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PATHOGENESIS-RELATED PROTEINS: identification, evolution and functional analysis in pear (*Pyrus bretschneideri*)

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Abstract

Pathogenesis-related proteins (PRs) are essential for plant defense against both biotic and abiotic stresses. However, knowledge regarding PRs in pears remains limited. In this study, 637 PRs, classified into 16 families, were identified in *Pyrus bretschneideri*. Phylogenetic, sequence similarity, and secondary structure analyses enabled the classification of several PRs with conserved sequences, including PR-11, PR-8, PR-15, and PR-16. Genomic analysis revealed that the expansion of most PR families resulted from recent whole-genome duplication (WGD) events, occurring approximately 30–45 million years ago (Mya). Various gene duplication mechanisms have collectively contributed to the proliferation of PR families, with purifying selection indicated by the low Ka/Ks ratios. Examination of apoplastic fluid composition showed that only a subset of PRs was present in the apoplast. Transcriptomic data from *Pyrus* infected with *Erwinia amylovora* and *Colletotrichum fructicola* revealed the expression profiles of PR genes post-infection, underscoring the complexity of PR regulation. This study elucidates the evolutionary expansion, regulation, and functional roles of PRs in disease resistance, highlighting certain PRs as reliable markers of pear disease resistance. These findings offer valuable insights for future functional analyses and breeding strategies to enhance pear resistance to pathogens.

Keywords Pathogenesis-related proteins, Pear, Classification, Evolution, Transcriptome sequencing, Biotic stress

Introduction

Plants employ a range of mechanisms to defend against stress factors, including various stress proteins that play protective roles (Fritig et al. 1998). Pathogenesis-related proteins (PRs) are plant-encoded proteins critical for pathogen defense and stress adaptation (Van Loon et al. 2006). They function as a defense system by forming barriers, producing antimicrobial compounds, and synthesizing defense-related proteins (Edreva 2005; Van Loon et al. 2006; Zribi et al. 2021).

Initially identified 50 years ago in tobacco plants responding to tobacco mosaic virus, PRs have since been extensively studied across diverse plant species (Van Loon et al. 1970). These proteins are typically induced by both biotic and abiotic stress factors (Van Loon et al. 1985). To standardize their classification, the term

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"pathogenesis-related proteins" was coined in 1980 to refer to "proteins coded for by the host plant but induced exclusively under pathological or related conditions" (Antoniw et al. 1980). According to this definition, any host protein activated by an infectious agent or a similar condition is considered a PR. Using molecular and genetic techniques, the 10 primary acidic PRs of tobacco were initially categorized into five families (PR-1 to PR-5) based on their decreasing electrophoretic mobility (Bol et al. 1990). In 1994, a unified nomenclature was established to include additional classes with diverse properties, grouping PRs into families based on shared amino acid sequences, serological relationships, and enzymatic or biological functions (Van Loon et al. 1994).

A total of 19 PR families (PR-1 to PR-19) have been identified, of which 17 are confirmed and 2 are putative, classified based on their biochemical properties (Van Loon et al. 2006; Islam et al. 2023). PR-1, the first identified PR family, is highly conserved across plants, making it a molecular marker for plant defense responses (Van Loon et al. 1970; Zribi et al. 2021). Despite extensive research, the precise functions of PR-1 proteins remain unclear. In both tomato and tobacco, PR-1 has been shown to inhibit *Phytophthora infestans* by reducing sporangial germination and germ-tube elongation (Niderman et al. 1995). In vivo experiments further demonstrated that tomato PR-1 possesses antifungal activity against *P. infestans*, suppressing spore germination and reducing leaf infection areas. PR-2, PR-3, PR-4, PR-8, and PR-11 are members of the glycoside hydrolase (GH) family. PR-2, known as β -1,3-glucanase (β Glu), belongs to the GH17 family and catalyzes the hydrolytic cleavage of 1,3- β -D-glucosidic bonds in fungal cell wall β -1,3-glucans (Filichkin et al. 2004; Shi et al. 2006). PR-3, PR-4, PR-8, and PR-11 are classified as chitinases, exhibiting the ability to hydrolyze β -1,4 bonds connecting N-acetylglucosamine units in chitin. Multiple nomenclatures have been used for these proteins, including GHs, chitinase classes, PRs, and gene families. To eliminate ambiguity, this study adopted gene family classification. PR-3 is classified in the GH19 family, PR-4 in the GH45 (Barwin) family, and PR-8 and PR-11 in the GH18 family (Gomez et al. 2002; Hong et al. 2002; Bravo et al. 2003; Guevara-Morato et al. 2010). Despite both being members of the GH18 family, PR-8 and PR-11 exhibit significant sequence divergence, with only two adjacent β -strands sharing conserved elements, including catalytic residues (Bravo et al. 2003). In addition to their roles in weakening and degrading fungal cell walls, PRs contribute to other plant-microbe interactions. PR-7, PR-9, and PR-10 display protease, peroxidase, and ribonuclease activities, respectively (Vera and Conejero 1988; Lo et al. 1999; Passardi et al. 2005; Sinha et al. 2020). PR-6 and

PR-14 exhibit protease-inhibitory properties. Most PRs contain an N-terminal signal peptide (SP), enabling their secretion into the apoplast (Doares et al. 1995; Douliez et al. 2001). These proteins accumulate in the extracellular space and can be easily harvested from apoplastic fluid (intercellular washing fluid), with the exception of PR-10, which is not secreted outside the cell (Van Loon et al. 2006).

The recently identified putative PR families, PR-18 and PR-19, are classified based on distinct biochemical characteristics. PR-18 was initially found in the leaf extracts of *Lactuca sativa* and *Helianthus annuus* treated with salicylic acid (SA). These are carbohydrate oxidases—specifically, *Helianthus annuus* (Ha-CHOX) and *Lactuca sativa* (Ls-CHOX) oxidases—that are induced by SA and fungal pathogens. They promote extracellular H_2O_2 production and enhance plant disease resistance in vivo (Custers et al. 2004). PR-19 consists of small, disulfide-rich proteins (Sp-AMPs) identified in Scots pine (*Pinus sylvestris*). These proteins bind to fungal cell wall glucans, disrupting the cell wall structure and causing hyphal morphological distortion (Manners 2009; Sooriyaarachchi et al. 2011). Consequently, PR-18 and PR-19 can be considered two newly defined PR families.

In addition to being induced by various biotic and abiotic stresses, PRs can accumulate during plant growth, development, and physiological processes. This phenomenon was first observed in healthy tobacco leaves, where PRs accumulate partially during flowering and senescence (Fraser 1981; Hanfrey et al. 1996). Some PRs are secreted and accumulate in senescing leaves during the yellowing stage and in ripening fruits (Quirino et al. 2000; Davoine et al. 2001; Buchanan-Wollaston et al. 2003). They can also be detected in the medium of cell suspension cultures, even in the absence of visible necrosis, suggesting that PRs can be produced under specific physiological conditions (Takeda et al. 1990). PR-9, a heme-dependent peroxidase, catalyzes phenolic compounds and their hydrogen peroxide derivatives. This protein is inducible by both internal and external stimuli and is ubiquitous across all stages of plant growth. It is involved in various physiological processes, such as lignin formation, cell differentiation, and auxin breakdown (Passardi et al. 2005). PR-14, also known as lipid transfer proteins (LTPs), is found in nearly all plant organs and can be transported to the extracellular space (Kielbowicz-Matuk et al. 2008). It exhibits antifungal and antibacterial activities and is involved in cutin synthesis, β -oxidation, and somatic embryogenesis (Tsuboi et al. 1992; García-Olmedo et al. 1995; Han et al. 2001; Debono et al. 2009).

The increasing availability of plant genomes has greatly facilitated the identification of PRs through domain-based approaches (Zhang et al. 2018; Meng

et al. 2022). Initially, the term "PR-like proteins" was proposed to describe homologous PRs from other plants based on their amino acid or cDNA sequences (Edreva et al. 2005). However, the term did not gain widespread adoption, as some proteins classified as "PR-like" due to sequence similarity were later found to be strongly induced during infections, blurring the distinction between PRs and PR-like proteins. Consequently, these proteins are more appropriately classified as genuine PRs. To standardize the terminology, the term "PR proteins" became widely used to encompass all microbe-induced proteins and their homologs (Van Loon et al. 1994; Edreva et al. 2005). Building on this understanding, the present study provides an annotation of the full-length PRs in Chinese white pear (*Pyrus bretschneideri*), examines their expansion and evolutionary history, and explores their expression changes following fungal and bacterial infections. This research offers a comprehensive overview of PR families in pear, aiming to elucidate their role in disease resistance. The findings are expected to provide valuable insights into the involvement of PRs in pear's defense mechanisms. These PRs can serve as reliable markers for evaluating pear disease resistance and hold significant potential for application in crop improvement through genetic engineering strategies.

Materials and methods

Plant materials, fungal strains, and bacterial strains

Pear seedlings were cultivated in a greenhouse under controlled conditions, with a 16-h light/8-h dark cycle, 75% relative humidity, and a temperature of 25°C. The *Colletotrichum fructicola* strain NC40 was cultured on PDA at 28°C, following the procedure outlined by Li et al. (2022). Fresh conidia were obtained by inoculating a 5-mm mycelial plug into 50 mL of sterilized PDB in a 100-mL flask, which was then shaken at 180 rpm at 28°C for 4 days. Conidial concentrations were determined using a hemocytometer, and the suspension was diluted to 1×10^4 conidia/mL in sterilized water. The fire blight pathogen *Erwinia amylovora* strain KL20-28, stored at -80°C (Han et al. 2022), was streaked onto NA agar plates and cultured in LB medium at 28°C for 16 h. Bacterial cells were suspended in distilled water and adjusted to 10^9 CFU/mL using a SmartSpec Plus Spectrophotometer (Bio-Rad, Hercules, CA, USA). Leaves were inoculated with either the *C. fructicola* conidial suspension or the bacterial suspension using a non-injury inoculation method, soaked for 15 min, and incubated at 26°C with 80% humidity. Five leaf samples were collected, flash-frozen in liquid nitrogen, and stored at -80°C.

Identification of PRs in Chinese white pear

Sequences of previously reported PRs from *Nicotiana tabacum*, *Solanum lycopersicum*, *Sorghum bicolor*, *Triticum aestivum*, *Arabidopsis thaliana*, *Raphanus raphanistrum*, *Hordeum vulgare*, *Helianthus annuus*, and *Pinus sylvestris* were retrieved from UniProt (<https://www.uniprot.org/>) (Table 1). Domain analysis was performed using the InterPro Scan tool (<http://www.ebi.ac.uk/interpro>) (Paysan-Lafosse et al. 2022), focusing on conserved domains identified based on Pfam. The corresponding Hidden Markov Model (HMM) profiles were downloaded for further examination. PRs in the *P. bretschneideri* (Chinese white pear) genome were identified using HMM searches and BLASTP. The Conserved Domains Database and the InterPro protein database were utilized to assess the integrity of the domains in the candidate PRs.

Phylogenetic analysis and prediction of conserved motifs

PRs in *P. bretschneideri* were aligned using MUSCLE, and the results were used to generate NJ phylogenetic trees with 1,000 bootstrap replicates in MEGA 7.0. Conserved motifs were identified using MEME (http://meme.sdsc.edu/meme4_3_0/intro.html), setting the motif count to 10–15 and motif length between 6 and 200 amino acids.

Chromosomal distribution, gene synteny, and gene duplication analysis

Chromosomal location data for *P. bretschneideri* were obtained from genome annotations, and synteny analysis was performed using a method similar to PGDD (<http://chibba.agtec.uga.edu/duplication/>) (Lee et al. 2012). Orthologous gene pairs in the *P. bretschneideri* genome were first identified using BLASTP to establish similarity relationships. MCScanX was then employed to detect collinear gene blocks among the homologous gene pairs using its collinearity detection algorithm (Wang et al. 2012). CircOS plots displaying these identified collinear gene blocks and their relationships across the genomes were visualized using Tbtools (Chen et al. 2020). Finally, DupGen_finder was used to categorize the detected gene pairs into various forms of gene duplication events, including dispersed duplication (DSD), proximal duplication (PD), tandem duplication (TD), transposed duplication (TRD), and whole-genome duplication (WGD) (Qiao et al. 2019).

Calculation of Ka and Ks

The Ka, Ks, and Ka/Ks ratios were calculated using the calculate_Ka_Ks_pipeline (Qiao et al. 2019). This pipeline involved preparing the coding sequences and gene pairs, followed by running the computing_Ka_Ks_pipe.

Table 1 Classification, protein family ID, and source of recognized pathogenesis-related proteins (PRs) from UniProt, along with the count of PRs in pear proposed in this study

Family	Source	Uniprot ID	PFAM	Protein domin	Number in Pear	References
PR-1	<i>Nicotiana tabacum</i>	P08299	PF00188	Cysteine-rich secretory protein family	31	Cutt et al. 1988
PR-2	<i>Nicotiana tabacum</i>	P15797	PF00332	Glycosyl hydrolases family 17	68	Shinshi et al. 1988
PR-3	<i>Nicotiana tabacum</i>	P24091	PF00182	Glycosyl hydrolases family 19	15	Shinshi et al. 1987
PR-4	<i>Nicotiana tabacum</i>	P29062	PF00967	Barwin family	1	Friedrich et al. 1991
PR-5	<i>Nicotiana tabacum</i>	P07052	PF00314	Thaumatoin family	35	Cornelissen et al. 1986
PR-6	<i>Solanum lycopersicum</i>	Q43710	PF00280	Potato inhibitor I family	8	Young et al. 1994
PR-7	<i>Solanum lycopersicum</i>	Q3Y590	PF00082	Subtilase family	74	Tornero et al. 1996
PR-8	<i>Nicotiana tabacum</i>	P29060	PF00704	Glycosyl hydrolases family 18	13	Lawton et al. 1992
PR-9	<i>Nicotiana tabacum</i>	P11965	PF00141	Peroxidase	100	Lagrimini et al. 1987
PR-10	<i>Sorghum bicolor</i>	Q4VQB5	PF00407	Bet v 1 family	59	Lo et al. 1999
PR-11	<i>Nicotiana tabacum</i>	Q43576	PF00704	Glycosyl hydrolases family 18	11	Heitz et al. 1994
PR-12	<i>Triticum aestivum</i>	W5FHQ6	PF00304	Gamma-thionin family	29	Mendez et al. 1990
PR-13	<i>Arabidopsis thaliana</i>	Q42596	PF00321	Plant thionin	0	Bohlmann et al. 1998
PR-14	<i>Hordeum vulgare</i>	Q43767	PF00234	Protease inhibitor/LTP family	81	Molina et al. 1993
PR-15	<i>Hordeum vulgare</i>	P45851	PF00190	Cupin (germin)	0	Hurkman et al. 1994
PR-16	<i>Hordeum vulgare</i>	Q43487	PF00190	Cupin (germin-like protein)	51	Carter et al. 1999
PR-17	<i>Nicotiana tabacum</i>	Q9XIY9	PF04450	Peptidase of plants and bacteria	9	Okushima et al. 2000
PR-18	<i>Helianthus annuus</i>	Q8SA59	PF01565	FAD binding domain	52	Custers et al. 2004
PR-19	<i>Pinus sylvestris</i>	Q71QE0	PF09117	MiAMP1	0	Sooriyaarachchi et al. 2011

pl script to perform multiple sequence alignments using MAFFT. The output was then converted into the AXT format for input into KaKs_Calculator (Wang et al. 2010). The GMYN model was applied to calculate Ka and Ks, and the final results, including Ka, Ks, Ka/Ks ratios, and P-values, were obtained for further analysis.

SP prediction and apoplastic protein localization

To predict secretion signals for the PRs, the complete protein sequences were submitted to the SignalP (version 5.0) server for SP prediction. The apoplast proteins of pear leaves have been previously described (Han et al. 2024).

Expression pattern analysis of PRs

The RNA-seq data of pear infected with *E. amylovora* and *C. fructicola* were retrieved from the CNGB Sequence Archive (CNSA) of the China National GeneBank Data-Base (CNGBdb), with accession numbers CNP0001995 and CNP0004466. The RNA-Seq data from leaf inoculation and mock inoculation at 12, 24, and 48 h were used for gene expression analysis. Differentially expressed genes (DEGs) were identified using the thresholds $|\log_2(\text{Fold change})| > 1$ and $\text{adj.pval} < 0.05$. A heatmap was generated using TBtools (Chen et al. 2020).

Based on the transcriptomic data, 15 candidate PR genes were selected for qRT-PCR validation. At 9 weeks old, pear seedlings were inoculated with *E. amylovora*,

C. fructicola, or water (mock inoculation) using a non-invasive soaking method. The inoculated leaves were collected 12 h after inoculation, flash-frozen in liquid nitrogen, and stored for RNA extraction. Total RNA was extracted, and cDNA synthesis was performed according to the protocols of the RNA kit (Tiangen, Beijing, China) and the PrimeScript RT reagent kit (TransGen). qRT-PCR was performed using a LightCycler 480 SYBR-GREEN I Master (Roche, Indianapolis, IN, USA), with tubulin (Tub) as the reference gene. Primers for all candidate genes were designed using Primer Premier 6.0 (Table S4).

Results

Identification of PRs in Chinese white pear

The sequences of 19 PR families were retrieved from previous studies in *A. thaliana*, *H. vulgare*, *H. annuus*, *N. tabacum*, *P. sylvestris*, *S. lycopersicum*, *S. bicolor*, and *T. aestivum* (Table 1). HMM profiles were generated based on these sequences (Fig. 1). HMM searches and BLASTP were then employed to identify PRs in the genome of *P. bretschneideri* (Chinese white pear; pear hereafter). A total of 637 PRs from 16 families were identified in pear, confirmed by the integrity of their conserved domains (Table 1, Table S1). The largest family, PR-9, comprises 100 members and is classified as secretory plant peroxidases, while the smallest, PR-4, belongs to the Barwin

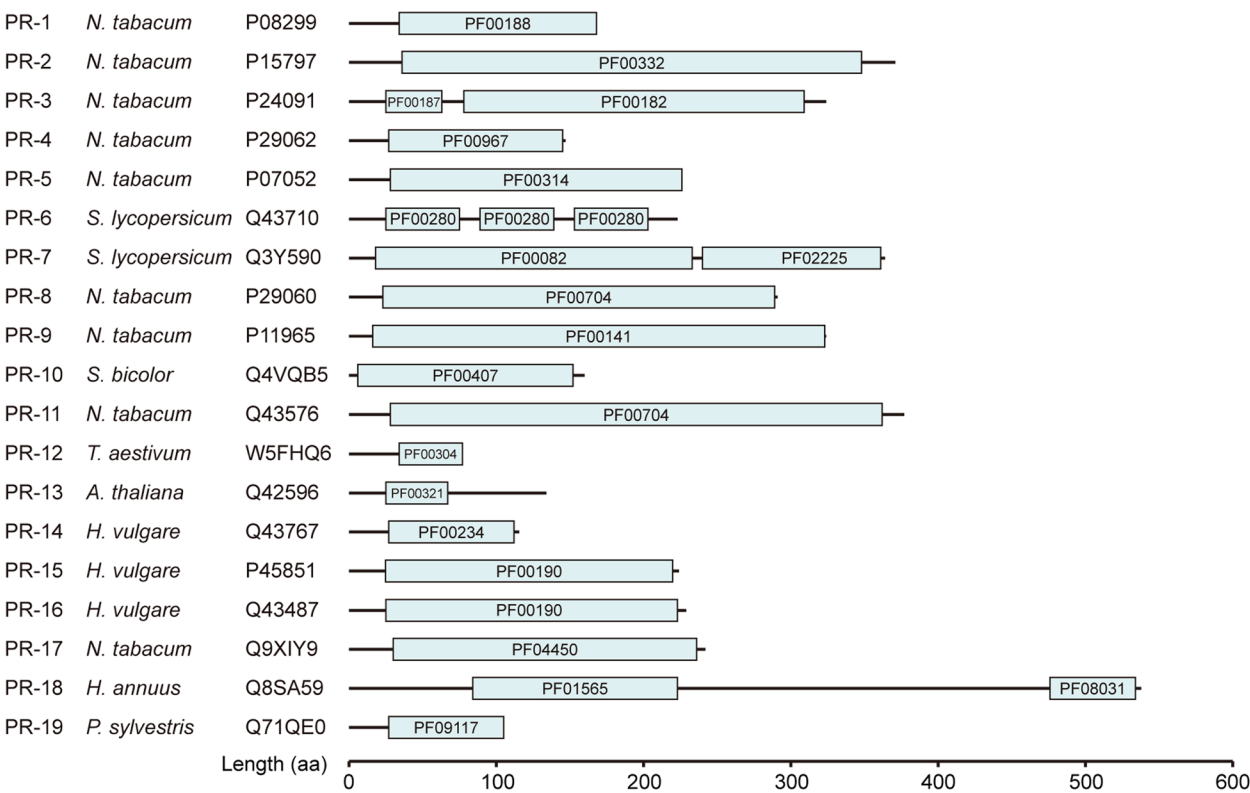


Fig. 1 Gene structure analysis of pathogenesis-related proteins (PRs). Domain analyses were performed using the InterPro database

family, with only one member. Notably, PR-13, PR-15, and PR-19 were absent in the pear genome.

Phylogenetic and gene characterization analyses of PRs

Although most PR families exhibit distinct domains (Table 1, Table S1), some, such as PR-8 and PR-11, as well as PR-15 and PR-16, share common domains. These proteins were differentiated through multiple sequence alignment, phylogenetic analysis, and gene structure analysis. A phylogenetic tree was constructed using the protein sequences of 24 GH18 family proteins from pear and two known PRs (PR-8 and PR-11) from *N. tabacum* via the Neighbor-Joining (NJ) method. The GH18 proteins in pear clustered into two clades: PR-8 and PR-11 (Fig. 2). In support of this, ten conserved motifs were identified using the MEME online tool, revealing distinct motif structures between PR-8 and PR-11. Similarly, Germins (PR-15) and Germin-like proteins (GLPs; PR-16) were distinguished using the same method. Germins, a highly conserved group with over 90% identity (Bernier et al. 2001), were initially identified in germinating wheat but have remained undetected in dicotyledonous plants, suggesting a potential restriction to the Poaceae family (Melchers et al. 1994; Carter et al. 1999). Despite sharing structural domains with GLPs, Germins and GLPs

exhibit striking sequence divergence. GLPs, although present across all angiosperm families, show minimal amino acid homology to Germins, often as low as 25% (Carter et al. 1999; Bernier et al. 2001). Our findings confirm that authentic Germins form a distinct clade in the phylogenetic tree, with unique motifs compared to other proteins (Fig. 3). Furthermore, the sequence diversity of GLPs in pear is extensive, with a total of 15 detected motifs.

Collinearity analysis of PRs

Gene duplication plays a critical role in expanding gene families and is a defining feature of plant genomes (Freeling 2009). To investigate the expansion of PRs in pear, the corresponding cDNA sequences and location information were extracted from the pear genome annotation files (Wu et al. 2013). The chromosomal locations and collinearity of PRs were mapped to the pear chromosomes. PRs in pear were randomly distributed across 17 chromosomes and contigs (Fig. 4; Fig. S1). A total of 165 syntenic gene pairs were identified, with PR-9 proteins showing the highest number of syntenic pairs (30), while PR-6 proteins had only one pair. The numbers of syntenic pairs for other PRs were as follows: PR-1, 4; PR-2, 18; PR-3, 3; PR-5, 7; PR-7, 12; PR-8, 3; PR-10, 21; PR-11, 10;

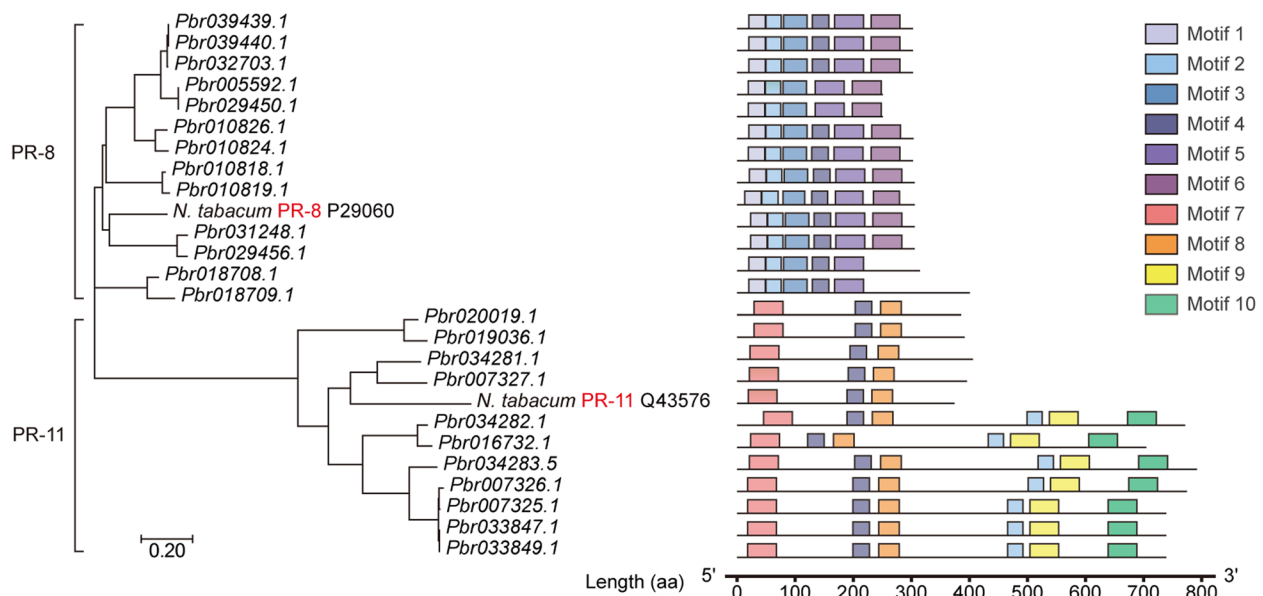


Fig. 2 Phylogenetic relationships and conserved domains of glycoside hydrolase 17 family proteins from *Pyrus bretschneideri* and two recognized pathogenesis-related proteins (PRs) (PR-8 and PR-11) from tobacco. The proteins were clustered into two groups: PR-8 and PR-11

PR-12, 3; PR-14, 26; PR-16, 18; and PR-18, 9. No syntenic gene pairs were found for PR-4 and PR-17 due to their lower gene counts.

The evolution of PRs in pear has been shaped by various gene duplication patterns. As outlined in previous studies (Qiao et al. 2019), these patterns can be categorized into five types: WGD or segmental duplication, TD, PD, TRD, and DSD (Fig. 5a). The expansion of different PR families is driven by distinct mechanisms. WGDs primarily contributed to the expansion of PR-2, PR-5, PR-7, PR-8, PR-9, PR-10, PR-11, PR-14, PR-16, and PR-18. PD was the main driver for PR-1, while TD played a significant role in the evolution of PR-12. The prevalence of DSDs, likely due to genomic rearrangements and gene loss, notably contributed to the expansion of the PR-17 family.

Gene duplication and evolution of PRs families in pear

The evolutionary timeframe of the WGD event is often approximated by K_s values (Lemoine et al. 2007). Besides the ancient WGD ($K_s=1.5-1.8$), which occurred around 140 Mya and is referred to as the γ -paleohexaploidization event shared among core eudicots (Fawcett et al. 2009), a more recent WGD event, estimated to have occurred 30–45 Mya ($K_s=0.15-0.3$), has been identified in pear (Wu et al. 2013). Several studies suggest that WGD events significantly influenced the evolutionary history of gene families in pear (Wu et al. 2013; Han et al. 2023). Among the identified PR gene pairs, 51 gene pairs have K_s values ranging from 0.15 to 0.30, indicating their

origin from the recent WGD event. In contrast, only 6 gene pairs exhibit K_s values between 1.5 and 1.8, suggesting they originated from the more ancient WGD event (Fig. 5b; Table S2). The K_s values differ notably among PR families. Specifically, 73.33% of PR-2 gene pairs, 58.33% of PR-7 gene pairs, 27.78% of PR-10 gene pairs, 34.78% of PR-14 gene pairs, and 50.00% of PR-16 gene pairs stem from the recent WGD event, highlighting its critical role in the expansion of these families, whereas older duplication events contributed less significantly.

However, gene duplication does not always result in the immediate fixation of new gene copies. Detrimental mutations arising from duplicated genes can be purged over time by negative (purifying) selection, which eliminates non-beneficial variations. Conversely, beneficial mutations that confer an advantage can accumulate through positive (Darwinian) selection, eventually becoming fixed in the population (Qiao et al. 2019). To further examine the selective pressures on PR families in pear, the K_a (non-synonymous substitution rate) and K_a/K_s ratios for the 14 PR families were evaluated (Fig. 5c; Table S2). The K_a/K_s ratio serves as an indicator of the evolutionary dynamics of these genes ($K_a/K_s > 1$: positive selection; $K_a/K_s = 1$: neutral evolution; $K_a/K_s < 1$: purifying selection). Our analysis revealed that, except for a single gene pair (*Pbr012071.1* and *Pbr004502.1* from the PR-10 family), which exhibited a K_a/K_s ratio greater than 1, all other PR gene pairs displayed K_a/K_s ratios below 1, indicating that most of these genes have evolved under purifying selection. This suggests that PR genes are highly

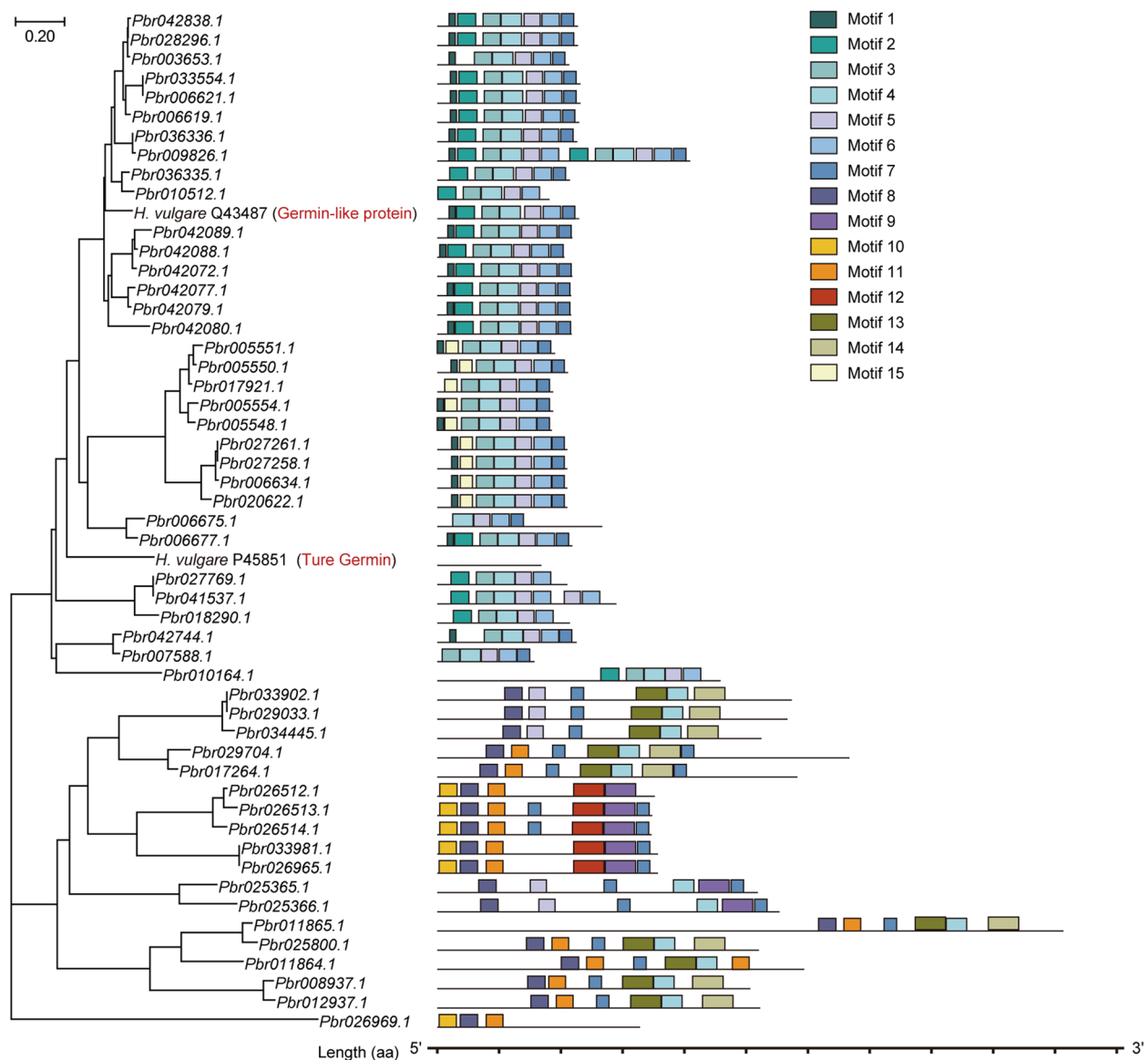


Fig. 3 Phylogenetic relationships and conserved domains of Germin-like proteins from pear and two recognized pathogenesis-related proteins (PRs), PR-15 and PR-16, from barley

conserved, with negative selection maintaining their functional integrity. The box plots of Ka/Ks ratios further revealed distinct clustering, particularly for PR-7, PR-10, and PR-18, suggesting these families are subject to similar selective pressures, likely due to their involvement in specific aspects of the plant immune response.

The apoplast is the main region where PRs accumulate

N-terminal SPs direct proteins to the secretory pathway in plants. Proteins destined for secretion carry an N-terminal SP that is recognized by the signal recognition particle (SRP) and its receptor (SR). The SRP-SR complex

then targets the ribosome-nascent chain complex to the endoplasmic reticulum (ER) membrane (Goder and Spiess 2003). Many PRs possess an N-terminal SP, which directs them into the ER before secretion into the apoplast. Examination of SPs in the 637 predicted PRs revealed that, with the exception of the PR-6 and PR-10 families, most members of the other 18 PR families carry SPs (Fig. 6). Notably, all members of the PR-3, PR-8, and PR-12 families contain SPs.

PRs accumulate in the extracellular space and can be readily collected from apoplast fluid (Van Loon et al. 2006). In a recent study, apoplast fluid was successfully

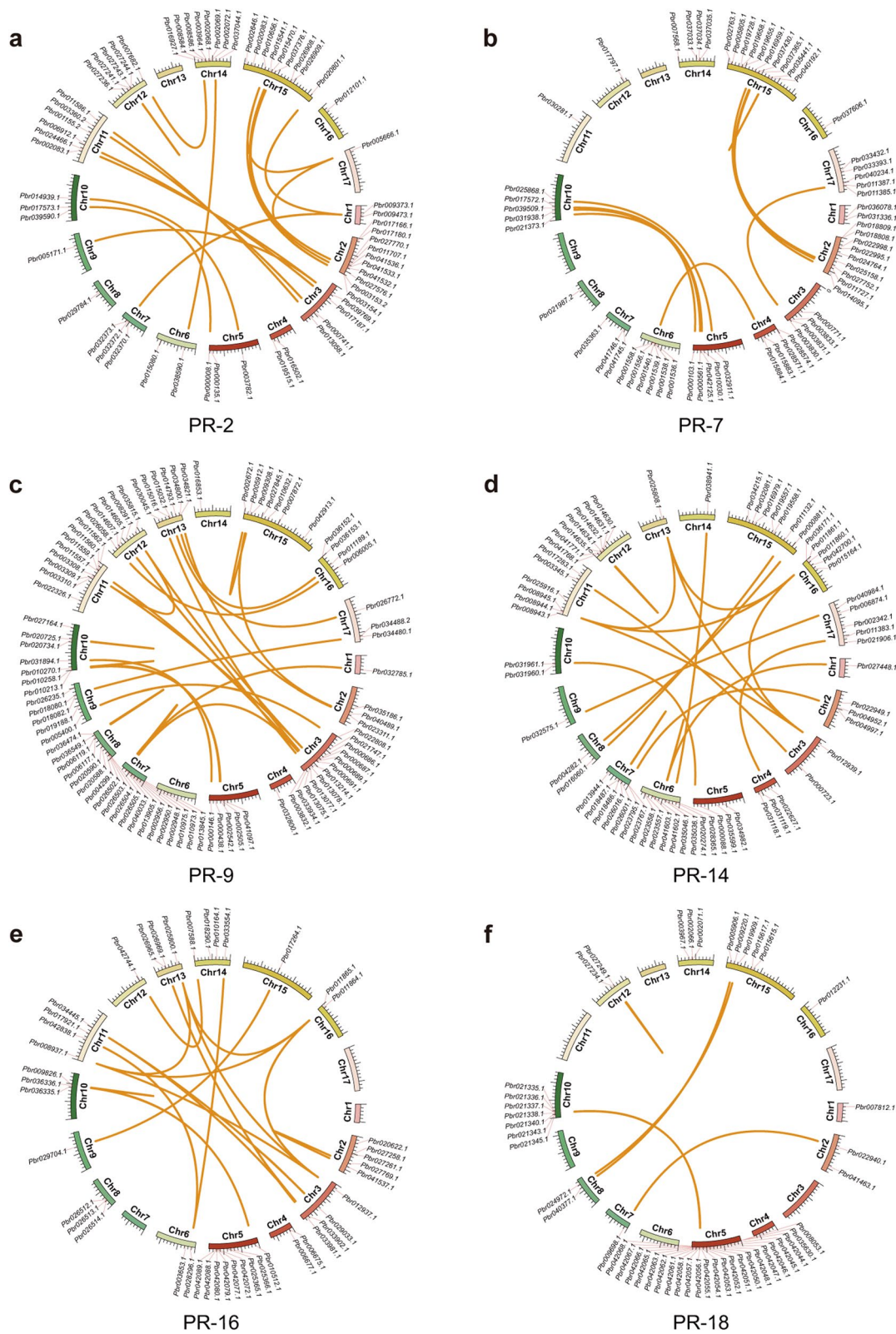


Fig. 4 Gene location and collinearity analysis of the pathogenesis-related proteins (PRs) families in pear. Chromosome numbers are indicated on the inner side, and co-linear gene pairs are connected by lines. PR-2 (a); PR-7 (b); PR-9 (c); PR-14 (d); PR-16 (e); PR-18 (f)

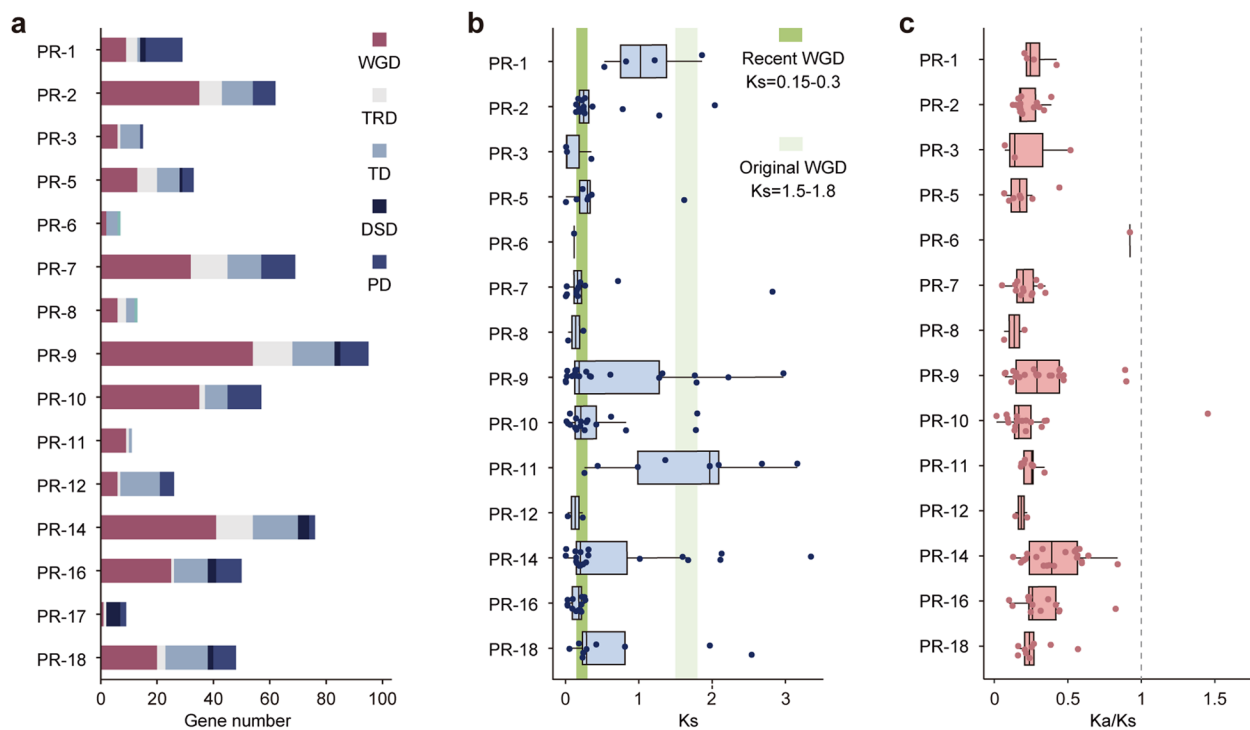


Fig. 5 Gene duplication and evolution in pathogenesis-related (PR) genes of pear. **a** The number of pear PR genes derived from various duplication modes, including Whole Genome Duplication (WGD), Transposed Duplication (TRD), Tandem Duplication (TD), Dispersed Duplication (DSD), and Proximal Duplication (PD). **b** The Ks distribution of gene pairs in each PR family. Ancient WGDs are plotted with light green squares ($K_s = 1.5\text{--}1.8$, ~140 million years ago), and recent WGDs are plotted with dark green squares ($K_s = 0.15\text{--}0.3$, 30–45 million years ago). **c** The Ka/Ks distribution of gene pairs derived from different PR families

isolated from pear leaves (Han et al. 2024). The identified proteins were compared to the predicted PRs from this study (Fig. 6). The results showed that, except for PR-6, representatives from all PR families were found in the apoplast fluid, accounting for 15.10% (44) of the total 414 identified proteins. Interestingly, two members of the PR-10 family (*Pbr034852.1* and *Pbr042265.1*) were detected in the apoplast fluid. However, PR-10 is widely reported not to function extracellularly (Van Loon et al. 2006).

Expression of PR genes in response to infection by *C. fructicola* and *E. amylovora*

To investigate the response of PRs to biotic stress, the pear leaf transcriptome was analyzed following infection with *C. fructicola* and *E. amylovora* (Fig. 7a). *C. fructicola* is a typical hemibiotrophic fungus from the *Colletotrichum* genus, while *E. amylovora* is a highly destructive pathogenic bacterium affecting plants in the Rosaceae family (Malnoy et al. 2012; Gan et al. 2013). During the early stages of infection (12 hpi), a greater number of DEGs were observed in leaves infected with *E. amylovora* compared to those infected with *C. fructicola*. In contrast, at later stages (48 hpi), the opposite trend was seen.

Across all time points, the number of upregulated DEGs consistently exceeded the downregulated DEGs (Fig. 7b). Analysis of the log₂ fold change (log₂FC) revealed a significant disparity at 24 hpi and 48 hpi (Fig. 7c). Notably, while changes in the expression levels of predicted PRs were observed, these variations were less pronounced compared to those of the overall gene expression (Fig. 7d).

Of the 637 predicted PRs, 331 were expressed in pear leaves, and 214 showed differential expression during infections by *C. fructicola* or *E. amylovora* at one or more time points (Fig. 8). The number of upregulated DEGs was substantially greater than that of downregulated DEGs (Fig. S2). Consistent with previous findings, no significant differences were observed in the responses of PRs to fungal versus bacterial infections. Expression differences among PR families were highly variable. Most PR families exhibited a distinct cluster of genes that were significantly upregulated, with the PR-10 family having the largest cluster, containing 30 genes. However, certain families, such as PR-7, PR-12, and PR-14, lacked upregulated members across all time points. Moreover, even within the same family, individual members exhibited diverse expression patterns. Except for PR-11

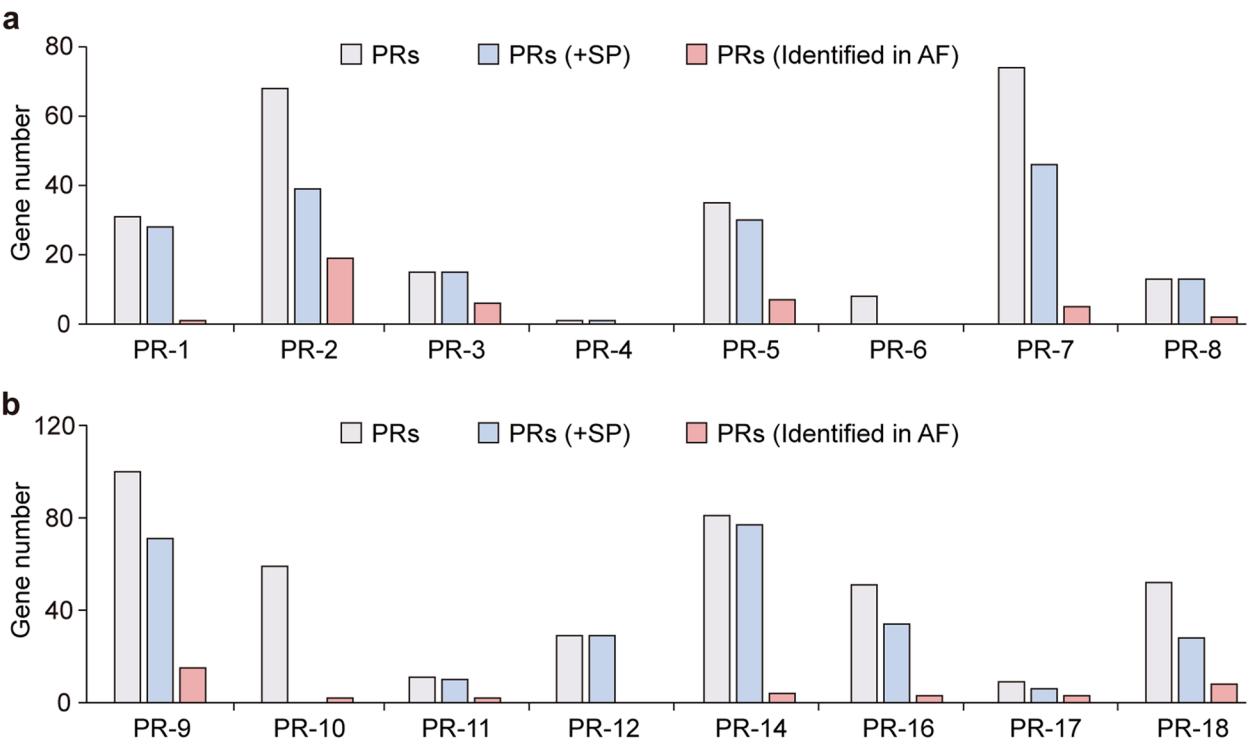


Fig. 6 Prediction of signal peptides and apoplastic localization analysis of pathogenesis-related proteins (PRs). The blue squares represent the number of proteins containing SPs, while the red squares represent the number of proteins detected in the apoplastic fluid (AF). PR1-8 (a); PR9-12, PR14, PR16-18 (b)

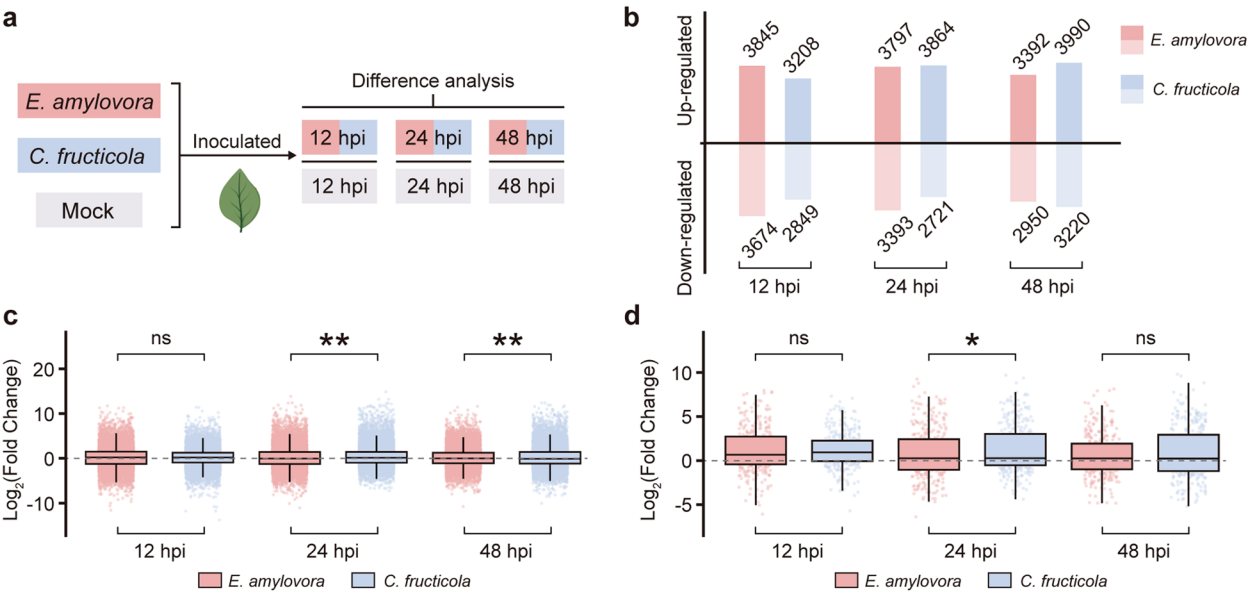


Fig. 7 Expression patterns of pear upon fungal and bacterial infections. **a** Elaborate illustration of the experimental design for RNA-seq analysis. **b** The number of DEGs in pear leaves after 12 h, 24 h, and 48 h of infection with *Colletotrichum fructicola* and *Erwinia amylovora*. **c** The log₂ fold change of total genes in pear upon inoculation of *Colletotrichum fructicola* and *Erwinia amylovora* compared to the mock inoculation. Asterisks indicate significant differences (**P* < 0.05, ***P* < 0.01). **d** The log₂ fold change of pathogenesis-related (PR) genes

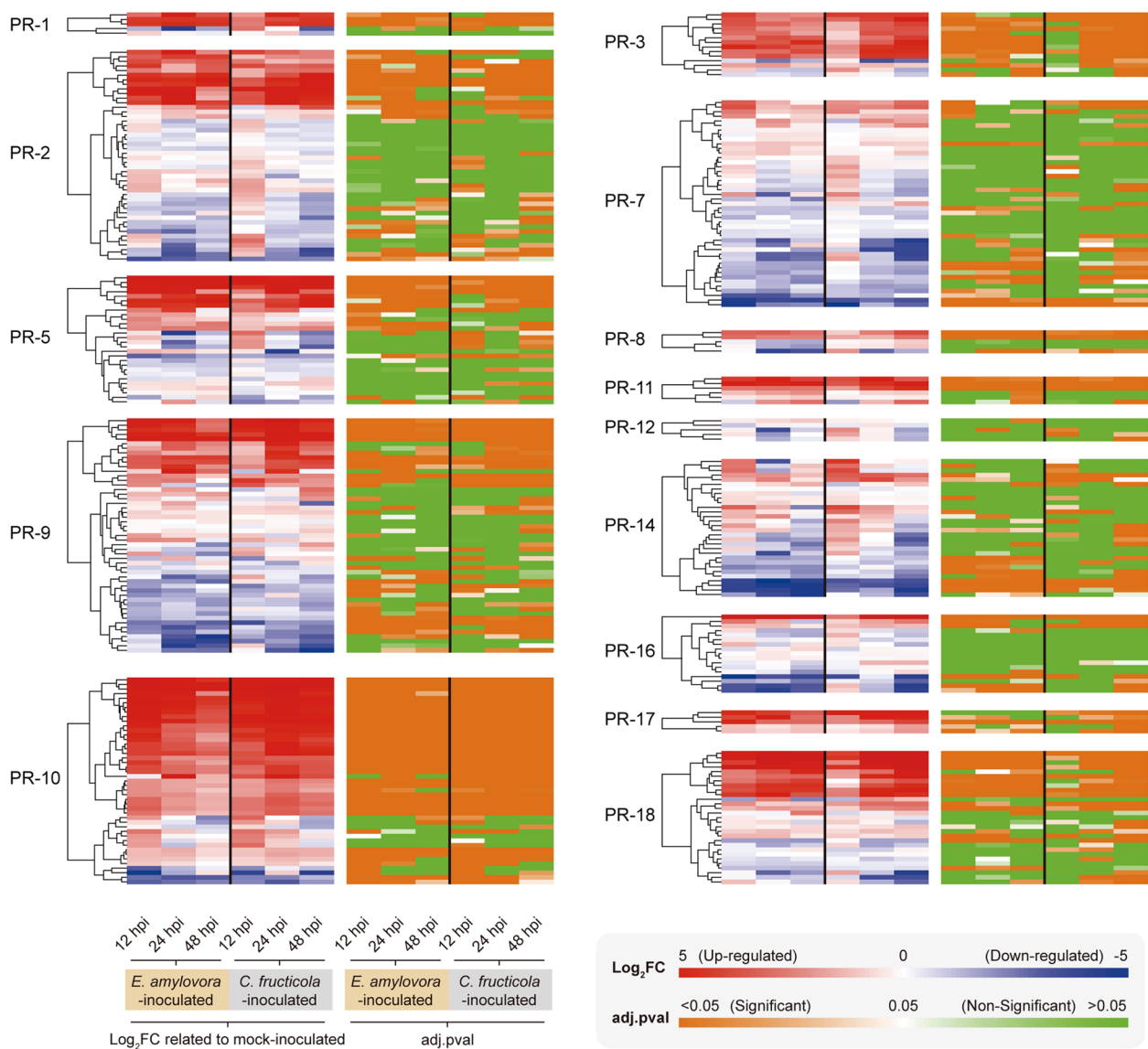


Fig. 8 Expression profile of pear pathogenesis-related (PR) genes after fungal and bacterial infections. The colors in the red-blue heatmap represent $\log_2$ -fold change, with red indicating significantly upregulated genes and blue indicating significantly downregulated genes. The colors in the orange-green heatmap represent the Benjamini–Hochberg FDR two-sided adjusted p -value. The gradient of orange-green color indicates the significance of the adj. pval. Adj. pval < 0.05 is considered significant. For comprehensive details on the DEGs, refer to Table S3

and PR-7, several PR families contained genes that were significantly downregulated. No DEGs displayed opposite regulation patterns between infections caused by *E. amylovora* and *C. fructicola* (Fig. S3). Notably, in the PR-7, PR-14, and PR-16 families, downregulated genes outnumbered upregulated ones. Based on the transcriptome data, eight candidate PRs were selected for validation by qRT-PCR (Fig. 9). These genes, whose corresponding proteins were detected in the apoplast fluid, showed significant upregulation, with *Pbr039286.1*

(PR-5) and *Pbr042058.1* (PR-18) exhibiting the highest expression levels.

Discussion

PRs were identified as a multi-gene family in the pear genome. Standard sequence homology analysis has typically been used to predict PRs in non-model plants (Bernier et al. 2001), but significant sequence variations have rendered ancestral PR family members unsuitable as reference sequences. Utilizing HMM searches, a more precise exploration of protein families and

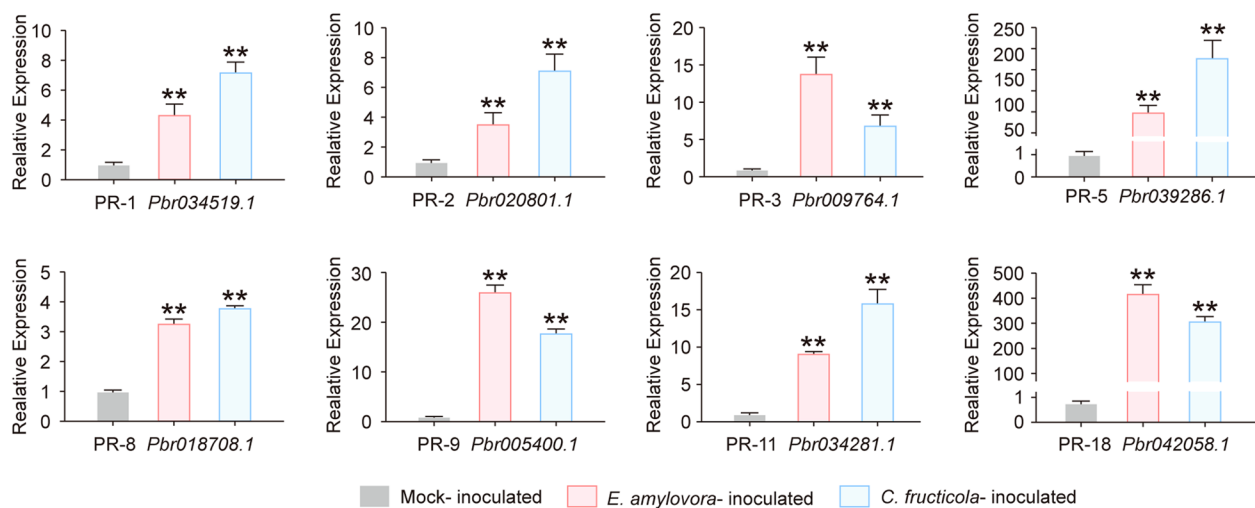


Fig. 9 The relative expression levels of several pear pathogenesis-related (PR) genes after infection of *Erwinia amylovora* and *Colletotrichum fructicola*. Bars represent the means $\pm$ standard error of three biological replicates. Asterisks indicate significant differences (** $P < 0.01$)

structural domains is possible, particularly for detecting distant homologous relationships (Eddy 2011). Although our gene identification method differs from previous approaches, the overall size of the PR family in pear is consistent with that observed in other dicotyledonous plants. Notably, PR-13, PR-15, and PR-19 were absent from the pear genome. PR-13 is also missing in *Manihot esculenta* and *Populus trichocarpa*, but it is present in *Arabidopsis* (Irigoyen et al. 2020). PR-19, initially discovered in macadamia (Order Proteales) and arum lilies (Order Arales), species representing early branches of monocot and eudicot lineages post-divergence, has not been identified in higher eudicots such as *Arabidopsis* or certain monocots like rice (Manners 2009). This absence, consistent with previous studies, is also evident in pears.

Gene duplication plays a pivotal role in plant evolution and diversity, with five main patterns of duplication identified: WGD, PD, TD, DSD, and TRD (Qiao et al. 2019). Previous studies have shown that large gene families, such as the Ethylene Responsive Factor (ERF) family in *Vitis vinifera* and the WRKY family in *Prunus mume*, are more prone to expansion via TDs (Du et al. 2013; Guo et al. 2014). In the pear genome, notable gene clusters resulting from TDs are evident within several major PR families, including PR-2, PR-9, PR-14, PR-16, and PR-18. Genome-wide analyses have revealed that the pear genome underwent at least two rounds of duplication, one ancient and the other preceding the divergence between pear and apple (Wu et al. 2013). This study further demonstrates that WGDs are the primary mechanism driving the expansion of most PR families, such as PR-2, PR-5, PR-7, PR-8, PR-9, PR-10, PR-11, PR-14, PR-16, and PR-18. Ka, Ks, and Ka/Ks analyses indicate

that the recent WGD event played a dominant role in PR family expansion, with only a few genes traced back to ancient WGDs. Notably, the majority of gene pairs in PR families, especially PR-2, PR-7, PR-10, PR-14, and PR-16, are derived from the recent WGD event. The PR-2 family, involved in the degradation of β -glucans (a component of fungal cell walls), contains the highest proportion of gene pairs from this recent duplication (Filichkin et al. 2004; Shi et al. 2006), suggesting that the expansion of PR-2 is particularly critical for defending against fungal pathogens, a significant threat to pear plants. These findings imply that recent duplications have had a greater influence on shaping pear's defense mechanisms, likely enhancing immune response diversity and efficiency. In contrast, the limited number of gene pairs from ancient WGDs suggests these older duplications contribute less to the current defense capabilities of pear. This supports the hypothesis that while ancient WGDs established genomic stability, recent duplications are primarily responsible for the functional diversification required to combat contemporary pathogens. The retention of genes from ancient duplications may reflect conserved immune functions that provide a foundational defense system. Variations in Ks values across different PR families likely stem from distinct evolutionary pressures and functional adaptations, influencing their evolutionary rates and timing. Almost all PR gene pairs in pear exhibit Ka/Ks ratios below 1, indicating strong purifying selection, which maintains the stability and integrity of the gene family (Wang et al. 2010).

Gene family amplification arises from gene duplication, generating sets of paralogous genes that exhibit similarity but not exact identity. This duplication can lead

to functional divergence, redundancy, and the formation of pseudogenes, complicating the identification of genes with distinct roles within the family (Lynch et al. 2000; Soltis et al. 2009). Most PRs functioning extracellularly possess an N-terminal SP, although many members across various PR families have lost this SP. This loss may correlate with gene functional diversification, mutations, and relaxation of selective pressures during gene family expansion. Protein analysis of pear leaf apoplast fluid following *C. fructicola* infection revealed the presence of only a few PRs, which are likely the functional components involved in the response. The PR-1 protein, part of the pathogen-induced dominant PR group, is widely used as a marker for the enhancement of systemic acquired resistance (SAR) against pathogens (Van Loon et al. 2006). Of the 32 members in the PR-1 gene family, 29 contain SPs; however, only one member (*Pbr034519.1*) was identified in the apoplast fluid, suggesting that most PRs may not play an active role in resisting pathogen invasion in pear leaves. This implies that the functionality of PRs could be under stringent regulatory control and selectivity. Beyond pathogen defense, PR-like proteins expressed in healthy tissues during plant development, such as seed formation and flowering, might serve unexpected roles in morphogenesis or symbiosis (Fraser et al. 1981; Vögeli-Lange et al. 1994; Hanfrey et al. 1996). This could explain why many proteins are not detected in the apoplast fluid, as they may perform different functions in other tissues. Interestingly, PR-10, which operates intracellularly and lacks an SP, has two members identified in the apoplast fluid (Van Loon et al. 2006). However, it remains unclear whether these proteins were secreted into the apoplast or if their presence is due to experimental errors, possibly resulting from excessive accumulation.

The initial definition of PRs was "proteins coded for by the host plant but induced only in pathological or related situations" (Antoniw et al. 1980). Research on these proteins has largely shifted towards analyzing the expression patterns of their corresponding genes. Analysis of RNA-seq data from pear leaves infected with *C. fructicola* and *E. amylovora* revealed numerous genes with significantly induced expression following infection. The expression patterns of PR genes in pear show little variation between fungal and bacterial infections, including PR families targeting fungal cell walls, such as PR-3, PR-8, and PR-11. However, this alone does not support the conclusion that these genes mediate broad-spectrum resistance. It is hypothesized that the timing of sampling may influence these outcomes. Upon recognition of Microbe-Associated Molecular Patterns (MAMPs) from external

microorganisms or microbial effectors within host cells, rapid defense responses are activated, including extensive transcriptional reprogramming (Ramirez-Prado et al. 2018; Zhou et al. 2020). At 12 hpi, for instance, the host has already initiated SAR. Some families, including PR-7, PR-9, PR-14, and PR-16, display clusters of genes consistently downregulated across all time points. This substantial downregulation is unexpected, as PR genes are typically upregulated following pathogen infection. The regulation of PR genes may be influenced by various factors. In addition to intrinsic regulatory mechanisms, pathogens can suppress disease resistance genes via effectors (Wang et al. 2019, 2022). For example, RXLR effectors from *P. infestans* target StMAP3K β 2 to inhibit the immune response in potatoes (Ren et al. 2019). However, this is unlikely to explain the downregulation of these PR genes, as similar patterns were observed in both *C. fructicola* and *E. amylovora* infections. Given the considerable differences in the internal microbial effectors between fungi and bacteria, this expression pattern may result from intrinsic regulation within pear. A similar phenomenon has been observed in cassava-pathogen interactions (Irigoyen et al. 2020). Our findings further suggest that most PRs were not detected in the apoplast fluid, indicating that these PRs may not play a significant role in plant immunity. Instead, they may be subject to negative feedback regulation and involved in resource redistribution within the pear.

Conclusions

This study identified 637 PRs across 16 PR families in pear. Collinearity analysis of the pear genome annotation revealed the pivotal role of recent WGD in driving the extensive expansion of the PR family. Purifying selection was found to be the primary evolutionary force shaping these genes. By integrating mass spectrometry and transcriptomic data, this study determined that only a small subset of PR family members are actively involved in disease resistance in pear, with several core members validated through qRT-PCR. Furthermore, a significant number of PR genes, typically regarded as "inducible", were downregulated in response to *C. fructicola* and *E. amylovora* infections. This finding highlights the functional diversity within the expanded PR family and suggests a complex regulatory network in the pear-pathogen interaction. In conclusion, these results enhance our understanding of pear-pathogen interactions and provide a foundation for further exploration of the roles of various PRs in pear development and immunity.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44281-025-00064-9>.

Supplementary Material 1. Fig. S1 Gene location and collinearity analysis of the PR-1, PR-3, PR-5, PR-6, PR-10, PR-11, and PR-12 families in pear. PR, pathogenesis-related protein

Supplementary Material 2. Fig. S2 The number of differentially expressed genes (DEGs) within pathogenesis-related (PR) genes in pear leaves at 12 hours, 24 hours, and 48 hours post-infection with *Colletotrichum fructicola* and *Erwinia amylovora*.

Supplementary Material 3. Fig. S3 Expression levels of pathogenesis-related (PR) genes in pear following infection with *Colletotrichum fructicola* and *Erwinia amylovora*. A regression line was fitted using linear regression.

Supplementary Material 4. Table S1 Gene list and basic features of the identified pathogenesis-related proteins (PRs) in pear

Supplementary Material 5. Table S2 Ka/Ks ratios of paralogous genes within pathogenesis-related protein (PR) families in pear

Supplementary Material 6. Table S3 Log₂FC and adjusted p-value of pathogenesis-related (PR) genes in pear.

Supplementary Material 7. Table S4 Primers used in this study

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Not applicable.

Authors' contributions

Conceived and designed the experiments: SLZ, FQL and CYH. Performed the experiments: QW, ZYS, and CHL. Analyzed the data: CYH, ZYS, FHZ, BDG and QW. Wrote the paper: CYH, ZYS, BT, YCZ and FQL. And all authors have read and approved the manuscript.

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Data availability

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://db.cngb.org/cnsa/>, CNP0004466; <https://db.cngb.org/cnsa/>, CNP0001995.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- Antoniw JF, Ritter CE, Pierpoint WS, Van Loon LC. Comparison of three pathogenesis-related proteins from plants of two cultivars of tobacco infected with TMV. *J Gen Virol*. 1980;47:79–87. <https://doi.org/10.1099/0022-1317-47-1-79>.
- Bernier F, Anne B. Germins and germin-like proteins: plant do-all proteins. But what do they do exactly? *Plant Physiol Biochem*. 2001;39:545–54. [https://doi.org/10.1016/S0981-9428\(01\)01285-2](https://doi.org/10.1016/S0981-9428(01)01285-2).
- Bohlmann H, Vignutelli A, Hilpert B, Miersch O, Wasternack C, Apel K. Wounding and chemicals induce expression of the *Arabidopsis thaliana* gene Thi2.1, encoding a fungal defense thionin, via the octadecanoid pathway. *FEBS Lett*. 1998;437:281–6. [https://doi.org/10.1016/S0014-5793\(98\)01251-4](https://doi.org/10.1016/S0014-5793(98)01251-4).
- Bol JF, Linthorst HJM, Cornelissen BJC. Plant pathogenesis-related proteins induced by virus infection. *Annu Rev Phytopathol*. 1990;28:113–38. <https://doi.org/10.1146/annurev.py.28.090190.000553>.
- Bravo JM, Campo S, Murillo I, Coca M, San SB. Fungus- and wound-induced accumulation of mRNA containing a class II chitinase of the pathogenesis-related protein 4 (PR-4) family of maize. *Plant Mol Biol*. 2003;52:745–59. <https://doi.org/10.1023/A:1025016416951>.
- Buchanan-Wollaston V, Earl S, Harrison E, Mathas E, Navabpour S, Page T, et al. The molecular analysis of leaf senescence—a genomics approach. *Plant Biotechnol J*. 2003;1:3–22. <https://doi.org/10.1046/j.1467-7652.2003.00004.x>.
- Carter C, Thornburg RW. Germin-like proteins: structure, phylogeny, and function. *J Plant Biol*. 1999;42:97–108. <https://doi.org/10.1007/BF03031017>.
- Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, et al. TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Mol Plant*. 2020;13:1194–202. <https://doi.org/10.1016/j.molp.2020.06.009>.
- Cornelissen BJ, Hooft van Huijsduijnen RA, Bol JF. A tobacco mosaic virus-induced tobacco protein is homologous to the sweet-tasting protein thaumatin. *Nature*. 1986;321:531–2. <https://doi.org/10.1038/321531a0>.
- Custers JH, Harrison SJ, Sela-Buurlage MB, Van Deventer E, Lageweg W, Howe PW, et al. Isolation and characterisation of a class of carbohydrate oxidases from higher plants, with a role in active defence. *Plant J*. 2004;39:147–60. <https://doi.org/10.1111/j.1365-313X.2004.02117.x>.
- Cutt JR, Dixon DC, Carr JP, Klessig DF. Isolation and nucleotide sequence of cDNA clones for the pathogenesis-related proteins PR1a, PR1b and PR1c of *Nicotiana tabacum* cv. Xanthi nc induced by TMV infection. *Nucleic Acids Res*. 1988;16:9861. <https://doi.org/10.1093/nar/16.20.9861>.
- Davoine C, Le Deunff E, Ledger N, Avic JC, Billard JP, Dumas B, et al. Specific and constitutive expression of oxalate oxidase during the ageing of leaf sheaths. *Plant Cell Environ*. 2001;24:1033–43. <https://doi.org/10.1046/j.1365-3040.2001.00757.x>.
- DeBono A, Yeats TH, Rose JK, Bird D, Jetter R, Kunst L, et al. Arabidopsis LTPG is a glycosylphosphatidylinositol-anchored lipid transfer protein required for export of lipids to the plant surface. *Plant Cell*. 2009;21:1230–8. <https://doi.org/10.1105/tpc.108.064451>.
- Doares SH, Syrovets T, Weiler EW, Ryan CA. Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. *Proc Natl Acad Sci USA*. 1995;92:4095–8. <https://doi.org/10.1073/pnas.92.10.4095>.
- Douliet JP, Pato C, Rabesona H, Molle D, Marion D. Disulfide bond assignment, lipid transfer activity and secondary structure of a 7-kDa plant lipid transfer protein, LTP2. *Eur J Biochem*. 2001;268:1400–3. <https://doi.org/10.1046/j.1432-1327.2001.02007.x>.
- Du D, Hao R, Cheng T, Pan H, Yang W, Wang J, et al. Genome-wide analysis of the AP2/ERF gene family in *Prunus mume*. *Plant Mol Biol Rep*. 2013;31:741–50. <https://doi.org/10.1007/s11105-012-0531-6>.
- Eddy SR. Accelerated profile HMM searches[J]. *PLoS Comput Biol*. 2011;7:e1002195. <https://doi.org/10.1371/journal.pcbi.1002195>.
- Edreva A. Pathogenesis-related proteins: research progress in the last 15 years. *Gen Appl Plant Physiol*. 2005;31:105–24.
- Fawcett JA, Maere S, Van de Peer Y. Plants with double genomes might have had a better chance to survive the Cretaceous-Tertiary extinction event. *Proc Natl Acad Sci U S A*. 2009;7;106(14):5737–42. <https://doi.org/10.1073/pnas.0900906106>.

- Filichkin SA, Leonard JM, Monteros A, Liu PP, Nonogaki H. A novel endo- β -mannanase gene in tomato LeMAN5 is associated with anther and pollen development. *Plant Physiol.* 2004;134:1080–7. <https://doi.org/10.1104/pp.103.035998>.
- Fraser R. Evidence for the occurrence of the “pathogenesis-related proteins” in leaves of healthy tobacco plants during flowering. *Physiol Plant Pathol.* 1981;19:69–76. [https://doi.org/10.1016/S0048-4059\(81\)80009-4](https://doi.org/10.1016/S0048-4059(81)80009-4).
- Freeling M. Bias in plant gene content following different sorts of duplication: tandem, whole-genome, segmental, or by transposition. *Annu Rev Plant Biol.* 2009;60:433–53. <https://doi.org/10.1146/annurev.arplant.043008.092122>.
- Friedrich L, Moyer M, Ward E, Ryals J. Pathogenesis-related protein 4 is structurally homologous to the carboxy-terminal domains of hevein, Win-1 and Win-2. *Mol Gen Genet.* 1991;230:113–9. <https://doi.org/10.1007/BF00290658>.
- Fritig B, Heitz T, Legrand M. Antimicrobial proteins in induced plant defense. *Curr Opin Immunol.* 1998;10:16–22. [https://doi.org/10.1016/S0952-7915\(98\)80025-3](https://doi.org/10.1016/S0952-7915(98)80025-3).
- Gan P, Ikeda K, Irieda H, Narusaka M, O'Connell RJ, Narusaka Y, et al. Comparative genomic and transcriptomic analyses reveal the hemibiotrophic stage shift of *Colletotrichum* fungi. *New Phytol.* 2013;197:1236–49. <https://doi.org/10.1111/nph.12085>.
- García-Olmedo F, Molina A, Segura A, Moreno M. The defensive role of non-specific lipid-transfer proteins in plants. *Trends Microbiol.* 1995;3:72–4. [https://doi.org/10.1016/S0966-842X\(00\)88879-4](https://doi.org/10.1016/S0966-842X(00)88879-4).
- Goder V, Spiess M. Molecular mechanism of signal sequence orientation in the endoplasmic reticulum. *EMBO J.* 2003;22:3645–53. <https://doi.org/10.1093/emboj/cdg361>.
- Gomez L, Allona I, Casado R, Aragoncillo C. Seed Chitinases Seed Sci Res. 2002;12:217–30. <https://doi.org/10.1079/SSR2002113>.
- Guevara-Morato MA, de Lacoba MG, García-Luque I, Serra MT. Characterization of a pathogenesis-related protein 4 (PR-4) induced in *Capsicum chinense* L3 plants with dual RNase and DNase activities. *J Exp Bot.* 2010;61:3259–71. <https://doi.org/10.1093/jxb/erq148>.
- Guo C, Guo R, Xu X, Gao M, Li X, Song J, et al. Evolution and expression analysis of the grape (*Vitis vinifera* L.) WRKY gene family. *J Exp Bot.* 2014;65:1513–28. <https://doi.org/10.1093/jxb/eru007>.
- Han GW, Lee JY, Song HK, Chang C, Min K, Moon J, et al. Structural basis of non-specific lipid binding in maize lipid-transfer protein complexes revealed by high-resolution X-ray crystallography. *Mol Biol J.* 2001;308:263–78. <https://doi.org/10.1006/jmbi.2001.4559>.
- Han C, Zhang F, Qiao X, Zhao Y, Qiao Q, Huang X, et al. Multi-omics analysis reveals the dynamic changes of RNA N⁶-Methyladenosine in pear (*Pyrus bretschneideri*) defense responses to *Erwinia amylovora* pathogen infection. *Front Microbiol.* 2022;12: 803512. <https://doi.org/10.3389/fmicb.2021.803512>.
- Han C, Dong H, Qiao Q, Dai Y, Huang X, Zhang S. Comparative genomic analysis of N⁶-methyladenosine regulators in nine rosaceae species and functional characterization in response to drought stress in pear. *Hortic Plant J.* 2023;9:693–704. <https://doi.org/10.1016/j.hpj.2022.09.008>.
- Han C, Su Z, Zhao Y, Li C, Guo B, Wang Q, et al. Uncovering the mechanisms underlying pear leaf apoplast protein-mediated resistance against *Colletotrichum fructicola* through transcriptome and proteome profiling. *Phytopathol Res.* 2024;6:3. <https://doi.org/10.1186/s42483-023-00220-x>.
- Hanfrey C, Fife M, Buchanan-Wollaston V. Leaf senescence in *Brassica napus*: expression of genes encoding pathogenesis-related proteins. *Plant Mol Biol.* 1996;30:597–609. <https://doi.org/10.1007/BF00049334>.
- Heitz T, Segond S, Kauffmann S, Geoffroy P, Prasad V, Brunner F, et al. Molecular characterization of a novel tobacco pathogenesis-related (PR) protein: a new plant chitinase/lysozyme. *Mol Gen Genet.* 1994;245:246–54. <https://doi.org/10.1007/BF00283273>.
- Hong JK, Hwang BK. Induction by pathogen, salt and drought of a basic class II chitinase mRNA and its in situ localization in pepper (*Capsicum annuum*). *Physiol Plant.* 2002;114:549–58. <https://doi.org/10.1034/j.1399-3054.2002.1140407>.
- Hurkman WJ, Lane BG, Tanaka CK. Nucleotide sequence of a transcript encoding a germin-like protein that is present in salt-stressed barley (*Hordeum vulgare* L.) roots. *Plant Physiol.* 1994;104:803–4. <https://doi.org/10.1104/pp.104.2.803>.
- Irigoyen ML, Garceau DC, Bohorquez-Chaux A, Lopez-Lavalle LAB, Perez-Fons L, Fraser PD, et al. Genome-wide analyses of cassava pathogenesis-related (PR) gene families reveal core transcriptome responses to whitefly infestation, salicylic acid and jasmonic acid. *BMC Genomics.* 2020;21:93. <https://doi.org/10.1186/s12864-019-6443-1>.
- Islam MM, El-Sappah AH, Ali HM, Zandi P, Huang Q, Soaud SA, et al. Pathogenesis-related proteins (PRs) countering environmental stress in plants: a review. *S Afr J Bot.* 2023;160:414–27. <https://doi.org/10.1016/j.sajb.2023.07.003>.
- Kielbowicz-Matuk A, Rey P, Rorat T. The organ-dependent abundance of a Solanum lipid transfer protein is up-regulated upon osmotic constraints and associated with cold acclimation ability. *J Exp Bot.* 2008;59:2191–203. <https://doi.org/10.1093/jxb/ern088>.
- Lagrimini LM, Burkhardt W, Moyer M, Rothstein S. Molecular cloning of complementary DNA encoding the lignin-forming peroxidase from tobacco: molecular analysis and tissue-specific expression. *Proc Natl Acad Sci USA.* 1987;84:7542–6. <https://doi.org/10.1073/pnas.84.21.7542>.
- Lawton K, Ward E, Payne G, Moyer M, Ryals J. Acidic and basic class III chitinase mRNA accumulation in response to TMV infection of tobacco. *Plant Mol Biol.* 1992;19:735–43. <https://doi.org/10.1007/BF00027070>.
- Lee TH, Tang H, Wang X, Paterson AH. PGDD: a database of gene and genome duplication in plants. *Nucleic Acids Res.* 2012;41:D1152–8. <https://doi.org/10.1093/nar/gks1104>.
- Lemoine F, Lespinet O, Labedan B. Assessing the evolutionary rate of positional orthologous genes in prokaryotes using synteny data. *BMC Evol Biol.* 2007;29:7:237. <https://doi.org/10.1186/1471-2148-7-237>.
- Li C, Sun W, Cao S, Hou R, Li X, Ming L, et al. The CfMK1 gene regulates reproduction, appressorium formation, and pathogenesis in a pear anthracnose-causing fungus. *J Fungi.* 2022;8:77. <https://doi.org/10.3390/jof8010077>.
- Lo SC, Hipskind JD, Nicholson RL. cDNA cloning of a sorghum pathogenesis-related protein (PR-10) and differential expression of defense-related genes following inoculation with *Cochliobolus heterostrophus* or *Colletotrichum sublineolum*. *Mol Plant Microbe Interact.* 1999;12:479–89. <https://doi.org/10.1094/MPMI.1999.12.6.479>.
- Van Loon LC, Van Kammen A. Polyacrylamide disc electrophoresis of the soluble leaf proteins from *Nicotiana tabacum* var. ‘Samsun’ and ‘Samsun NN’: II. Changes in protein constitution after infection with tobacco mosaic virus. *Virology.* 1970;40:199–211. [https://doi.org/10.1016/0042-6822\(70\)90395-8](https://doi.org/10.1016/0042-6822(70)90395-8).
- Lynch M, Conery JS. The evolutionary fate and consequences of duplicate genes. *Science.* 2000;290:1151–5. <https://doi.org/10.1126/science.290.5494.1151>.
- Malnoy M, Martens S, Norelli JL, Barny MA, Sundin GW, Smits TH, et al. Fire blight: applied genomic insights of the pathogen and host. *Annu Rev Phytopathol.* 2012;50:475–94. <https://doi.org/10.1146/annurev-phyto-081211-172931>.
- Manners JM. Primitive defence: the MiAMP1 antimicrobial peptide family. *Plant Mol Biol Rep.* 2009;27:237–42. <https://doi.org/10.1007/s11105-008-0083-y>.
- Melchers LS, Apotheker-de Groot M, van der Knaap JA, Ponstein AS, Sela-Buurlage MB, Bol JF, et al. A new class of tobacco chitinases homologous to bacterial exo-chitinases displays antifungal activity. *Plant J.* 1994;5:469–80. <https://doi.org/10.1046/j.1365-3113.1994.05040469.x>.
- Mendez E, Moreno A, Colilla F, Pelaez F, Limas GG, Mendez R, et al. Primary structure and inhibition of protein synthesis in eukaryotic cell-free system of a novel thionin, γ -hordothionin, from barley endosperm. *Eur J Biochem.* 1990;194:533–9. <https://doi.org/10.1111/j.1432-1033.1990.tb15649.x>.
- Meng X, Qiao X, Wu X, Zeng W, Hu Y, Liu C, et al. Genomic characterisation, phylogenetic comparison and candidate gene identification of the lipid transfer proteins gene family in pear (*Pyrus bretschneideri* Rehd.) and other Rosaceae species. *J Hort Sci Biotechnol.* 2023;98:178–93. <https://doi.org/10.1080/14620316.2022.2109519>.
- Molina A, García-Olmedo F. Developmental and pathogen-induced expression of three barley genes encoding lipid transfer proteins. *Plant J.* 1993;4:983–91. <https://doi.org/10.1046/j.1365-3113.1993.04060983.x>.
- Niderman T, Genetet I, Bruyere T, Gees R, Stintzi A, Legrand M, et al. Pathogenesis-related PR-1 proteins are antifungal (isolation and characterization of three 14-kilodalton proteins of tomato and of a basic PR-1 of tobacco with inhibitory activity against *Phytophthora infestans*). *Plant Physiol.* 1995;108:17–27. <https://doi.org/10.1104/pp.108.1.17>.
- Okushima Y, Koizumi N, Kusano T, Sano H. Secreted proteins of tobacco cultured BY2 cells: identification of a new member of pathogenesis-related proteins. *Plant Mol Biol.* 2000;42:479–88. <https://doi.org/10.1023/A:1006393326985>.

- Passardi F, Cosio C, Penel C, Dunand C. Peroxidases have more functions than a Swiss army knife. *Plant Cell Rep.* 2005;24:255–65. <https://doi.org/10.1007/s00299-005-0972-6>.
- Paysan-Lafosse T, Blum M, Chuguransky S, Grego T, Pinto BL, Salazar GA, et al. InterPro in 2022. *Nucleic Acids Res.* 2023;51:D418–27. <https://doi.org/10.1093/nar/gkac993>.
- Qiao X, Li Q, Yin H, Qi K, Li L, Wang R, et al. Gene duplication and evolution in recurring polyploidization-diploidization cycles in plants. *Genome Biol.* 2019;20:1–23. <https://doi.org/10.1186/s13059-019-1650-2>.
- Quirino BF, Noh YS, Himmelblau E, Amasino RM. Molecular aspects of leaf senescence. *Trends Plant Sci.* 2000;5:278–82. [https://doi.org/10.1016/S1360-1385\(00\)01655-1](https://doi.org/10.1016/S1360-1385(00)01655-1).
- Ramirez-Prado JS, Abulfaraj AA, Rayapuram N, Benhamed M, Hirt H. Plant immunity: from signaling to epigenetic control of defense. *Trends Plant Sci.* 2018;23:833–44. <https://doi.org/10.1016/j.tplants.2018.06.004>.
- Ren Y, Armstrong M, Qi Y, McLellan H, Zhong C, Du B, et al. *Phytophthora infestans* RXLR effectors target parallel steps in an immune signal transduction pathway. *Plant Physiol.* 2019;180:2227–39. <https://doi.org/10.1104/pp.18.00625>.
- Shi Y, Zhang Y, Shih DS. Cloning and expression analysis of two β -1,3-glucanase genes from strawberry. *J Plant Physiol.* 2006;163:956–67. <https://doi.org/10.1016/j.jplph.2005.09.007>.
- Shinshi H, Mohnen D, Meins F. Regulation of a plant pathogenesis-related enzyme: inhibition of chitinase and chitinase mRNA accumulation in cultured tobacco tissues by auxin and cytokinin. *Proc Natl Acad Sci USA.* 1987;84:89–93. <https://doi.org/10.1073/pnas.84.1.89>.
- Shinshi H, Wenzler H, Neuhaus JM, Felix G, Hofsteenge J, Meins F. Evidence for N- and C-terminal processing of a plant defense-related enzyme: primary structure of tobacco prepro- β -1,3-glucanase. *Proc Natl Acad Sci USA.* 1988;85:5541–5. <https://doi.org/10.1073/pnas.85.15.5541>.
- Sinha RK, Verma SS, Rastogi A. Role of pathogenesis-related protein 10 (PR10) under abiotic and biotic stresses in plants. *Phyton.* 2020;89:167. <https://doi.org/10.32604/phyton.2020.09359>.
- Soltis DE, Albert VA, Leebens-Mack J, Bell CD, Paterson AH, Zheng C, et al. Polyploidy and angiosperm diversification. *Am J Bot.* 2009;96:336–48. <https://doi.org/10.3732/ajb.0800079>.
- Sooriyaarachchi S, Jaber E, Covarrubias AS, Ubhayasekera W, Asiegbu FO, Mowbray SL. Expression and β -glucan binding properties of Scots pine (*Pinus sylvestris* L.) antimicrobial protein (Sp-AMP). *Plant Mol Biol.* 2011;77:33–45. <https://doi.org/10.1007/s11103-011-9791-z>.
- Takeda S, Sato F, Ida K, Yamada Y. Characterization of polypeptides that accumulate in cultured *Nicotiana tabacum* cells. *Plant Cell Physiol.* 1990;31:215–21. <https://doi.org/10.1093/oxfordjournals.pcp.a077895>.
- Tornero P, Conejero V, Vera P. Primary structure and expression of a pathogen-induced protease (PR-P69) in tomato plants: similarity of functional domains to subtilisin-like endoproteases. *Proc Natl Acad Sci USA.* 1996;93:6332–7. <https://doi.org/10.1073/pnas.93.13.6332>.
- Tsuboi S, Osafune T, Tsugeki R, Nishimura M, Yamada M. Nonspecific lipid transfer protein in castor bean cotyledon cells: subcellular localization and a possible role in lipid metabolism. *J Biochem.* 1992;111:500–8. <https://doi.org/10.1093/oxfordjournals.jbchem.a123787>.
- Van Loon LC. Pathogenesis-related proteins. *Plant Mol Biol.* 1985;4:111–6. <https://doi.org/10.1007/BF02418757>.
- Van Loon LC, Pierpoint WS, Boller TH, Conejero V. Recommendations for naming plant pathogenesis-related proteins. *Plant Mol Biol Reporter.* 1994;12:245–64. <https://doi.org/10.1007/BF02668748>.
- Van Loon LC, Rep M, Pieterse CM. Significance of inducible defense-related proteins in infected plants. *Annu Rev Phytopathol.* 2006;44:135–62. <https://doi.org/10.1146/annurev.phyto.44.070505.143425>.
- Vera P, Conejero V. Pathogenesis-related proteins of tomato: P-69 as an alkaline endoproteinase. *Plant Physiol.* 1988;87:58–63. <https://doi.org/10.1104/pp.87.1.58>.
- Vögeli-Lange R, Fründt C, Hart CM, Beffa R, Nagy F, et al. Evidence for a role of β -1,3-glucanase in dicot seed germination. *Plant J.* 1994;5:273–8. <https://doi.org/10.1046/j.1365-3113.1994.05020273.x>.
- Wang Y, Tang H, Debarry JD, Tan X, Li J, Wang X, et al. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res.* 2012;40: e49. <https://doi.org/10.1093/nar/gkr1293>.
- Wang Y, Tyler BM, Wang Y. Defense and counterdefense during plant-pathogenic oomycete infection. *Annu Rev Microbiol.* 2019;73:667–96. <https://doi.org/10.1146/annurev-micro-020518-120022>.
- Wang Y, Pruitt RN, Nürnberger T, Wang Y. Evasion of plant immunity by microbial pathogens. *Nat Rev Microbiol.* 2022;20:449–64. <https://doi.org/10.1038/s41579-022-00710-3>.
- Wang D, Zhang Y, Zhang Z, Zhu J, Yu J, KaKs_Calculator 2.0: a toolkit incorporating gamma-series methods and sliding window strategies. *Genom Proteom Bioinf.* 2010;8:77–80. [https://doi.org/10.1016/S1672-0229\(10\)60008-3](https://doi.org/10.1016/S1672-0229(10)60008-3).
- Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu S, et al. The genome of the pear (*Pyrus bretschneideri* Rehd.). *Genome Res.* 2013;23:396–408. <https://doi.org/10.1101/gr.144311.112>.
- Young RJ, Scheuring CF, Harris-Haller L, Taylor BH. An auxin-inducible proteinase inhibitor gene from tomato. *Plant Physiol.* 1994;104:811. <https://doi.org/10.1104/pp.104.2.811>.
- Zhang N, Li R, Shen W, Jiao S, Zhang J, Xu W. Genome-wide evolutionary characterization and expression analyses of major latex protein (MLP) family genes in *Vitis vinifera*. *Mol Genet Genomics.* 2018;293:1061–75. <https://doi.org/10.1007/s00438-018-1440-7>.
- Zhou JM, Zhang Y. Plant immunity: danger perception and signaling. *Cell.* 2020;181:978–89. <https://doi.org/10.1016/j.cell.2020.04.028>.
- Zribi I, Ghorbel M, Brini F. Pathogenesis related proteins (PRs): from cellular mechanisms to plant defense. *Curr Protein Pept Sci.* 2021;22:396–412.

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