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Comparative transcriptome analysis reveals defense responses against soft rot induced by *Pectobacterium aroidearum* and *Pectobacterium carotovorum* in *Pinellia ternata*

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Abstract

Pectobacterium carotovorum and *Pectobacterium aroidearum* represent the primary pathogens causing variable soft rot disease. However, the fundamental defense responses of *Pinellia ternata* to pathogens remain unclear. Our investigation demonstrated that the disease produced by *P. carotovorum* is more serious than *P. aroidearum*. RNA-seq analysis indicated that many cell wall-related genes, receptor-like kinase genes, and resistance-related genes were induced by *P. aroidearum* and *P. carotovorum* similarly. But many different regulatory pathways exert a crucial function in plant immunity against *P. aroidearum* and *P. carotovorum*, including hormone signaling, whereas more auxin-responsive genes were responsive to *P. carotovorum*, while more ethylene and gibberellin-responsive genes were responsive to *P. aroidearum*. 12 GDSL esterase/lipase genes and 3 fasciclin-like arabinogalactan protein genes were specifically upregulated by *P. carotovorum*, whereas 11 receptor-like kinase genes and 8 disease resistance genes were up-regulated only by *P. aroidearum*. Among them, a lectin gene (part1transcript/39001) was induced by *P. carotovorum* and *P. aroidearum* simultaneously. Transient expression in *N. benthamiana* demonstrated that the lectin gene improves plant resistance to *P. carotovorum*. This study offers a comprehensive perspective on *P. ternata* immunity produced by different soft rot pathogens and reveals the importance of lectin in anti-soft rot of *P. ternata* for the first time.

Keywords *Pinellia ternata*, Soft rot, Defense response, *Pectobacterium aroidearum*, *Pectobacterium carotovorum*, Lectin gene

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Background

Pinellia ternata (Thunb.) Breit (*P. ternata*), whose dry tuber is a traditional Chinese medicine (TCM) in Chinese Pharmacopoeia, has an important function in the treatment of cough, vomiting, infection, inflammation, early pregnancy, and tumors [1–3]. It has been employed as a TCM for millennia, traced back to the Han Dynasty in many notable prescriptions. With increased demand, *P. ternata* has been produced via artificial planting, causing continuous cropping barriers and frequent disease [4].

Among the frequent diseases identified, soft rot is one of the most destructive in *P. ternata*. Symptomatic plants have small water-soaked spots on leaves that progress into widespread translucent spots, spreading to the stems and tubers [5, 6]. Soft rot is responsible for significant agricultural losses in *P. ternata*. The pathogens responsible for soft rot in *P. ternata* were found to be *Dickeya fangzhongdai* (*D. fangzhongdai*), *Pectobacterium carotovorum* (*P. carotovorum*), *Pectobacterium atrosepticum* (*P. atrosepticum*), and *Pectobacterium aroidearum* (*P. aroidearum*) [6–9]. To limit the invasion of soft rot, increasing chemical fungicides were employed to protect *P. ternata*, leading to pesticide residues and environmental pollution. Therefore, it is essential to understand the defense responses of *P. ternata* to soft rot invasion to develop green, effective, and economical strategies to control soft rot and breed for disease resistance.

Plant immunity produced by microorganisms can be described by a "zigzag model" [10]. In the early stage of infection, pathogen-associated molecular patterns (PAMPs), recognized by pattern recognition receptor (PRRs) complexes at the plasma membrane, activate downstream immune regulation of the host plant, called PAMPs triggered immunity (PTI) [11]. Recognition of PAMPs leads to a series of signaling events, commonly referred to as the basal defense response, which activates the plant's systemically acquired resistance [12]. In this process, mitogen-activated protein kinase (MAPK), receptor kinase, and phosphorylase are also activated, reactive oxygen species (ROS) are generated, accompanied by hormone biosynthesis and callose deposition [13]. However, when pathogens exhibit a tendency to adapt to the host plant, a series of virulence factors that interfere with the PTI of the plant, called effectors, contribute to pathogen infection. Meanwhile, a series of resistance proteins (R proteins) shows a predominant role in plant immunity. R proteins activate downstream immune regulation by recognizing pathogen effectors, limiting the infection and diffusion of pathogens [14]. The recognition of effectors is specific in effector-triggered immunity (ETI), which supports the hypothesis of "gene to gene" to defend different pathogens specifically.

Plant resistance to soft rot does not necessarily depend on a single resistance gene but activates defense systems mediated by SA, JA, and ethylene following recognition of damage-associated molecular patterns (DAMPs), including oligogalacturonide (OG) fragments [15]. PRRs identify the invasion of pathogens, and activate the hormone signal to promote the expression of downstream defense genes (CPK, CML, RBOH, MPK3, and MPK4) alongside the biosynthesis of indole-thioglucoside and other secondary metabolites, promoting plant resistance [16, 17]. Phenylalanine ammonia lyase-dependent and salicylic acid-mediated host resistance is the core of plant immunity [18]. Throughout this process, ferredoxin-like protein (pflp) is essential in conferring resistance against soft rot disease by accelerating the rapid production of H_2O_2, callose deposition, and hypersensitivity reaction [19–21]. Transcription factors also play important roles in plant resistance. Overexpression of *WRKY12* increases the resistance to soft rot pathogen by transcriptional activation of defense-related genes [22, 23], but could also be a negative regulator of plant immunity against pathogens [24]. Transcription factors, plant hormones, MAPK signal transduction, and resistance-related genes form a comprehensive defense system in plants against soft rot disease.

Plant lectins are proteins with functions in immune regulation, bactericidal responses, and anti-inflammatory reactions, and can be separated into ten classes according to motif conformation, namely the Agaricus bisporus agglutinin (ABA), the Amaranthin domain, the chitinase-related agglutinin (CRA), the Cyanovirin domain, the Galanthus nivalis agglutinin (GNA), the Hevein domain, the Jacalin-related domain, the Legume lectin domain, the Lys M domain, and the Ricin-B domain [25]. Lectins bind to carbohydrates on their surface to damage the cell walls of pathogens, especially bacteria, fungi, and protozoa, preventing the microorganism from attaching to the host cell [26]. A unique mannose-binding plant lectin from *Narcissus tazetta* bulbs, NTL-125, is a potential antiviral compound of natural origin against SARS-CoV-2 [27]. A lectin extracted from the fish species *Misgurnus anguillicaudatus* possessed significant agglutinating activity against gram-negative bacteria, resulting from its relationship with lipopolysaccharides (LPS) [28]. Additionally, lectin could also interact with chitin to exert antifungal effects [29]. These mechanisms are common to most lectins and explain how these molecules inhibit the formation of biofilms and bacterial aggregates [30]. Jacalin-like lectins (JRLs) are involved in mediating broad-spectrum disease resistance to monocotyledonous plants by binding to oligosaccharide signatures characteristic of the infection process to relocate the protein towards the location of pathogen attack [31]. Lectin is an important feature of Araceae plants [32],

belonging to mannose-binding modules in monocotyledonous plants, which can bind specifically to mannooligosaccharides [33]. However, there is limited research on lectins in *P. ternata* and their role in plant immunity and resistance to pathogens.

In this study, RNA-seq was employed to characterize candidate genes of *P. ternata* "Ying Shan" induced by virulent bacteria *P. carotovorum* QJ-1 and mild virulent bacteria *P. aroidearum* QS-1. Differentially expressed genes (DEGs) analysis and Gene Ontology (GO) analysis were conducted to identify key genes and core signal pathways in plant immunity. Quantitative real-time PCR (qRT-PCR) and transient expression were conducted to explore the induced expression patterns and potential biological function of key genes. This study provides integrated and basic data regarding the immunity of *P. ternata* induced by *Pectobacterium*, laying the foundation for further exploration of the *P. ternata-Pectobacterium* molecular interaction.

Results

Disease produced by *P. carotovorum* is more severe than by *P. aroidearum*

P. carotovorum and *P. aroidearum* are soft rot pathogens in *P. ternata*. In our prior work, *P. carotovorum* QJ-1 and *P. aroidearum* QS-1 were extracted from symptomatic plants and then configured as a bacterial suspension with a concentration of $OD_{600}=1.0$. Infection of whole *P. ternata* "Yingshan" plants were conducted to examine their virulence. Water-soaked spots and beating down were observed at 48 hpi following treatment with *P. aroidearum* and *P. carotovorum*, respectively, compared to water treatment (Fig. 1A). Additionally, inoculation on detached leaves of *P. ternata* "Yingshan" was performed, and the disease spot was observed and calculated every 24 h. The results indicated that larger disease lesions were observed with exposure to *P. carotovorum* compared to *P. aroidearum* (Fig. 1B and C). It demonstrated that although both *P. carotovorum* QJ-1 and *P. aroidearum* QS-1 are pathogens producing soft rot, the pathogenicity of *P. carotovorum* QJ-1 is significantly higher than *P. aroidearum* QS-1.

RNAseq and differentially expressed gene analysis

The virulence of *P. carotovorum* is stronger than *P. aroidearum*, motivating us to evaluate the similarities and differences in immunity response triggered by *P. carotovorum* and *P. aroidearum*. To investigate gene expression changes under the treatment of *P. aroidearum* and *P. carotovorum*, leaves from inoculated plants for 48 h were collected for RNA isolation and RNA-seq. In total, 269.69 million raw paired-end reads were acquired from

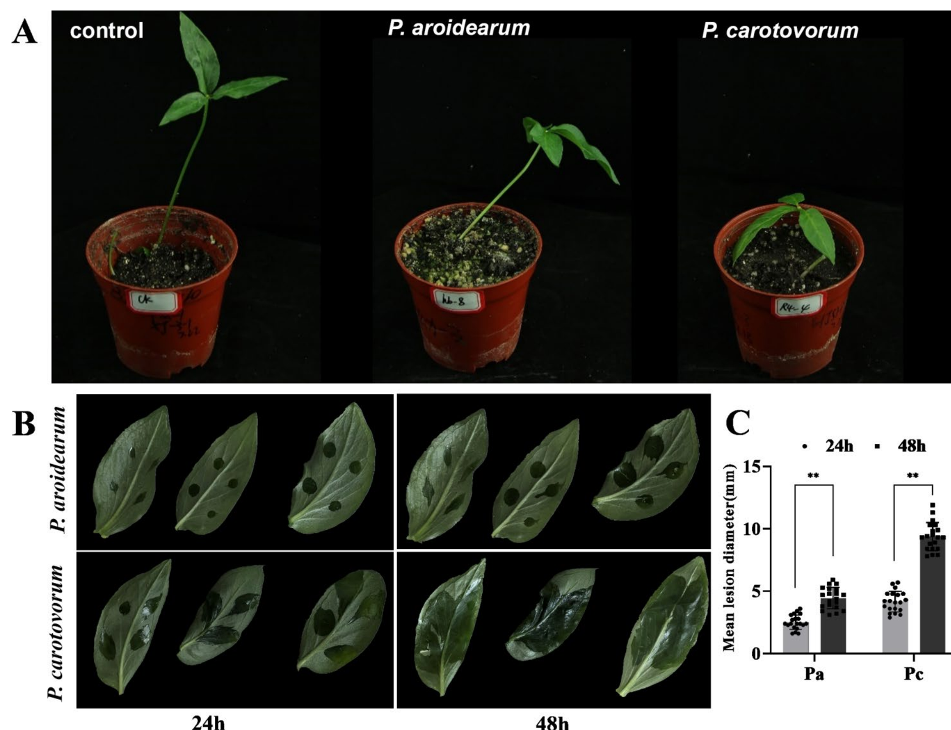

Fig. 1 Infection with *P. aroidearum* and *P. carotovorum* of *P. ternata*. (**A**) Whole plant of *P. ternata* infected with *P. aroidearum* and *P. carotovorum* for 48 h. (**B**) Detached leaves of *P. ternata* inoculated with *P. aroidearum* and *P. carotovorum*. Images were photographed at 24 hpi and 48 hpi under normal light. (**C**) Statistic of mean lesion diameter. The disease lesion diameter was measured every 24 h from at least 20 leaves with three replication (one-way ANOVA, **, $p<0.01$). Error bars indicate SD. Pa means the infection with *P. aroidearum*, Pc means the infection with *P. carotovorum*

12 RNA samples. Following quality assessment and filtering, 264.4 million clean paired-end reads were retained (Table S1). All clean reads were mapped to the full-length transcript assembled in our previous work using bowtie2, and 21,464 transcripts were constructed in total. The expression of transcripts was computed using rsem. The FPKM value was calculated and shown in Table S1, and the correlation between the 12 samples was shown with a heatmap (Fig. S1). Nine genes were randomly selected for qRT-PCR to validate the expression (Fig. S2).

To investigate the differentially expressed genes triggered by *P. aroidearum* and *P. carotovorum*, DEGs were identified by EBSeq. A total of 1968 transcripts were upregulated and 570 transcripts were downregulated by *P. aroidearum*, while 1339 upregulated transcripts and 662 downregulated transcripts were triggered by *P. carotovorum* (Fig. 2A and B). Many common immunity responses were triggered by *P. aroidearum* and *P. carotovorum*, 628 transcripts were upregulated, and 262 transcripts were downregulated commonly (Fig. 2C). To clarify common immunity induced in the progress, the classification of common DEGs was shown in Fig. 2D and Table S2. Many cell wall-related genes were upregulated by the infection of pathogen to against the damage of cell wall, several receptor kinase genes and resistance-related

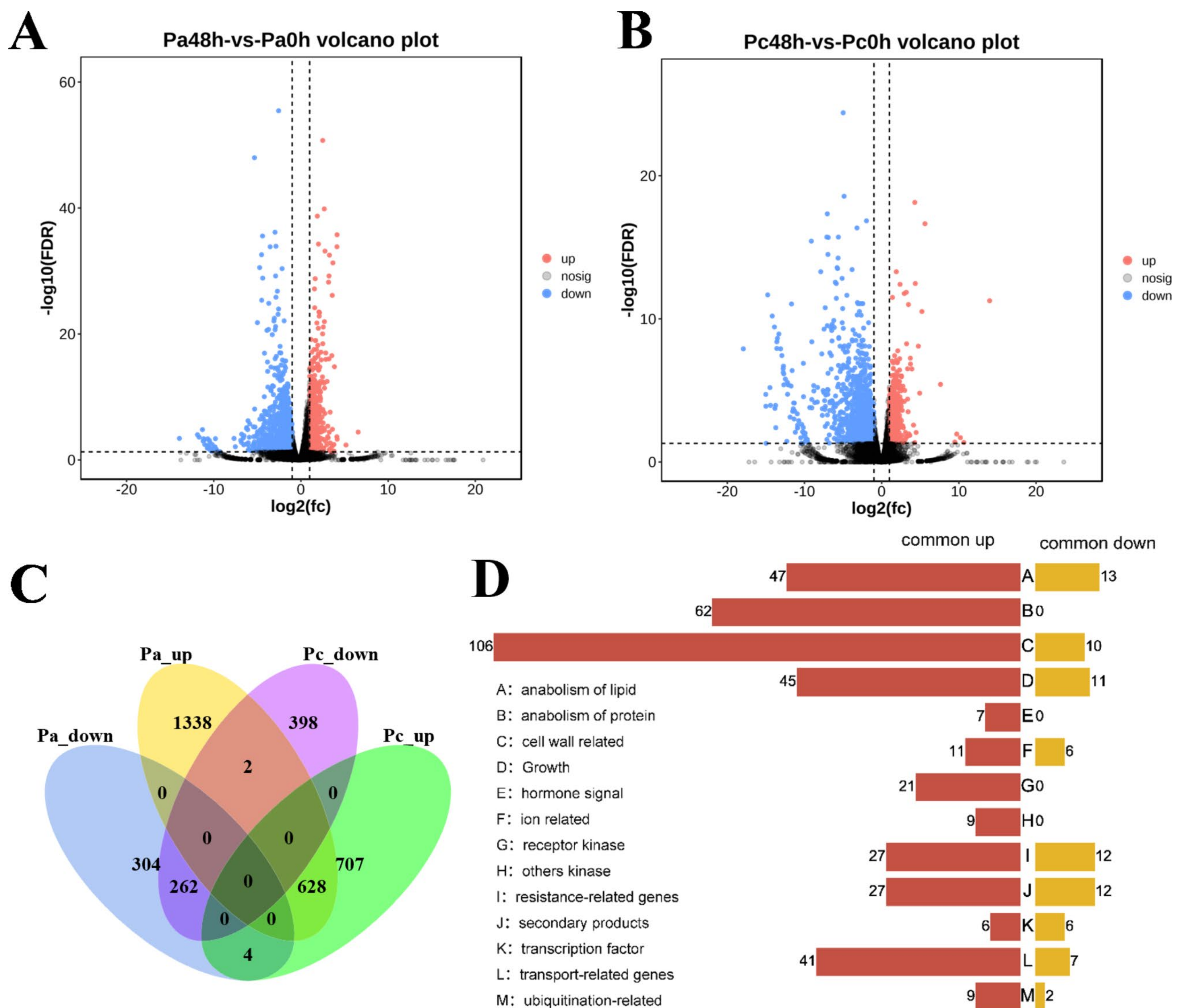

Fig. 2 The DEGs of *P. ternata* induced by *P. aroidearum* and *P. carotovorum*. (**A**) Volcano plot of the differentially expressed genes induced by *P. aroidearum*. (**B**) Volcano plot of the differentially expressed genes induced by *P. carotovorum*. The red spots mean up-regulated genes, blue spots mean down-regulated genes, black spots mean no-diff genes. Pa means *P. aroidearum* QS-1 infection and Pc means *P. carotovorum* QJ-1 infection. (**C**) Venn graph of DEGs inoculated by *P. aroidearum* and *P. carotovorum*. (**D**) The classification of common DEGs induced by *P. aroidearum* and *P. carotovorum*. Pa means *P. aroidearum* QS-1and Pc means *P. carotovorum* QJ-1. Common up means common up-regulated genes induced by *P. aroidearum* and *P. carotovorum*. Common down/up means common down/up-regulated genes induced by *P. aroidearum* and *P. carotovorum*

genes were upregulated to defend against the invasion of pathogens.

GO and KEGG analysis of the DEGs triggered by *P. aroidearum* and *P. carotovorum*

To assess the common signal pathways regulated by soft rot, GO analysis of 628 common DEGs was conducted (Table S3). Genes involved in cellulose biosynthetic (GO:0030244) and fatty acid biosynthetic (GO:0006633) processes were induced by pathogen infection, and genes associated with channel activity (GO:0015267), transferase activity, transferring acyl groups other than amino-acyl groups (GO:0016747), and cellulose synthase (UDP-forming) activity (GO:0016760) were activated to enhance plant immunity. Moreover, genes associated with membranes (GO:0031225), apoplasts (GO:0048046), and cell walls (GO:0005618) were also highly expressed to defend the pathogen invasion (Fig. 3A). In contrast, Rho guanyl-nucleotide exchange factor activity (GO:0005089), catalase activity (GO:0004096), and oxidoreductase activity, acting on NAD(P)H (GO:0016651) were suppressed under the perception of pathogens (Fig. 3B). These findings demonstrated that various different types of genes and related signaling pathways constitute the basic immune defense of *P. ternata* to soft rot, genes involved in cell wall, membrane, and oxidoreductase functions are the core of plant immunity.

GO analysis was also conducted on specific DEGs induced by *P. carotovorum* and *P. aroidearum* (Fig. S3). The findings indicated that genes involved in hydrolase, hydrolyzing O-glycosyl compounds, xyloglucan: xyloglucosyl transferase activity, and cell wall were upregulated, and genes involved in enzyme inhibitor activity and translation initiation factor activity were downregulated with exposure to *P. carotovorum*. Hydrolase and xyloglucan: xyloglucosyl transferase plays an essential role in the degradation of the cell walls. Large watery lesions appear on the leaves of *P. ternata* at 48 hpi, the cells of which are collapsed, releasing enzymes to degrade the cell walls of *P. ternata*. Additionally, genes involved in fatty acid beta-oxidation, calcium-dependent phospholipid binding, and catalase activity were upregulated upon treatment with *P. aroidearum*, while genes involved in carbohydrate metabolic process, and integral components of membranes were downregulated. These results suggested that many defense pathways of *P. ternata* were induced by *P. aroidearum* to resist pathogen infection, and genes related to the membrane were inhibited by *P. aroidearum* to facilitate destruction.

KEGG analysis was also conducted to explore the secondary metabolites biosynthesis, the result found that genes involved in ko01130 (Biosynthesis of antibiotics) were significantly induced by *P. aroidearum*. Genes involved in ko00940 (phenylpropanoid biosynthesis), ko00941 (Flavonoid biosynthesis), ko00945 (Stilbenoid, diarylheptanoid and gingerol biosynthesis), were significantly induced by *P. carotovorum* (Table S4).

Differential signally pathways were induced by *P. aroidearum* and *P. carotovorum*

In addition to these common defense genes, 398 genes were downregulated and 707genes were upregulated by *P. carotovorum*, while 1338 upregulated genes and 304 downregulated genes were induced by *P. aroidearum* (Fig. 2C and Table S5). Plant hormones emerged as cellular signaling molecules with crucial functions in regulating immune responses to microbial pathogens. In our study, different hormone signaling was induced by *P.*

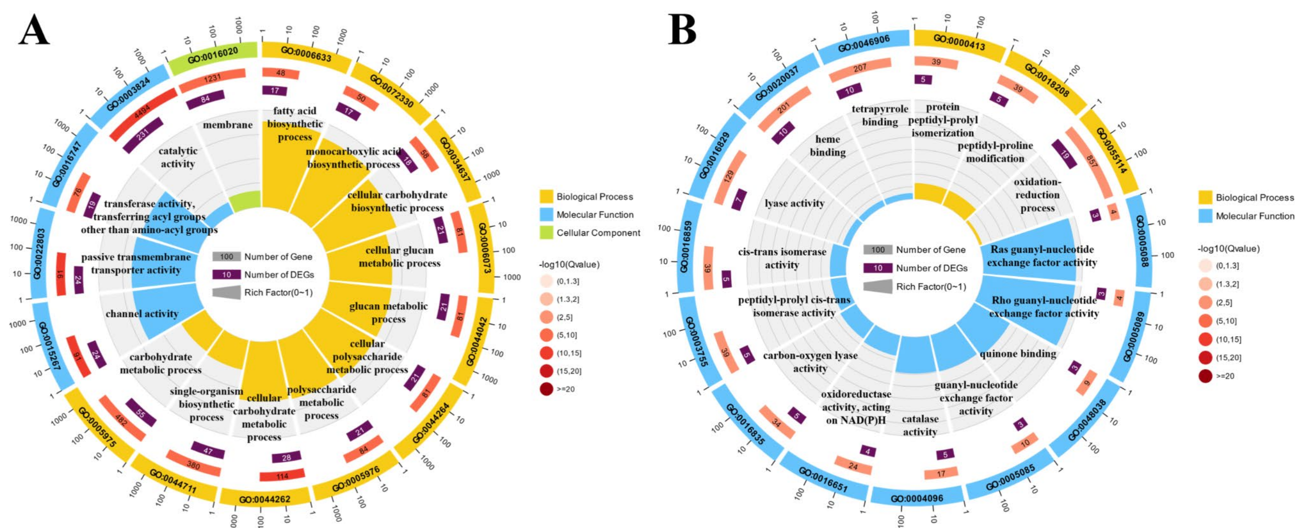

Fig. 3 GO analysis of common DEGs triggered by *P. aroidearum* and *P. carotovorum*. (**A**) GO analysis of the 628 common up-regulated DEGs. (**B**) GO analysis of 262 the common down-regulated DEGs

aroidearum and *P. carotovorum*. Eight auxin-responsive proteins were upregulated significantly by *P. carotovorum*, indicating that auxin-mediated immunity plays an important role in defending against *P. carotovorum*. Additionally, five ethylene-responsive genes and four gibberellin-responsive genes were specifically upregulated by *P. aroidearum* (Fig. 4A and Table S5), and qRT-PCR was conducted to verified the expression (Fig. S4).

In addition to hormone signaling, many other specific immune signals were induced by *P. carotovorum* and *P. aroidearum*. A total of 12 GDSL esterase/lipase genes and three fasciclin-like arabinogalactan protein genes were upregulated by exposure to *P. carotovorum*, whereas 11 receptor-like kinases (RLKs), and eight disease resistance genes were upregulated only by *P. aroidearum* (Fig. 4B). This finding indicated that different degrees of disease occurred under the infection by two pathogens due to the expression of different genes.

34 lectin genes differentially expressed in *P. ternata* induced by *P. carotovorum* and *P. aroidearum*

Lectin is an important ingredient in *P. ternata*, and it inhibits bacteria and kills insects. In our study, 34 lectin genes were differentially expressed in response to the invasion of pathogens. The structure of the 34 lectin proteins was analyzed, demonstrating that these lectin genes were primarily separated into three classes: class I, which consists of one B-lectin domain; class II, which consists of two similar B-lectin domains; class III, which consists of two different B-lectin domains (Fig. 5). We also found that most lectin genes were highly expressed upon *P. aroidearum* infection, which may be due to the participation of lectins in plant immunity against pathogen infection, while half of them were suppressed under infection by *P. carotovorum* (Fig. 5), resulting from the damage of plant tissues, causing a decrease in lectin content.

Lectin gene inhibited the infection of *P. carotovorum*

A lectin gene (part1transcript/39001) was chosen for functional verification, which is up-regulated by *P. aroidearum* and *P. carotovorum* (Fig. 6A). *Agrobacterium*-mediated transient expression of the lectin gene was conducted in *N. benthamiana* leaves, with the empty vector (EV) expressing GFP protein used as a control. After 48 h of agro-infiltration, the lectin gene and empty vector were expressed in *N. benthamiana* leaves at 48 hpi (Fig. 6B). The leaves were detached and inoculated with *P. carotovorum* QJ-1 at a concentration of $OD_{600} = 1$. The disease lesion diameter was evaluated at 48 hpi. The findings showed that transient expression of the lectin gene significantly inhibited *P. carotovorum* colonization, reflected by smaller lesion diameters compared to the EV control (Fig. 6C and D). The above results demonstrated that the lectin gene positively regulates plant defense response against soft rot.

Discussion

Soft rot is a critical disease in *P. ternata* production, which is primarily caused by *P. carotovorum* and *P. aroidearum*. However, different virulence is exerted by these two pathogens, which has been confirmed in our study (Fig. 1). Comparative genomics determined that *P. carotovorum* encodes more plant cell wall degrading enzymes and effectors than *P. atrosepticum*, including hrpK-like type III secretion system-dependent effector protein, resulting in the stronger pathogenicity of *P. carotovorum*

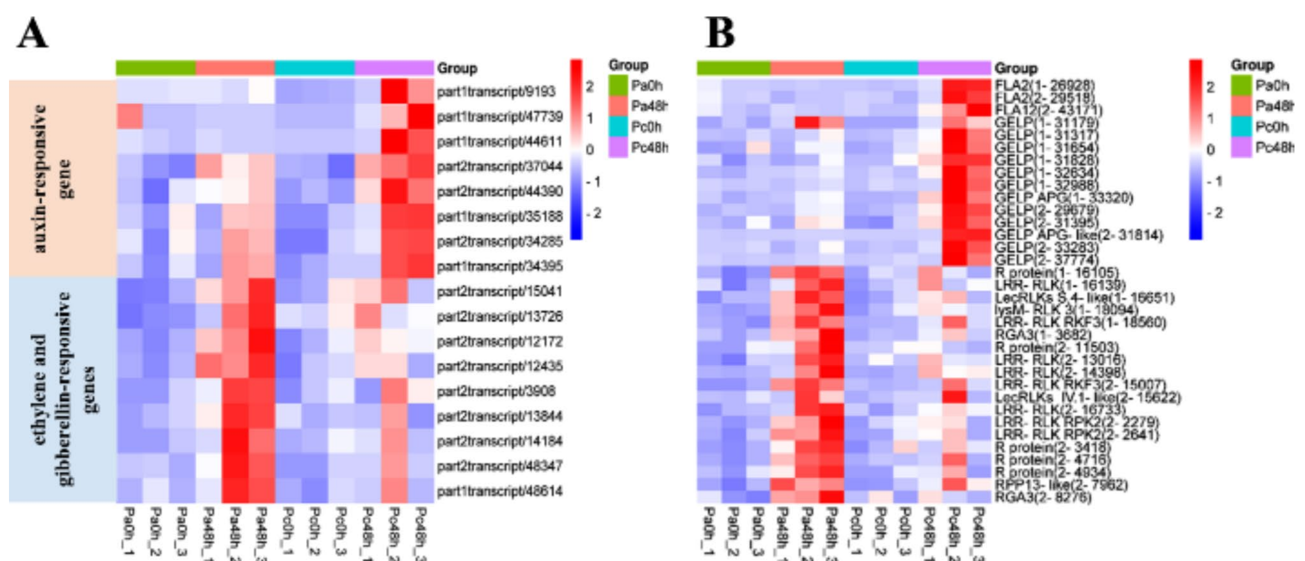

Fig. 4 Heatmap of genes involved in different pathway induced by *P. aroidearum* and *P. carotovorum*. (**A**) Heatmap of genes involved in different hormone signaling pathway. (**B**) Heatmap of key genes involved in other specific resistance signaling pathway. FPKM value was used for the heatmaps, and Z-score normalization method was applied

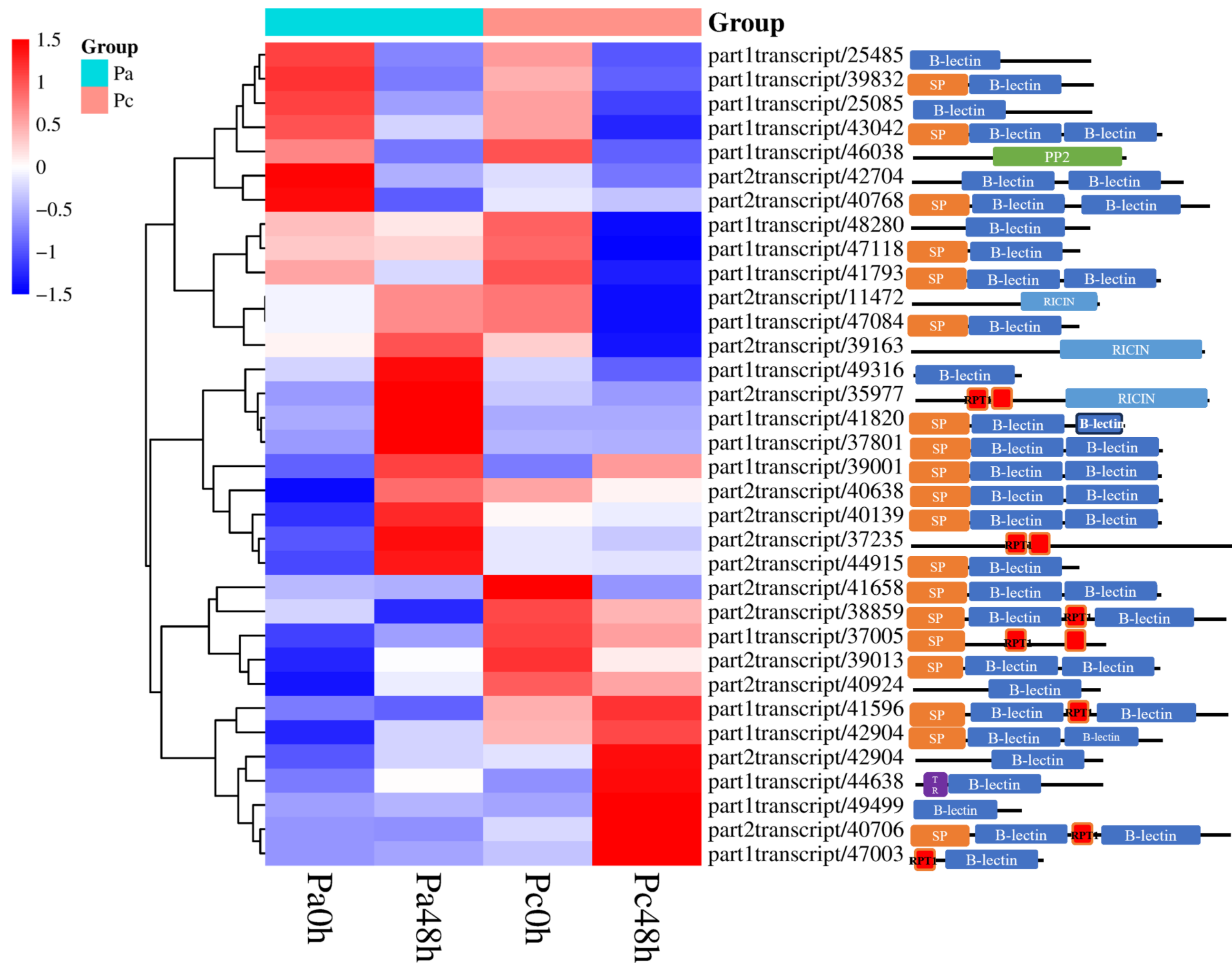

Fig. 5 Heatmap and structure of 34 lectin genes differentially expressed in *P. ternata* induced by *P. carotovorum* and *P. aroidearum*. FPKM value was used and Z-score method was applied

[34]. The same phenomenon appears in potatoes, with more damage caused by *P. carotovorum* than by *P. atrosepticum* [35]. One of the most important pathogenic factors are exoenzymes, including pectate lyase (pel), polygalacturonase (PG), protease (Prt), and cellulase (cel), that adhere to and destroy plant cell walls by degrading pectin, resulting in the water stain presentation [36].

To investigate the basic and variable immunity of *P. ternata* induced by *P. carotovorum* and *P. aroidearum*, RNA-seq was performed in this study. It found that genes involved in the regulation of cell walls, receptor-like kinase genes, and resistance-related genes were induced by both pathogens, forming the basic defense system of plants. Receptor-like kinase genes are elements of a critical signaling pathway for plant immunity (Table S1). For instance, BAK1, belonging to the LRR receptor-like protein kinase family, interacts with BRI1 to modulate brassinosteroid signaling [37]. FLS2 is an LRR receptor-like protein kinase that senses bacterial flagellin to activate plant immunity [38]. EDS1-PAD4-ADR1, a kind of LRR receptor kinase, mediates pattern-triggered immunity in *Arabidopsis* [39]. OsLRP, a leucine-rich repeat (eLRR) domain protein, has been introduced into Chinese cabbage, exhibiting enhanced disease resistance to bacterial soft rot [40]. Different classes of genes, such as WRKY transcription factors, receptor-like protein kinase, LRR domain proteins, and genes involved in the MAPK pathway, form the basis of plant immunity against soft rot. In this study, 219 R genes expressed in our study, and most of them have the same expression pattern (Table S1). 8 R genes were upregulated only by *P. aroidearum* (Table S5). And 64 WRKY genes were identified in RNAseq data (Table S1). *WRKY40* and *WRKY76* were induced by *P. carotovorum*. *WRKY50*, *WRKY75*, *WRKY24-like* were induced by *P. aroidearum* (Table S5).

Plant hormones have critical roles in the regulation of plant growth and development. They also have key functions in the regulation of immune responses to microbial

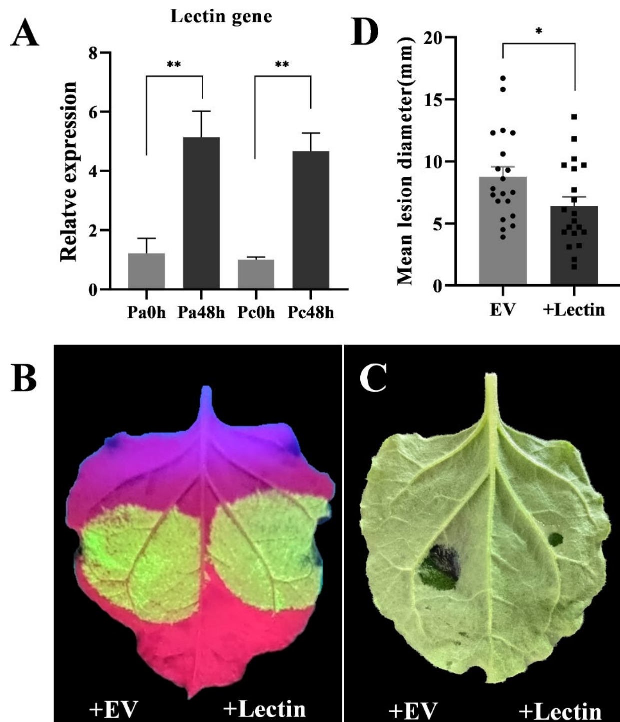

Fig. 6 Transient expressing the lectin gene to verify their function in regulation of soft rot resistance. (**A**) qRT-PCR verified that the lectin gene was higher expressed with *P. aroidearum* and *P. carotovorum* induction. Gene expression levels were analyzed by the $2^{-\triangle\triangle CT}$ method with *18S* as the reference gene. One-way ANOVA, **, $p<0.01$. Error bars represent mean ± SD of 3 biological replicates. (**B**) Lectin protein and GFP protein expressed in *N. benthamiana* leaves at 48 hpi, the leaf photos were taken under UV-light. (**C**) The infection with *P. carotovorum* QJ-1 on leaves transiently expressed the lectin gene and EV for 48 h, the leaf photos were taken at 48 hpi. (**D**) Disease lesion on *N. benthamiana* leaves was measured 2 d after *P. carotovorum* QJ-1 inoculation, one-way ANOVA, *, $p<0.05$, 3 biological replicates with 20 leaves from 6–7 plants at least for each replicate. Error bars represent mean ± SD. Pa means *P. aroidearum* QS-1 and Pc means *P. carotovorum* QJ-1

pathogens [41]. Li et al. have demonstrated that MeJA could display improved performance in enhancing the resistance to disease in kiwifruit by regulating the phenylpropanoid and jasmonate pathways [42]. Jasmonate regulates plant resistance to *P. brasiliense* and *D. dadantii* by regulating indole glucosinolate biosynthesis [17, 43]. Abscisic acid deficiency causes rapid activation of tomato defense responses upon infection with *Erwinia chrysanthemi* [44]. N-3-Oxo-Octanoyl Homoserine Lactone primes plant resistance against the necrotrophic pathogen *P. carotovorum* by coordinating jasmonic acid and auxin-signaling pathways [45]. In our study, many genes responsive to plant hormones were substantially induced. Notably, some differences appeared in the hormone signaling induced by *P. aroidearum* and *P. carotovorum*. Elevated amounts of auxin-responsive protein genes were induced by *P. carotovorum*, and more ethylene and gibberellin-responsive protein genes were induced by *P. aroidearum* (Table S1). The conclusion that the auxin-signaling pathway figures prominently in plant resistance to *P. carotovorum* mirrors previous works [16, 46]. In addition, several studies suggest that ethylene and gibberellin pathways play a necessary role in plant immunity against other *Pectobacterium* species. Narváez-Barragán et al. have demonstrated that Expansin-like Exl1 from *P. resilience* and *P. atrosepticum* is a virulence factor, producing a plant immunity response in ROS, and jasmonate, ethylene, and salicylic acid signaling pathways in *Arabidopsis thaliana* [47]. *ERF96* is a key player in the ERF network that positively regulates *Arabidopsis* resistance responses to necrotrophic pathogens [48]. *GLIP1* functions independently of salicylic acid but requires ethylene signaling [49]. Potato gibberellin stimulated-like 2 (*GSL2*) gene in transgenic potatoes enhances resistance to blackleg disease produced by *P. atrosepticum* [50]. Meanwhile, many genes in JA and SA signalling pathways were identified, part1transcript/44,968 (23 kDa jasmonate-induced protein) was expressed higher under the infection of *P. atrosepticum*. But part2transcript/15,083, part2transcript/15,903 (jasmonic acid-amido synthetase JAR1-like), part2transcript/34,625 (jasmonate O-methyltransferase), part1transcript/44,416 (salicylic acid-binding protein 2-like) were identified without significantly difference.

GDSL esterase/lipase and asciclin-like arabinogalactan (FLA) genes, contributing to plant growth, were induced by *P. carotovorum* specifically to repair the severe damage to plants. Receptor-like kinases and disease-resistance genes, promoting plant immunity against pathogens, were induced by *P. aroidearum* specifically to avoid the further invasion of *P. aroidearum*, resulting in smaller spots on leaves (Fig. 1B). Many studies have confirmed that GDSL esterase/lipase (GELP) modulates plant immunity through lipid homeostasis by fatty acid degradation [51, 52]. Meanwhile, *FLA* genes, specifically situated in sclerenchyma cells [53], were required for stem development [54]. These genes were induced by *P. carotovorum* to defend against the pathogen infection by lipid homeostasis and stem development. RLKs play a central role in signaling during pathogen recognition, subsequent activation of plant defense mechanisms, and developmental control [55]. Disease-resistance protein genes are necessary for plants to avoid further infection by pathogens, playing a crucial role in plant immunity.

Lectins are fundamental to plant life and have necessary roles in cell-to-cell communication, constituting versatile recognition systems at the cell surface and contributing to the detection of symbionts and pathogens [56]. In this study, a lectin gene was chosen from common DEGs to validate its defensive function with *P. carotovorum* treatment. RNA-seq and qRT-PCR demonstrated that the lectin gene is highly expressed upon treatment

with *P. aroidearum* and *P. carotovorum* (Fig. 6A). Functional verification indicated that the transient expression of the lectin gene in *N. benthamiana* decreases the lesion damage by *P. carotovorum*, demonstrating that the lectin gene elevates the immune response against *P. carotovorum* (Fig. 6C and D). Furthermore, a down regulated lectin gene (part1transcript25085) was also selected to verify the function, and the same result was obtained (Fig. S5). Expression of the *Pinellia pedatisecta* lectin gene in transgenic wheat enhances resistance to wheat aphids [57]. Rice, wheat, and barley plants overexpressing OsJAC1, a member of Jacalin-like lectins, are resistant to several fungal pathogens [58]. These results demonstrate that the lectin genes benefit plant immunity against *P. aroidearum* and *P. carotovorum*, providing guidance and promising practices to unravel the molecular foundation of plant immunity.

Conclusion

Soft rot is a devastating disease in *P. ternata* and other plants, with a great impact on their yield and quality. The soft rot pathogens in *P. ternata* included *P. aroidearum* and *P. carotovorum*, causing varying degrees of disease. RNA-seq analysis of *P. ternata* following exposure to each pathogen showed that they could cause the differential expression of a large number of cell wall membrane-related genes, transport-related genes, MAPK pathway-related genes, and hormone response genes, and these regulatory networks formed the broad-spectrum immune mechanism of *P. ternata* in response to bacterial diseases. However, many functional genes are specifically regulated by *P. aroidearum* and *P. carotovorum*. For instance, more auxin-responsive genes and growth-related genes are induced by *P. carotovorum*, while more ethylene-responsive genes, gibberellin-responsive genes, and disease-resistance-related genes are regulated by *P. aroidearum*, which may be responsible for the variable degrees of soft rot in *P. ternata*. Lectins are a class of important proteins in *P. ternata*. The transient expression of the lectin gene indicated that the lectin protein could enhance plant resistance to soft rot. In this study, the role of lectins in plant resistance to bacterial diseases was investigated for the first time. Future studies will focus on the molecular mechanisms involved in how lectin gene regulate host immunity to promote the green prevention and control of *P. ternata* production.

Materials and methods

Plant and pathogen material

P. ternata "Yingshan" specimens were collected on April 25, 2022, at Hubei University of Chinese Medicine and authenticated by Prof. Liu Dahui (voucher No. Yingshan202204), and stored in the medical plants garden of Hubei University of Chinese Medicine. Tissue culture seedlings were acclimated for 15 days in floating dishes, then transferred to pots and grown under controlled conditions (24 °C; 12 h of light/12 hours of dark; 60% humidity) for 30 days.

P. carotovorum QJ-1 and *P. aroidearum* QS-1 were previously isolated and identified [9], and stored at -80 ℃.

The *N. benthamiana* seeds used in this work were preserved in our laboratory in Hubei University of Chinese Medicine. The seeds of *Nicotiana benthamiana* were grown under standard conditions (24 °C; 12 h light/12 h dark photoperiod; and 60% relative humidity) in a chamber for approximately 4 weeks for transient expression and pathogen inoculation.

The extraction of RNA and inverse transcription

Total RNA was extracted using TRIzol reagent and quality-assessed using an RNA Nano 6000 Assay Kit (Bioanalyzer 2100, Agilent Technologies, CA, USA). Reverse transcription was performed using Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV RT; Promega, USA).

Library preparation and transcriptome sequencing

mRNA was purified from total RNA using poly-T oligo-attached magnetic beads and fragmented using divalent cations under elevated temperatures in First Strand Synthesis Reaction Buffer (5×). cDNA fragments 370 to 420 bp in length were selected using the AMPure XP approach (Beckman Coulter, Beverly, USA). Libraries were PCR-amplified, purified using AMPure XP beads, pooled according to their concentration, and sequenced on an Illumina NovaSeq 6000 (150-bp paired-end reads).

Raw reads in fastq format were quality-checked using FastQC and trimmed using Trimmomatic [59]. Clean reads were obtained by discarding reads containing adapters, N bases, and low-quality reads. Simultaneously, Q20, Q30, and GC content of the clean data were calculated. All downstream analyses were based on clean, high-quality data.

Differentially expressed gene (DEG) analysis and enrichment analysis

Paired-end clean reads were mapped to the full-length transcript (PRJNA893095) from our previous work using bowtie2 with a 1% mismatch [60]. Calculations and differentialy expression analysis (FDR < 0.05 and the |log2 (fold change)| > 1) were performed with rsem [61]. The GO and KEGG analysis were conducted using the OmicShare tools, a free online platform for data analysis (http://www.omicshare.com/ tools).

PCR and qRT-PCR

Standard PCR was performed using 2 x Es Taq MasterMix (CWBIO, China). For qRT-PCR, cDNA was

synthesized using reverse transcriptase M-MLV (RNase H-) (Code No.: 2641 A) from TaKaRa with 2 μg of total RNA in the 20 μL reaction for reverse transcription. qRT-PCR was conducted using Gloria Nova HS 2X Master Mix (RK20717; ABclonal) on a QuantStudio 12 K Flex Real-Time PCR system. A total of 10 μL of the mixture was used for qRT-PCR, encompassing 5 μL of 2 × ChamQ SYBR qPCR Master Mix, 1 μL of cDNA, 0.5 μL of forward primer, 0.5 μL of reverse primer, and 3 μL of H_2O. The annealing temperature was 60 °C with a total of 35 amplification cycles in triplicate for each sample. The expression level of each gene was computed using the $2^{-\Delta\Delta Ct}$ approach using *18S* as an internal reference gene [62], Primers for this analysis (Table S6) were designed using NCBI primer designing tools.

Plasmid constructs for transient expression

The full-length CDS of the lectin gene from *P. ternata* "Yingshan" cDNA was cloned using specific primers with recombinant adaptors (Table S6) according to sequences deposited in NCBI. An overexpression plasmid, pH7Lic-GFP, driven by the *35S* promoter (Fig. S6), underwent digestion with *Stu*I, and the full-length CDS with adaptors was inserted into the vector with SE recombinase. The constructs were verified by sequencing. The empty vector with GFP was used as a negative control.

Agrobacterium-mediated transient gene expression assays

Constructs were transformed into the *Agrobacterium* strain GV3101. A sample of 1 μg of plasmid mixture was added to 50 μL GV3101, mixed and placed on ice for 10 min, before being flash-frozen in liquid nitrogen for 5 min. The mixtures were placed in a water bath at 37 ℃ for 5 min and placed on ice again for 5 min. This mixture was then added to 500 μL of liquid LB medium and cultured at 28 ℃ for 2 to 3 h. The liquid was evenly applied to a solid LB agar medium. The plates were cultured on inverted plates at 28 ℃ for 2 to 3 days. Colonies were selected from plates and inoculated in liquid YEB overnight at 28 °C while being shaken. *Agrobacterium* cultures were centrifuged at 4000 rpm for 10 min, and the pellet was resuspended in 10 mM MES and 10 mM $MgCl_2$ buffer. The OD_{600} was adjusted to 0.2 for agroinfiltration, with acetosyringone being added at a concentration of 200 mM. *N. benthamiana* leaves were infiltrated using a 1 mL syringe following wounding with a needle. The agroinfiltration approach followed the procedure outlined by Luo et al. [63].

Pathogen inoculation

P. carotovorum QJ-1 and *P. aroidearum* QS-1 were utilized for inoculation to infect *Pinellia ternata* "Yingshan" plants, detached leaves, and *N. benthamiana* leaves. Pathogens were cultured on LB solid medium, then grown in LB liquid medium, centrifuged at 4000 rpm for 10 min, and resuspended with ddH_2O to $OD_{600}=1.0$. "Yingshan" plants were sprayed with the *P. carotovorum* and *P. aroidearum* solutions for 0 h and 48 h. The detached leaves of *P. ternata* were inoculated with the *P. carotovorum* and *P. aroidearum* solutions using 10 μL droplets pipetted onto the surfaces of detached leaves, which were maintained in sealed boxes with moist tissue paper. Boxes were kept in the dark for the first 24 h before being transferred to normal light conditions. Lesion diameters were assessed every 24 h. When used for infection after transient expression, *P. carotovorum* was inoculated 48 h after infiltration with *Agrobacterium* suspension to transiently express selected genes. Each group underwent triplicate experimentation, with leaves flash-frozen in liquid nitrogen for total RNA extraction.

Statistical analysis

One-way ANOVA was employed to analyze statistical significance using GraphPad Prism 8.0 software (GraphPad Prism Software Inc.). The lesion-size calculation was represented by the mean ± SD from three independent experiments, while the qRT-PCR results are shown as the mean ± SD from three biological replicates.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10746-9.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7
Supplementary Material 8
Supplementary Material 9
Supplementary Material 10
Supplementary Material 11
Supplementary Material 12
Supplementary Material 13
Supplementary Material 14

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Author contributions
Conceptualization, D.L.; methodology, Y.M.; software, M.L.; validation, M.W. and M.L.; formal analysis, M.L. and J.X.; investigation, M.L. and K.Q.; resources, M.W. and J.X.; data curation, Y.M.; writing—original draft preparation, M.L.; writing—review and editing, Y.M.; visualization, M.L.; supervision, D.L.; project

administration, D.L.; funding acquisition, D.L. and M.L. All authors have read and agreed to the published version of the manuscript.

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Data availability
The datasets supporting the conclusions of this article are included within the article (and its supplemental files). The Illumina sequence data generated during the current study are accessible through BioProject accession number PRJNA932221. The sequence of the lectin gene (part1transcript/39001) is accessible through Genebank (OR394647).

Declarations

Ethical approval and informed consent
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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