., 3rd. (2006). Phylogenetic and structural relationships of the PR5 gene family reveal an ancient multigene family conserved in plants and select animal taxa. *Journal of Molecular Evolution*, *63*(1), 12-29. https://doi.org/10.1007/s00239-005-0053-z

Stec, B. (2006). Plant thionins-the structural perspective. *Cellular and Molecular Life Sciences*, *63*(12), 1370-1385. https://doi.org/10.1007/s00018-005-5574-5

Sucher, J., Mbengue, M., Dresen, A., Barascud, M., Didelon, M., Barbacci, A., & Raffaele, S. (2020). Phylotranscriptomics of the pentapetalae reveals frequent regulatory variation in plant local responses to the fungal pathogen *Sclerotinia sclerotiorum*. *Plant Cell*, *32*(6), 1820-1844. https://doi.org/10.1105/tpc.19.00806

Vaghela, B., Vashi, R., Rajput, K., & Joshi, R. (2022). Plant chitinases and their role in plant defense: A comprehensive review. *Enzyme and Microbial Technology*, *159*, 110055. https://doi.org/10.1016/j.enzmictec.2022.110055

Wang, L., Xu, Z., Yin, W., Xu, K., Wang, S., Shang, Q., Sa, W., Liang, J., & Wang, L. (2022). Genome-wide analysis of the Thaumatin-like gene family in Qingke (*Hordeum vulgare* L. var. *nudum*) uncovers candidates involved in plant defense against biotic and abiotic stresses. *Frontiers in Plant Science*, *13*, 912296. https://doi.org/10.3389/fpls.2022.912296

Wolfgang, A., Temme, N., Tilcher, R., & Berg, G. (2023). Understanding the sugar beet holobiont for sustainable agriculture. *Frontiers in Microbiology*, *14*, 1151052. https://doi.org/10.3389/fmicb.2023.1151052

Yu, B., Chen, M., Grin, I., & Ma, C. (2020). Mechanisms of sugar beet response to biotic and abiotic stresses. *Advances in Experimental Medicine and Biology*, *1241*, 167-194. https://doi.org/10.1007/978-3-030-41283-8_10

Zhang, Y., Chen, W., Sang, X., Wang, T., Gong, H., Zhao, Y., Zhao, P., & Wang, H. (2021). Genome-wide identification of the thaumatin-like protein family genes in *Gossypium barbadense* and analysis of their responses to *Verticillium dahliae* infection. *Plants (Basel)*, *10*(12), 2647. https://doi.org/10.3390/plants10122647

Zhu, Y., & Gao, F. (2025). Involvement of pathogenesis-related proteins and their roles in abiotic stress responses in plants. *Biomolecules*, *15*(8), 1103. https://doi.org/10.3390/biom15081103