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ORIGINAL RESEARCH

Uncovering Proteomic Insights Into Cell Wall Thickening Genes for Phytophthora Blight Resistance in Pigeon Pea

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ABSTRACT

Phytophthora blight is a destructive disease affecting pigeon pea, often leading to significant yield losses. Resistance to this disease is complex, quantitative, and controlled by multiple genes, although the molecular mechanisms involved remain poorly understood. Cell wall thickening is a crucial aspect of quantitative resistance, regulated by receptors, transcriptional networks, and phytohormones. In this study, we conducted a comparative proteomic analysis on two contrasting pigeon pea genotypes—ICPL99010 (resistant) and ICPL7119 (susceptible)—to explore plant-pathogen interactions at the molecular level. The analysis identified key differentially expressed proteins associated with receptors, transcription factors, phytohormones, and secondary biosynthetic pathways, all of which play roles in lignin and callose deposition. Higher accumulation of phenylpropanoids (phenylalanine ammonia-lyase and caffeoyl-CoA O-methyltransferase), ATP-Binding Cassette G (ABCG) transporters, 1,3-β-glucan synthase, and respiratory burst oxidase homologs (RBOHs) was observed in the resistant genotype. Lignin and callose were deposited as polymers and contribute to cell wall thickening to prevent pathogen establishment. Histochemical staining further confirmed secondary cell wall thickening through lignin and callose accumulation. Quantitative real-time PCR studies revealed higher expressions of *caffeoyl-CoA O-methyltransferase, NAC72, cysteine-rich receptor-like kinase 29, phospholipase D, calcium-dependent protein kinase 1*, *abscisic-aldehyde oxidase,* and *phospholipid-transporting ATPase 1* in the pathogen-inoculated resistant genotype than in the susceptible genotype. This study is the first to use comparative proteomics to investigate the molecular mechanisms of resistance in the Phytophthora–Pigeonpea interaction, offering valuable insights into the plant's defense strategies.

1 | Introduction

Pigeon pea (*Cajanus cajan* (L.)) is an important pulse crop for small and marginal farmers in tropical and subtropical regions across the world (Sinha et al. 2024). Valued for its high-protein split pulses (Murtujasab et al. 2024), pigeon pea encounters various abiotic and biotic stresses throughout its lifecycle, which constrain its yield potential (Dhanorkar et al. 2024). Phytophthora

blight, caused by *Phytophthora cajani*, is a devastating disease affecting pigeon pea, especially in hot and humid conditions. Early infections resemble damping-off disease, leading to seedling mortality, while later stages present as water-soaked lesions on leaves, brown to black spots, and slightly sunken lesions on stems and petioles (Sharma and Ghosh 2016). The yield loss was recorded to be up to 98 to 100% under favorable conditions during the seedling stage (Bala et al. 2021; Sharma and Ghosh 2018). Further, yield losses are more pronounced in early-maturing pigeon pea varieties than in medium- and long-maturing varieties (Satheesh Naik et al. 2020). The disease occurrence has spread to most of the pigeon pea growing areas in Asia, Africa, Australia, America, Kenya, Dominican Republic, Panama, and Puerto Rico (Jadesha et al. 2022). Despite extensive research, the development of resistant varieties remains limited due to high environmental variability among pathotypes and the absence of highly resistant donors within the cultivated species (Keerthi et al. 2022). A deeper understanding of the molecular mechanisms governing Phytophthora blight resistance is essential for effectively integrating these insights into pigeon pea breeding programs. Plants have developed a highly intricate and well-organized defense system to protect themselves from various diseases. Upon infection, pathogens are identified by a wide array of cell-surface and intracellular immune receptors in the host (Kourelis and Van Der Hoorn 2018; Van De Weyer et al. 2019). These receptors, known as Pattern Recognition Receptors (PRRs), detect conserved molecular signatures of invading pathogens, known as PAMPs or DAMPs, derived from the host. This recognition triggers pattern-triggered immunity (PTI), the plant's first line of defense, which enables a rapid stress response (Rao et al. 2018; Sun et al. 2020; Wang et al. 2022). To overcome PTI responses, plant pathogens secrete small molecules called effectors into the host cell, facilitating infection and disrupting the plant defenses (Toruño et al. 2016; Collemare et al. 2019; Ceulemans et al. 2021). These effectors are recognized by resistance (R) proteins in plants, which activate a secondary defense mechanism called effector-triggered immunity (ETI), also referred to as qualitative resistance (Peng et al. 2018; Alhoraibi et al. 2019). Additionally, PAMP recognition triggers the production of phytohormones and various transcription factors that regulate plant defense responses, leading to the up- or down-regulation of resistance genes and resulting in quantitative resistance (Kushalappa et al. 2016). Despite extensive research on plant-pathogen interactions, the disease resistance mechanisms in pigeon pea plants remain relatively limited. Previous studies indicate that resistance to Phytophthora blight is monogenic, with contributions from minor genes (Gupta et al. 1997), whereas in *C. platycarpus* (ICPW 61), the nature of resistance is governed by a single recessive gene (Mallikarjuna et al. 2006). However, the continuous emergence of new pathogen variants and the lack of durable resistance to Phytophthora blight have posed significant challenges in breeding resistant pigeon pea varieties (Keerthi et al. 2022). Given these challenges, it is crucial to investigate the molecular mechanisms of resistance further by identifying key proteins and signalling pathways involved in host-pathogen interactions.

The proteome network within a cell determines its functional state and shapes its phenotypes by dynamically adapting to stress conditions (Liu et al. 2019). Without prior information on the mechanism of resistance or host-plant interactions, untargeted proteomics analysis offers a powerful approach to identifying proteins that are either constitutively expressed or induced in response to biotic stress (Feussner and Polle 2015). Among the various techniques available, liquid chromatography-mass spectrometry (LC–MS/MS) has become the most widely used method for untargeted proteomics analysis. This technology helps decipher cellular signalling networks and provides molecular insights into plant responses to biotic stress (Lundberg and Borner 2019; Liu et al. 2019; Aebersold and Mann 2016; Zhang et al. 2013). Recent studies have successfully identified proteins involved in host-pathogen interactions. In pigeon pea, LC–MS/MS-based label-free proteomics revealed that introducing rice *oxalate oxidase 4 (Osoxo4)* significantly enhanced resistance to *Fusarium udum*-induced wilt by modulating key downstream proteins and upregulating metabolites associated with stress response (Karmakar et al. 2024). Similarly, a comparative proteomic analysis of contrasting groundnut genotypes infected with *Aspergillus flavus* identified phytohormones and transcription factors involved in regulating downstream secondary metabolic pathways that suppress pathogen growth and development (Bhatnagar-Mathur et al. 2021). In maize, resistance to *Fusarium graminearum* was linked to the upregulation of proteins involved in phenylpropanoid, flavonoid, and terpenoid biosynthesis, along with the WRKY transcription factor ZmWRKY83, which was shown to enhance resistance (Bai et al. 2021). Proteins synthesizing phenylpropanoid compounds like lignin enhance resistance to various pathogens through secondary cell wall thickening. These compounds have been reported to be synthesized in response to infection by various pathogens in different crops, such as in wheat against *F. graminearum* (Yang et al. 2021), in groundnut in response to *A. flavus* (Avuthu et al. 2024), and in cotton against *Verticillium dahliae* (Guo et al. 2016). Thus, considering the crucial role of host-pathogen interactions in plant immunity, proteomics has emerged as an indispensable tool for unraveling the molecular mechanisms underlying disease resistance.

Our study investigates proteomic changes in two contrasting pigeon pea genotypes (ICPL99010-resistant and ICPL7119-susceptible) with differing resistance to *P. cajani* infection using an LC–MS/MS approach. The two genotypes exhibited a distinct activation of signalling pathways, transcription factors, and secondary biosynthetic pathways under constitutive and induced conditions, contributing to resistance by upregulating proteins involved in the thickening of the secondary cell wall. Gaining a deeper molecular understanding of these resistance mechanisms will be invaluable for integrating these insights into pigeon pea breeding programs to develop varieties with long-lasting resistance to this disease.

2 | Materials and Methods

2.1 | Plant Materials

The genotypes ICPL 99010 (resistant) and ICP 7119 (susceptible) were obtained from the Pigeonpea Breeding Unit, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India. ICPL 99010 is a cleistogamous line derived from a pedigree selection (9901040-SW8-SW1-SW1-SW1) from the sterility mosaic and wilt nursery (SW) at ICRISAT, Patancheru, India

and was developed from a cross between ICPL87119 and ICPL 87159, and is reported to be consistent in performance as moderately resistant in previous studies (Sharma et al. 2023). ICP 7119 is a germplasm line and is reported to be highly susceptible to Phytophthora blight (Reddy et al. 1991; Sharma et al. 2015). Accordingly, these genotypes were selected based on their consistency in performance from previous studies.

2.2 | *P. cajani* Growth and Inoculum Preparation

A 5 mm plug of actively growing mycelial culture (5–7 days old) of the fungal isolate was obtained from 20% tomato extract broth and incubated in the dark at 25 C for 72 h to promote mycelial growth. The resulting mycelial mat was then transferred to sterilized pond water to induce sporangial development. Zoospore concentration was determined using a hemocytometer (1/400 mm^2, 1/10 mm depth; Sigma-Aldrich) and adjusted to 1.5×10^5 zoospores ml^{-1} using sterilized deionized water. For disease resistance screening, genotypes were evaluated for Phytophthora blight resistance in a Completely Randomized Block Design (CRBD), following the recommended agronomic practices. Inoculation was carried out on both leaves and stems using the prepared zoospore suspension (1.5×10^5 zoospores mL^{-1}). Leaf inoculation was performed on 15-day-old plants using the paper disc method, while stem inoculation involved drenching seven-day-old seedlings for 1 week with the suspension.

The experiment was conducted with two treatments—pathogen-inoculated and mock-inoculated—each with three replications. Treatments were categorized as follows: RP (resistant genotype with pathogen inoculation), RM (resistant genotype with mock inoculation), SP (susceptible genotype with pathogen inoculation), and SM (susceptible genotype with mock inoculation). All samples were immediately frozen in liquid nitrogen and stored at −80 C for subsequent analyses.

2.3 | Fungal Biomass Quantification

Leaf samples from mock- and *P. cajani*-infected plants were collected at 120 h post-infection (hpi) from both resistant and susceptible genotypes for fungal biomass estimation. Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen). The relative fungal biomass in infected leaves was quantified by qPCR using Phytophthora-specific primers (Sharma et al. 2015). Reactions were performed with SYBR Green Master Mix (Bioline) on a CFX96 Real-Time PCR System (Bio-Rad), following the manufacturer's protocol. Specific primers targeting *P. cajani* and pigeon pea DNA were used for amplification and detection (Table S1).

2.4 | Protein Extraction

Protein extraction from the leaf and stem samples was carried out as previously reported (Bhatnagar-Mathur et al. 2021). Approximately 100 mg of frozen leaf and stem tissue ($n = 3$ biological replicates) was finely ground into a uniform powder and suspended in 1 mL of extraction buffer containing 0.9 M sucrose, 0.1 M Tris–HCl (pH 8.8), 10 mM EDTA, 50 mM DTT, 1 mM PMSF, and 25 μL of protease inhibitor cocktail (Sigma–Aldrich). An equal volume of Tris-saturated phenol was added, and the mixture was incubated with continuous mixing at 4°C for 30 min. After centrifugation at 5000 *g* for 20 min at 4°C, the upper phenolic phase was collected and re-extracted twice. Proteins were precipitated by adding five volumes of 0.1 M ammonium acetate containing 10 mM DTT and incubated overnight at −20°C. The resulting pellet was recovered by centrifugation at 5000 g for 30 min at 4°C and washed sequentially with methanol and acetone, both containing 10 mM DTT. The final protein pellet was either dissolved in rehydration buffer for immediate downstream processing or stored at −80°C for later use.

2.5 | In-Solution Digestion

RapiGest SF surfactant (Waters) was dissolved in ammonium bicarbonate and mixed in equal volumes with the protein sample. The mixture was incubated at 65°C for 30 min. Following this, 10 μL of 100 mM DTT was added, and the sample was further incubated at 65°C for an additional 30 min. 100 mM iodoacetamide (IAM) was added, following which the reaction was incubated for 30 min in the dark for alkylation. Trypsin was added at a 1:20 enzyme-to-protein ratio, followed by incubation of the mixture overnight at 37°C for 16 h. To terminate the digestion, formic acid was added to a final concentration of 5%. The digested samples were completely dried using a vacuum centrifuge, reconstituted in Buffer-A, and purified using a C18 spin column prior to LC–MS/MS analysis.

2.6 | Mass Spectrometry and Data Acquisition

Tryptic peptides were separated using an Acquity BEH C18 UPLC column (75 μm × 150 cm × 1.7 μm; Waters) over a 60-min gradient. Mobile phases A and B consisted of LC–MS/MS grade water and acetonitrile, respectively, along with 0.1% formic acid. The column temperature was maintained at 60°C. MS and MS/MS analyses were conducted using an Xevo G2-XS QTOF mass spectrometer (Waters) equipped with an ESI source in positive ion mode. The Data was acquired in resolution mode with a 0.5 s scan time over a 50–2000 Da range. Leucine enkephalin was used at a concentration of 200 pg. μL^{-1} for lock mass calibration every 30 s. Spectra were acquired using MassLynx v4.0 and processed for protein identification and quantification.

2.7 | Data Analysis

Proteins were identified and quantified using Progenesis QI for Proteomics Software version 4.0 (Nonlinear Dynamics), based on raw spectral data. The software aligned MS scans according to retention time and generated peak lists containing m/z values and abundance for each replicate. To enhance peak detection sensitivity, parameters were configured to include proteins with charges greater than 5, within an m/z range of 100–1600, and retention times between 5 and 80 min. Prior to statistical analysis, triplicate MS scans were grouped by treatment, followed by normalization and abundance comparison. Peptides assigned to multiple proteins were excluded from quantification

to ensure data accuracy and reliability. Protein identification was performed using Progenesis' built-in ion accounting algorithm, referencing the pigeon pea proteome database obtained from UniProt (http://www.uniprot.org/). The peptide modification applied carbamidomethylation of Cys was considered as a fixed modification, and oxidation of Met and Deamidation (N-term) as variable modifications. Search parameters included tryptic digestion with up to two missed cleavages, a maximum precursor mass error of 20 ppm, at least two fragment ions per peptide, five fragment ions per protein, and a minimum of two peptides per protein. The proteins were identified by a minimum of two unique peptides and were considered valid. The false discovery rate (FDR) for statistically significant proteins was set at 5% (Benjamini and Hochberg, Benjamini and Hochberg 1995). Quantification was done based on comparing the protein ion count ratios across different samples or treatments. Only proteins meeting the criteria for having two or more unique peptides were included in the final analysis. The proteomics data obtained from mass spectrometry have been deposited via jPOSTrepo (Okuda et al. 2025) to the ProteomeXchange consortium with the database identifier PXD063337 (https://repository.jpostdb.org/entry/JPST003774.0).

The Minimum Information About a Proteomics Experiment (MIAPE) was developed in accordance with guidelines established by the Proteomics Standards Initiative (Table S2; Taylor et al. 2007; Martínez-Bartolomé et al. 2014).

2.8 | Histochemical Analysis of Pigeon Pea Stem Samples

Pigeon pea stem samples, both pathogen-inoculated and mock-inoculated, were collected 120 h post-inoculation (hpi), rapidly frozen in liquid nitrogen, and stored at −80°C for subsequent histochemical staining of lignin and callose. Stem sectioning was done using the CryoStar NX50 Cryostat (Thermo Scientific). The cross-sections thus obtained were approximately 10 μm thick and were then mounted onto glass slides. For lignin staining, the sections were treated with a mixture of 3% phloroglucinol in absolute ethanol and concentrated HCl (37 N) in a 3:1 ratio. The stained sections were then visualized under a bright-field filter using a Nikon fluorescent microscope. To detect callose deposition, the stem sections were stained with 0.01% (w/v) aniline blue in 0.01 M K_3PO_4 buffer (pH 12), following the previous protocol (Zavaliev and Epel 2015). Fluorescence was observed using a DAPI filter with blue laser diode excitation at 405 nm and an HQ442/45 emission filter.

2.9 | RNA Isolation and Gene Expression Analysis by qRT-PCR

Total RNA was extracted from frozen leaf tissues of both pathogen-inoculated and control samples at 48 hpi using the RNeasy Plant Mini Kit (Qiagen), with three biological replicates. Approximately 2.0 μg of purified RNA from each sample was used for cDNA synthesis, following the manufacturer's instructions (Thermoscript RT-PCR System, Invitrogen). Quantitative PCR (qPCR) was done using gene-specific primers (Table S1) on a CFX96 Real-Time PCR System (Bio-Rad). Cycle threshold (Ct) values were normalized against the housekeeping gene G6Pd (EG030635), and relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

2.10 | Statistical Analysis

Proteins identified across four treatments—RP, RM, SP, and SM—each with three biological replicates, were averaged for further analysis. Pairwise Student's t-tests were performed for four key comparisons: RP vs. RM, RP vs. SP, RM vs. SM, and SP vs. SM. Fold changes were calculated as the ratio of average protein abundance in resistant versus susceptible samples. Proteins upregulated in the resistant genotype were categorized as either Resistant Related Constitutive (RRC) or Resistant Related Induced (RRI). RRC proteins were identified by comparing RM and SM treatments, with a fold change ≥ 1.0 and a statistically significant ANOVA p-value ($p \leq 0.05$). In contrast, RRI proteins were determined from RP vs. RM and SP vs. SM comparisons, with a fold change ≥ 1.0 and a significant ANOVA p-value ($p \leq 0.05$).

To visualize differential protein accumulation, volcano plots were generated using the SR plot online tool (https://www.bioinformatics.com.cn/en?keywords=volcano). Venn diagrams illustrating shared and unique proteins across treatments were created using the Venn Diagram web tool (https://bioinformatics.psb.ugent.be/webtools/Venn/). Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) plots were produced via MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml). Differentially expressed proteins (DEPs) were annotated and classified into Gene Ontology (GO) categories using UniProt (http://www.uniprot.org/) and the DAVID Bioinformatics Resources (https://davidbioinformatics.nih.gov/), facilitating functional interpretation. The subcellular localization of significant proteins was predicted using Plant-mPLoc (Chou and Shen 2010). The proteomics analysis workflow for the contrasting pigeon pea genotypes is outlined in Figure S1.

3 | Results

3.1 | Reconfirmation of Resistance to *P. cajani* Infection and Fungal Biomass Quantification

Phytophthora blight severity was evaluated in both the resistant line (ICPL 99010) and the susceptible line (ICP 7119) following infection with *P. cajani* (Figure 1). Early symptoms appeared as brown lesions on the inoculated leaves by 3 dpi in both genotypes (Figure 1A). Quantitative PCR analysis further showed that fungal biomass was significantly higher in the susceptible genotype compared to the resistant one (Figure 1B), confirming differential responses to the pathogen.

3.2 | Proteomic Alterations Caused by *P. cajani* Infection

To study the proteome changes in leaf and stem tissue of resistant and susceptible genotypes, a label-free quantitative

proteomics analysis was performed at 72 hpi of *P. cajani* using UPLC-ESI-MS/MS. The comparison was made between RP, RM, SP, and SM. Over a period of 72 hpi, 2604 proteins were identified in the stem, and 2223 proteins were identified in the leaf. About 826 and 455 proteins were identified as RRI and RRC, respectively, in the stem, whereas in the leaf, a total of 288 and 599 proteins were differentially expressed as RRC and RRI over a period of 72 hpi.

Comparative protein expression analysis was done to identify the proteins responding to *P. cajani* inoculation, having at least two unique peptides with 1.0-fold change at $p \leq 0.05$ for RP/RM, RP/SP, RM/SP, and SP/SM. The significantly changed proteins thus identified were observed to be unique or shared between the treatments (Table 1). Venn diagrams were constructed to compare proteins in RP/RM, RP/SP, RM/SP, and SP/SM in both leaves and stems, and the proteins significantly expressed were observed to be unique or shared between the treatments. About 2534 and 1895 proteins were differentially expressed in the stems and leaves, respectively. A maximum number of unique proteins was observed in RP/RM (51, Figure S2A) and RM/SM (223) in the stems and leaves, respectively (Figure S2B). In stems, the highest accumulation of DEP was observed in RP/SP (1988), followed by SP/SM (1926), RP/RM (1892), and RM/SM (1368), whereas in leaves, the highest accumulation of DEP was observed in RM/SM (1568), followed by SP/SM (1288), RP/SP (1083), and RP/RM (913).

A principal component analysis (PCA) was carried out to check the reliability and reproducibility of the experimental design. In the stems, principal component-1 (PC1) explained 44.4% variance, thus distinguishing between the resistant and the susceptible genotypes. The proteins from each genotype formed separate clusters, with a minor overlap among the biological replicates. About 27.3% variance was explained by PC2, distinguishing the samples based on pathogenesis and separating the pathogen inoculation from mock inoculation. Similarly, PC1 and PC2 in the leaves explained 48.8% and 27%, respectively (Figure 2A,B). In addition, a partial least-squares discriminant analysis (PLS-DA) was carried out to distinguish the differences in protein profiles between groups. The score plots show variation between the two genotypes under pathogen-inoculated and mock treatments, indicating minimal experimental error. The PLS-DA score plots thus verified the results obtained through PCA. This analysis clearly distinguished the samples under treatment with pathogen inoculation (RP and SP) and mock inoculation (RM and SM; Figure 2C,D).

3.3 | Functional Classification of the Identified Proteins Based on GO Annotation

Gene Ontology (GO) annotation was carried out to categorize the identified proteins based on their roles in biological

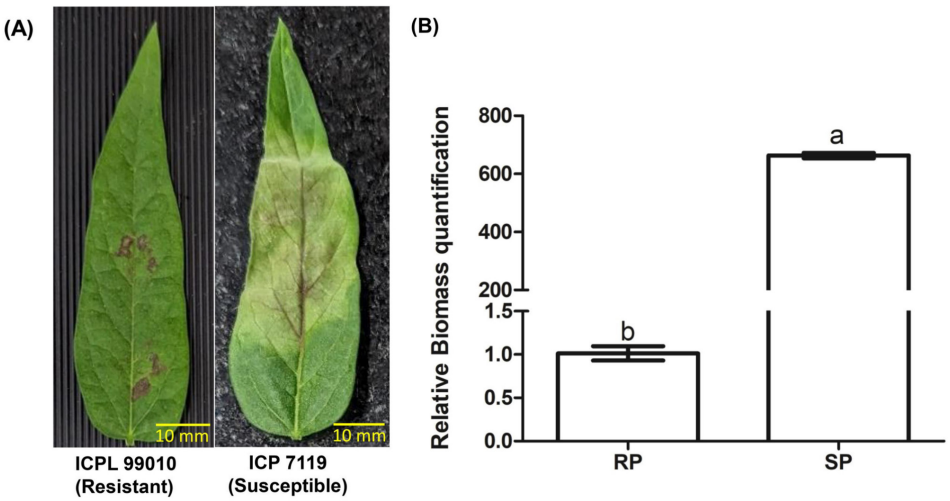

FIGURE 1 | Response of contrasting pigeon pea genotypes to *Phytophthora cajani* infection. (A) Comparison of the effect of pathogen infection on leaves of resistant (ICPL 99010) and susceptible (ICP 7119) at 72 hpi. (B) Fungal biomass at 120 h post-infection in resistant line (RP) and susceptible genotypes (SP) following *P. cajani* infection.

TABLE 1 | Summary of total, unique, and shared differentially expressed proteins in resistant and susceptible genotypes under *Phytophthora cajani* and/or mock inoculation in leaf and stem samples.

		Total number of proteins		Unique proteins		Shared proteins	
SI No	Comparison	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	RP_RM	913	1892	63	51	850	1841
2	RM_SM	1568	1368	223	22	1345	1346
3	RP_SP	1083	1988	34	48	1049	1940
4	SP_SM	1288	1926	40	40	1248	1886

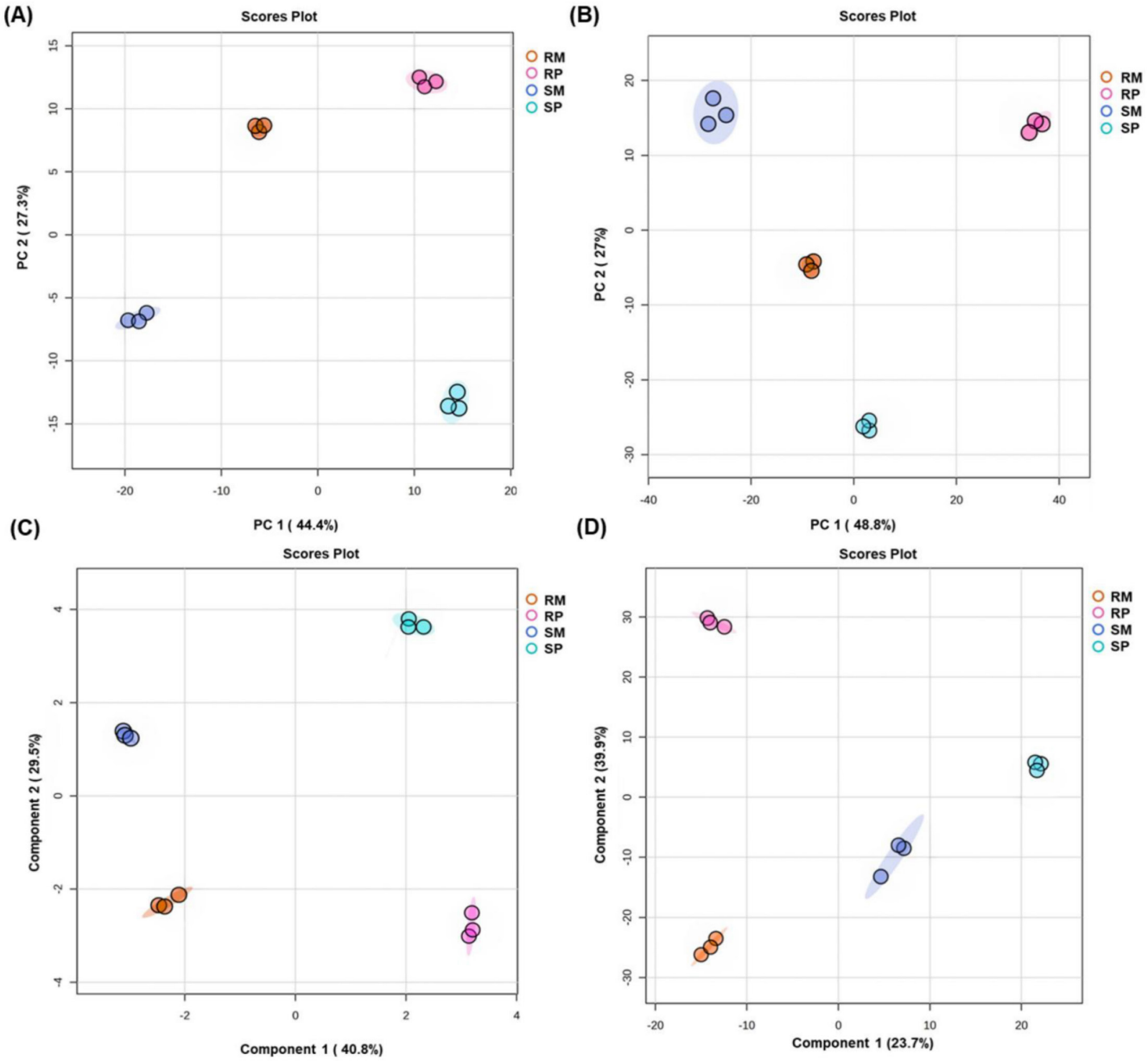

FIGURE 2 | Insights into protein changes in pigeon pea in response to Phytophthora infection. (A, B) Principal component analysis (PCA) of protein profiles in (A) stems and (B) leaf tissues. (C, D) Partial Least Squares Discriminant Analysis (PLS-DA) of protein profiles in (C) stems and (D) leaf tissues from resistant (ICPL 99010) and susceptible (ICPL 7119) pigeon pea genotypes following Phytophthora or mock inoculation. Distinct clustering of mock- and pathogen-treated samples indicates differential protein accumulation in response to infection.

processes (BP), molecular functions (MF), and cellular components (CC), detailing their hierarchical relationships within these classifications. Analysis of putative functions revealed 495 GO terms for BP in the stem, among which 252 were unique to RRI, and 94 were unique to RRC. About 465 GO terms were related to MF, among which 203 and 96 GO functions were unique to RRI and RRC, respectively. Further, 177 GO terms were identified as CC, of which 76 were unique to RRI, and 115 were unique to RRC. About 404 GO terms for BP were identified in the leaf, among which 98 were unique to RRI and 214 to RRC. About 402 GO terms were related to MF, among which 98 were only present in RRI, 181 were only annotated in RRC, and 145 GO terms for CC, among which 66 were in RRC and 25 were present in RRI.

Functional enrichment analysis for GO terms was performed to identify significantly represented BP, MF, and CC associated with the dataset in stem and leaf (Figure 3). In stems, BP such as response to biotic stimulus, defense response, response to viruses and other organisms, signal transduction, and 1,3-β-glucan biosynthetic process were most enriched. The 1,3-β-glucan synthase complex was most enriched in the CC for GO. Under MF, ATP binding, ABC transporter activity, and 1,3-β-glucan synthase activity were more enriched (Figure 3A). In leaves, in terms of BP such as response to biotic stimulus, defense response, defense response to viruses and other organisms, signal transduction, and 1,3-β-glucan biosynthetic process, cell wall biosynthetic process, cell wall organization, and primary cell wall biogenesis were more

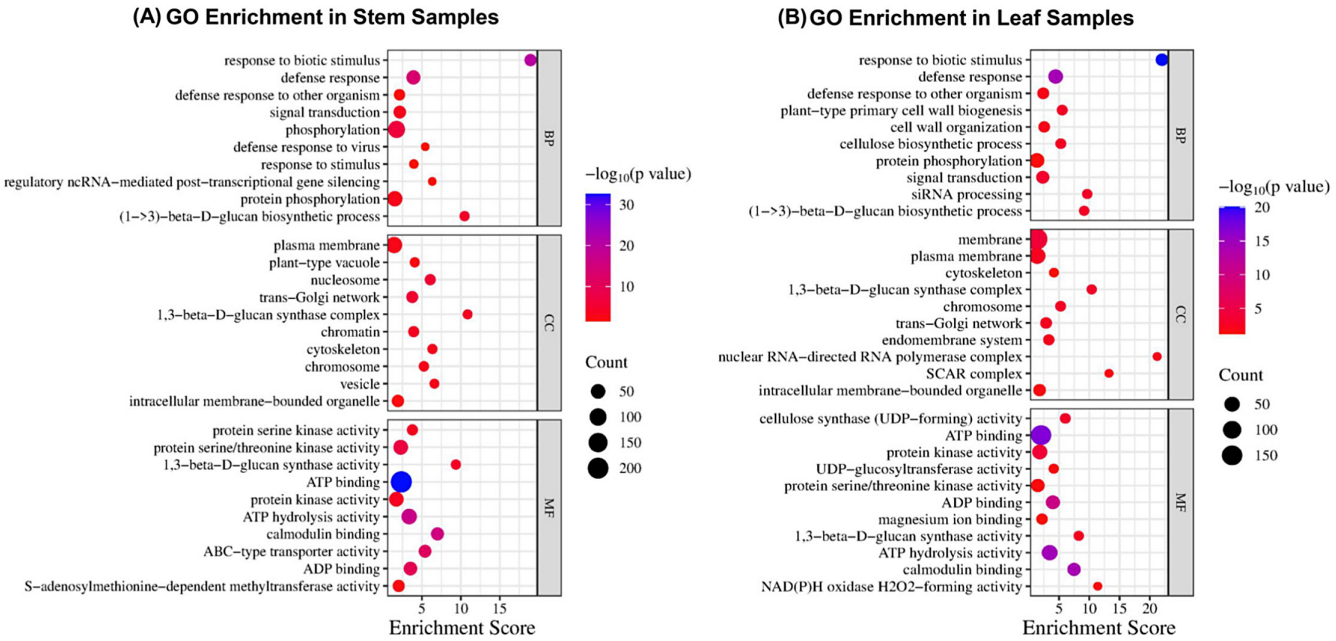

FIGURE 3 | Gene Ontology (GO) enrichment analysis and classification of differentially expressed proteins in pigeon pea stems and leaf tissues. (A) Stem and (B) Leaf: Proteins were categorized based on GO terms under three major domains—Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). The size of each circle represents the number of proteins associated with the respective GO term, while the color scale indicates the statistical significance (*p*-value) of enrichment.

enriched. In the CC, the nuclear RNA–directed RNA polymerase complex, the SCAR complex, and the 1,3-β-glucan synthase complex were most enriched. Under MF, ATP binding, cellulose synthase activity, UDP glucosyl transferase activity, and 1,3-β-glucan synthase activity were more enriched (Figure 3B).

3.4 | Differentially Expressed Proteins in Contrasting Pigeon Pea Genotypes Following *P. cajani* Infection

The up-regulation and down-regulation of differentially expressed proteins were highlighted by a volcano plot following either pathogen or mock treatments (Figure 4). In terms of upregulated proteins, RP/SP comparison in stems (↑1122 and ↓118) was higher when compared to leaves (↑625 and ↓438; Figure 4A,C). In contrast, the RM/SM comparison showed the highest number of upregulated proteins in the leaves (↑771 and ↓606) when compared to the stems (↑587 and ↓728; Figure 4B,D). This showed a clear early response and changes at the protein levels, indicating a significant change in the accumulation of resistance-associated proteins following *P. cajani* infection.

3.4.1 | Resistance-Related Constitutive Proteins (RRC)

A total of 1371 constitutive proteins were differentially accumulated in the RM/SM comparison in the stem and 1568 in the leaves. Among these, 455 and 599 proteins were significantly upregulated with FC ≥1.0 in the resistant genotype in the stems and leaves, respectively. These proteins are identified as resistance-related constitutive (RRC) proteins (Table S3). RRC metabolites were classified into different chemical groups to identify the biological pathways that were significantly altered following treatment with either *P. cajani* or water (Figure 5).

Among the prominent proteins with significant fold changes were those related to the receptor kinases, including receptor protein kinases—CLAVATA1 (23.64 FC), FERONIA (32.84 FC), LRR-RLK At1g14390 family (44.02 FC), CRK29 (14.24 FC), CRK10 (5.12 FC), CRK25 (19.97 FC), 1-phosphatidylinositol-3-phosphate-5-kinase (11.14 FC), histidine kinase (41.67 FC). CRK29 (2.67 FC), CRK25 (19.97 FC), CDPK17 (7.12 FC), WAKL20 (3.45 FC), and WAK2 (1.50 FC). Several ROS related proteins showed notable upregulation, such as Heat shock cognate 70 kDa protein 2 (13.56 FC), Heat shock protein 82 (1.72 FC), and respiratory burst oxidase isogeny proteins—RBOHA (2.40), RBOHF (2.26), RBOHB (4.67 FC). Cell wall-related proteins were elevated, including cellulose synthase-like protein H1 (35.71 FC), pectinesterase (15.40 FC), cellulose synthase-like protein D3 (10.60 FC), and rhamnogalacturonate lyase (17.05 FC). The proteins related to the transcription factors, including NAC78 (3.01 FC), WRKY32 (1.23 FC), and MYB44 (4.26 FC), and phytohormones, such as auxin response factor (10.12 FC), and abscisic-aldehyde oxidase (2.32) were demonstrating increased abundance. Lastly, callose biosynthesis proteins comprised of 1,3-β-glucan synthase (16.93 FC), and fatty acid pathway proteins including phospholipase D (8.90 FC), lipoxygenase (13.24 FC), and CDP-diacylglycerol-glycerol-3-phosphate3-phosphatidyltransferase (12.26 FC), were among those notably upregulated.

3.4.2 | Resistance-Related Induced Proteins (RRI)

Following *P. cajani* infection, 1893 proteins in the stems and 912 in the leaves were significantly expressed in resistant compared to susceptible genotypes. A total of 827 proteins in

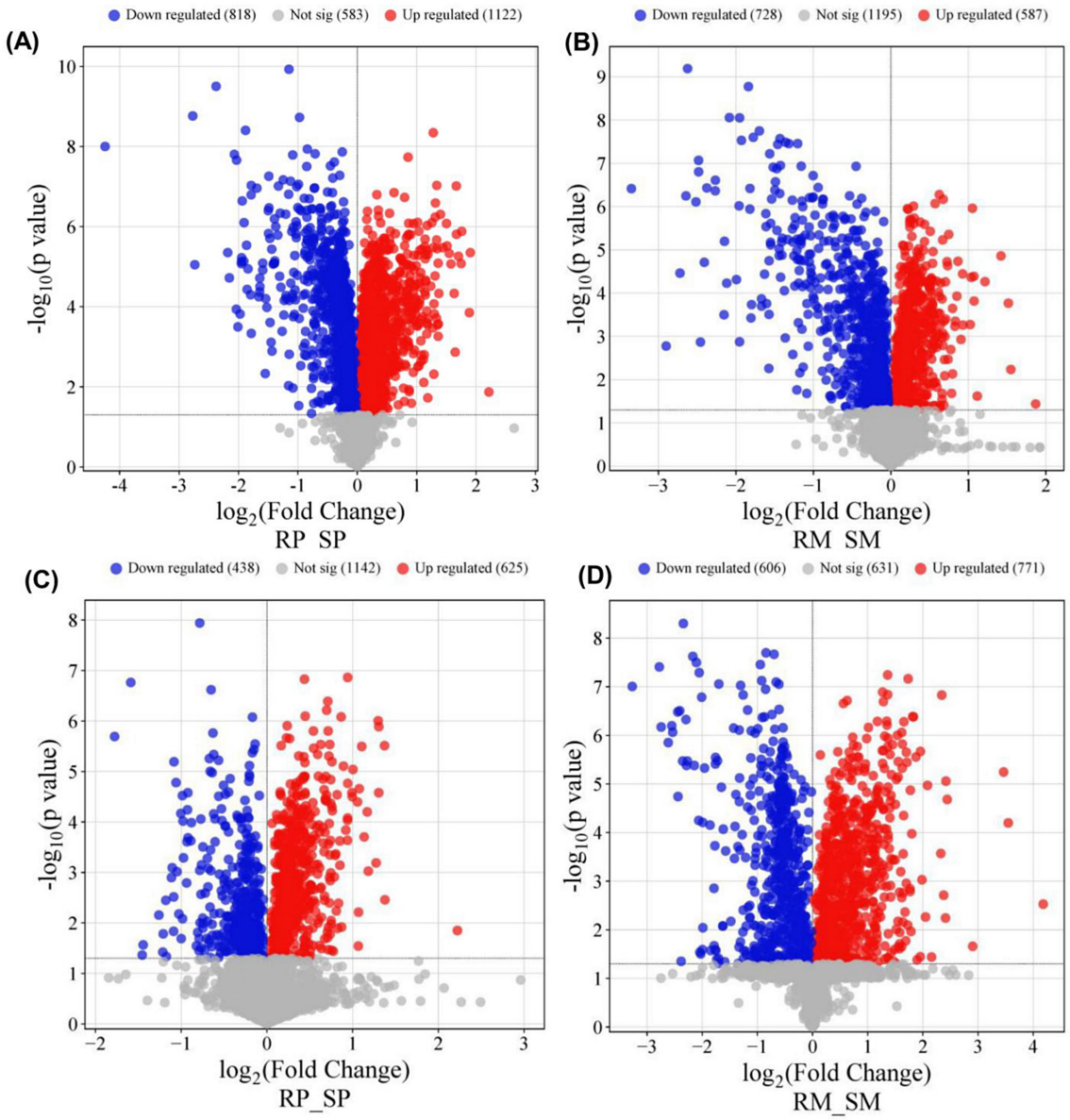

FIGURE 4 | Volcano plots of differentially accumulated proteins in resistant and susceptible pigeonpea genotypes. Volcano plots representing significantly accumulated proteins with a cut-off at $p \leq 0.05$ and $\log_2$ FC > 1 in (A) Stem RP_SP, (B) Stem RM_SM, (C) Leaf RP_SP, and (D) Leaf RM_SM. Red and blue dots indicate upregulated and downregulated proteins, respectively.

the stems and 288 proteins in the leaves were higher FC ≥ 1.0 in the resistant genotype and were designated as resistance-related induced (RRI) proteins (Table S4, Figure 5). Among these, receptor proteins such as, LRR RLK—At5g48740 (58.93 FC) and At4g08850 (MIK2) family (16.58 FC), WAK3 (1.46 FC), TMK1 (12.89 FC), histidine kinase (9.79 FC), ERL1 (7.12 FC), serine/threonine-protein kinase PBL7 (3.06), and calcium-dependent protein kinase 20 (1.93) were predominantly upregulated.

ROS-related Proteins, including Heat shock cognate 70 kDa protein 2 (30.90), and respiratory burst oxidase isogeny protein F (1.45), and Cell wall-related proteins, including pectinesterase (15.01 FC), and protein WAX2 (2.48 FC) showed increased expression. Key transcription factors, including NAC72 (2.82 FC), homeobox-leucine zipper protein meristem L1 (26.99 FC). NAC 76 (3.49 FC), AP2/ERF and B3 domain-containing transcription factor RAV1 (2.02 FC), and MYB family transcription factor At1g14600 (6.34 FC), and proteins related to phytohormone, such as auxin response factor (3.03 FC), abscisic-aldehyde oxidase (9.44 FC), and chitin-inducible gibberellin-responsive protein 1 (6.13 FC), were elevated in the resistant genotype. The secondary metabolic pathway proteins, Phenylpropanoid pathway, such as caffeoyl-CoA O-methyltransferase (1.54 FC), and Callose biosynthesis proteins, including 1,3-β-glucan synthase (10.94 FC), and callose synthase 7 (9.68 FC), were significantly upregulated.

3.5 | Proteins Related to Lignin and Callose Deposition Promoting Cell Wall Reinforcement

We identified differentially expressed proteins with a high fold change in both RRI and RRC, associated with lignin

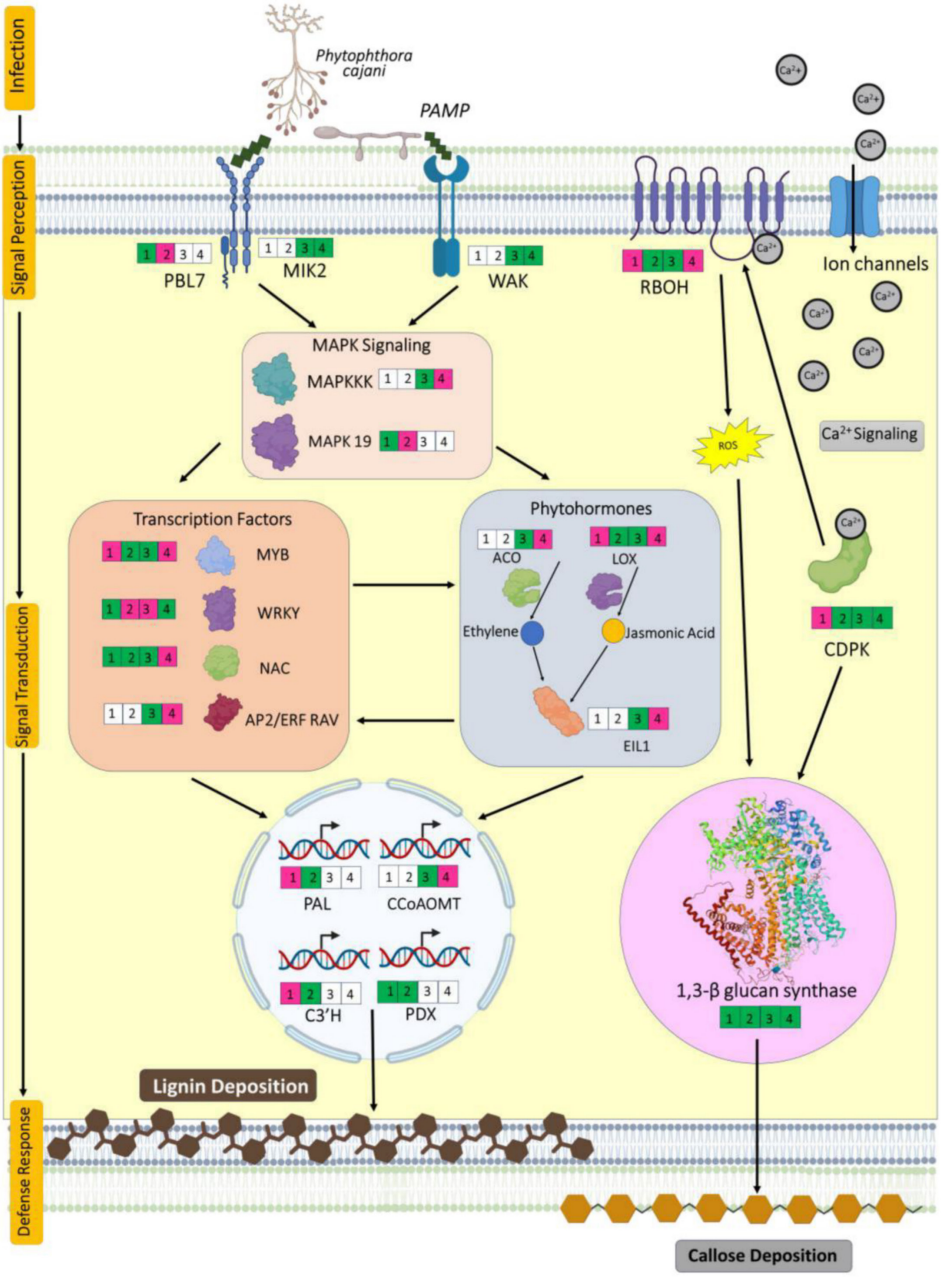

FIGURE 5 | Overview of the *Phytophthora cajani*–Pigeon pea interaction, highlighting the hierarchical involvement of proteins in signal perception, signal transduction, and defense response, ultimately contributing to cell wall thickening and disease resistance in resistant lines inoculated with *P. cajani*. The schematic illustrates: Signal perception via receptors (e.g., WAK, PBL7, MIK2); Signal transduction involving MAPK and calcium signalling pathways, transcription factors, phytohormones, reactive oxygen species (ROS), and activation of lignin and callose biosynthetic genes; Defense response through proteins involved in lignin and callose deposition. Color coding: Green boxes: Upregulated proteins; Magenta boxes: Downregulated proteins; White boxes: Absence of proteins. Numbers within boxes represent tissue-specific resistance types: 1—Leaf RRI, 2—Leaf RRC, 3—Stem RRI, 4—Stem RRC. ACO—1-aminocyclopropane-1-carboxylate oxidase; C3′H—*p*-coumaroyl shikimate/quinate 3′-hydroxylase; CCoAOMT—caffeoyl-coa o-methyltransferase; CDPK—calcium-dependent protein kinase; LOX –lipoxygenase; MAPK—mitogen-activated protein kinase; MIK2—LRR receptor-like serine/threonine-protein kinase At4g08850 family (MIK2); PAL—phenylalanine ammonia lyase; PBL7—Serine/threonine protein-like kinase PBL7; PDX—peroxidase; RBOH—respiratory burst oxidase homolog; WAK—wall associated kinase.

and callose deposition (Table 2). Several proteins involved in lignin biosynthesis, such as phenylalanine ammonia-lyase, p-coumaroyl shikimate/quinate 3′-hydroxylase (C3′H), and caffeoyl-CoA O-methyltransferase, were found to be up-regulated in the resistant genotype. Additionally, various ABCG transporters (ABCG9, ABCG10, ABCG11, ABCG14, ABCG26, ABCG28, ABCG31, ABCG32, ABCG34, ABCG36, and ABCG39) displayed increased expression, indicating their role in lignin deposition.

Proteins associated with callose deposition, including 1,3-β-glucan synthases and respiratory burst oxidase homologs (RBOHA, RBOHB, and RBOHF), were also highly expressed in resistant genotypes (Table 2). Furthermore,

TABLE 2 | Resistance-related proteins involved in cell wall reinforcement by lignin and callose deposition *Phytophthora cajani* or mock inoculation.

Accession	Mass (KDa)	Protein name	Leaf		Stem	
			RRI	RRC	RRI	RRC
A. Lignin deposition						
A0A151TIW6	70.90	Phenylalanine ammonia-lyase		238.47*		
A0A151QNQ1	30.30	Caffeoyl-CoA O-methyltransferase			1.54*	
A0A151TAP4	57.69	p-coumaroyl shikimate/quinate 3′-hydroxylase		2.96*		
A0A151R6G2	34.33	Peroxidase		4.57*		
A0A151S4P8	77.71	ABC transporter G family member 26			2.67*	
A0A151U2G2	77.48	ABC transporter G family member 26			2.54*	
A0A151S095	75.46	ABC transporter G family member 14			2.29*	
A0A151T2B4	77.21	ABC transporter G family member 11			2.42	
A0A151S297	152.61	ABC transporter G family member 39			1.66*	
A0A151T207	57.29	ABC transporter G family member 14			2.10*	
A0A151U9Y5	162.03	ABC transporter G family member 31		1.46*	2.32*	
A0A151SBX7	138.41	ABC transporter G family member 31			1.94*	
A0A151SZD8	90.43	ABC transporter G family member 28			1.89*	
A0A151S9A1	57.64	ABC transporter G family member 10		6.77*		
A0A151QRD4	66.42	ABC transporter G family member 9		5.02*		
A0A151TVI2	163.06	ABC transporter G family member 34		3.61*		
A0A151SHB4	169.53	ABC transporter G family member 36		10.16*		1.17*
A0A151SLF3	163.45	ABC transporter G family member 32				11.28*
B. Callose deposition						
A0A151SBP6	217.07	1,3-β-glucan synthase				1.51*
A0A151SF34	192.86	1,3-β-glucan synthase		16.93*	1.74*	
A0A151T7C1	177.03	1,3-β-glucan synthase				3.00*
A0A151TLC4	215.16	1,3-β-glucan synthase		1.63*		1.45*
A0A151RX23	225.69	1,3-β-glucan synthase	2.45*			
A0A151ST57	220.68	1,3-β-glucan synthase			1.63*	
A0A151T7C1	177.03	1,3-β-glucan synthase			10.94*	
A0A151SBT9	99.80	Callose synthase 7	9.68*			
A0A151R5J1	105.77	Respiratory burst oxidase isogeny protein A		2.40*		
A0A151U4U3	101.1	Respiratory burst oxidase isogeny protein B		4.67*		
A0A151SPI8	92.77	Respiratory burst oxidase isogeny protein F			1.12*	
A0A151SV30	106.47	Respiratory burst oxidase isogeny protein F		2.26*		
A0A151T587	94.14	Respiratory burst oxidase isogeny protein F			1.45*	

*FC > 1, $p \leq 0.05$.

histochemical staining was performed to visually confirm and strengthen the proteomics findings (Figure 6). The resistant genotype inoculated with the pathogen displayed significantly higher lignin (Figure 6A—visualized as red-pink staining) and callose (Figure 6B—indicated by increased blue fluorescence) deposition compared to the resistant mock-treated, susceptible pathogen-treated, and susceptible mock-treated samples.

3.6 | Validation of Protein Expression

The expression patterns of eight selected genes, previously reported to play roles in biotic or abiotic stress responses, were validated through quantitative RT-PCR (Figure 7). These genes, involved in phenylpropanoid biosynthesis, calcium signalling, phytohormone signalling, and fatty acid biosynthesis, were found to be directly or indirectly associated with plant resistance. Notably, the expression levels of *caffeoyl-CoA O-methyltransferase* (3.5 FC), *cysteine-rich receptor-like protein kinase 29* (2.3 FC), *calcium-dependent protein kinase 1* (1.5 FC), *NAC 72* (1.5 FC), *phospholipase D α-1* (1.75 FC), *phospholipid-transporting ATPase 1* (3.2 FC), and *abscisic-aldehyde oxidase* (2.0 FC) were significantly upregulated ($p \leq 0.05$) in the resistant line compared to the susceptible line. These results aligned closely with pathogen-induced differential expression in

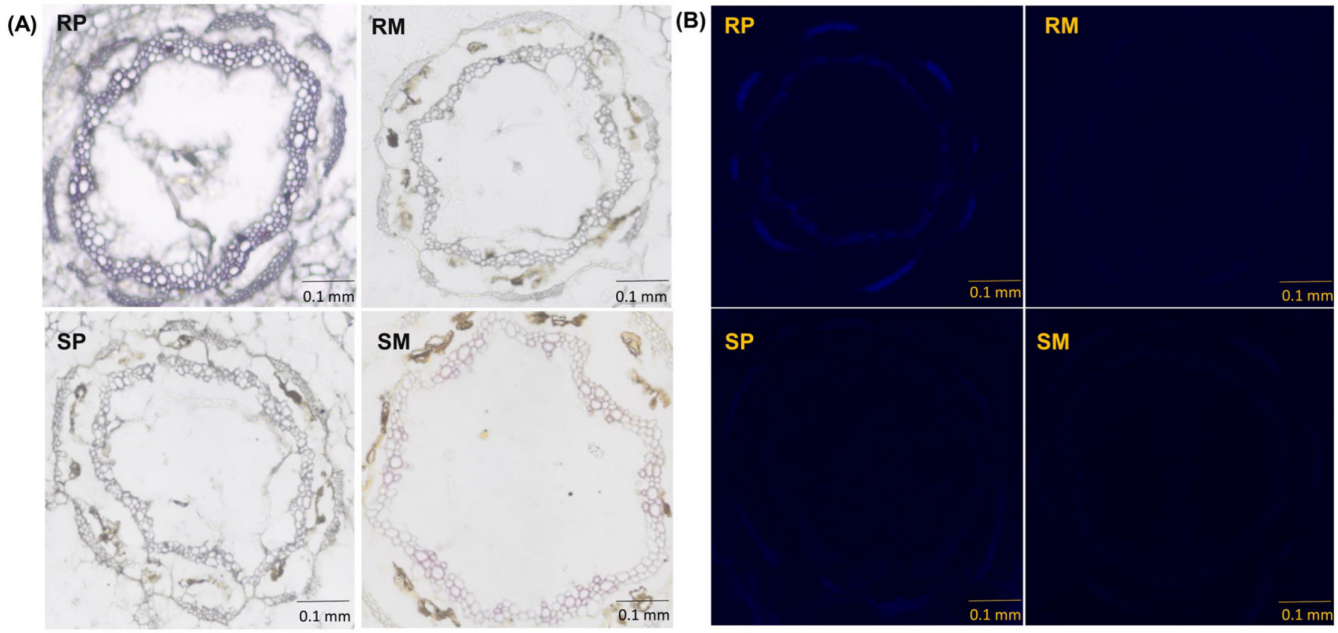

FIGURE 6 | Histochemical analysis of lignin and callose deposition in pigeon pea stem cross-sections. Fluorescence microscopy images of pigeon pea stem cross-sections (Magnification—20X, Scale—0.1 mm) showing cell wall reinforcement due to (A) lignin deposition (red-pink coloration) and (B) callose deposition (blue fluorescence). RP and RM represent pathogen- and mock-inoculated samples of the resistant genotype, respectively, while SP and SM represent pathogen- and mock-inoculated samples of the susceptible genotype.

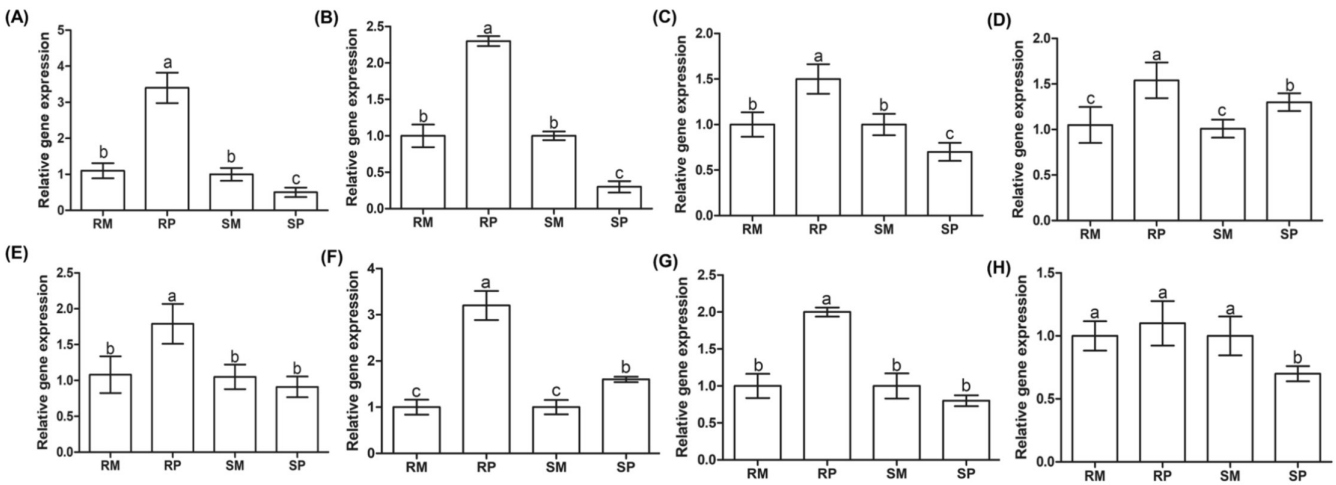

FIGURE 7 | Relative transcript expression levels in the resistant genotype compared to the susceptible genotype at 48 h post-inoculation (hpi) with *P. cajani* and mock treatments, measured by qRT-PCR using *GAPDH* as the reference gene. Expression analysis includes: (A) Caffeoyl-CoA O-methyltransferase, (B) Cysteine-rich receptor-like protein kinase 29, (C) Calcium-dependent protein kinase 1, (D) NAC domain-containing protein 72, (E) Phospholipase D alpha 1, (F) Phospholipid-transporting ATPase 1, (G) Abscisic-aldehyde oxidase, and (H) Chitin-inducible gibberellin-responsive protein 1. RP and RM represent resistant genotypes with *P. cajani* and mock inoculation, respectively; SP and SM represent susceptible genotypes with *P. cajani* and mock inoculation, respectively. Different letters above bars indicate statistically significant differences in expression levels between RP and SP ($p \leq 0.05$).

resistant genotypes and highlighted distinct patterns between contrasting genotypes.

4 | Discussion

Plants have developed complex and sophisticated innate immune systems that can recognize and respond to pathogen attacks (Bhatnagar-Mathur et al. 2021; Dodds et al. 2024). Proteomics offers a powerful method for investigating plant-pathogen interactions, providing insights into how plants respond at the protein level and how pathogens manipulate host processes. This approach has been applied to crops such as groundnut (Bhatnagar-Mathur et al. 2021), maize (Corrêa et al. 2025), and rice (Keerthana et al. 2025) to explore these interactions. Earlier studies have identified a range of proteins involved in secondary cell wall thickening, spanning from receptors to proteins associated with secondary biosynthetic pathways (Kishi-Kaboshi et al. 2018; Li et al. 2023). However, research on Pigeon pea–Phytophthora interactions remains limited. Using an untargeted proteomics approach, we have identified a hierarchy of proteins involved in the cell wall-mediated defense mechanism at 72 hpi. At this point, defense responses are fully activated, providing an optimal window to assess protein-level changes linked to stress signalling, pathogen recognition, and defense mechanisms. Examining this stage allows for the identification of key proteins involved in the plant's response, offering valuable insights into the molecular pathways underlying resistance and susceptibility.

4.1 | Regulatory Roles of Receptors, Transcription Factors, and Phytohormone-Related Proteins Involved in Cell Wall Thickening

4.1.1 | Receptor-Like Kinases

Plants detect pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs) through the activation of pattern recognition receptors (PRRs) located on the plasma membrane, triggering defense responses that lead to pattern-triggered immunity (PTI; Li et al. 2023). Receptor-like kinases (RLKs), a type of PRR, possess an intracellular kinase domain (Zhou et al. 2023). In our study, we observed the upregulation of several RLK families that included wall-associated receptor kinases (WAKs), leucine-rich repeat (LRR) receptor-like serine/threonine-protein kinases, cysteine-rich receptor-like kinases (CRKs), and receptor-like cytoplasmic kinases (RLCKs) in the resistant genotype following *P. cajani* infection. The LRR receptor-like serine/threonine-protein kinase At4g08850 family (*MIK2*) is known to sense cell wall integrity by detecting conserved pathogen signatures, particularly in response to *Fusarium* infection in Arabidopsis (Van Der Does et al. 2017; Hou et al. 2021). Similarly, the FLS2 receptor, another LRR receptor-like serine/threonine-protein kinase, recognizes the bacterial flagellin epitope flg22 (Cheng et al. 2020) and has been reported to detect *Fusarium sulphureum* in potato dry rot, initiating defense signalling (Fan et al. 2021). Upon pathogen recognition, PRRs activate RLCKs to trigger immune signalling (Liang and Zhou 2018; Liu et al. 2023). MIK2 has been shown to interact with RLCK serine/threonine-protein kinase PBL1 to initiate downstream signalling. In our study, the serine/threonine-protein kinase PBL7 was upregulated, suggesting that MIK2 and PBL7 may collaboratively contribute to defense-induced cell wall signalling. Additionally, CRKs have been implicated in perceiving extracellular stimuli and transmitting signals intracellularly to mediate disease resistance against phytopathogens (Zhao et al. 2022). CRK5 and CRK22 have been reported to recognize toxins from *V. dahliae*, activate downstream signalling, and confer resistance in Arabidopsis (Zhao et al. 2022). The breakdown of pectin facilitates pathogen penetration into the host and serves as a carbon source, acting as a major damage-associated molecular pattern (DAMP) (Cantu et al. 2008; De Lorenzo et al. 2011; Levesque-Tremblay et al. 2015). The wall-associated receptor kinase (WAK) family proteins are reported to interact with pectin and mediate defense signalling. For instance, *GmWAK1* has been shown to enhance resistance against *P. sojae*, with its overexpression in transgenic soybean plants promoting the downstream signalling of *GmPKK4* and *GmMPK6*, thereby inducing a defense response (Zhao et al. 2023). In wheat, *TaWAK2* has been reported to detect *F. graminearum* and suppress the expression of *Pectin Methyl-Esterase-1 (PME1)* through the activation of intracellular signalling. This leads to the formation of a more rigid and thickened cell wall, limiting fungal penetration and spread (Gadaleta et al. 2019). However, recent studies suggest that WAKs are not essential for OG-induced signal transduction or the initiation of immune responses in Arabidopsis (Herold et al. 2025). Instead, WAKs might play a role in fine-tuning oxidative burst-related proteins or in modulating the activity of primary receptors through alterations in the cell wall. Genetic evidence from other species highlights the role of WAK-like (WAKL) proteins in pathogen resistance, suggesting a potential interaction between WAKs and WAKLs. However, the exact mechanisms through which WAKs contribute to immunity remain to be fully elucidated (Yao et al. 2025). All the above-mentioned RLKs—CRKs, MIK2-PBL1, FLS2 (Zhao et al. 2022; Hou et al. 2021; Fan et al. 2021)—and WAKs (Zhao et al. 2023) have been implicated in inducing downstream immune signalling via the MAPK signalling pathway. In this study, proteins associated with MAPK signalling, such as MAPKKK and MAPK19, were upregulated, supporting the hypothesis that these RLKs and WAKs can activate MAPK signalling. This pathway is known to regulate various downstream transcription factors and phytohormonal responses (Soltabayeva et al. 2020).

4.1.2 | Transcription Factors

Transcription factors (TFs), including WRKY, MYB, AP2/ERF, and NAC, are key regulators of plant defense by detecting stress signals and activating defense genes, notably those involved in lignin biosynthesis and secondary cell wall thickening to enhance pathogen resistance (Meraj et al. 2020). Similar roles have been observed across species, such as AtMYB15 upregulating *AtPAL1* and *AtC3′H* in Arabidopsis (Chezem et al. 2017), OscWRKY1 enhancing PAL1 expression in Arabidopsis infected with *Pseudomonas syringae* (Joshi et al. 2022), TaNAC032 promoting *TaLAC4* and *TaCAD* in wheat against *F. graminearum* (Soni et al. 2021), and MdERF114 inducing MdPRX63 in apple responding to *Fusarium solani* (Liu et al. 2023). In switchgrass, NAC TFs SWN1/SWN2 activate MYB TFs (MYB46/83, MYB58/63, MYB42/85), forming a feedback loop that drives

lignification (Rao et al. 2019). In apple, MdWRKY75 binds the promoter of MdERF114, enhancing its activation of *MdPRX63* to promote lignin deposition (Liu et al. 2023). In our study, upregulation of WRKY32, WRKY39, MYB At1g14600, AP2/ERF RAV1, and NACs NAC72 and NAC76 suggests their involvement in lignin gene regulation. NAC activation via ethylene signalling by ERF TFs (Kim et al. 2014) and WRKY29-mediated upregulation of ethylene biosynthetic genes (Wang et al. 2023) further support hormone-mediated regulation of secondary cell wall thickening (Yogendra et al. 2017). These TFs likely coordinate to enhance lignin biosynthesis, though further protein interaction and co-expression studies are needed.

4.1.3 | Phytohormone-Related Proteins

Phytohormones regulate a complex signalling network coordinating pathogen defense and secondary cell wall thickening through hormonal crosstalk (Khan 2025). Detection of PAMPs activates ethylene biosynthesis, increasing expression of ethylene-related genes (Vidhyasekaran 2015). Ethylene acts as a stress hormone in both biotic and abiotic responses (Perata 2020; Zhao et al. 2021). In our study, ethylene biosynthesis proteins such as ACO and EIL1 were upregulated; ACO catalyzes the conversion of ACC to ethylene, while EIL1 is central to ethylene signalling (Chang et al. 2013; Zhang et al. 2014). EIL1 activity is regulated by JA-responsive JAZ proteins, which suppress it under non-stress conditions; stress-induced JA-Ile promotes JAZ degradation, activating EIN3/EIL1 (Zhu et al. 2011; Samanta and Roychoudhury 2025). Upregulation of lipoxygenase, a key JA biosynthetic enzyme, suggests crosstalk between JA and ethylene pathways, which can be synergistic or antagonistic (Zhang et al. 2014; Samanta and Roychoudhury 2025). EIL1 and EIN3 also bind ERF promoters to regulate their expression (Huang, Zhao, et al. 2021), and ERFs activate genes involved in lignin biosynthesis and cell wall thickening. For example, GbERF1-like TF upregulates phenylpropanoid pathway enzymes (*GbPAL, GbC4H, GbC3H, GbHCT, GbCCoAOMT, GbCCR, GbF5H*), enhancing lignification and resistance to *V. dahliae* in cotton (Guo et al. 2016).

4.2 | Differentially Expressed Proteins Involved in Lignin Biosynthesis for Enhanced Secondary Cell Wall Thickening

We observed the upregulation of key proteins from the phenylpropanoid pathway—PAL, p-C3′H, CCoAOMT, and peroxidase—in the resistant genotype in response to *P. cajani* infection. These enzymes are crucial for lignin deposition, a process derived from the phenylpropanoid pathway (Ninkuu et al. 2022). The upregulation of these enzymes has also been reported in various plant-pathogen interactions, such as pepper-*P. capsici* (Li et al. 2020), wheat-*F. graminearum* (Yang et al. 2021), peanut-*A. flavus* (Avuthu et al. 2024), and cotton-*V. dahlia* (Guo et al. 2016). In these cases, defense-induced lignification contributed to pathogen resistance by reinforcing the secondary cell wall.

Additionally, several ABCG transporters, including ABCG9, ABCG10, ABCG11, ABCG14, ABCG26, ABCG28, ABCG31, ABCG32, ABCG34, ABCG36, and ABCG39, were found to be upregulated in resistant genotypes following *P. cajani* infection. The ABCG transporter family, known for its functional diversity, plays a crucial role in transporting various defense metabolites, signalling molecules, and hormones across cellular membranes (Cheng et al. 2025). Notably, the overexpression of *LkABCG36* in tobacco has been shown to enhance lignin deposition, suggesting its role in transporting lignin precursors to the cell wall (Sun et al. 2024). Similarly, *ABCG29* has been reported to facilitate the transport of monolignols for lignin biosynthesis (Alejandro et al. 2012). Moreover, silencing *MtABCG10* in *Medicago truncatula* hairy roots led to increased pathogen progression and reduced accumulation of phenylpropanoid-derived medicarpin and its precursors in response to *Fusarium oxysporum* (Banasiak and Jasiński 2014). The upregulation of these proteins from the phenylpropanoid pathway and ABCG transporters in our study suggests their potential involvement in defense-induced lignification.

4.3 | Differentially Expressed Proteins Mediating Callose Deposition for Cell Wall Thickening

Callose (1,3-β-glucan) is synthesized by callose synthases (1,3-β-glucan synthases) and is reported to be deposited as a form of pathogen-induced defense mechanism, thus preventing pathogen invasion and cell wall reinforcement (Wang et al. 2021). In our study, 1,3-β-glucan synthases and callose synthases were observed to be upregulated, indicating deposition of callose in response to *P. cajani*. Similarly, in potato, callose synthesis was enhanced in response to *P. infestans* by the upregulation of 1,3-β-glucan synthase, which led to a significant inhibition of the pathogen's growth and expansion in the host cells (Yang et al. 2020). Also, in avocado, callose deposition played a key role in resistance to *P. cinnamoni* (Van Den Berg et al. 2021). These findings suggest that callose deposition enhances resistance to pathogens, which was also observed in response to *P. cajani* in our study. In addition, ROS plays a pivotal role in plant defense mechanisms, including the reinforcement of cell walls through callose deposition. The NADPH oxidase family, particularly the respiratory burst oxidase homologs (RBOHs), are key producers of ROS in plants (Hino et al. 2024). In our study, RBOHA, RBOHB, and RBOHF were observed to be upregulated. Overexpression of RBOHA enhanced resistance (Soliman et al. 2021). Similarly, overexpression of RBOHB and RBOHF imparted resistance to *Xanthomonas axonopodis* in cassava (Huang, Tang, et al. 2021). In cassava, the overexpression of specific RBOH genes, such as *RBOHB* and *RBOHF*, has been associated with increased ROS production, leading to enhanced callose deposition. This structural reinforcement of the cell wall contributes to improved resistance against pathogens like *X. axonopodis*, which causes cassava bacterial blight (Huang, Tang, et al. 2021). Thus, the upregulation of these RBOH proteins in our study depicts the importance of ROS signalling in callose-mediated defense response.

5 | Conclusions

Our study highlights the dynamic changes in the proteome and uncovers key molecular mechanisms underlying the resistance

of pigeon pea to *P. cajani*. By integrating proteomic analysis, histochemical assays, and gene expression profiling, we identified several significantly upregulated proteins, such as receptor-like kinases, transcription factors, phytohormonal regulators, ROS-mediated signalling components, and ABCG transporters. These regulators influence the expression of downstream proteins, including *PAL*, *CCOAMT*, *C3′H*, and *1,3-β-glucan synthases*, which are crucial for strengthening the cell wall through lignin and callose deposition, respectively. This reinforces a cell wall-mediated defense mechanism against the pathogen. With further validation, the proteins identified in this study may serve as potential biomarkers for enhancing resistance to *P. cajani*. The insights gained from the Phytophthora–Pigeon pea interaction will support the development of pigeon pea varieties resistant to Phytophthora blight through targeted breeding strategies.

Author Contributions

A.A.B. carried out the investigation and validation and prepared the original draft. B.R.P. contributed to the conceptualization and methodology and reviewed and edited the manuscript. H.S. was responsible for visualization, formal analysis, and data curation. S.K.D., S.J., R.G., M.S., and P.I.G. contributed to resources, methodology, data curation, and reviewing and editing the manuscript. K.Y. conceptualized and supervised the study, provided resources, administered the project, and contributed to reviewing and editing. All authors have reviewed and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting this study's findings are available in Figures S1 and S2 and Tables S1–S4 of this article. The proteomics data obtained from mass spectrometry has been deposited via jPOSTrepo to the ProteomeXchange consortium with the database identifier PXD063337.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Workflow for the comparative proteomics analysis of lignin and callose deposition in response to *Phytophthora cajani* in pigeon pea. **Figure S2:** Venn diagrams showing the overlap of significantly accumulated proteins among. **Table S1:** Primer sequences used for gene expression analysis. **Table S2:** Minimal Information About a Proteomics Experiment (MIAPE) used in comparative proteomics analysis of contrasting pigeonpea genotypes against *Phytophthora cajani* infection. **Table S3:** List of Resistance-related constitutive (RRC) proteins identified in contrasting pigeonpea lines following *P.cajani* or mock inoculation. **Table S4:** List of Resistance-related induced (RRI) proteins identified in contrasting pigeonpea lines following *P.cajani* or mock inoculation.