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Transcriptome Profiling Reveals Mungbean Defense Mechanisms Against Powdery Mildew

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Abstract

Powdery mildew (PM), caused by *Sphaerotheca phaseoli*, severely threatens mungbean (*Vigna radiata*) productivity and quality, yet the molecular basis of resistance remains poorly defined. This study employed transcriptome profiling to compare defense responses in a resistant genotype, SUPER5, and a susceptible variety, CN84-1, following pathogen infection. A total of 1755 differentially expressed genes (DEGs) were identified, with SUPER5 exhibiting strong upregulation of genes encoding pathogenesis-related (PR) proteins, disease resistance proteins, and key transcription factors. Notably, genes involved in phenylpropanoid and flavonoid biosynthesis, pathways associated with antimicrobial compound and lignin production, were markedly induced in SUPER5. In contrast, CN84-1 showed limited activation of defense genes and downregulation of essential regulators such as MYB14. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses highlighted the involvement of plant–pathogen interaction pathways, MAPK signaling, and reactive oxygen species (ROS) detoxification in the resistant response. Quantitative real-time PCR validated 11 candidate genes, including *PAL3*, *PR2*, *GSO1*, *MLO12*, and *P21*, which function in pathogen recognition, signaling, the biosynthesis of antimicrobial metabolites, the production of defense proteins, defense regulation, and the reinforcement of the cell wall. Co-expression network analysis revealed three major gene modules linked to flavonoid metabolism, chitinase activity, and responses to both abiotic and biotic stresses. These findings offer valuable molecular insights for breeding PM-resistant mungbean varieties.

Keywords: defense response genes; resistance mechanisms; *Sphaerotheca phaseoli*; transcriptomics; *Vigna radiata*



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1. Introduction

Mungbean (*Vigna radiata* (L.) Wilczek), a crucial leguminous crop in tropical and subtropical regions, serves as a major source of dietary protein, making significant contributions to food security and nutrition worldwide. Its role in sustainable agricultural practices is further underscored by its inherent ability to fix atmospheric nitrogen, enhancing soil fertility and reducing the need for synthetic fertilizers [1]. In Thailand, mungbean was

grown on approximately 609,756 rai, with a total production of 94,236 tons [2]. However, the viability and productivity of mungbean crops are significantly hampered by both abiotic and biotic stresses. Biotic factors include insect-pests, especially bruchids, whitefly, thrips, aphids, and pod borers, and diseases, particularly yellow mosaic, anthracnose, dry root rot, *Cercospora* leaf spot (CLS), and powdery mildew (PM), posing a paramount challenge [3,4].

PM, primarily caused by the obligate biotrophic pathogen *Sphaerotheca phaseoli*, is a ubiquitous and severe affliction affecting numerous crops globally [5]. The disease affects aerial plant parts, inducing chlorosis, necrosis, and premature leaf senescence. This leads to reduced seed quality and yield losses of 20–40% worldwide, with severe cases in Maharashtra State, India, suffering losses of up to 100% [6]. Therefore, breeding mungbean varieties with resistance to PM is critically important to ensure food security and advance sustainable agriculture. Previous studies indicated that PM resistance is a quantitatively inherited trait, characterized by high heritability and primarily governed by additive genetic effects [7]. In addition, Khajudparn [8] reported that PM resistance in mungbean lines, V4718, V4758, and V4785, is controlled by a distinct single dominant gene, and these resistance loci are non-allelic, suggesting that they are located at different positions in the genome. At the molecular level, numerous studies have identified MLO proteins as key factors contributing to PM susceptibility [9] or resistance [10], and the nucleotide-binding site and leucine-rich repeat domain (NBS-LRR)-type gene, *Recognition of Peronospora parasitica 13-like protein (RPP13L)*, has been proposed as a candidate gene conferring PM resistance as well [11]. These genetic insights are highly valuable for mungbean improvement through molecular breeding. Recently, our research group successfully developed new backcross progenies of the recurrent parent Thai mungbean variety SUT1, which exhibit resistance to PM along with favorable agronomic traits, using marker-assisted backcross breeding [12]. A deeper understanding of the genetic mechanisms underlying PM resistance is crucial, as it offers durable, broad-spectrum, and environmentally sustainable strategies for long-term disease management. The complex interaction between PM pathogens and their host plants encompasses diverse defense responses and pathogen virulence strategies, revealing a sophisticated biological function [13]. Plants have evolved a robust defense apparatus, which includes physical barriers, pattern-triggered immunity (PTI), effector-triggered immunity (ETI), and the synthesis of antimicrobial compounds [14]. However, the precise molecular frameworks governing mungbean defense responses to PM infection remain inadequately characterized. Uncovering these underlying mechanisms is critical for devising effective resistance strategies and mitigating the impact of this disease.

Transcriptome profiling has emerged as a vital technique for elucidating the molecular foundation of plant–pathogen interactions. Capturing a comprehensive genome-wide expression landscape, transcriptomics enables the identification of crucial genes, signaling cascades, and regulatory networks involved in defensive responses to pathogens [15,16]. Recent advancements in high-throughput sequencing methods, particularly RNA sequencing (RNA-seq), have transformed the landscape of plant transcriptomic studies, enabling precise assessments of gene expression modulation under the influence of biotic and abiotic stresses. In mungbean, transcriptome analysis presents a unique opportunity to investigate the dynamic gene expression shifts during PM infection and to pinpoint candidate genes linked to resistance.

Insights from transcriptomic investigations in related crops such as melon [17,18], barley [19,20], wheat [21], and Arabidopsis [22] have significantly advanced our understanding of PM resistance mechanisms [23,24]. These studies underscore the roles of pathogenesis-related (PR) proteins, hormonal signaling pathways, including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), along with reactive oxygen species (ROS) production and scavenging, and the biosynthesis of secondary metabolites and lignin, in fortifying plant

defenses [13,25,26]. Despite these advancements, the specific transcriptional responses of mungbean to PM remain largely uncharted. A detailed understanding of how mungbean orchestrates its defense at the transcriptional level may provide valuable insights into its unique resistance mechanisms and illuminate potential targets for genetic enhancement.

This study endeavors to delineate the transcriptomic alterations in mungbean during PM infection through the application of RNA-Seq technology. By juxtaposing gene expression profiles between resistant and susceptible mungbean genotypes, we aim to identify differentially expressed genes (DEGs) and unravel the molecular pathways implicated in the defense response. Our objectives are to characterize gene expression dynamics during PM infection and to elucidate candidate genes and regulatory networks associated with resistance mechanisms against PM. Additionally, this research serves as a foundational framework for subsequent functional genomics investigations aimed at validating the roles of these candidate genes in PM resistance.

The findings of this study will not only enhance our grasp of the molecular basis of mungbean's defense to PM but also yield crucial data for the development of molecular markers relevant for marker-assisted breeding strategies. By identifying and harnessing key genetic determinants of resistance, this research will facilitate the development of PM-resistant mungbean varieties, ultimately enhancing crop productivity and resilience to biotic stresses. Moreover, the insights derived from this study may have broader implications for elucidating plant–pathogen interactions across diverse crop species, contributing to global efforts aimed at promoting agricultural sustainability and food security.

2. Materials and Methods

2.1. Plant Materials

The resistant line SUPER5 was derived through the genetic integration of three Indian disease-resistant lines: V4718, V4758, and V4785. This homozygous line demonstrates enhanced resistance to CLS, PM, and Mungbean Yellow Mosaic Virus (MYMV), exhibiting greater stability across a range of environmental conditions when compared to its parental lines [27]. In contrast, the susceptible variety CN84-1 was developed from the Chai Nat Field Crops Research Center in Thailand and is widely cultivated among Thai farmers as a certified variety. This cultivar is recognized for its large seed size, high yield potential, and notable nutritional values; however, it remains vulnerable to CLS, PM, and MYMV diseases.

2.2. Methods

2.2.1. Phenotyping of SUPER5 and CN84-1 Inoculated with *Sphaerotheca phaseoli*

In November 2023, the experiments were conducted at Suranaree University of Technology, Nakhon Ratchasima, Thailand. The resistant line SUPER5 and susceptible variety CN84-1 were grown at 25 °C with 12 light hours/day (8000–9000 lux) in the growth chamber. Subsequently, 21-day-old plants of resistant and susceptible mungbean genotypes with control (non-inoculated) and PM-inoculated conditions in three biological and technical replications per treatment (one plant/replication) were evaluated using a whole-plant assay. Conidia were collected from infected mungbean leaves in the experimental field. PM inoculation was performed by uniformly distributing infected leaves around the evaluation area, facilitating natural dissemination through wind dispersal at 20 °C and 55–60% humidity. Control plants were treated with non-infected leaf material under identical environmental conditions. A tightly sealed plastic partition was used to physically isolate the non-inoculated condition from the PM-inoculated condition, thereby preventing any potential cross-contamination. Disease severity was recorded at 0, 3, 6, 11, and 16 days after inoculation (DAI). The disease severity scoring was adapted from [28], employing a 1–5

scale based on the percentage of leaf area affected by PM (Table S1). Statistical analysis of disease severity scores was conducted using SPSS version 16.0 [29].

2.2.2. Sample Collection and RNA Isolation

At 21 days post-planting, leaves from two mungbean genotypes were inoculated with *S. phaseoli*. At 11 DAI, leaf samples were collected, flash-frozen in liquid nitrogen, and stored at -80°C for subsequent analysis. Total RNA was extracted from both genotypes under control and inoculated conditions, with three biological replicates per treatment in each genotype, using the Favor Prep Plant Total RNA Mini Kit (Favorgen, Ping Tung, Taiwan), following the manufacturer's protocol. RNA quality was assessed via 1% agarose gel electrophoresis and quantified using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA).

2.2.3. RNA Library Constructing, RNA Sequencing, and Data Analysis

For RNA library preparation, 1 μg of total RNA was used as input material. Library construction and indexing were performed, followed by multiplexing and sequencing on an Illumina HiSeq platform using a 2×150 bp paired-end (PE) configuration, following the manufacturer's guidelines (Azenta Life Sciences, Beijing, China). Raw image data were processed using Bcl2fastq (version 2.20.0.422) for base calling and initial quality assessment. The first 25 bases of each sequencing read were evaluated using Illumina's built-in software to determine retention or exclusion. High-quality reads that passed filtering were stored in FASTQ format, containing both sequence data and corresponding quality scores. Low-quality reads and adapter contaminants were removed using Cutadapt (version 1.9.1) prior to further analysis.

DEGs were identified using DESeq2, applying a fold change threshold of ≥ 2 and a q -value (false discovery rate [FDR]; p -adjusted) ≤ 0.05 . The number of upregulated and downregulated genes was quantified and summarized. Functional enrichment analysis of DEGs was performed using Goseq (version 1.34.1), which mapped enriched Gene Ontology (GO) terms against the genomic background to elucidate biological functions associated with the DEGs. A significance threshold of $p \leq 0.05$ was applied. Additionally, pathway enrichment analysis was carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. KEGG pathway units and the hypergeometric test were employed to identify pathways significantly enriched with DEGs relative to the transcriptomic background [30], with a q -value cutoff of ≤ 0.05 . Gene expression profiles were visualized through heatmaps. The interactions among candidate genes were elucidated by co-expression network analysis using the STRING (version 12).

2.2.4. Validation of RNA Sequencing Results by Quantitative Real-Time Polymerase Chain Reaction

To validate the RNA-seq findings, quantitative real-time polymerase chain reaction (qRT-PCR) was conducted on a subset of DEGs. Complementary DNA (cDNA) synthesis was performed using 1 μg of total RNA, confirmed to be free of genomic DNA contamination in duplicate, with the iScript Reverse Transcription Supermix (Bio-Rad, Hercules, CA, USA), according to the manufacturer's protocol. Gene-specific primers were designed using Primer3 Plus software (version 3.3.0), and the *Actin* gene was used as an internal reference for normalization. The primer sequences were detailed in Table S2 [31]. qRT-PCR amplification was carried out using the CFX Opus 96 Real-Time PCR System (Bio-Rad, Hercules, CA, USA). Each 10 μL reaction mixture comprised $1 \times$ Luna Universal qPCR Mix (New England Biolabs Inc., Ipswich, MA, USA), 0.1 pmol of each primer, and 67.4 ng of cDNA. The thermal cycling conditions, adapted from [32], included an initial denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 5 s, 60°C for 30 s, and a final extension

at 60 °C for 5 s. Threshold cycle (Ct) values were averaged across three biological replicates, and relative gene expression levels were determined using the comparative Ct ($2^{-\Delta\Delta CT}$) method. The concordance between RNA-seq and qRT-PCR results was evaluated through Pearson correlation analysis using SPSS version 16.0 [29].

3. Results and Discussions

3.1. Differential Responses Between Resistant Mungbean Line SUPER5 and Susceptible Variety CN84-1 to Powdery Mildew Infection Induced by *Sphaerotheca phaseoli*

The assessment of PM infection in the resistant mungbean line SUPER5 and the susceptible variety CN84-1, following inoculation with *S. phaseoli*, revealed distinct disease responses between the two genotypes. The susceptible variety CN84-1 exhibited more pronounced disease symptoms compared to the resistant line SUPER5. No visible symptoms were observed in either genotype at 0, 3, or 6 DAI, with an average disease severity score of 1. However, by 11 DAI, PM symptoms were evident on the leaves of CN84-1 but remained absent in SUPER5, with average disease scores of 2.50 and 1.00, respectively. Disease progression was more pronounced at 16 DAI, with severity scores increasing to 5.00 in CN84-1 while remaining at 1.33 in SUPER5 (Table 1). Abundant hyphae were visibly observed on the leaves of CN84-1, whereas no hyphae were detected on SUPER5, which exhibited a hypersensitive response (HR) (Figure 1).

Table 1. PM disease severity scores in mungbean leaves of SUPER5 and CN84-1 at 0, 3, 6, 11, and 16 DAI.

Days Post Inoculation	SUPER5		CN84-1	
	Severity Score	Response ³	Severity Score	Response
0	1.00 ± 0.00 ²	R	1.00 ± 0.00 c ²	R
3	1.00 ± 0.00	R	1.00 ± 0.00 c	R
6	1.00 ± 0.00	R	1.00 ± 0.00 c	R
11	1.00 ± 0.00	R	2.50 ± 0.29 b	MR
16	1.33 ± 0.33	R	5.00 ± 0.00 a	S
F-test ¹	ns		**	

¹ ns and ** indicate non-significant and highly significant differences among the five infection times of each genotype at $p \leq 0.01$, respectively. ² mean ± SE in the same column with different letters are significantly different ($p < 0.05$) based on Duncan's Multiple Range Test (DMRT). ³ disease response refers to PM resistance levels based on severity scores as follows: resistance (R) = 1.0–2.3, moderate resistance (MR) = 2.4–3.6, and susceptibility (S) with score ratings of 3.7–5.00.



Figure 1. PM disease symptoms at 16 DAI in resistant line SUPER5 and susceptible line CN84-1.

To elucidate the molecular mechanisms underlying PM resistance, leaf samples from both genotypes were collected at 11 DAI for RNA extraction. At this point, fungal hyphae were observed in the susceptible line but not in the resistant line, suggesting that critical upstream gene(s) involved in resistance regulation may be differentially expressed. Transcriptomic analysis was performed by comparing RNA sequences from inoculated and non-inoculated control leaves, enabling the identification of DEGs associated with PM resistance responses in mungbean.

3.2. Transcriptome Analysis Using RNA Sequencing Technique

3.2.1. RNA Sequencing Analysis

Transcriptome profiling was conducted using RNA-seq on RNA samples extracted from the resistant mungbean line SUPER5 and the susceptible variety CN84-1, collected at 11 DAI, as well as from non-inoculated control plants. Sequencing data were processed using the Cutadapt software (version 1.9.1) to ensure quality trimming and adapter removal. A total of 12 libraries were constructed, yielding approximately 529.77 million raw reads, with individual libraries generating between 39.7 and 50.5 million high-quality reads (HQRs) possessing a Phred quality score of ≥ 30 . Of these, approximately 94% of the reads were successfully aligned to the mungbean reference genome, demonstrating high mapping efficiency. The average GC content across the libraries was determined to be 43%, as summarized in Table 2. This analysis provides a robust foundation for subsequent differential gene expression studies between resistant and susceptible genotypes under pathogen stress conditions.

Table 2. The number of raw reads and clean sequences of the resistant mungbean line (SUPER5) and the susceptible variety (CN84-1) under non-inoculated (control) and inoculated conditions with the pathogen *S. phaseoli*.

Samples	Total Sequences		Sequence Length		GC Content (%)	
	Raw Read	Clean Read	Raw Read	Clean Read	Raw Read	Clean Read
CN84-1 (control)-1	51,054,240	50,532,360	150	144.12	44.18	43.86
CN84-1 (control)-2	43,313,750	42,895,648	150	144.83	44.00	43.75
CN84-1 (control)-3	43,260,030	42,713,682	150	144.38	44.22	43.92
CN84-1 (inoculated)-1	42,844,270	42,383,432	150	145.22	44.14	43.87
CN84-1 (inoculated)-2	43,655,424	43,103,460	150	144.26	44.29	43.94
CN84-1 (inoculated)-3	45,585,534	45,125,966	150	144.20	44.13	43.79
SUPER5 (control)-1	43,307,702	42,866,758	150	144.70	43.62	43.31
SUPER5 (control)-2	40,001,488	39,660,530	150	145.72	43.65	43.43
SUPER5 (control)-3	47,896,438	47,366,050	150	145.23	43.93	43.65
SUPER5 (inoculated)-1	43,388,166	42,902,528	150	143.91	44.21	43.87
SUPER5 (inoculated)-2	41,040,500	40,562,014	150	143.13	43.75	43.33
SUPER5 (inoculated)-3	50,155,254	49,657,154	150	144.32	44.19	43.91
Total	535,502,796	529,769,582	-	-	-	-

RNA-seq was performed on 12 libraries derived from mungbean RNA samples, including the resistant line SUPER5 and the susceptible variety CN84-1, under both inoculated and non-inoculated (control) conditions. Transcript abundance was quantified using the fragments per kilobase of transcript per million mapped reads (FPKM) metric, calculated with the HT-Seq software (version 0.6.1). DEGs were identified based on a log2 fold change threshold of ≥ 2 and a statistically significant q -value (false discovery rate, FDR; p -adj) of < 0.05 . Comparative analysis revealed a total of 1755 genes with significant differential expression between the treatments: SUPER5 (control) vs. SUPER5 (inoculated) and CN84-1 (control) vs. CN84-1 (inoculated). These DEGs were further analyzed using the Bioinformatics & Evolutionary Genomics tool to generate a Venn diagram, facilitating the visualization of shared and unique gene expression patterns.

Among the upregulated genes at 11 DAI compared to the control, 18 genes were significantly upregulated in both SUPER5 and CN84-1. However, when analyzed separately, SUPER5 exhibited 1198 significantly upregulated genes, whereas CN84-1 displayed only 63 significantly upregulated genes (Figure 2A,C,D). In contrast, 13 genes were significantly downregulated in both varieties. Individually, SUPER5 showed 373 significantly downregulated genes, while CN84-1 had 121 significantly downregulated genes (Figure 2B–D).

These findings highlight distinct transcriptional responses between the resistant and susceptible genotypes under pathogen stress, providing insights into the molecular mechanisms underlying resistance and susceptibility in mungbean.

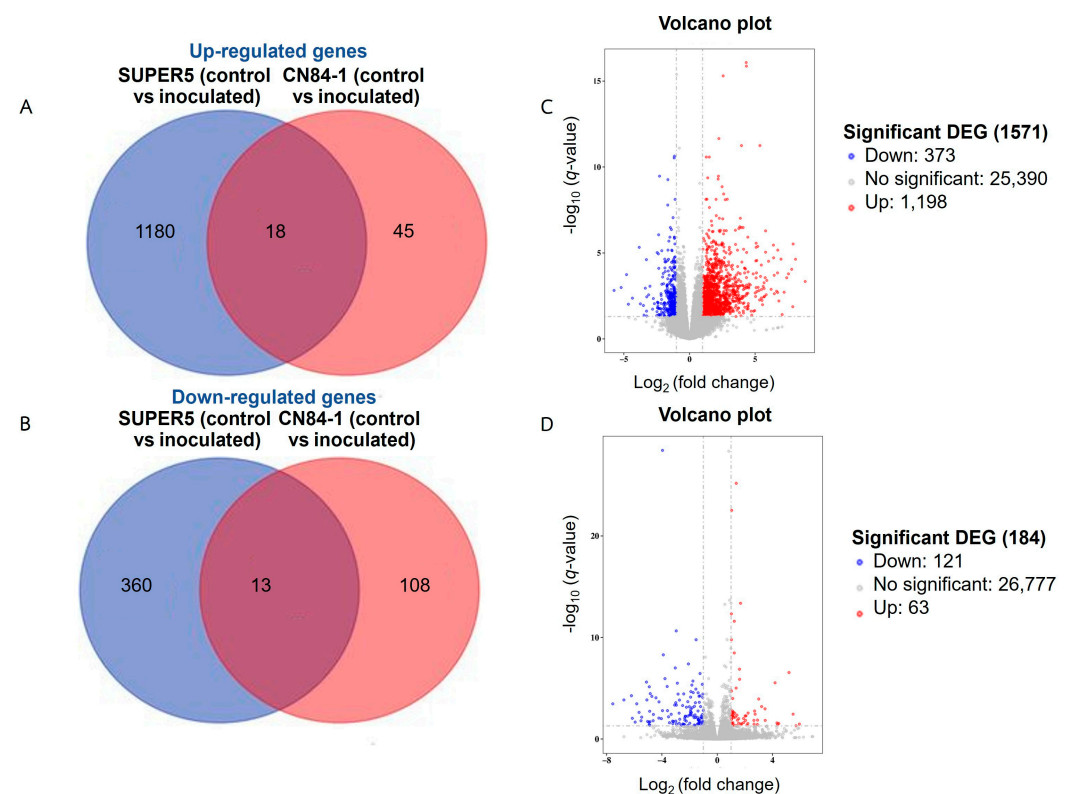


Figure 2. Venn diagram and volcano plots of gene expression in resistant (SUPER5) and susceptible (CN84-1) mungbean genotypes. **(A)** Representing the Venn diagram showing upregulated genes in the resistant line SUPER5 and the susceptible variety CN84-1. **(B)** Representing the Venn diagram showing downregulated genes in the resistant line SUPER5 and the susceptible variety CN84-1. **(C)** Representing the volcano plot illustrating the interaction between SUPER5 (control) and SUPER5 (inoculated). **(D)** Representing the volcano plot illustrating the interaction between CN84-1 (control) and CN84-1 (inoculated). Red and blue dots represent significantly upregulated and downregulated genes, respectively. The horizontal axis represents the log₂ fold change in gene expression, and the vertical axis represents the statistical significance of gene expression differences in log₁₀ (*q*-value (FDR, *p*-adj)).

3.2.2. Analysis of Gene Ontology and Kyoto Encyclopedia of Genes and Genome Pathway Enrichment of DEGs Responding to Powdery Mildew Infection in Mungbean

GO and KEGG pathway analyses were conducted to elucidate the functional attributes of DEGs responding to PM infection in mungbean. The GO analysis, which categorizes genes based on biological attributes, classified the DEGs from both the resistant line SUPER5 and the susceptible variety CN84-1 into three functional groups: molecular function, cellular component, and biological process. In SUPER5, 599, 727, and 448 DEGs were assigned to these categories, respectively, while CN84-1 exhibited 67, 18, and 62 DEGs in the corresponding groups (Figure 3). Additionally, the top 30 most significantly enriched biological attributes and gene functions were identified, with thresholds set at a *p*-value ≤ 0.05, log₂ FC ≥ 1, and FDR < 0.05.

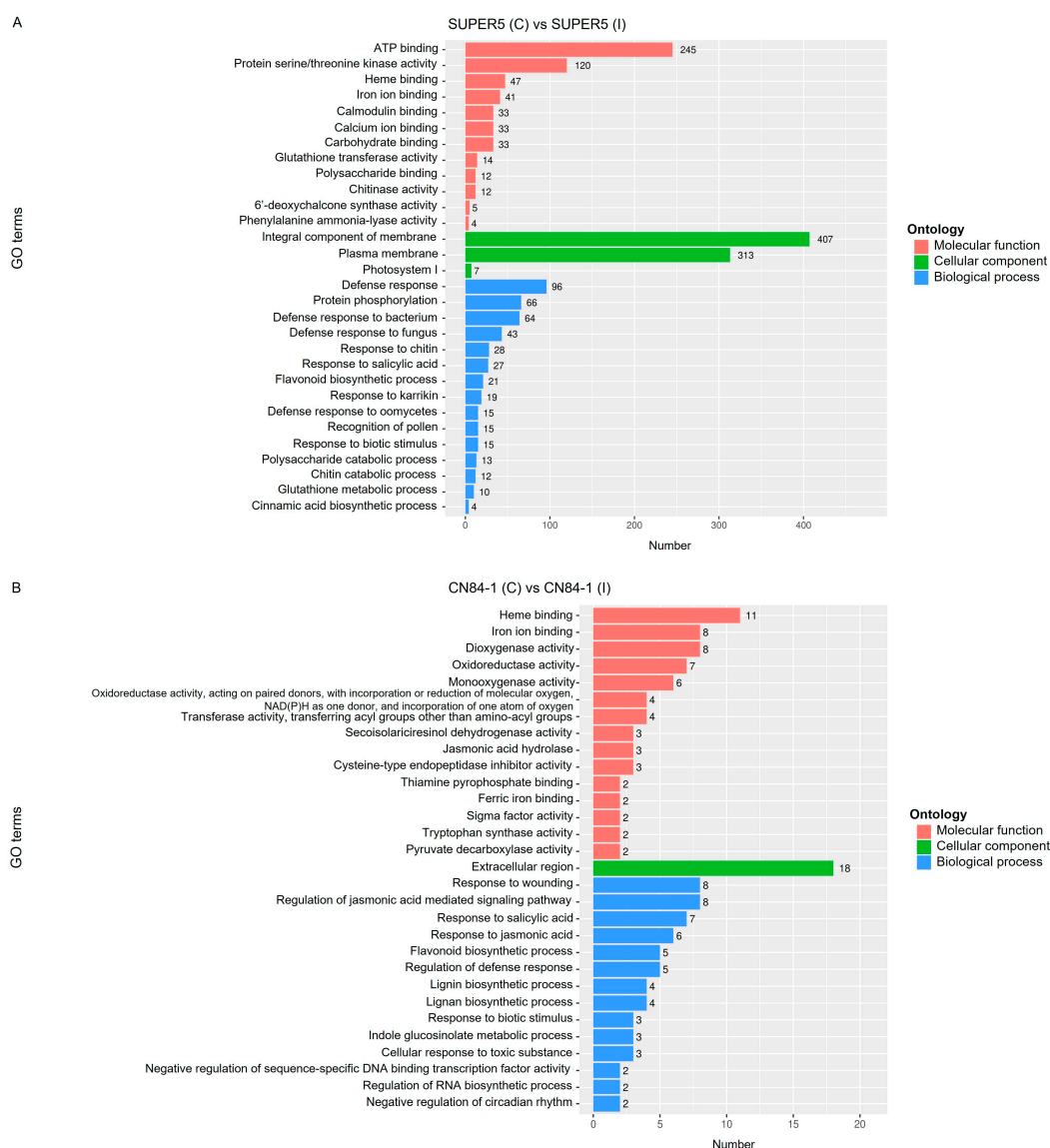


Figure 3. GO analysis of DEGs in the resistant line SUPER5 (SUPER5 (control) vs. SUPER5 (inoculated)) (A) and the susceptible variety CN84-1 (CN84-1 (control) vs. CN84-1 (inoculated)) (B).

Within the biological process category, DEGs in SUPER5 were of particular interest due to their potential roles in disease resistance. These included 96 genes associated with plant defense mechanisms, 66 genes involved in phosphorylation processes, and 28 genes responsive to chitin. Notably, 58 genes implicated in plant defense against fungal infection were identified, along with genes responsive to the signaling molecule SA. The majority of these genes exhibited upregulation in the resistant line SUPER5 (Figure 3A). Consistently, numerous previous studies have reported that DEGs associated with plant defense responses to fungal pathogens are predominantly enriched in GO biological process terms [33–35].

In the susceptible variety CN84-1, DEGs were linked to processes such as wound response, lignin biosynthesis, plant signaling, and defense mechanisms. However, most of these genes displayed downregulation in CN84-1, suggesting a diminished defensive capacity compared to SUPER5 (Figure 3B). These findings highlight the differential transcriptional responses between the resistant and susceptible genotypes and provide insights into the molecular mechanisms underlying mungbean response to PM infection. The

identification of these DEGs and their functional annotations underscores their potential roles in mediating resistance or susceptibility to PM.

Functional analysis of DEGs using the KEGG database (p -value < 0.05) revealed distinct metabolic and signaling pathways in mungbean genotypes under both inoculated and non-inoculated conditions with PM. Among the 1755 DEGs identified across both genotypes, the resistant line SUPER5 (SUPER5 (control) vs. SUPER5 (inoculated)) displayed a broader range of functional categories, including organismal systems, cellular processes (metabolism), and environmental information processing (Figure 4). In contrast, the susceptible variety CN84-1 (CN84-1 (control) vs. CN84-1 (inoculated)) exhibited genes primarily associated with fundamental metabolic processes, such as starch and sucrose metabolism and nitrogen metabolism (Figure 5).

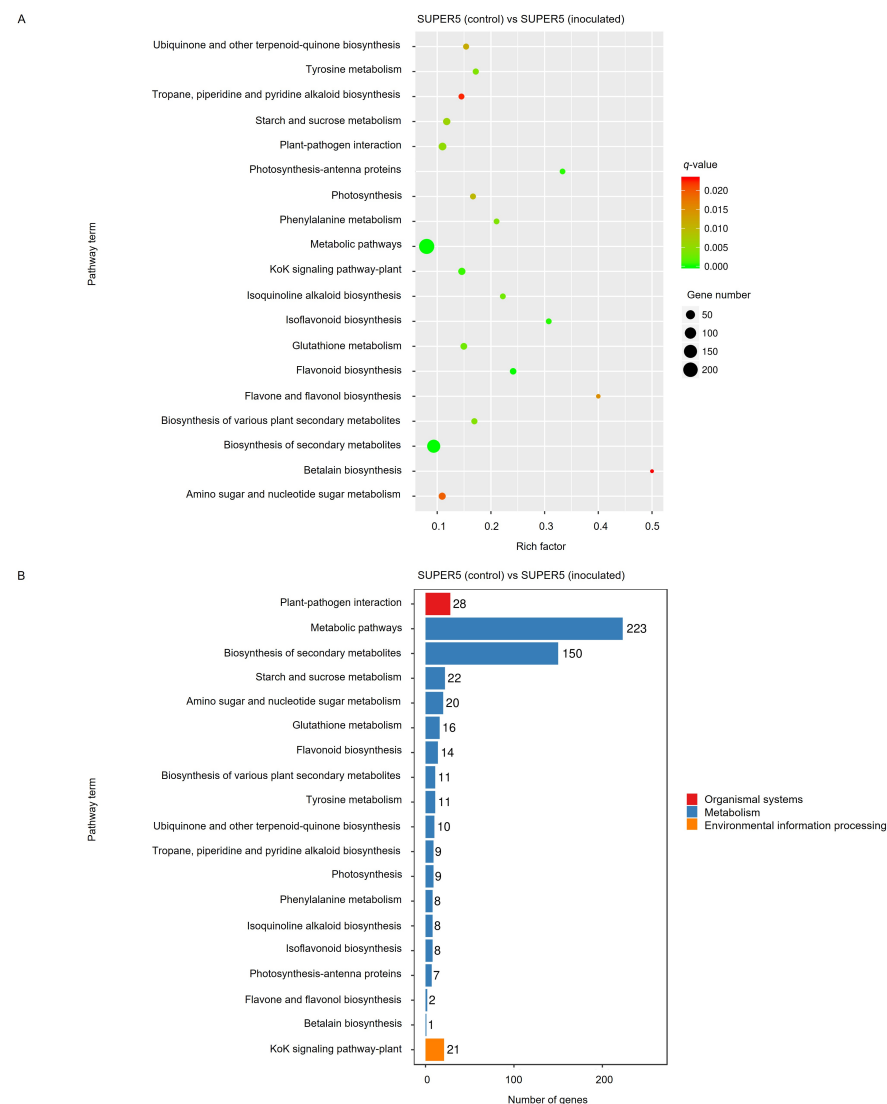


Figure 4. KEGG pathways significantly enriched DEGs in an interaction between control and inoculated conditions in SUPER5 (**A**). The size of each circle represents the number of enriched DEGs, while the color indicates the q -value range. The number of DEGs enriched in an interaction between control and inoculated conditions in SUPER5 (**B**). Red indicates the organismal systems, blue indicates the metabolism, and orange indicates the environmental information processing. The rich factor represents the ratio of DEGs in a pathway relative to the total genes in that pathway. The q -value is the p -value after multiple hypothesis testing and ranges between 0 and 1; the closer to zero, the more significant the enrichment.

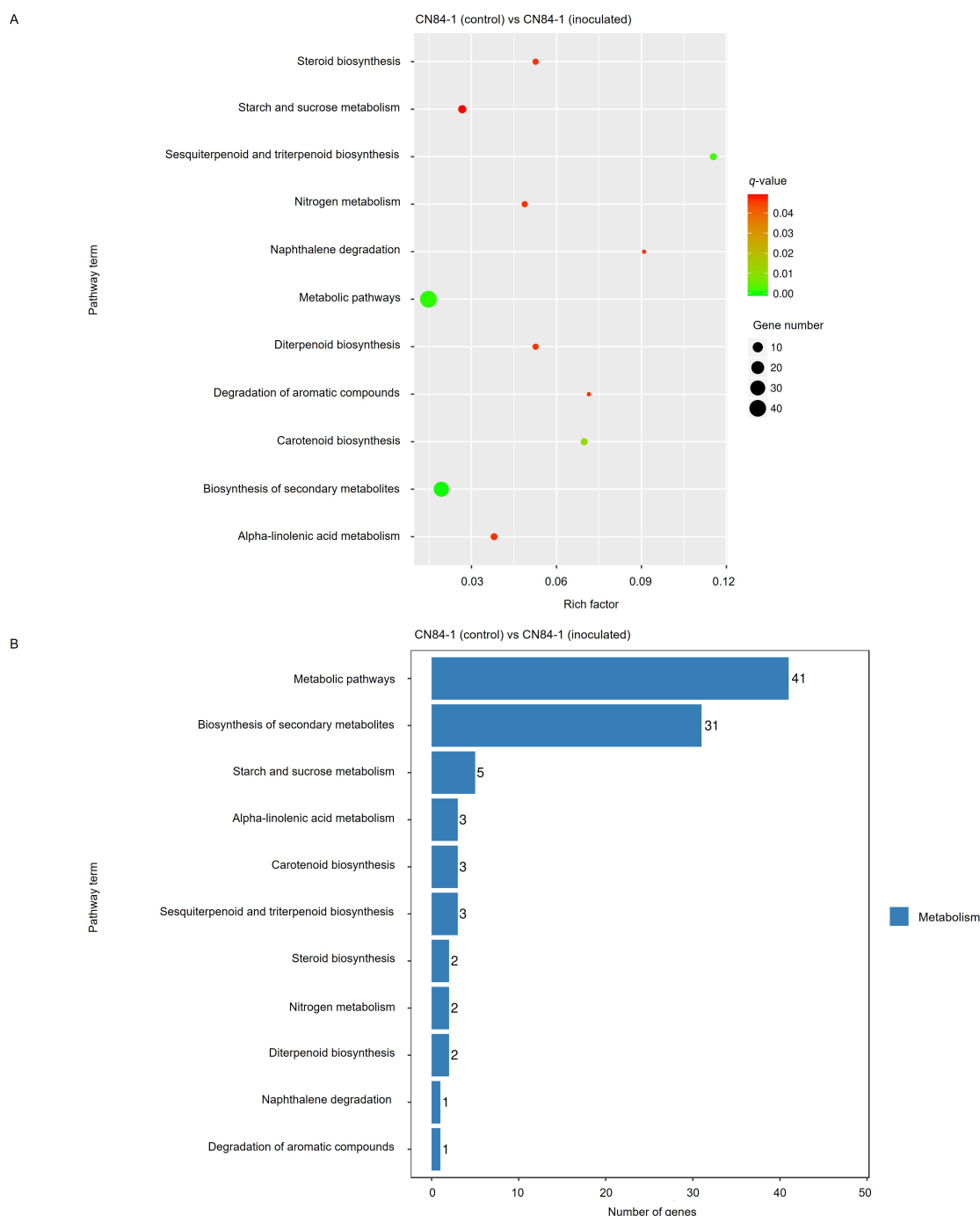


Figure 5. KEGG pathways significantly enriched DEGs in an interaction between control and inoculated conditions in CN84-1 (**A**). The size of each circle represents the number of enriched DEGs, while the color indicates the q -value range. The number of DEGs enriched in an interaction between control and inoculated conditions in CN84-1 (**B**). Blue indicates the metabolism. The rich factor represents the ratio of DEGs in a pathway relative to the total genes in that pathway. The q -value is the p -value after multiple hypothesis testing and ranges between 0 and 1; the closer to zero, the more significant the enrichment.

Specifically, genes related to organismal systems in SUPER5 included the 28 DEGs involved in plant–pathogen interactions, while metabolic processes encompassed pathways such as secondary metabolite biosynthesis, starch and sucrose metabolism, and photosynthesis. Additionally, genes associated with environmental information processing, particularly those involved in signaling pathways that activate plant resistance (e.g.,

plant signaling pathway), were identified (Figure 4). These genes play critical roles in activating disease resistance mechanisms in crops, including disease resistance proteins, PR proteins, and transcription factors. Key enzymes such as phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) in secondary metabolite and lignin biosynthesis, as well as signaling components like mitogen-activated protein kinase kinase kinase (MAPKKK), serine/threonine-protein kinase, and brassinosteroid insensitive 1-associated receptor kinase 1, were also implicated. Notably, these genes were predominantly upregulated in the resistant line SUPER5, except for secondary metabolite biosynthesis-related genes, which were expressed in both genotypes. However, while 125 genes were upregulated in SUPER5, only 25 genes showed reduced expression. In contrast, CN84-1 exhibited upregulation of only 4 genes, with 25 genes showing downregulation (Figures 4 and 5). Our results indicated that the DEGs involved in the biosynthesis of secondary metabolites showed the highest significant difference in expression (p -value = 0.0000) after PM infection. However, genes associated with plant–pathogen interactions and plant signaling pathways were also of particular interest, as they are directly involved in plant defense mechanisms and were exclusively detected in the resistant line SUPER5 but absent in the susceptible variety CN84-1.

These findings highlight the differential transcriptional regulation between resistant and susceptible genotypes, emphasizing the role of specific pathways and genes in conferring resistance to PM infection in mungbean.

Transcriptome analysis via RNA-seq revealed the identification of several candidate genes potentially implicated in PM resistance mechanisms. These genes encompass diverse functional categories, including pathogen recognition and signal transduction, hormone signaling pathways, transcription factors, defense-related gene activation, including PR genes, genes involved in ROS-related scavenging, phenylpropanoid metabolism and flavonoid biosynthesis, and defense regulation, and genes associated with the HR were identified as differentially expressed. A comprehensive summary of these genes and their functional annotations is provided in Table S3 and Figure 6.

In SUPER5, 1571 DEGs were identified as significantly DEGs through RNA-seq analysis. Notably, the upregulation of genes encoding resistance (R) proteins such as *RPM1* (3.08-fold), *RPP5-like* (5.32-fold), and *RPP13-like* (3.55-fold) further supports the activation of ETI and HR in SUPER5 [11,14,36,37]. Moreover, the *RPP13-like* gene is known to play a role in pathogen recognition [38], leading to the activation of disease resistance mechanisms. It has been widely reported as a candidate gene conferring PM resistance in several plant species, for instance, in barley [39], wheat [40–42], and common bean (*Phaseolus vulgaris* L.) [38]. Interestingly, Waengwan [11] conducted fine mapping and QTL analysis, identifying *RPP13L* as a key candidate gene associated with PM resistance at the *qPMRUM5-2* locus in mungbean. This finding supported our results, suggesting that *RPP13-like* may serve as a key functional gene underlying PM resistance in the SUPER5. However, further functional validation is required to confirm its precise role.

The pathogen recognition by R proteins might rapidly activate signals through *NDR1* [43]. It has been shown that transient overexpression of *NDR1/HIN1-like protein 6*, which was upregulated 18.28-fold in SUPER5, increases the expression of SA-related and JA-related genes, enhances ROS accumulation, and inhibits pathogen infection [44]. Moreover, upregulation of *probable linoleate 9S-lipoxygenase 5* in SUPER5 (18.39-fold) may be involved in JA-independent oxylipin signaling, potentially enhancing defense gene activation, cell wall modification, or localized HR [45]. Small auxin upregulated RNAs (SAURs) are a family of early auxin-responsive genes primarily known for their roles in cell elongation and growth, as well as regulators of disease resistance. The differential expression of SAUR genes in SUPER5 may contribute to PM resistance in mungbean by

mediating auxin-defense hormone crosstalk, altering cell wall properties, and fine-tuning growth-defense trade-offs, or modulating ROS dynamics [46]. Furthermore, genes encoding protein kinases and receptor-like kinases (RLKs), such as *serine/threonine-protein kinase OX11* and *LRR receptor-like serine/threonine-protein kinase GSO1*, play critical roles in signal transduction, further amplifying defense responses upon pathogen recognition [47].

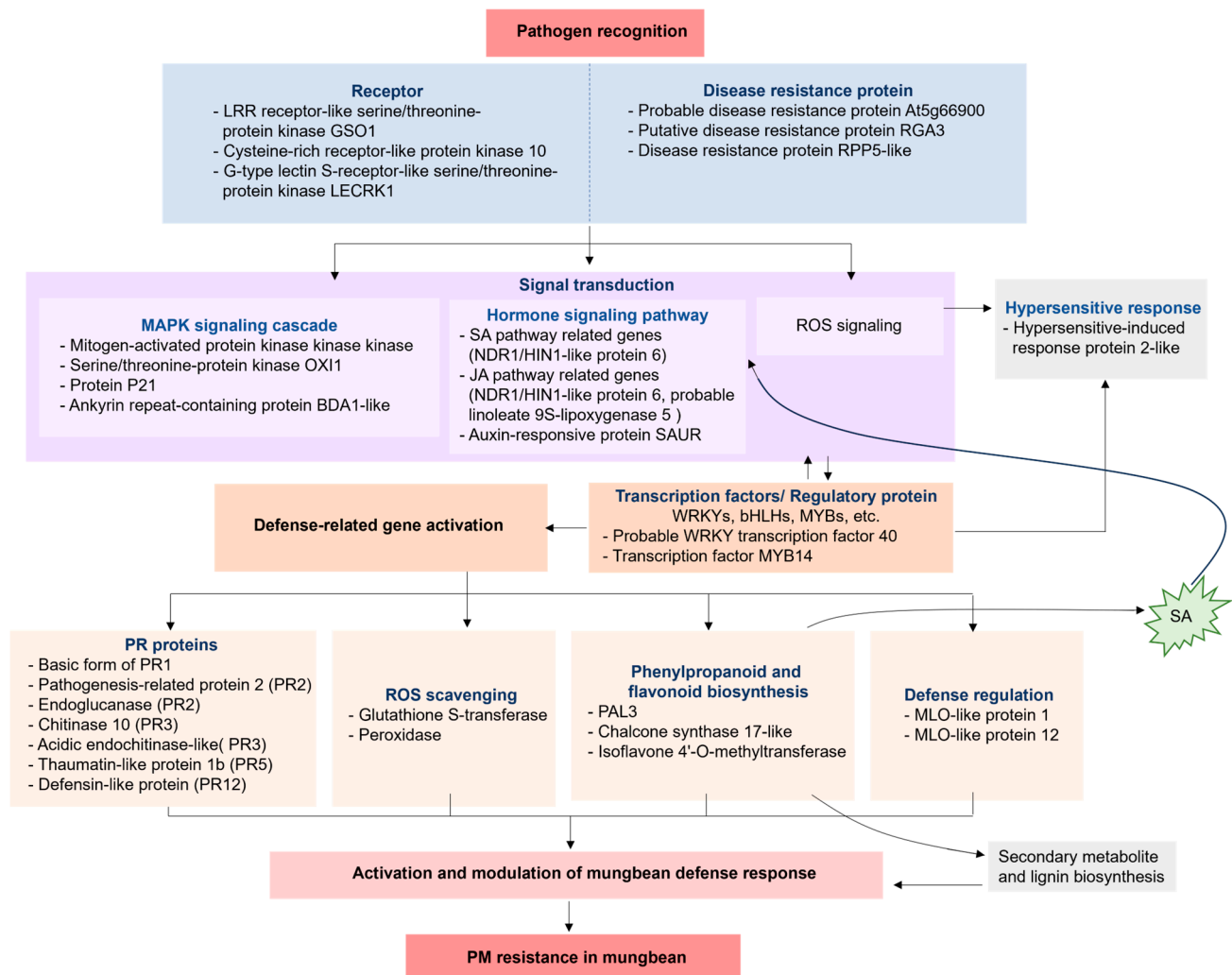


Figure 6. Model representing the DEGs related to defense mechanisms leading to PM resistance in mungbean.

The significant upregulation of transcription factors like *WRKY40* (14.05-fold) and *bHLH113* (57.37-fold) indicates a coordinated transcriptional defense program against PM by modulating key signaling and metabolic pathways. *WRKY40* may fine-tune SA signaling, ROS homeostasis, and HR-related gene expression to balance pathogen resistance with controlled cell death. In parallel, *bHLH113* enhances flavonoid biosynthesis and antioxidant defense, contributing to antimicrobial compound accumulation and redox stability. Together, they may orchestrate a multilayered defense that limits fungal invasion while preserving cellular integrity [48–51].

The upregulation of PR genes included *pathogenesis-related protein 2* (PR2) (237.80-fold), *chitinase* (5.18-fold), *acidic endochitinase* (34.91-fold), and *thaumatin-like protein 1b* (55.70-fold). These genes are involved in degrading fungal cell walls and producing antimicrobial compounds, directly inhibiting pathogen growth and proliferation [52–54]. Moreover, *peroxidase* was induced 5.96-fold, substantiating its significant dual role in plant defense by both scavenging excess ROS to prevent cellular damage and catalyzing lignin

biosynthesis to strengthen cell walls, thereby limiting pathogen invasion [55]. Other ROS-related genes found to be upregulated (2.57- to 5.52-fold) in our study were *glutathione S-transferases* (GSTs), which may contribute to PM resistance by detoxifying ROS and maintaining redox balance. Their upregulation in resistant line SUPER5 suggests a role in early defense, including the regulation of HR and antimicrobial secondary metabolite conjugation [56]. Additionally, the upregulation of genes involved in phenylpropanoid metabolism and flavonoid biosynthesis, such as *PAL class 3* (*PAL3*; 12.73-fold) and *CHS 17-like* (22.04-fold), highlights the production of antimicrobial compounds and lignin that reinforce physical and chemical barriers against pathogen invasion, and/or are related to ROS scavenging [57] (Table S3; Figure 6). Interestingly, our previous investigation into CLS resistance mechanisms in mungbean, utilizing RNA-seq analysis, also revealed that these genes were significantly upregulated in the resistant line SUPER5, but not in the susceptible variety CN84-1 [35]. Therefore, these genes appear to play pivotal roles in conferring broad-spectrum resistance against multiple fungal pathogens in mungbean. Furthermore, the genes involved in defense regulation, *Mildew locus O* (*Mlo*), play a crucial role in susceptibility to PM in legumes (*Medicago truncatula*) [9]. *Mlo* encodes an integral membrane protein in the form of seven transmembrane helices and is a classic S-gene that facilitates fungi to penetrate plant cells. The structural disruption of the *Mlo* protein through mutation disrupts fungal penetration of the epidermal cell wall without eliciting a host cell death response [58]. *MLO1*, whether naturally occurring or induced through mutagenesis, has been shown to confer resistance to PM in legume crops caused by *Erysiphe pisi*. In the present study, *MLO-like protein 1* and *MLO-like protein 12* were upregulated by 2.5- and 8.36-fold, respectively, while *MLO-like protein 6* was downregulated in the resistant line SUPER5. These genes may have an important role in PM resistance in mungbean.

In contrast, the limited upregulation of defense-related genes in CN84-1, along with the downregulation of key genes like transcription factor *MYB14*, suggests a compromised defense response, rendering this variety more susceptible to PM infection. The downregulation of *MYB14*, a regulator of secondary metabolite biosynthesis, likely impairs the production of antimicrobial compounds, further exacerbating susceptibility [59]. The differential expression patterns between SUPER5 and CN84-1 underscore the importance of a coordinated and multifaceted defense strategy, involving pathogen recognition, signal transduction, antimicrobial compound and defense protein production, and ROS regulation, in conferring resistance to PM. These findings provide valuable insights into the molecular mechanisms underlying PM resistance in mungbean and highlight potential genetic targets for breeding resistant varieties (Figure 6).

RNA-seq-based transcriptomic analysis facilitated the comprehensive classification of genes and the identification of DEGs, distinguishing between resistant (SUPER5) and susceptible (CN84-1) genotypes. Among the DEGs, 15 candidate genes were identified as potentially associated with resistance to PM, based on their significant differential expression patterns between the two genotypes (Table 3). Most of these genes exhibited notably higher expression levels in the resistant line SUPER5, while their expression remained unchanged in the susceptible variety CN84-1, or their expression was downregulated in CN84-1 while unchanged in SUPER5 (Table 3). Given their potential role in disease resistance mechanisms, these genes were selected for further co-expression network analysis and validation of gene expression using qRT-PCR.

3.2.4. Gene Expression Evaluation Using Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The qRT-PCR analysis was conducted using *Actin* as a reference gene (housekeeping gene) to normalize the amplification of target DNA across each PCR cycle. Ct values were determined to quantify the relative expression levels of the candidate genes. This approach provided a detailed assessment of gene expression changes in mungbean genotypes under both control and PM-inoculated conditions, offering insights into the molecular basis of resistance and susceptibility to PM infection.

Among the 15 candidate genes, 11 were amplifiable; the expression patterns of these were validated using qRT-PCR. It was found that the genes encoding PR2, LRR receptor-like serine/threonine-protein kinase GSO1, the probable disease resistance protein At5g66900, acidic endochitinase-like, PAL3, and MLO-like protein 12 exhibited a highly significant upregulation in the resistant line SUPER5 ($p \leq 0.01$), with a 15.80-, 9.73-, 16.28-, 13.10-, 7.24-, and 3.58-fold increase in expression, respectively, but showed no significant change in the susceptible variety CN84-1. Additionally, genes encoding proteins associated with disease resistance, including thaumatin-like protein 1b, serine/threonine-protein kinase OXI1, protein P21, and probable WRKY transcription factor 40, were significantly upregulated only in SUPER5 ($p \leq 0.05$), with fold changes of 13.48, 13.64, 4.86, and 5.64, respectively (Figure 8), while the genes encoding transcription factor MYB14 were highly significantly downregulated only in CN84-1 by 22.33-fold ($p \leq 0.01$), but not in SUPER5. Correlation of these 11 DEGs as identified from RNA-seq with those from qRT-PCR revealed a significant correlation with a coefficient of 0.64 ($p < 0.05$). These results validated RNA-seq findings and suggest a robust and coordinated defense response against PM infection. A functional characterization of each protein encoded by these genes is provided below.

The probable disease resistance protein At5g66900 is likely involved in recognizing pathogen-associated molecular patterns (PAMPs) and initiating downstream immune signaling cascades, which may account for its elevated expression in SUPER5 [14]. Upon pathogen recognition, key components of signal transduction were also upregulated. Notably, serine/threonine-protein kinases OXI1 and GSO1 play critical roles in amplifying defense signaling [47]. The marked upregulation of GSO1 in SUPER5 upon CLS infection [35] further underscores its importance in conferring resistance to multiple fungal pathogens.

At the transcriptional regulation level, WRKY40, a transcription factor known to regulate diverse defense-related gene expression, was activated in SUPER5, further amplifying defense gene networks to ensure robust pathogen defense without triggering widespread cellular damage [60]. In contrast, the MYB14 transcription factor, which regulates the biosynthesis of secondary metabolites such as flavonoids and phenylpropanoids, was significantly downregulated in CN84-1 (22.33-fold, $p \leq 0.01$), while remaining unchanged in SUPER5. This downregulation in CN84-1 likely impairs the accumulation of antimicrobial secondary metabolites, contributing to its heightened susceptibility [59]. Genes encoding enzymes involved in the phenylpropanoid pathway were also prominently expressed. PAL3, a key enzyme in the biosynthesis of SA, phytoalexins, and lignin, was upregulated in SUPER5 [61], facilitating the accumulation of antimicrobial compounds and lignin that reinforce both physical and biochemical barriers to pathogen invasion [57].

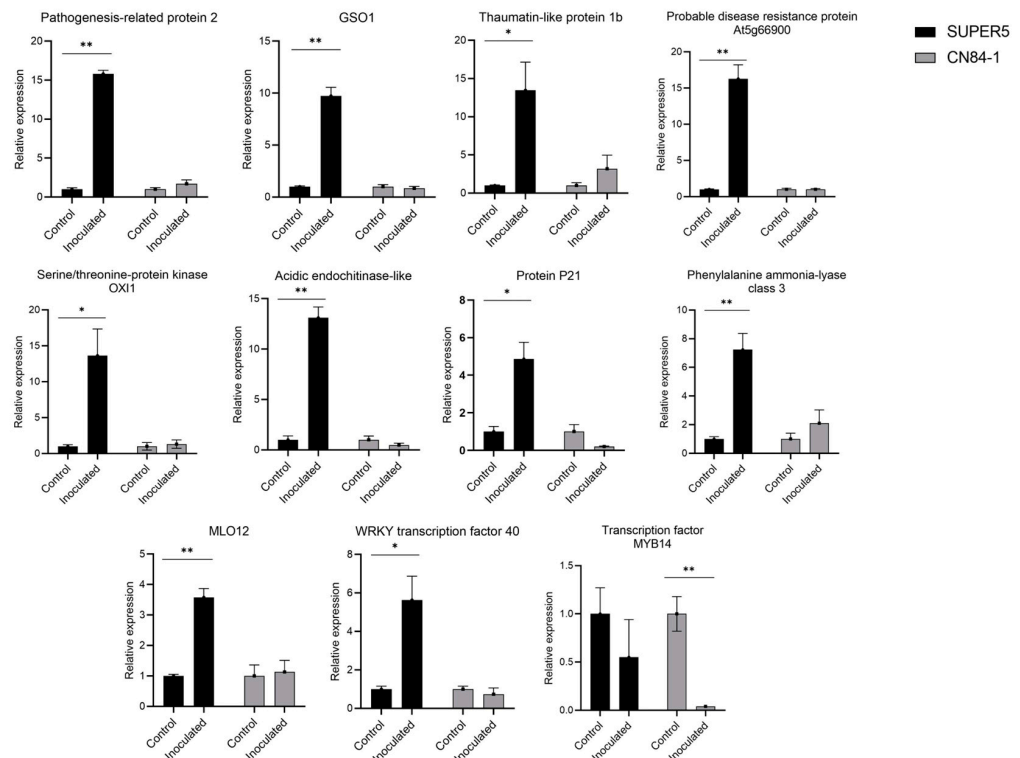


Figure 8. The expression analysis of putative genes involved in PM resistance by qRT-PCR in mungbean. The y-axis indicates the relative expression of control and PM-inoculated mungbean. The X-axis represents the resistant line, SUPER5 (■) and the susceptible variety, CN84-1 (▒). * and ** indicate significant differences between control and inoculated groups of each variety/line, $n = 3$, $p < 0.05$ and 0.01 , respectively.

Several PR genes were also highly expressed in SUPER5. A gene encoding acidic endochitinase-like protein, known to degrade fungal cell walls and generate antimicrobial compounds, was significantly upregulated [52–54]. These findings are consistent with [62], who reported increased chitinase expression in a moderately resistant *Poa pratensis* variety in response to *Blumeria graminis*, an obligate fungal pathogen that causes PM in most Poaceae crops and grasses. The PR2 protein, which functions similarly by degrading fungal cell walls, directly inhibiting pathogen growth, was also induced in SUPER5 [52]. In addition, genes encoding thaumatin-like proteins (TLPs), members of the PR5 family, were strongly expressed in the resistant genotype. These multifunctional proteins exhibit a wide range of antifungal activities, including glucan-binding, glucanase activity, xylanase inhibition, and cytokinin- and Actin-binding properties. Legume TLPs have demonstrated antifungal efficacy against diverse pathogens such as *Fusarium* spp., *Phytophthora* spp., *Verticillium* spp., *Alternaria alternata*, and *Rhizoctonia solani*. In addition, TLPs also confer tolerance to abiotic stresses like cold, drought, and salinity [54].

Interestingly, genes encoding MLO-like proteins, known regulators of PM susceptibility/resistance, exhibited differential expression in SUPER5. The induction of *MLO* expression in response to PM infection, along with its involvement in modulating intracellular calcium levels and facilitating PM invasion, has been well-documented [13]. However, a previous study reported that *VrMLO12* is related to PM resistance in mungbean [10]. This gene may play a different role from other *MLO* genes generally associated with PM susceptibility, in which a loss of function typically results in recessively inherited resistance [11]. Our results also showed that the *MLO12* transcript was significantly induced only in the resistant genotype SUPER5 through qRT-PCR analysis, substantiating a potential involvement of *MLO12* in resistance to PM in mungbean. This suggests a complex, fine-tuned

regulatory mechanism in the resistant genotype that balances the suppression and activation of defense pathways [22]. Another gene of interest is protein P21, a cyclin-dependent kinase inhibitor involved in cell-cycle regulation [63]. Its significant upregulation in SUPER5, but not in CN84-1, suggests a possible involvement in defense responses against PM. Although its precise role in plant immunity remains to be characterized, P21 may contribute to resistance by modulating cell-cycle progression or promoting localized cell death, which are key components of the HR. Further functional studies are needed to clarify whether P21 acts directly in ETI or as part of broader defense-associated signaling pathways.

Importantly, these defense-related genes were not significantly induced in CN84-1, as confirmed by both RNA-seq and qRT-PCR analyses. The lack of activation of these genes in the susceptible genotype likely compromises its ability to mount an effective immune response, rendering it more vulnerable to PM infection.

The gene expression patterns evaluated from qRT-PCR analysis were generally consistent with those obtained from RNA-seq data. These findings highlight the distinct transcriptional responses between the resistant and susceptible genotypes, underscoring the potential roles of these genes in conferring resistance to PM infection in mungbean. The coordinated upregulation of these genes in SUPER5 suggests a robust and multifaceted defense mechanism, involving pathogen recognition, signal transduction, antimicrobial compound and defense protein production, defense regulation, and cell wall reinforcement, which collectively contribute to enhanced resistance against PM.

The results of this study identified several key pathways integral to disease resistance mechanisms in mungbean. These pathways include plant–pathogen interactions, characterized by the activation of RLKs, R proteins, and PR proteins, which play critical roles in recognizing and responding to pathogen attacks. Additionally, phenylpropanoid metabolism and flavonoid biosynthesis pathways were implicated, contributing to the enhanced production of antimicrobial compounds and lignin that bolster plant defense. Hormone signaling pathways, involving the coordinated regulation of SA and JA, were also identified as essential for orchestrating defense responses. Furthermore, ROS signaling and scavenging pathways were highlighted for their role in managing oxidative stress during pathogen defense. Together, these pathways provide a comprehensive framework for understanding the molecular mechanisms underlying PM resistance in mungbean.

4. Conclusions

This study elucidated the molecular defense mechanisms of mungbean against PM caused by *S. phaseoli* through transcriptome profiling of the resistant line SUPER5 and the susceptible variety CN84-1. Fifteen candidate genes were identified, belonging to the genes involved in pathogen recognition, signal transduction, antimicrobial compound and defense protein production, defense regulation, and cell wall fortification. However, their precise functional roles should be validated in future studies using gene knockout, overexpression, or other functional genomics approaches. These findings advance our understanding of PM defense mechanisms in mungbean and provide a genetic foundation for improving disease resistance in legume crops.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15081871/s1>, Table S1: Powdery mildew disease severity scores of mungbean as modified from [28]; Table S2: Primers for qRT-PCR validation; Table S3: Genes differentially expressed in the resistant line SUPER5 after 11 DAI with the pathogen *S. phaseoli* causing powdery mildew; Table S4: Details of co-expression network analysis; Table S5: Annotation of co-expression genes.

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Data Availability Statement: Data is provided within the manuscript or supplementary information files. Reference genome files were downloaded from https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000741045.1/ (accessed on 1 December 2023) with NCBI RefSeq assembly GCF_000741045.1. The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found below: NCBI BioProject repository under the accession PRJNA1293791.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CLS	Cercospora leaf spot
PM	Powdery mildew
PTI	Pattern-triggered immunity
ETI	Effector-triggered immunity
RNA-seq	RNA sequencing
PR protein	Pathogenesis-related protein
SA	Salicylic acid
JA	Jasmonic acid
ET	Ethylene
ROS	Reactive oxygen species
DEGs	Differentially expressed genes
MYMV	Mungbean Yellow Mosaic Virus
DAI	Days after inoculation
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
qRT-PCR	Quantitative real-time polymerase chain reaction
cDNA	Complementary DNA
Ct	Threshold cycle
PAL	Phenylalanine ammonia lyase
CHS	Chalcone synthase
MAPKKK	Mitogen-activated protein kinase kinase kinase
GST	Glutathione S-transferases
RLKs	Receptor-like kinases
PR2	Pathogenesis-related protein 2
PAMPs	Pathogen-associated molecular patterns
TLPs	Thaumatococcus-like proteins
MLO12	MLO-like protein 12

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