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RNAseq Reveals Differential Gene Expression Contributing to *Phytophthora nicotianae* Adaptation to Partial Resistance in Tobacco

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Abstract: *Phytophthora nicotianae* is a devastating oomycete plant pathogen with a wide host range. On tobacco, it causes black shank, a disease that can result in severe economic losses. Deployment of host resistance is one of the most effective means of controlling tobacco black shank, but adaptation to complete and partial resistance by *P. nicotianae* can limit the long-term effectiveness of the resistance. The molecular basis of adaptation to partial resistance is largely unknown. RNAseq was performed on two isolates of *P. nicotianae* (adapted to either the susceptible tobacco genotype Hicks or the partially resistant genotype K 326 *Wz/Wz*) to identify differentially expressed genes (DEGs) during their pathogenic interactions with K 326 *Wz/Wz* and Hicks. Approximately 69% of the up-regulated DEGs were associated with pathogenicity in the K 326 *Wz/Wz*-adapted isolate when sampled following infection of its adapted host K 326 *Wz/Wz*. Thirty-one percent of the up-regulated DEGs were associated with pathogenicity in the Hicks-adapted isolate on K 326 *Wz/Wz*. A broad spectrum of over-represented gene ontology (GO) terms were assigned to down-regulated genes in the Hicks-adapted isolate. In the host, a series of GO terms involved in nuclear biosynthesis processes were assigned to the down-regulated genes in K 326 *Wz/Wz* inoculated with K 326 *Wz/Wz*-adapted isolate. This study enhances our understanding of the molecular mechanisms of *P. nicotianae* adaptation to partial resistance in tobacco by elucidating how the pathogen recruits pathogenicity-associated genes that impact host biological activities.

Keywords: RNAseq; *Phytophthora nicotianae*; adaptation; partial resistance; tobacco

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1. Introduction

Plant diseases are estimated to cause crop losses of 13% annually, imposing a major constraint on global crop production [1]. Deployment of complete and partial resistance in host plants is one of the most effective means of managing plant diseases and is an integral part of sustainable disease management that reduces the use of fungicides and other management inputs [2]. However, wide distribution of cultivars with complete resistance places strong selection pressure on pathogen populations to overcome that resistance [3]. Partial resistance selects for isolates that are more aggressive than isolates produced on susceptible cultivars, which can erode the effectiveness of partial resistance over time [4–6].

Various mechanisms utilized by plant pathogens to overcome complete resistance have been recognized, including loss of avirulence (*Avr*) gene products that trigger plant immunity, transposon insertions or mutations to the *Avr* gene sequence, acquisition of additional epistatic effectors that suppress the plant immune system without disrupting the original *Avr* gene [7], and endogenous small RNAs silencing *Avr* genes [8]. Despite our rapid improvement in understanding the molecular basis underlying complete resistance

and how pathogens overcome it, mechanisms of plant pathogen adaptation to partial resistance remains largely unknown.

Phytophthora nicotianae is a widely prevalent plant pathogen with hosts in 255 genera from 90 plant families [9]. When infecting tobacco (*Nicotiana tabacum* L.), the pathogen causes black shank, a potentially devastating disease with losses reaching 100% in some fields [10]. The use of host resistance provides an effective system for reducing yield losses due to black shank, but isolates of *P. nicotianae* can rapidly adapt to genetic resistance in tobacco. Populations of *P. nicotianae* rapidly shifted from race 0 (wild type) to race 1 after deployment of tobacco varieties with the *Php* gene [11]. Deployment of partial resistance is generally thought to be a sustainable approach to managing plant diseases. However, adaptation to partial resistance in *P. nicotianae* has also been observed. A significant increase in pathogen aggressiveness was documented in isolates of *P. nicotianae* exposed to a tobacco variety with a high level of partial resistance [12–14]. A greenhouse study demonstrated that isolates of *P. nicotianae* were able to overcome partial resistance QTLs derived from cigar cultivar Florida 301 and the *Wz* genomic region from *Nicotiana rustica* after exposure for only a few host generations [14,15]. Phenotypically, isolates of *P. nicotianae* adapted to sources of partial resistance exhibited increased infection efficiency and produced more sporangia on infected root tips, larger lesions on tobacco stems, and more aggressive asexual progeny than isolates not adapted on the resistant hosts [16].

The goal of the present study was to explore the molecular mechanisms underlying *P. nicotianae* adaptation to *Wz*-mediated partial resistance in tobacco genotype K 326 *Wz/Wz*, which was developed using an elite flue-cured tobacco cultivar K 326 as the recipient of *Wz* with the backcross breeding method. RNA samples of two *P. nicotianae* isolates adapted on either partially resistant inbred tobacco parental line K 326 *Wz/Wz* or the very susceptible cultivar Hicks were collected following infection of their adapted and their non-adapted host genotypes and subjected to RNA sequencing (RNAseq). The changes in gene expression in the two isolates were investigated by comparing the DEGs identified in each of the two isolates when infecting K 326 *Wz/Wz* compared to infecting Hicks. In addition, DEGs were identified in infected root samples of K 326 *Wz/Wz* by comparing to gene expression in inoculated root samples of Hicks. The results from this study enhance our understanding of how pathogens adapt to partial resistance in host plants, which will help in the development of sound deployment strategies for partial resistance and help increase the durability of partial resistance in host plants in the future.

2. Materials and Methods

2.1. RNAseq Preparation

2.1.1. Collection of Pathogen Isolates

Two isolates of *P. nicotianae* were collected from a previous greenhouse study where a race 0 isolate of *P. nicotianae* adapted on tobacco genotype K 326 *Wz/*—(a genotype heterozygous for *Wz*) was continually exposed to either K 326 *Wz/Wz* or the susceptible host Hicks. Isolates of *P. nicotianae* presented a significantly lower aggressiveness on K 326 *Wz/Wz* after exposure to Hicks compared to the isolates maintained on a host with *Wz* resistance. The two isolates selected for current study represented a broad spectrum of aggressiveness on K 326 *Wz/Wz* in the greenhouse aggressiveness evaluation. One isolate (*Wz*-*Wz*) was from K 326 *Wz/Wz* and had an aggressiveness index of 9.25 out of a possible 10. The second isolate (*Wz*-H) was from Hicks and had an aggressiveness index of 1.25. The aggressiveness index was converted from disease severity value caused by a given isolate. A disease severity value of 0 was given to plants that did not have above ground symptoms 28 days after inoculation. The severity values were: 1–6 days = 0, 7–10 days = 8, 11–16 days = 6, 17–22 days = 4, 23–28 days = 2, and no symptoms at day 28 = 0 [17].

2.1.2. Pathogen Culture and Tobacco Infection

The two isolates were maintained on 5% V8 agar at 28 °C in the dark in an incubator. Mycelial plugs from the edge of the cultures grown on V8 were transferred to the center of

Petri dishes containing oatmeal agar (Difco, Detroit, MI, USA). Petri dishes were incubated in the dark at 28 °C for approximately 2 weeks until dense hyphal mats formed. Hyphal mats were peeled from the oatmeal agar surface and placed into Petri dishes containing 20 mL of sterile 5% sandy-loam soil extract. Five percent soil extract was prepared by mixing 50 g of soil with 1 L of deionized water and letting it sit at room temperature for 48 h. The suspension was filtered through Fisher Brand Qualitative P8 filter paper and Celite 545 (Fisher Scientific, Fair Lawn, NJ, USA), and sterilized by autoclaving for two consecutive days at 121 °C for 60 min. Petri dishes were placed under constant light at room temperature in laboratory for about 5 days at which time numerous sporangia had produced. Sterile 5% soil extract was replaced daily during incubation. Zoospores were released by incubating hyphal mats at 4 °C for 1 h, followed by incubation at 28 °C for 30 min. The concentration of zoospore suspension was determined and adjusted to a concentration of 1×10^5 zoospores/mL using a hemocytometer.

Tobacco seeds of K 326 *Wz/Wz* and Hicks were seeded in potting mix (Fafard 2 Mix; Conrad Fafard, Inc., Agawam, MA, USA) in plastic pots in a greenhouse with a 35 °C/26 °C day/night temperature regime and a 14 h photoperiod supplemented with high intensity lights. After two weeks, germinated seedlings were transplanted to cell trays (cell size 3.8 cm × 3.8 cm × 5.7 cm) containing calcined clay (TURFACE® All Sport™, PROFILE Products LLC, Buffalo Grove, IL, USA) and grown for about two weeks. Six seedlings of each genotype were removed from calcined clay, washed gently with sterile deionized water, and inoculated by immersing the roots for 3 h in 60 mL of zoospore suspension of one of the two isolates in a Petri dish (25 × 100 mm). After inoculation, seedlings were moved to a new Petri dish (25 × 100 mm) containing 60 mL of 5% soil extract and incubated under constant light at room temperature. Forty-eight hours post inoculation (hpi), roots of individual seedlings were flash frozen in liquid nitrogen in separate 1.7 mL centrifuge tubes and subjected to RNA extraction.

2.1.3. RNA Isolation and Transcriptome Sequencing

A total of 24 infected root samples (2 isolates × 2 tobacco genotypes × 6 biological replicates) were subjected to RNA isolation. Total RNA of infected roots of each seedling was extracted using Qiagen Plant RNeasy Kits (Qiagen, Hilden, Germany) following the manufacturer's instructions. Genomic DNA was removed by on-column digestion with DNase I (Zymo Research Corporation, Irvine, CA, USA). The concentration and quality of total RNA were determined by BioAnalyzer RNA analysis. Three of the six biological replicates of each treatment with the highest RNA quality were selected for RNA sequencing.

RNA library preparation and sequencing were conducted at the Genomic Science Library at North Carolina State University (Raleigh, NC, USA). Briefly, the RNAseq library was constructed using a NEBNext® Ultra™ Directional RNA library Prep Kit for Illumina (New England BioLabs, Ipswish, MA, USA) using 1 µg of each of the RNA samples followed by a 350–500 bp final library size selection. Libraries of the three biological replicates for each of the four treatments (2 isolates × 2 tobacco genotypes) were multiplexed and sequenced in a single Illumina NextSeq 500 lane, generating 75 bp paired-end reads.

2.2. Analyses of RNAseq Data from P. nicotianae

2.2.1. Detection of Differentially Expressed Genes (DEGs) in *P. nicotianae*

Sequence quality was assessed using FastQC v0.11.8 [18]. No trimming was performed since the Phred quality score of each sequenced base was above 30 for all samples. Reads of all samples were aligned to the *P. nicotianae* genome (phytophthora_parasitica_inra_310.3.scaffolds.fasta) using Hisat2 v2.1.0 [19] with default parameters and maximum intron length of 5000 bp.

Reads mapped to coding sequences (CDS) of annotated genes were counted using featureCounts [20] with default settings. DEGs in a given isolate were identified by comparing infected K 326 *Wz/Wz* samples to infected Hicks samples using edgeR [21] with

TMM normalization, a generalized linear model, and false discovery rate (FDR) calculations based on the Benjamini–Hochberg method. Genes with a false FDR < 0.05 were considered to be DEGs. DEGs were divided into up- and down-regulated groups for further analyses.

2.2.2. Gene Ontology Analysis, KEGG Pathway Enrichment Analysis and PHIB-Blast

Gene Ontology (GO) ID and protein sequences were linked to individual DEGs using the UniProt website (https://www.uniprot.org/. Accessed on 21 May 2019) [22]. GO term enrichment analysis was performed using the BiNGO plugin [23] in Cytoscape v3.7.1 [24]. Over-represented GO terms were evaluated against the *P. nicotianae* genome in the categories "biological process", "molecular function", and "cellular component". The DEGs were subjected to the Kyoto Encyclopedia of Genes and Genomes (KEGG) [25] pathway enrichment analysis to understand their roles in biological pathways using KOBAS [26] with background species set to *Phytophthora infestans*, statistical method set to Hypergeometric test/Fisher's exact test, and FDR correction method set to Benjamini and Hochberg. Protein sequences of the DEGs were subjected to a blast search in PHIB-Pathogen Host Interactions base [27] to identify DEGs associated with pathogenicity. A DEG was considered pathogenicity-associated if it or its ortholog was verified in association with "loss of pathogenicity", "reduced virulence", "lethal", or "effector" in pathogens with an Evalue cutoff of 1.0×10^{-5}.

2.2.3. Detection of Differential Transcript Usage

To detect differential transcript usage, transcripts in each sample were assembled and quantified using StringTie [28] by comparing the BAM file (aligned using Hisat2) to the annotated reference genome (phytophthora_parasitica_inra_310.3.genes.gtf). Analysis of alternative splicing and isoform switches was conducted using IsoformSwitchAnalyzeR [29] package in R. Isoforms in each isolate of *P. nicotianae* found by comparing infected samples of K 326 *Wz/Wz* to infected samples of Hicks were considered differentially switched if difference in isoform fraction (dIF) > 0.1 and FDR corrected q-value < 0.05. Genes with differential transcript usage were subject to GO, KEGG, and PHIB blast analyses as well.

2.2.4. Identification of Single-Nucleotide Polymorphisms (SNPs)

To identify SNPs in the two isolates of *P. nicotianae*, sorted bam files of individual samples were subjected to variant calling using samtools mpileup and filtered using bcftools [30]. SNPs identified between the two isolates were located in genes. Gene sequences and corresponding protein sequences were blasted in NCBI to further identify their potential roles in aggressiveness in each of the two isolates of *P. nicotianae*.

2.3. Analyses of RNAseq Data from N. tabacum

2.3.1. Detection of Differentially Expressed Genes (DEGs) in *N. tabacum*

Reads of all samples were aligned to the *Nicotiana tabacum* genome [31] using Hisat2 v2.1.0 [19]. Reads mapped to coding sequences (CDS) of annotated genes were counted using featureCounts [20] with default settings. DEGs in K 326 *Wz/Wz* inoculated with a given isolate were identified by comparing it to Hicks inoculated with the same isolate by edgeR [21] using TMM normalization, a generalized linear model, and false discovery rate (FDR) calculations based on the Benjamini–Hochberg method. Genes with a false FDR < 0.05 were considered DEGs. DEGs were divided into up- and down-regulated datasets for further analysis.

2.3.2. Gene Ontology and KEGG Enrichment Analyses

DEGs were subjected to GO enrichment analysis using AgriGO v2 [32] against Nitab4.5 ID (solgenomics) as background with default settings. Corresponding protein sequences of DEGs were extracted and subjected to KEGG enrichment analysis using KOBAS [26] with background species set to *Nicotiana tabacum*, statistical method set to Hypergeometric test/Fisher's exact test, and FDR correction method set to Benjamini and Hochberg.

2.4. Quantitative Real-Time PCR (qRT-PCR) Validations

Two up-regulated and two down-regulated DEGs detected in both of the two *P. nicotianae* isolates with largest fold changes (Table 1) were chosen for qRT-PCR quantification to validate the DEGs called in RNAseq analysis. RNA samples extracted from two biological samples of each treatment were used as templates in qRT-PCR validations. First strand cDNA synthesis was initiated using ProtoScript®II reverse transcriptase (New England Biolabs, Beverly, MA, USA) following first strand cDNA synthesis standard protocol NEB#M0277. The ubiquitin-conjugating enzyme (Ubc) and the 40S ribosomal protein S3A (WS21), constitutively expressed throughout *P. nicotianae* development stages, were used as internal control genes [33] (Table 1).

Table 1. Genes and corresponding primers used for qRT-PCR validation.

Gene	Regulation	Forward Primer	Reverse Primer
PPTG_10666	Up	CGTTCTCTTTTGCTCACGGA	CAGCTCCGACAAGTACACTG
PPTG_19949	Up	CAACACTGTCACTGCTGGAT	GATCCAGTTGCTAGCGAGAG
PPTG_20266	Down	CTCTCCGAAACAGAACCAACT	GTAGATCTCGGCAGTAACGC
PPTG_08585	Down	AACACCACTACTCCAGCACT	ACAACTTCACCACATCCGTC
Ubc (ubiquitin-conjugating enzyme)		CCACTTAGAGCACGCTAGGA	TACCGACTGTCCTTCGTTCA
WS21 (40S ribosomal protein S3A)		TACGCCAAGACGGCTCAGA	TTCCATCAGACGCACCAGG

A total of 20 µL of reaction solution, including 1 µL of cDNA, 10 µL of iTaq Universal SYBR Green SuperMix (BioRad, Hercules, CA, USA), 0.6 µL of forward and 0.6 µL of reverse primers (10 µM), and 7.8 µL of molecular grade water was used for qRT-PCR. qRT-PCR was performed on 96-well plates using the Applied Biosystems QuantStudioTM 6 Flex Real-Time PCR system with the following settings: one cycle of 95 °C for 20 s (hold stage), followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s (PCR stage), with a final melt curve stage: 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. Three technical replicates were performed for each sample and primer set combination.

3. Results

3.1. RNAseq Overview

3.1.1. Infected Tobacco Root Samples for RNAseq

Root tissue colonized by *P. nicotianae* was obtained for RNAseq by inoculating and harvesting the roots of seedlings 48 h post inoculation (hpi). At 48 hpi, slight browning of the roots was present and abundant sporangia were present around roots.

3.1.2. RNA Sequencing and Sequence Mapping to the Reference Genome

Approximately 33 million reads were obtained per sample. An average of 27% of the reads were mapped to the *P. nicotianae* genome and an average of 6% of the reads were mapped to the *N. tabacum* genome (Table 2).

3.2. Overview of DEGs in P. nicotianae

3.2.1. DEGs Identified in *P. nicotianae*

For each of the two isolates, DEGs were identified by comparing infected K 326 *Wz*/*Wz* samples to infected Hicks samples. The DEGs identified in the two isolates were compared to view the dynamics in gene expression in the two isolates after infecting their adapted and their non-adapted tobacco host genotypes.

Forty-six genes in *Wz*-*Wz* and 50 genes in *Wz*-H isolates were differentially expressed. Specifically, 16 up-regulated and 30 down-regulated genes were identified in the *Wz*-*Wz* isolate (Table 3), and 29 up-regulated and 21 down-regulated genes were detected in the *Wz*-H isolate (Table 4). qRT-PCR of the four selected DEGs indicated expression pattern consistent with those captured in RNAseq where PPTG_08585 and PPTG_20266 had a lower

expression, and PPTG_10666 and PPTG_19949 had a higher expression in the two isolates when infecting K 326 *Wz/Wz* comparing to when they were infecting Hicks (Figure 1).

Both isolates up-regulated genes PPTG_19949, PPTG_06767, PPTG_10666, PPTG_05470 that encode uncharacterized proteins in *P. nicotianae*, and down-regulated 8 genes including genes that encode 60S ribosomal protein L38, phosphoadenosine phosphosulfate reductase, and NAD(P)H:quinone oxidoreductase.

Thirty-four DEGs were detected exclusively in the *Wz-Wz* isolate. Most of these genes encoded for uncharacterized proteins in *P. nicotianae*. Up-regulated genes with known function included PPTG_02121, PPTG_08145, and PPTG_12158 that encode Hsp70-like protein, 4-aminobutyrate transaminase, and ULK/ULK protein kinase. Similarly, the majority of the 38 DEGs identified only in the *Wz*-H isolate encoded for uncharacterized proteins. Genes with known function included an up-regulated gene, PPTG_17442, that encodes protein-S-isoprenylcysteine O-methyltransferase and down-regulated genes, PPTG_17561, PPTG_00501, PPTG_21942, and PPTG_15084 predicted to encode for a glycine cleavage system H protein, homoserine O-acetyltransferase, phosphate acetyltransferase, and TKL/DRK protein kinase.

Table 2. Summary of RNAseq data and mapping results.

Host Genotype	Isolate for Inoculation	Replication	Total Reads	*P. nicotianae*		*N. tabacum*	
				No. and Rate of Reads Mapped	Overall Alignment Rate	No. and Rate of Reads Mapped	Overall Alignment Rate
Hicks	*Wz*-H	1	32,616,245	11,799,948 (36.18%)	38.50%	1,170,706 (3.59%)	4.77%
Hicks	*Wz*-H	2	37,590,753	8,954,805 (23.82%)	25.42%	691,568 (1.84%)	2.55%
Hicks	*Wz*-H	3	32,142,283	8,581,032 (26.70%)	28.55%	1,526,273 (4.75%)	6.21%
K 326 Wz/Wz	*Wz*-H	1	30,738,911	6,756,839 (21.98%)	23.62%	1,234,275 (4.02%)	5.84%
K 326 Wz/Wz	*Wz*-H	2	32,228,624	7,512,158 (23.31%)	24.89%	2,571,539 (7.98%)	10.15%
K 326 Wz/Wz	*Wz*-H	3	41,304,137	8,159,363 (19.75%)	21.14%	1,146,835 (2.78%)	3.63%
Hicks	*Wz-Wz*	1	32,441,520	8,583,661 (26.46%)	28.35%	1,003,943 (3.09%)	4.19%
Hicks	*Wz-Wz*	2	33,639,749	6,441,805 (19.15%)	20.54%	561,746 (1.67%)	2.95%
Hicks	*Wz-Wz*	3	30,538,468	10,333,429 (33.84%)	36.10%	557,494 (1.83%)	2.91%
K 326 Wz/Wz	*Wz-Wz*	1	32,424,684	9,437,437 (29.11%)	31.12%	5,499,898 (16.96%)	20.63%
K 326 Wz/Wz	*Wz-Wz*	2	34,550,232	7,129,753 (20.64%)	22.21%	412,999 (1.20%)	1.98%
K 326 Wz/Wz	*Wz-Wz*	3	30,323,291	7,477,321 (24.66%)	26.38%	852,994 (2.81%)	4.20%
		Average	33,378,241		27.23%		5.83%

Table 3. DEGs identified in the *Wz-Wz* isolate of *P. nicotianae* by comparing transcriptomes in inoculated K 326 *Wz/Wz* to inoculated Hicks.

Up-Regulated Gene	logFC	FDR	Annotation in NCBI
PPTG_02121	2.72729992	0.04116515	Nucleotide-Binding Domain of the sugar kinase/HSP70/actin superfamily
PPTG_10666	1.40170745	0.00461859	NADB_Rossmann
PPTG_12300	1.38934498	0.0113136	Elicitin protein RAL13D [Phytophthora nicotianae]
PPTG_06767	1.29785829	0.00975047	Cytochrome P450
PPTG_00731	1.14244371	0.02689335	Mitochondrial succinate-semialdehyde dehydrogenase and ALDH family members 5A1 and 5F1-like
PPTG_08145	1.06166001	0.03655436	4-aminobutyrate aminotransferase or related aminotransferase
PPTG_01316	1.01159768	0.0113136	Aspartate aminotransferase (AAT) superfamily (fold type I) of pyridoxal phosphate (PLP)-dependent enzymes
PPTG_06886	0.9770123	0.04116515	Mitochondrial carrier protein
PPTG_00433	0.95687542	0.04116515	Amino acid permease
PPTG_05470	0.94439287	0.00975047	SPRY domain in Ran binding proteins, SSH4, HECT E3 and SPRYD3
PPTG_19949	0.90621478	0.01386612	Peptidase domain in the S8 and S53 families
PPTG_08778	0.89782849	0.0287979	NA
PPTG_11182	0.89467137	0.04116515	GAF domain
PPTG_10595	0.88099898	0.02633833	Second domain of the pleiotropic drug resistance-like (PDR) subfamily G of ATP-binding cassette transporters
PPTG_05834	0.80783638	0.01128107	Putative lectin [Phytophthora palmivora var. palmivora]
PPTG_12158	0.63468318	0.02633833	Serine/Threonine protein kinases, catalytic domain
Down-Regulated Gene	**logFC**	**FDR**	**Annotation in NCBI**
PPTG_12754	−0.6308213	0.04116515	60S ribosomal protein L38
PPTG_19261	−0.6513629	0.01740912	WRKY transcription factor 19 [Phytophthora nicotianae]
PPTG_15145	−0.7844201	0.04116515	Scavenger mRNA decapping enzyme C-term binding
PPTG_00424	−0.8320101	0.04116515	Amino acid permease
PPTG_19041	−0.9101204	0.02968312	acetate kinase A/propionate kinase 2
PPTG_00957	−0.9268608	0.04550147	NA
PPTG_00099	−0.9276355	0.03028585	NA
PPTG_11197	−1.01735	0.03970974	3′-phosphoadenosine 5′-phosphosulfate sulfotransferase (PAPS reductase)/FAD synthetase or related enzyme
PPTG_09433	−1.2431276	0.02997457	Major Facilitator Superfamily (MFS) proteins
PPTG_17813	−1.2434395	0.04550147	Ankyrin repeats
PPTG_04568	−1.3541329	0.0459794	PQ-loop
PPTG_02974	−1.4004705	0.03655436	Major Facilitator Superfamily (MFS) proteins
PPTG_02595	−1.49923	0.03655436	NAD(P)+-dependent aldehyde dehydrogenase superfamily
PPTG_11386	−1.5047674	0.02633833	NA
PPTG_07126	−1.5248213	0.04304619	Short chain dehydrogenase
PPTG_12006	−1.5957463	0.01170615	Glycosyl hydrolase family 1
PPTG_08585	−1.6180257	0.00106403	Old yellow enzyme (OYE)-like FMN binding domain
PPTG_18570	−1.633993	0.01009357	Zinc finger, C2H2 type
PPTG_23779	−1.6347597	0.00427631	NAD(P)H:FMN oxidoreductases, oxygen-insensitive nitroreductase, flavin reductase P, dihydropteridine reductase, NADH oxidase or NADH dehydrogenase
PPTG_05530	−1.6929325	0.01258482	NADPH oxidase (NOX)
PPTG_02448	−1.7462909	0.00975047	Major Facilitator Superfamily (MFS) proteins
PPTG_18743	−1.7855609	0.03655436	NA
PPTG_13181	−2.0941462	0.01009357	Major Facilitator Superfamily (MFS) proteins
PPTG_08485	−2.1192135	0.02633833	Major Facilitator Superfamily (MFS) proteins
PPTG_09275	−2.1770096	0.0000271	NADPH-dependent FMN reductase
PPTG_00236	−2.2019316	0.02968312	TonB receptor activity [Phytophthora megakarya]
PPTG_13068	−2.3667825	0.03655436	Membrane-associating domain
PPTG_20266	-2.4697411	0.00888653	NADPH-dependent FMN reductase
PPTG_04065	−3.3756258	0.03655436	ZIP Zinc transporter
PPTG_10399	−3.9114676	0.01740912	D-arabinose 1-dehydrogenase, Zn-dependent alcohol dehydrogenase family

Table 4. DEGs identified in *Wz*-H isolate of *P. nicotianae* by comparing transcriptomes in inoculated K 326 *Wz/Wz* to inoculated Hicks.

Up-Regulated Gene	logFC	FDR	Annotation in NCBI
PPTG_01162	1.75973946	0.03399441	Exonuclease-Endonuclease-Phosphatase (EEP) domain superfamily
PPTG_01484	1.66331002	0.01294692	Amino_oxidase; Flavin containing amine oxidoreductase
PPTG_08721	1.36573335	0.03296512	SCP-like extracellular protein domain
PPTG_12693	1.35565787	0.0217849	NA
PPTG_15982	1.22889916	0.03661908	NAD(P)-dependent dehydrogenase
PPTG_17442	1.14815854	0.03856894	Isoprenylcysteine carboxyl methyltransferase (ICMT) family
PPTG_19949	1.09394216	0.00569986	Peptidase domain in the S8 and S53 families
PPTG_06767	1.09239542	0.02680104	Cytochrome P450
PPTG_10666	1.05056269	0.02831652	Rossmann-fold NAD(P)(+)-binding proteins
PPTG_23419	1.01740189	0.0217849	large tegument protein UL36;
PPTG_04377	0.94043542	0.03296512	Major Facilitator Superfamily (MFS) proteins
PPTG_05470	0.91187391	0.01702338	SPRY domain in Ran binding proteins
PPTG_22853	0.85943994	0.03296512	Putative storage protein LPV
PPTG_13013	0.8307265	0.0217849	Cyst germination specific acidic repeat protein
PPTG_20368	0.82758849	0.02281486	NA
PPTG_01588	0.82579718	0.02281486	Tetratricopeptide repeat
PPTG_04341	0.78702506	0.014592	Kazal type serine protease inhibitors
PPTG_00655	0.7658604	0.02490186	Iron-enterobactin transporter ATP-binding protein
PPTG_08559	0.76309934	0.03296512	NA
PPTG_00623	0.75665029	0.0217849	The Phox Homology domain, a phosphoinositide binding module
PPTG_22560	0.72235522	0.04236865	Ricin-type beta-trefoil
PPTG_07553	0.71836169	0.03296512	HAM34-like putative membrane protein
PPTG_01906	0.69845644	0.03303654	Cytochrome P450
PPTG_11777	0.69839919	0.03303654	Alpha-N-acetylglucosaminidase (NAGLU) tim-barrel domain
PPTG_13016	0.68630319	0.03147853	Glycosyltransferase (GlcNAc)
PPTG_17323	0.66267543	0.03303654	tRNA binding domain
PPTG_15053	0.66097703	0.04910001	NA
PPTG_03113	0.65586116	0.03147853	NA
PPTG_00340	0.54904534	0.04815318	Dynein heavy chain and region D6 of dynein motor
Down-Regulated Gene	**logFC**	**FDR**	**Annotation in NCBI**
PPTG_17561	−0.5858454	0.04815318	Biotinyl_lipoyl_domains
PPTG_12754	−0.6407469	0.03613858	60S ribosomal protein L38
PPTG_02651	−0.8343073	0.03303654	TLR4 regulator and MIR-interacting MSAP
PPTG_00501	−0.8933285	0.03147853	Abhydrolase
PPTG_21942	−0.9296731	0.03842764	Phosphate acetyltransferase
PPTG_11197	−1.0358966	0.03303654	3′-phosphoadenosine 5′-phosphosulfate sulfotransferase (PAPS reductase)/FAD synthetase or related enzyme
PPTG_08585	−1.0629125	0.02281486	Old yellow enzyme (OYE)-like FMN binding domain
PPTG_21974	−1.1383951	0.02281486	NA
PPTG_13205	−1.2268529	0.04815318	Elicitin
PPTG_21937	−1.2492039	0.03296512	START/RHO_alpha_C/PITP/Bet_v1/CoxG/CalC (SRPBCC) ligand-binding domain superfamily
PPTG_05327	−1.2837131	0.03147853	NA
PPTG_15084	−1.5159809	0.04674932	TKL/DRK protein kinase
PPTG_02595	−1.5567119	0.03303654	NAD(P)+-dependent aldehyde dehydrogenase superfamily
PPTG_09275	−1.6934963	0.00080536	NADPH-dependent FMN reductase
PPTG_18743	−1.7231432	0.03661908	NA
PPTG_15596	−1.7614863	0.02490186	START/RHO_alpha_C/PITP/Bet_v1/CoxG/CalC (SRPBCC) ligand-binding domain superfamily
PPTG_23779	−1.7704877	0.00245828	NAD(P)H:FMN oxidoreductases, oxygen-insensitive nitroreductase, flavin reductase P, dihydropteridine reductase, NADH oxidase or NADH dehydrogenase.
PPTG_05968	−2.0360954	0.02831652	Redox-sensitive bicupin YhaK, pirin superfamily
PPTG_20266	−2.1555007	0.0217849	NADPH-dependent FMN reductase
PPTG_05967	−2.4675984	0.00569986	Redox-sensitive bicupin YhaK, pirin superfamily
PPTG_16697	−3.3993324	0.04099675	Solute carrier families 5 and 6-like

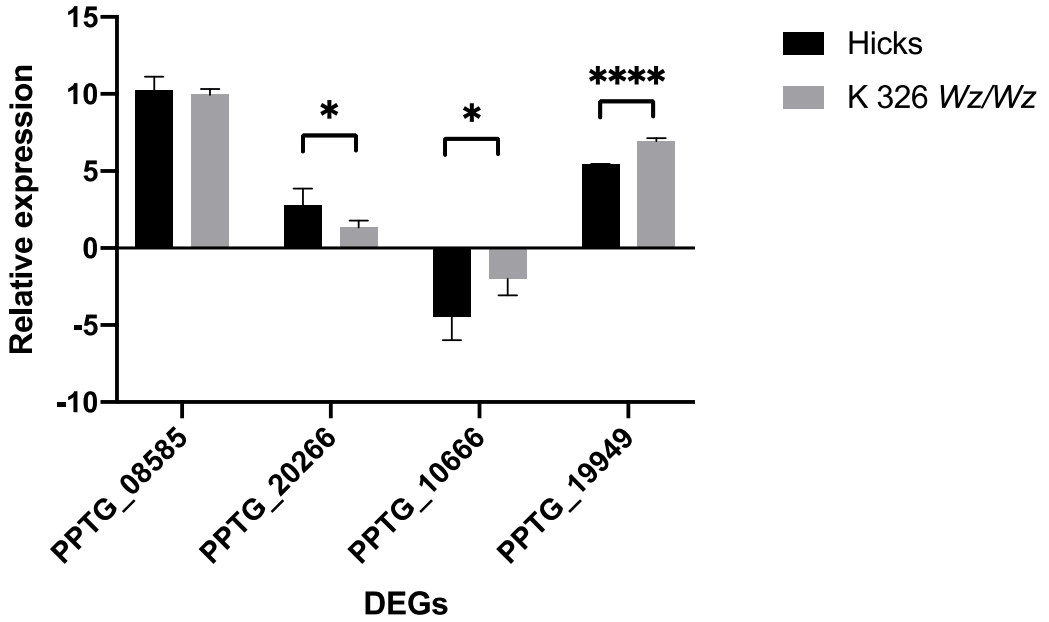


Figure 1. qRT-PCR validation of the DEGs identified in *P. nicotianae* by RNAseq analysis. Difference in relative gene expression between groups was determined using *t*-test in Prism 8. Significance is indicated using * for p value < 0.05, **** for p value < 0.0001. Genes PPTG_08585 and PPTG_20266 showed a lower relative expression in *P. nicotianae* when infecting K 326 *Wz/Wz* compared to infecting Hicks. Genes PPTG_10666 and PPTG_19949 showed a higher relative expression in *P. nicotianae* when infecting K 326 *Wz/Wz* compared to infecting Hicks. The expression of the genes was normalized to Ubc and WS21 as two internal controls.

3.2.2. Over-Represented Gene Ontology Analysis

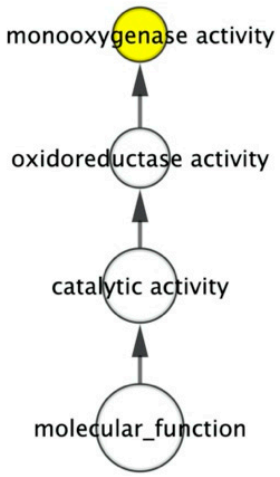
To obtain insight into the types of differentially expressed genes in the two isolates, GO enrichment analysis was performed using BiNGO to test over-representation for the DEGs against the annotated genes in *P. nicotianae*. Down-regulated genes in the *Wz-Wz* isolate were significantly enriched into three GO terms for the functional classes "sulfate reduction" (GO: 0019419), "sulfate assimilation, phosphoadenylyl sulfate reduction by phosphoadenylyl-sulfate reductase (thioredoxin)" (GO: 0019379) and "sulfate assimilation" (GO: 0000103). Up-regulated genes in the *Wz-Wz* isolate were enriched in "monooxygenase activity" (GO: 0004497) (Figure 2). In the *Wz*-H isolate, down-regulated genes were enriched into a broader spectrum of GO terms. In addition to the ones found in the *Wz-Wz* isolate, down-regulated genes in the *Wz*-H isolate were also enriched in "glycine cleavage complex" (GO: 0019419), "NAD(P)H dehydrogenase (quinone) activity" (GO: 0004604), "FMN binding" (GO: 0050662), and "oxidoreductase activity" (GO: 0016651, GO: 0000103, GO: 0009071; GO: 0016671). No GO terms were particularly enriched for up-regulated genes in the *Wz*-H isolate (Figure 3).

3.2.3. KEGG Analysis of DEGs

A KEGG pathway enrichment analysis was conducted on DEGs identified in the two isolates to help understand the interaction between isolates and their adapted and non-adapted tobacco host genotypes.

For up-regulated DEGs, "spliceosome" was enriched in the *Wz-Wz* isolate. No enriched pathway was identified for up-regulated DEGs in the *Wz*-H isolate. For down-regulated DEGs, "ubiquinone and other terpenoid-quinone biosynthesis" and "biosynthesis of secondary metabolites" were enriched in the *Wz-Wz* isolate. In addition to the two pathways enriched, another eight enriched pathways were identified in the *Wz*-H isolate including "biosynthesis of antibiotics", "carbon metabolism", and "sulfur metabolism" (Table 5).

(A)



(B)

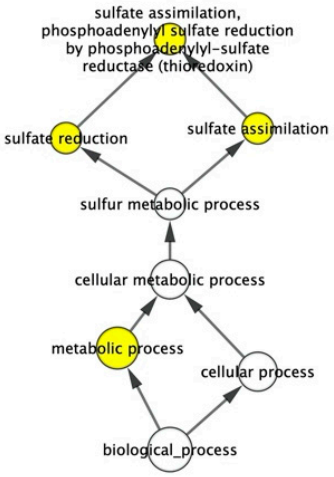


Figure 2. Enriched GO terms (colored in yellow, adjusted p value < 0.05) for (**A**) up-regulated and (**B**) down-regulated DEGs identified in the *Wz-Wz* isolate using BiNGO.

Table 5. Enriched KEGG pathways in the *Wz-Wz* and *Wz*-H isolates of *P. nicotianae* on K 326 *Wz/Wz*.

Isolate	DEG Regulation	Term	Rich-Factor	Corrected *p*-Value
Wz-Wz	up-regulated	Spliceosome	0.018	0.049
	down-regulated	Ubiquinone and other terpenoid-quinone biosynthesis	0.118	0.008
		Biosynthesis of secondary metabolites	0.010	0.040
Wz-H	down-regulated	Biosynthesis of secondary metabolites	0.015	0.000
		Ubiquinone and other terpenoid-quinone biosynthesis	0.118	0.002
		Biosynthesis of antibiotics	0.016	0.002
		Glyoxylate and dicarboxylate metabolism	0.053	0.005
		Glycine, serine and threonine metabolism	0.051	0.006
		Metabolic pathways	0.005	0.007
		Carbon metabolism	0.014	0.043
		Ascorbate and aldarate metabolism	0.067	0.049
		Sulfur metabolism	0.059	0.049
		Histidine metabolism	0.056	0.049

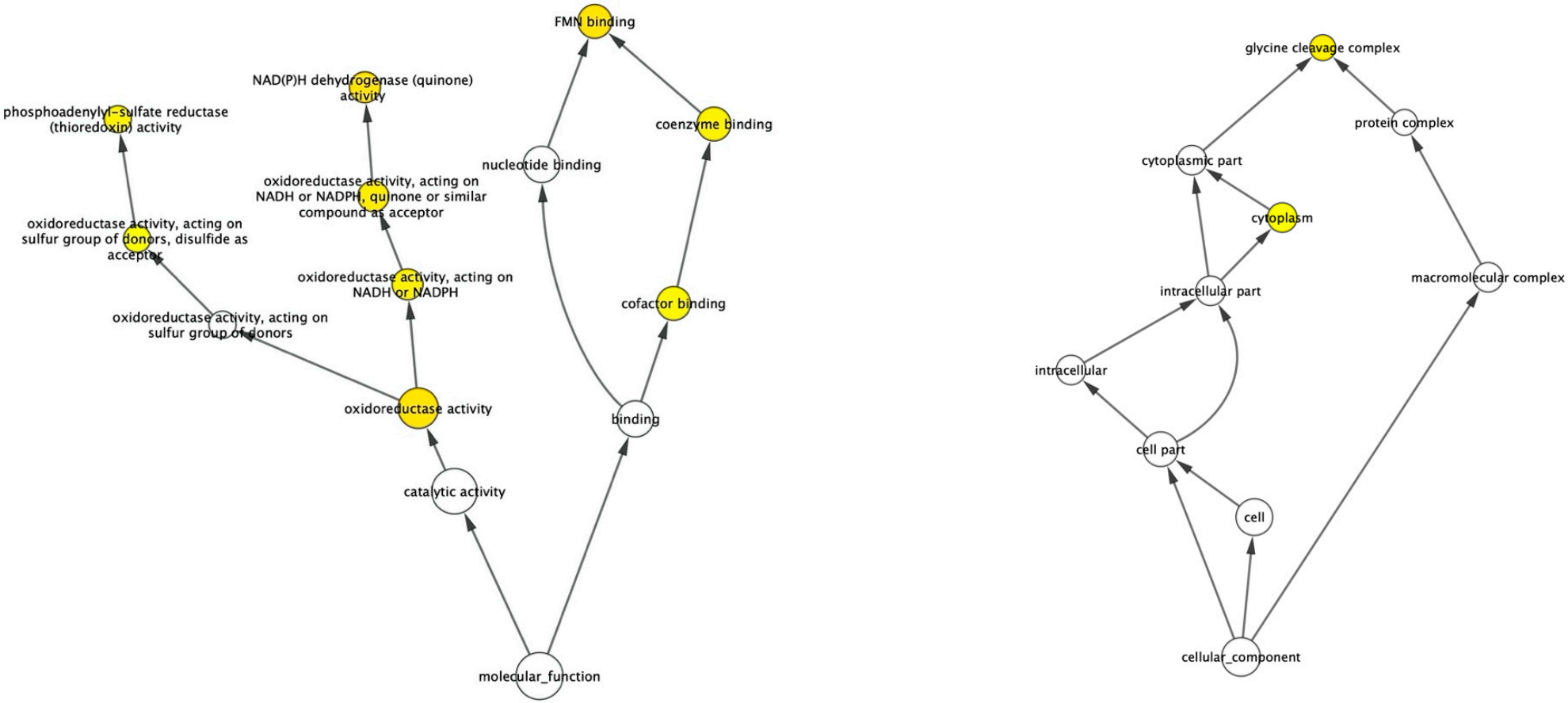


Figure 3. *Cont.*

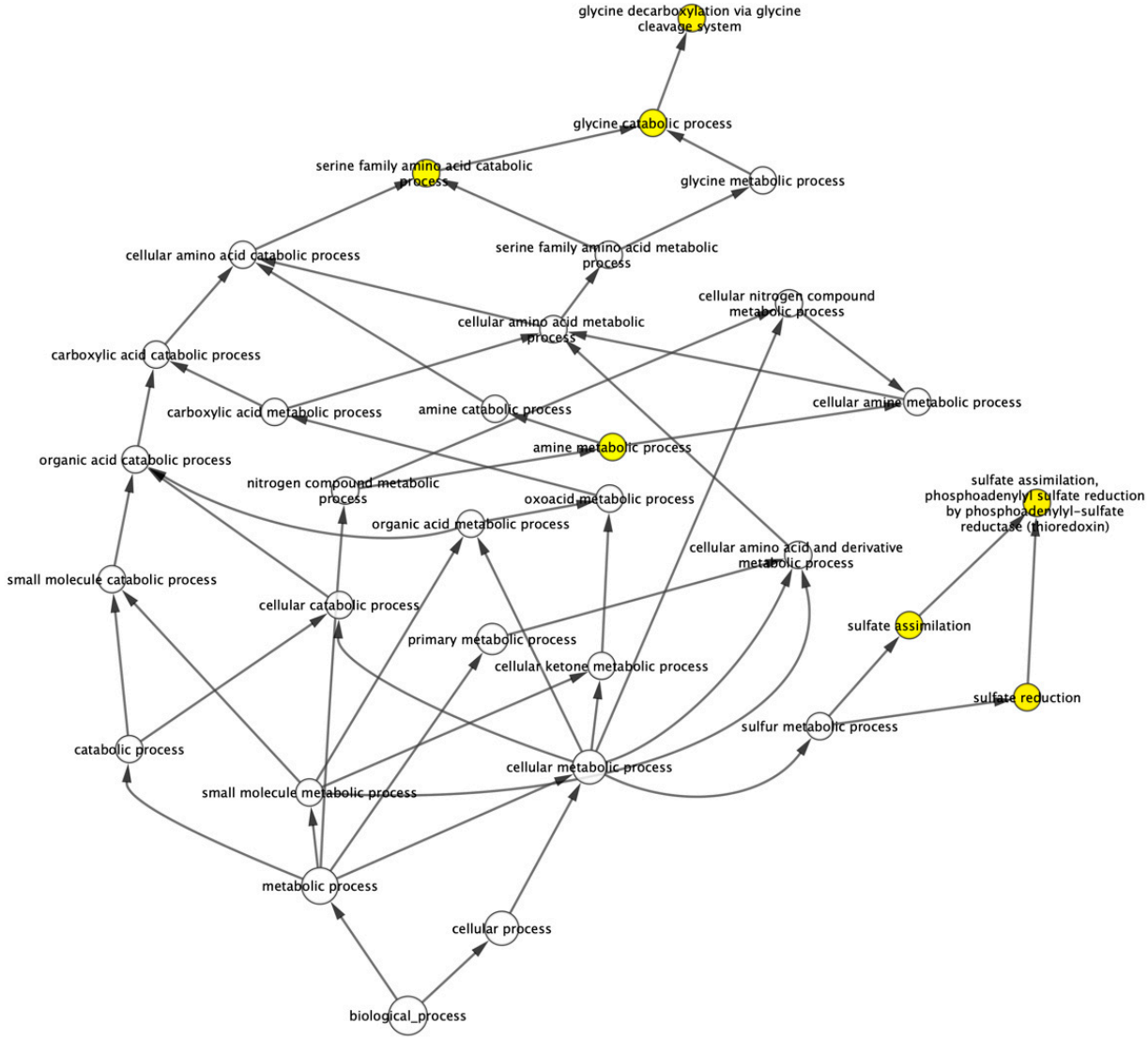


Figure 3. Enriched GO terms (colored in yellow, adjusted p value < 0.05) for down-regulated DEGs identified in the *Wz*-H isolate using BiNGO.

3.2.4. PHIB-Database Blast

The pathogen-host interactions database (PHI-base) stores curated molecular and biological information on genes experimentally proven to alter the outcome of pathogen-host interactions. Given that a majority of the DEGs were identified to encode uncharacterized proteins in the annotated *Phytophthora nicotianae* genome, the protein sequences of the identified DEGs were subjected to a blast search in the PHI-base to determine their role or the role of their orthologs to have a better knowledge of how they could potentially contribute to aggressiveness in individual isolates.

Among the up-regulated genes in the *Wz-Wz* isolate, 68.75% (11/16) were associated with pathogenicity. In contrast, 31.03% (9/29) of the up-regulated genes in the *Wz*-H isolate were found to have a role in pathogenicity. The two isolates showed a similar percentage of down-regulated genes involved in pathogenicity, with 46.67% (14/30) in the *Wz-Wz* isolate and 47.62% (10/21) in the *Wz*-H isolate (Table S1).

Specifically, the *Wz-Wz* isolate up regulated pathogenicity-associated genes including, but not limited to, PPTG_10595 encoding a protein belonging to ABC transporter superfamily, PPTG_12158 encoding ULK/ULK protein kinase, PPTG_02121 encoding a heat shock protein 70, and PPTG_06886 encoding a protein within solute carrier family. Down-regulated pathogenicity-associated genes included PPTG_19261 and PPTG_00236 encoding WRKY transcription factor, PPTG_02595 encoding aldehyde dehydrogenase, and PPTG_02595 encoding a sugar transport protein (Table S1).

The *Wz*-H isolate up regulated pathogenicity-associated genes including PPTG_22560 which matched to a gene encoding an effector protein in *P. infestans* and PPTG_15982 matched to a gene encoding a glycoside hydrolase in *P. palmivora*. The down regulated genes in association with pathogenicity in the *Wz*-H isolate included PPTG_15084 encoding a TKL/DRK protein kinase and PPTG_13205 matched to a gene encoding an elicitin-like protein in *P. infestans* (Table S1).

3.2.5. Genes with Differential Transcript Usage in *P. nicotianae*

Differential transcript usage allows a single gene to produce multiple transcript isoforms. To explore possible molecular mechanisms other than selectively expressing genes in a specific pathogen isolate—host genotype interaction, genes with differential transcript usage were identified and analyzed in *P. nicotianae*. Sixty-six and 128 annotated genes in the *Wz-Wz* and *Wz*-H isolates, respectively, were differentially transcribed when the given isolate was infecting K 326 *Wz/Wz* compared to its infecting Hicks. Intron-retention (IR), alternative transcription start sites (ATSS), and alternative transcription termination sites (ATTS) were the three most common alternative splicing types in the two isolates.

Twenty-seven and 60 differentially transcribed genes were predicted to have functional consequences in the *Wz-Wz* and *Wz*-H isolates, respectively (Table S2). These genes were subjected to GO and PHIB blast analyses. No GO terms were enriched for either of the two sets of genes identified in the two isolates. The PHIB blast results showed that 9 out of 27 (33.3%) genes with differential transcript usage in the *Wz-Wz* isolate were involved in pathogenicity, while 13 (21.7%) pathogenicity-associated genes were identified from the 60 genes in the *Wz*-H isolate (Table S3). For example, the *Wz-Wz* isolate alternatively spliced genes PPTG_10075 encoding a serine/threonine protein kinase and PPTG_00215 encoding eukaryotic translation initiation factor. The *Wz*-H isolate differentially transcribed genes such as PPTG_06129 encoding pre-mRNA 3′ end processing protein and PPTG_03522 encoding ankyrin repeat protein.

3.2.6. SNPs Identified in the *Wz-Wz* and *Wz*-H Isolates of *P. nicotianae*

A total of 8 SNPs were identified between the two isolates of *P. nicotianae* (Table 6). Three SNPs (CHROM: 7000000185249344 POS: 2066387; CHROM: 7000000185249344 POS: 2066377; CHROM: 7000000185249344 POS: 2066882) were located in gene *PPTG_03590* encoding a conserved hypothetical protein in *Phytophthora* species with unknown function.

A SNP (CHROM: 7000000185249172 POS: 182510) was located in *PPTG_17734* which encodes an effector protein in the Crinkler family. Another SNP (CHROM: 7000000185249382 POS: 599392) was found in *PPTG_05817* encoding transcription factor S.

Table 6. SNPs identified in thte *Wz-Wz* and *Wz*-H isolates of *P. nicotianae*.

CHROM	POS	Gene	Annotation	*Wz-Wz*	*Wz-H*
7000000185249081	1188712	PPTG_07972	Hypothetical protein	C/C	C/T
7000000185249382	599392	PPTG_05817	Transcription factor S	C/C	C/T
7000000185249084	546281	PPTG_05165	Hypothetical protein	T/C	T/T
7000000185249172	182510	PPTG_17734	Crinkler family	G/A	G/G
7000000185249344	2066387	PPTG_03590	Hypothetical protein	G/A	G/G
7000000185249061	1073008	NA	NA	A/T	T/T
7000000185249344	2066377	PPTG_03590	Hypothetical protein	A/G	G/G
7000000185249344	2066882	PPTG_03590	Hypothetical protein	C/T	T/T

3.3. Overview of DEGs in N. tabacum

3.3.1. DEGs Identified in *N. tabacum*

DEGs were identified in K 326 *Wz/Wz* by comparing it to Hicks inoculated with a given *P. nicotianae* isolate. When inoculated with the *Wz-Wz* isolate, K 326 *Wz/Wz* had 305 up-regulated and 303 down-regulated genes compared to 174 up-regulated and 393 down-regulated genes when inoculated with the *Wz*-H isolate. The DEGs identified in K 326 *Wz/Wz* inoculated with the two isolates were further analyzed for commonalities and differences. There were 94 up-regulated and 163 down-regulated genes in common from samples of K 326 *Wz/Wz* inoculated with the two isolates (Table 7; Table S4).

Table 7. Number of DEGs identified in K 326 *Wz/Wz* inoculated with either the *Wz-Wz* or the *Wz*-H isolate of *P. nicotianae* compared to Hicks inoculated with the same isolate.

Tobacco Genotype for DEG Identification	Isolate for Inoculation	Total No. of DEGs	Up-Regulated DEGs	Down-Regulated DEGs
K 326 *Wz/Wz*	*Wz-Wz*	608	305	303
K 326 *Wz/Wz*	*Wz*-H	567	174	393
	Shared DEGs	257	94	163

Among the commonly up-regulated genes were Nitab4.5_0007488g0040.1 and Nitab4.5_0001477g0080.1 that encode pathogenesis-related (PR) protein 1a. In addition, five genes, Nitab4.5_0003154g0030.1, Nitab4.5_0000754g0140.1, Nitab4.5_0003324g0100.1, Nitab4.5_0014015g0010.1, Nitab4.5_0013087g0020.1, were predicted to encode proteinase inhibitors. Of the 163 commonly down-regulated genes, 13 genes were found to encode Glutathione S-transferase or Glutathione S-transferase-like protein.

3.3.2. GO Analysis of DEGs in Tobacco

Up-regulated genes identified exclusively in K 326 *Wz/Wz* inoculated with the *Wz-Wz* isolate were enriched in 33 GO terms, while down-regulated genes were enriched in 63 GO terms. The GO terms enriched for down-regulated DEGs are involved in various aspects of nuclear biosynthesis including "nucleosome organization" (GO: 0034728), "nucleosome assembly" (GO: 0006334), "chromosome organization" (GO: 0051276), "chromatin assembly or disassembly" (GO: 0006333), "DNA packaging complex" (GO: 0044815), "DNA conformation change" (GO: 0071103), "DNA-templated transcription, initiation" (GO: 0006352), "RNA biosynthetic process" (GO: 0032774), "protein complex biogenesis" (GO: 0070271), "protein complex assembly" (GO: 0006461) (Table 8).

No significant GO terms were enriched for exclusively up-regulated genes in K 326 *Wz/Wz* inoculated with the *Wz*-H isolate, and 8 GO terms were enriched for down-regulated genes after inoculation with the *Wz*-H isolate (Table 9).

Table 8. GO terms enriched for DEGs exclusively identified in K326 *Wz/Wz* inoculated with the *Wz-Wz* isolate.

Up-Regulated DEGs in K326 *Wz/Wz*				
GO Accession	**Term**	**Term Type**	***p* Value**	**FDR**
GO: 0044710	single-organism metabolic process	P	3.40×10^{-6}	0.0019
GO: 0044281	small molecule metabolic process	P	0.00015	0.0063
GO: 0044712	single-organism catabolic process	P	0.00015	0.0063
GO: 0046031	ADP metabolic process	P	0.00018	0.0063
GO: 0009179	purine ribonucleoside diphosphate metabolic process	P	0.00018	0.0063
GO: 0046496	nicotinamide nucleotide metabolic process	P	0.00015	0.0063
GO: 1901575	organic substance catabolic process	P	0.00013	0.0063
GO: 0051186	cofactor metabolic process	P	6.50×10^{-5}	0.0063
GO: 0044723	single-organism carbohydrate metabolic process	P	0.00016	0.0063
GO: 0006757	ATP generation from ADP	P	0.00018	0.0063
GO: 0006732	coenzyme metabolic process	P	7.40×10^{-5}	0.0063
GO: 0009135	purine nucleoside diphosphate metabolic process	P	0.00018	0.0063
GO: 0009185	ribonucleoside diphosphate metabolic process	P	0.00018	0.0063
GO: 0072524	pyridine-containing compound metabolic process	P	0.00017	0.0063
GO: 0006096	glycolytic process	P	0.00018	0.0063
GO: 0019362	pyridine nucleotide metabolic process	P	0.00015	0.0063
GO: 0006733	oxidoreduction coenzyme metabolic process	P	0.00019	0.0063
GO: 0044724	single-organism carbohydrate catabolic process	P	0.00026	0.0075
GO: 0009056	catabolic process	P	0.00025	0.0075
GO: 0006090	pyruvate metabolic process	P	0.00029	0.0082
GO: 0006165	nucleoside diphosphate phosphorylation	P	0.00032	0.0086
GO: 0009132	nucleoside diphosphate metabolic process	P	0.00042	0.011
GO: 0016209	antioxidant activity	F	8.40×10^{-5}	0.011
GO: 0003824	catalytic activity	F	8.50×10^{-5}	0.011
GO: 0046939	nucleotide phosphorylation	P	0.00053	0.012
GO: 0005975	carbohydrate metabolic process	P	0.00053	0.012
GO: 0016052	carbohydrate catabolic process	P	0.00056	0.012
GO: 0016903	oxidoreductase activity, acting on the aldehyde or oxo group of'donors	F	0.00017	0.014
GO: 0006082	organic acid metabolic process	P	0.0014	0.03
GO: 0043436	oxoacid metabolic process	P	0.0016	0.033
GO: 0019752	carboxylic acid metabolic process	P	0.0016	0.033
GO: 0043168	anion binding	F	0.00057	0.035
GO: 1901135	carbohydrate derivative metabolic process	P	0.0025	0.048
Down-Regulated DEGs in K326 *Wz/Wz*				
GO Accession	**Term**	**Term Type**	***p* Value**	**FDR**
GO: 0000786	nucleosome	C	5.10×10^{-27}	2.10×10^{-25}
GO: 0032993	protein-DNA complex	C	5.10×10^{-27}	2.10×10^{-25}
GO: 0044815	DNA packaging complex	C	8.30×10^{-27}	2.20×10^{-25}
GO: 0000785	chromatin	C	3.40×10^{-26}	6.90×10^{-25}
GO: 0046982	protein heterodimerization activity	F	1.60×10^{-26}	2.40×10^{-24}
GO: 0031497	chromatin assembly	P	5.30×10^{-26}	7.70×10^{-24}
GO: 0065004	protein-DNA complex assembly	P	8.30×10^{-26}	7.70×10^{-24}
GO: 0034728	nucleosome organization	P	5.30×10^{-26}	7.70×10^{-24}
GO: 0006334	nucleosome assembly	P	5.30×10^{-26}	7.70×10^{-24}
GO: 0071824	protein-DNA complex subunit organization	P	8.30×10^{-26}	7.70×10^{-24}
GO: 0006333	chromatin assembly or disassembly	P	1.10×10^{-25}	8.80×10^{-24}
GO: 0006323	DNA packaging	P	1.70×10^{-25}	1.20×10^{-23}
GO: 0071103	DNA conformation change	P	2.60×10^{-24}	1.50×10^{-22}
GO: 0044427	chromosomal part	C	1.00×10^{-23}	1.70×10^{-22}
GO: 0005694	chromosome	C	1.90×10^{-22}	2.50×10^{-21}
GO: 0006325	chromatin organization	P	7.90×10^{-23}	4.10×10^{-21}
GO: 0034622	cellular macromolecular complex assembly	P	4.30×10^{-21}	2.00×10^{-19}
GO: 0051276	chromosome organization	P	1.00×10^{-20}	4.20×10^{-19}
GO: 0065003	macromolecular complex assembly	P	1.30×10^{-20}	5.00×10^{-19}
GO: 0070271	protein complex biogenesis	P	8.40×10^{-20}	2.80×10^{-18}

Table 8. *Cont.*

Up-Regulated DEGs in K326 *Wz*/*Wz*				
GO Accession	**Term**	**Term Type**	***p* Value**	**FDR**
GO: 0006461	protein complex assembly	P	8.40×10^{-20}	2.80×10^{-18}
GO: 0022607	cellular component assembly	P	4.90×10^{-19}	1.50×10^{-17}
GO: 0071822	protein complex subunit organization	P	1.40×10^{-18}	3.90×10^{-17}
GO: 0043933	macromolecular complex subunit organization	P	4.60×10^{-18}	1.20×10^{-16}
GO: 0044085	cellular component biogenesis	P	3.80×10^{-17}	9.70×10^{-16}
GO: 0006996	organelle organization	P	5.30×10^{-17}	1.30×10^{-15}
GO: 0046983	protein dimerization activity	F	1.70×10^{-13}	1.30×10^{-11}
GO: 0016043	cellular component organization	P	5.30×10^{-12}	1.20×10^{-10}
GO: 0071840	cellular component organization or biogenesis	P	3.10×10^{-11}	6.90×10^{-10}
GO: 0044422	organelle part	C	2.30×10^{-10}	2.30×10^{-9}
GO: 0044446	intracellular organelle part	C	2.30×10^{-10}	2.30×10^{-9}
GO: 0003677	DNA binding	F	5.50×10^{-11}	2.80×10^{-9}
GO: 0043234	protein complex	C	4.90×10^{-9}	4.50×10^{-8}
GO: 0043232	intracellular non-membrane-bounded organelle	C	1.30×10^{-8}	9.40×10^{-8}
GO: 0043228	non-membrane-bounded organelle	C	1.30×10^{-8}	9.40×10^{-8}
GO: 0005634	nucleus	C	3.00×10^{-6}	2.00×10^{-5}
GO: 0009987	cellular process	P	2.10×10^{-6}	4.30×10^{-5}
GO: 0003676	nucleic acid binding	F	1.30×10^{-6}	5.10×10^{-5}
GO: 0032991	macromolecular complex	C	8.20×10^{-6}	5.10×10^{-5}
GO: 0006352	DNA-templated transcription, initiation	P	6.00×10^{-6}	0.00012
GO: 0043231	intracellular membrane-bounded organelle	C	0.0002	0.0011
GO: 0043227	membrane-bounded organelle	C	0.0002	0.0011
GO: 0043229	intracellular organelle	C	0.00071	0.0034
GO: 0043226	organelle	C	0.00071	0.0034
GO: 1901576	organic substance biosynthetic process	P	0.00025	0.0046
GO: 0009058	biosynthetic process	P	0.00025	0.0046
GO: 1901363	heterocyclic compound binding	F	0.00019	0.005
GO: 0097159	organic cyclic compound binding	F	0.00019	0.005
GO: 0019438	aromatic compound biosynthetic process	P	0.00045	0.008
GO: 1901362	organic cyclic compound biosynthetic process	P	0.0006	0.009
GO: 0097659	nucleic acid-templated transcription	P	0.00059	0.009
GO: 0006351	transcription, DNA-templated	P	0.00059	0.009
GO: 0032774	RNA biosynthetic process	P	0.0006	0.009
GO: 0044249	cellular biosynthetic process	P	0.00057	0.009
GO: 0034654	nucleobase-containing compound biosynthetic process	P	0.0018	0.027
GO: 0044424	intracellular part	C	0.0061	0.028
GO: 0065007	biological regulation	P	0.0024	0.034
GO: 0050794	regulation of cellular process	P	0.0025	0.034
GO: 0005622	intracellular	C	0.0081	0.035
GO: 0044711	single-organism biosynthetic process	P	0.0029	0.039
GO: 0050789	regulation of biological process	P	0.0031	0.04
GO: 0065008	regulation of biological quality	P	0.0034	0.042
GO: 0018130	heterocycle biosynthetic process	P	0.0036	0.043

Table 9. GO terms enriched for DEGs exclusively identified in K326 *Wz*/*Wz* inoculated with the *Wz*-H isolate.

Down-Regulated DEGs in K326 *Wz*/*Wz*				
GO Accession	**Term**	**Term Type**	***p* Value**	**FDR**
GO: 0006457	protein folding	P	9.70×10^{-11}	3.60×10^{-8}
GO: 0051082	unfolded protein binding	F	1.30×10^{-9}	2.90×10^{-7}
GO: 0005488	binding	F	2.30×10^{-5}	0.0025
GO: 0043565	sequence-specific DNA binding	F	0.00035	0.026
GO: 1901363	heterocyclic compound binding	F	0.0011	0.048
GO: 0001071	nucleic acid binding transcription factor activity	F	0.0015	0.048
GO: 0097159	organic cyclic compound binding	F	0.0011	0.048
GO: 0003700	transcription factor activity, sequence-specific DNA binding	F	0.0015	0.048

To investigate the potential resistance mechanism in K 326 *Wz/Wz*, commonly up-regulated and down-regulated DEGs found across samples of K 326 *Wz/Wz* inoculated with each of the two isolates were analyzed for enriched GO terms. In particular, GO terms in the functional categories "serine-type endopeptidase inhibitor activity" (GO: 0004867), "peptidase inhibitor activity" (GO: 0030414), "peptidase regulator activity" (GO: 0061134), "endopeptidase inhibitor activity" (GO: 0004866), "endopeptidase regulator activity" (GO: 0061135) were enriched for the up-regulated genes (Table 10). GO terms enriched for the down-regulated genes in the functional categories included "transferase activity, transferring hexosyl groups" (GO: 0016758), "transferase activity, transferring glycosyl groups" (GO: 0016757), "ATPase activity, coupled to transmembrane movement of substances" (GO: 0042626), "ATPase activity, coupled to movement of substances" (GO: 0043492), "ATPase activity" (GO: 0016887), "ATPase activity, coupled" (GO: 0042623) (Table 10).

Table 10. GO terms enriched for DEGs identified in K326*Wz*/*Wz* across inoculations using the *Wz*-*Wz* and *Wz*-H isolates.

GO Terms Enriched for Up-Regulated DEGs in K326 *Wz*/*Wz*				
GO Accession	**Term**	**Term Type**	***p* Value**	**FDR**
GO: 0004867	serine-type endopeptidase inhibitor activity	F	2.60×10^{-11}	4.20×10^{-9}
GO: 0030414	peptidase inhibitor activity	F	1.40×10^{-8}	4.40×10^{-7}
GO: 0061134	peptidase regulator activity	F	1.40×10^{-8}	4.40×10^{-7}
GO: 0004866	endopeptidase inhibitor activity	F	1.40×10^{-8}	4.40×10^{-7}
GO: 0061135	endopeptidase regulator activity	F	1.40×10^{-8}	4.40×10^{-7}
GO: 0044710	single-organism metabolic process	P	2.10×10^{-6}	0.00012
GO: 0004857	enzyme inhibitor activity	F	1.30×10^{-5}	0.00036
GO: 0050660	flavin adenine dinucleotide binding	F	8.80×10^{-5}	0.0018
GO: 0030234	enzyme regulator activity	F	7.70×10^{-5}	0.0018
GO: 0098772	molecular function regulator	F	0.00014	0.0025
GO: 0044699	single-organism process	P	0.00015	0.0043
GO: 0016491	oxidoreductase activity	F	0.00035	0.0057
GO: 0055114	oxidation-reduction process	P	0.00032	0.0063
GO: 0050662	coenzyme binding	F	0.00085	0.012
GO: 0048037	cofactor binding	F	0.0021	0.029
GO Terms Enriched for Down-Regulated DEGs in K326 Wz/Wz				
GO Accession	**Term**	**Term Type**	***p* Value**	**FDR**
GO: 0016758	transferase activity, transferring hexosyl groups	F	3.9×10^{-5}	0.01
GO: 0016757	transferase activity, transferring glycosyl groups	F	0.00029	0.013
GO: 0015405	P-P-bond-hydrolysis-driven transmembrane transporter activity	F	0.00043	0.013
GO: 0042626	ATPase activity, coupled to transmembrane movement of substances	F	0.00021	0.013
GO: 0015399	primary active transmembrane transporter activity	F	0.00043	0.013
GO: 0043492	ATPase activity, coupled to movement of substances	F	0.00023	0.013
GO: 0016491	oxidoreductase activity	F	0.00048	0.013
GO: 0016820	hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	F	0.00042	0.013
GO: 0016667	oxidoreductase activity, acting on a sulfur group of donors	F	0.00036	0.013
GO: 0016887	ATPase activity	F	0.00073	0.018
GO: 0006457	protein folding	P	0.00031	0.032
GO: 0042592	homeostatic process	P	0.0004	0.032
GO: 0019725	cellular homeostasis	P	0.00022	0.032
GO: 0045454	cell redox homeostasis	P	0.00017	0.032
GO: 0042623	ATPase activity, coupled	F	0.0021	0.048

3.3.3. KEGG Pathway Enrichment Analysis of DEGs

DEGs were subjected to KEGG pathway enrichment analysis to identify their biological roles in K 326 *Wz/Wz* in response to the two *P. nicotianae* isolates. Up-regulated DEGs in the *Wz*-*Wz*- and *Wz*-H-inoculated samples were enriched in 35 and 18 KEGG

pathways, respectively (Figures 4 and 5). Sixteen KEGG pathways were commonly enriched for up-regulated DEGs identified in K 326 *Wz*/*Wz* inoculated with each of the two isolates, with the most significantly enriched pathway being "valine, leucine and isoleucine degradation" in both *Wz*-*Wz*- and *Wz*-H-inoculated samples. Down-regulated DEGs in the *Wz*-*Wz*- and *Wz*-H-inoculated samples were enriched in 9 and 12 KEGG pathways, respectively (Figures 6 and 7). KEGG pathways that included "sulfur metabolism", "metabolic pathways", "glutathione metabolism", "ascorbate and aldarate metabolism", and "ABC transporters" were commonly enriched for down-regulated DEGs in K 326 *Wz*/*Wz* inoculated with each of the two isolates.

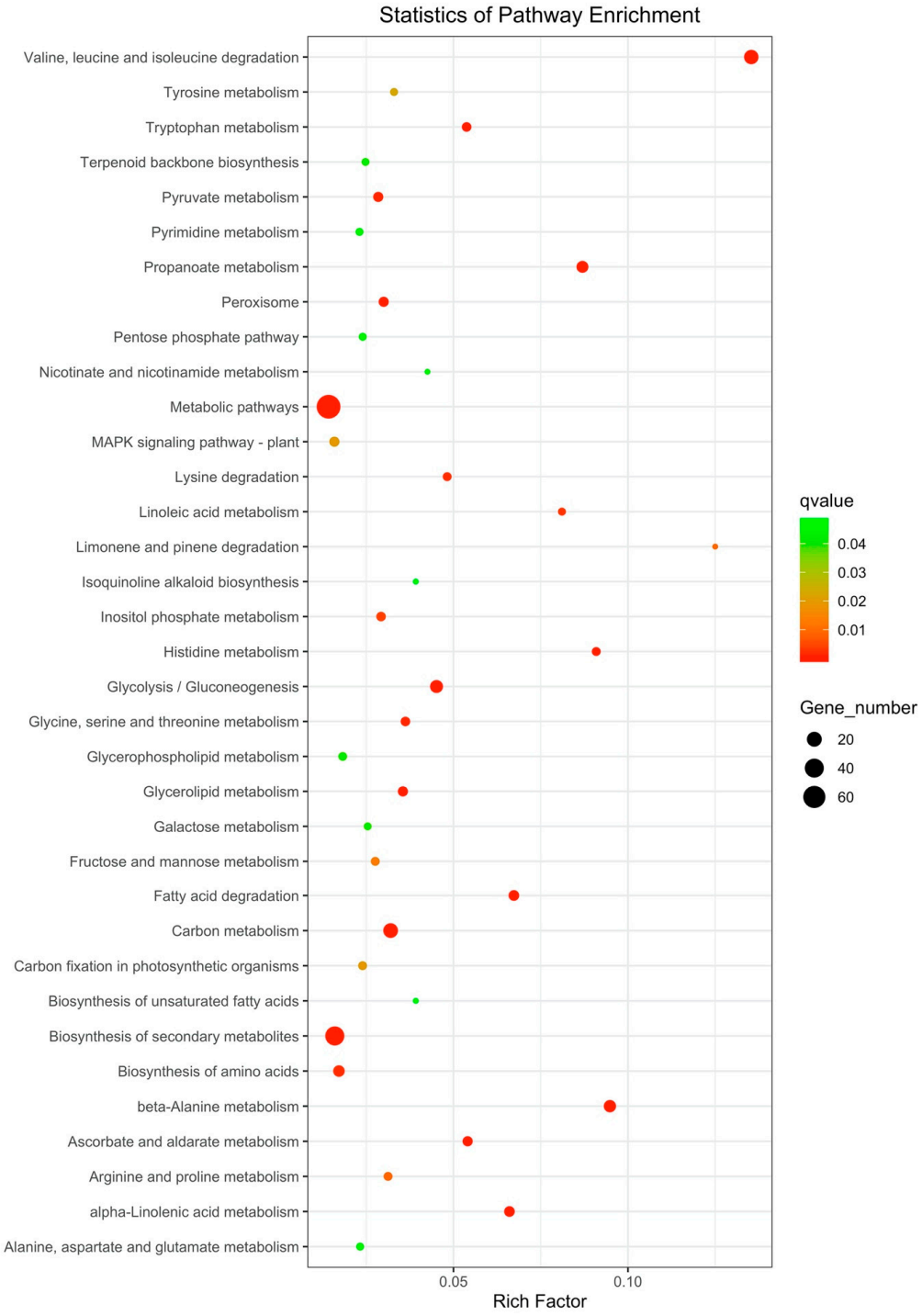


Figure 4. KEGG pathways enriched for up-regulated DEGs in K 326 *Wz*/*Wz* inoculated with the *Wz*-*Wz* isolate of *P. nicotianae* compared to inoculated Hicks. The *y*-axis indicates the name of the KEGG pathway. The dot size means the gene number. The dot color indicates the *q*-value.

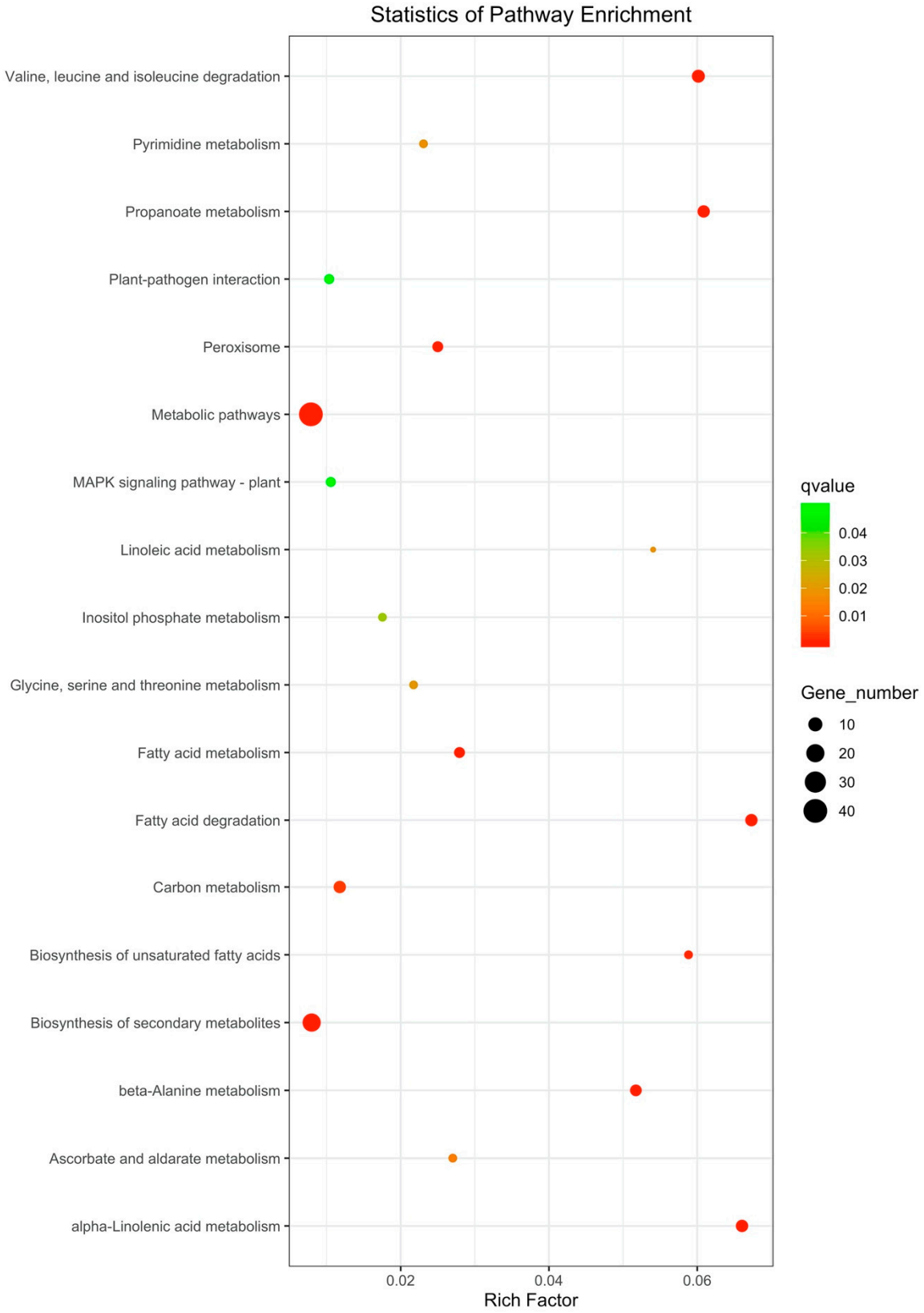


Figure 5. KEGG pathways enriched for up-regulated DEGs in K 326 *Wz*/*Wz* inoculated with the *Wz*-H isolate of *P. nicotianae* compared to inoculated Hicks. The *y*-axis indicates the name of the KEGG pathway. The dot size means the gene number. The dot color indicates the *q*-value.

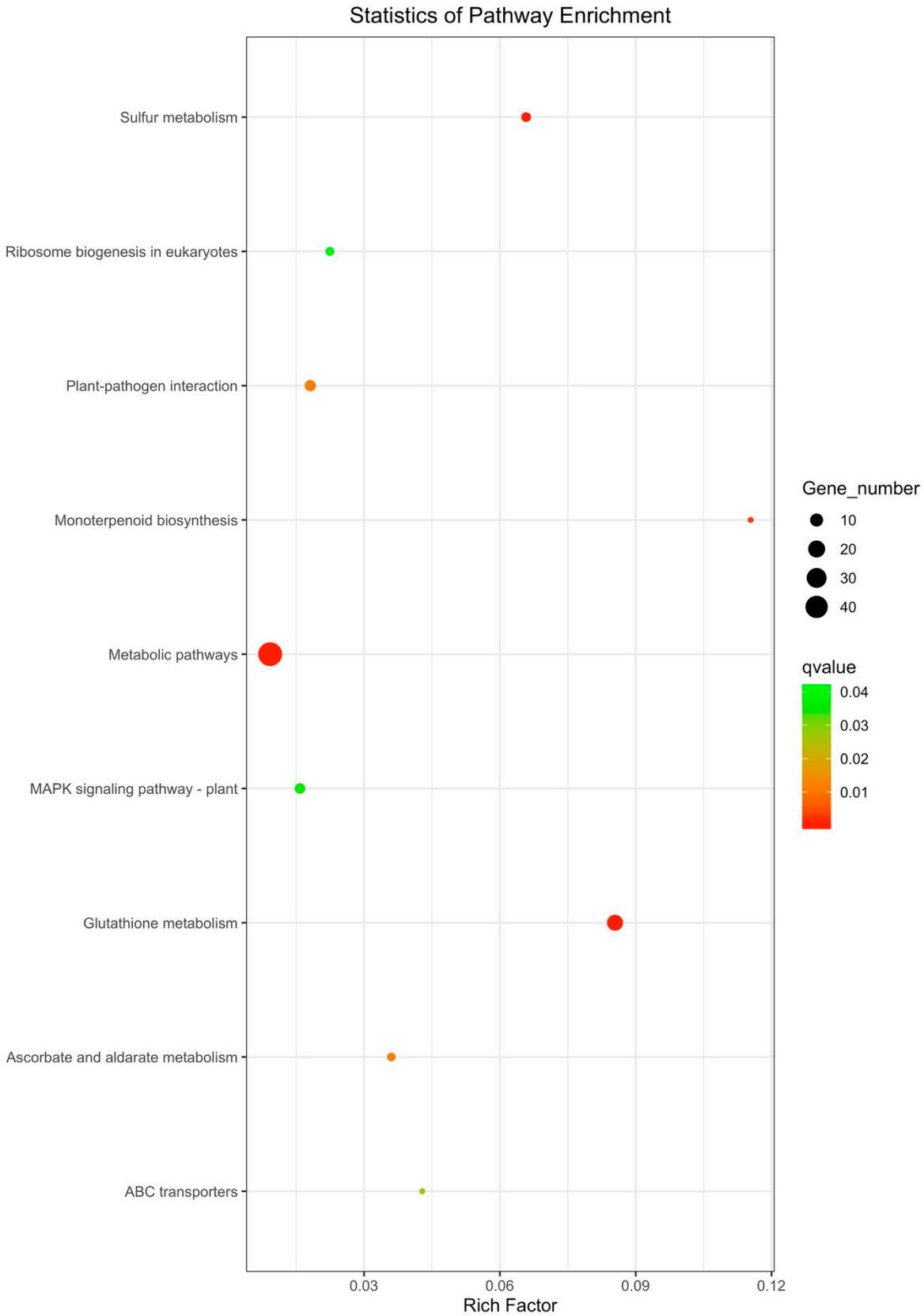


Figure 6. KEGG pathways enriched for down-regulated DEGs in K 326 *Wz*/*Wz* inoculated with the *Wz*-*Wz* isolate of *P. nicotianae* compared to inoculated Hicks. The *y*-axis indicates the name of the KEGG pathway. The dot size means the gene number. The dot color indicates the *q*-value.

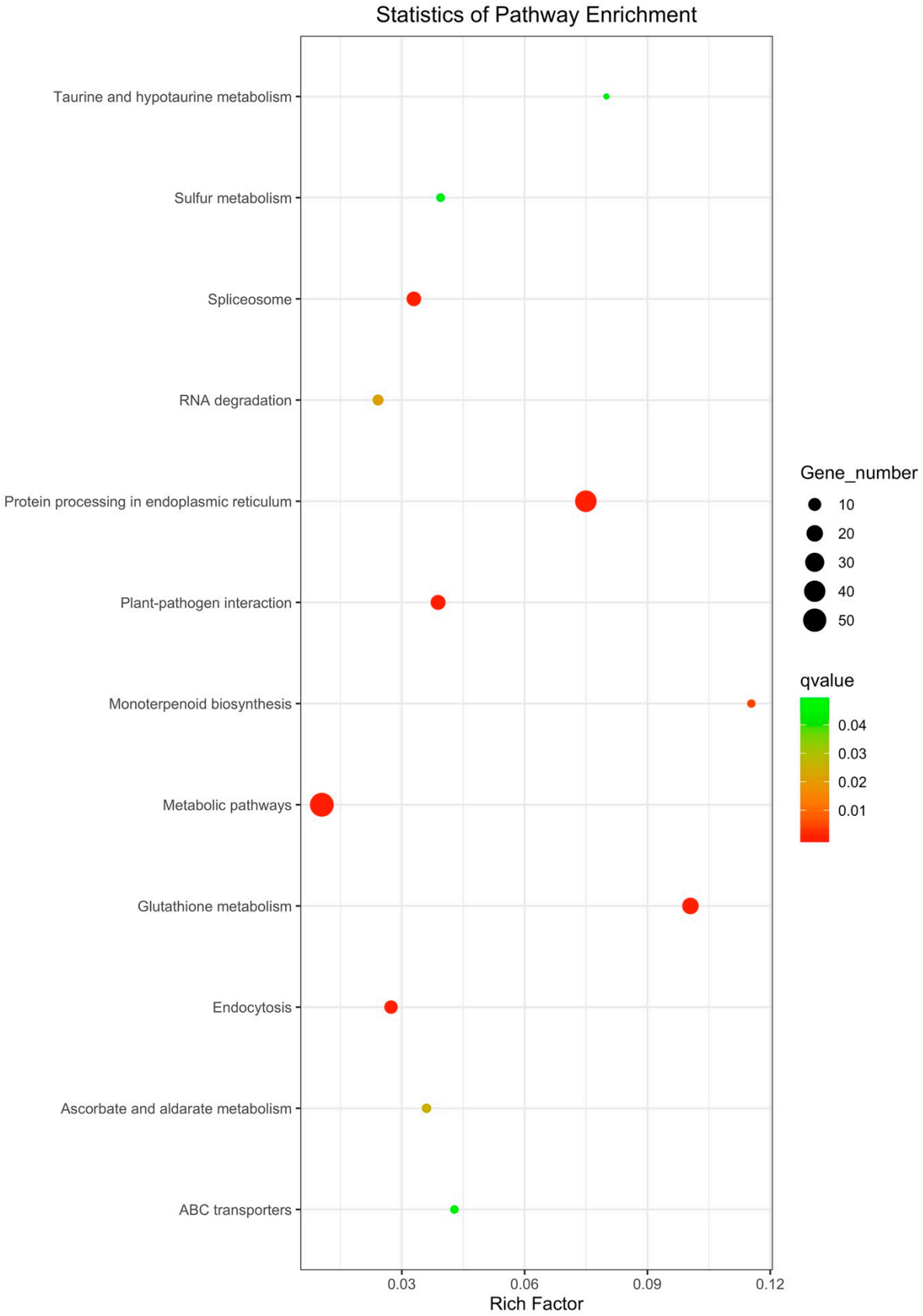


Figure 7. KEGG pathways enriched for down-regulated DEGs in K 326 *Wz*/*Wz* inoculated with the *Wz*-H isolate of *P. nicotianae* compared to inoculated Hicks. The *y*-axis indicates the name of the KEGG pathway. The dot size means the gene number. The dot color indicates the *q*-value.

4. Discussion

Black shank is one of the most devastating tobacco diseases globally. The use of host resistance is the most important and effective way to manage the disease worldwide, but with reduced value of complete resistance, an integrated approach using partial resistance is needed to effectively manage the disease [10]. Partial resistance is vital to the management of black shank, like many other root diseases, due to the absence of complete resistance genes or the loss of complete resistance due to pathogen new race development. Pathogen adaptation to partial resistance has been observed in various pathosystems, including *P. nicotianae* and tobacco, resulting in a pathogen population that is more aggressive than wild type populations [12–14]. It is urgent to better understand how pathogens overcome partial resistance in host plants so that resistance deployment strategies can be optimized to preserve the durability of the resistance.

The major goal of this study was to explore the molecular mechanisms underlying adaptation by *P. nicotianae* to partial resistance in tobacco [14,15]. Adaptation to partial resistance involves many genes and is generally considered to be more complex than overcoming complete resistance, which can result from a single nucleotide mutation in an *Avr* gene [34]. To obtain a holistic view of genetic differences that occur during adaptation to partial resistance and between pathogen isolates with distinctly different aggressiveness levels on a single source of partial resistance, we kept DEGs with a false FDR < 0.05 without a specified fold change of gene expression. Aggressiveness and partial resistance are two quantitative traits involving various biological activities supported by a broad spectrum of genes in the pathogen and host plant. The cumulative effect of slight changes in multiple genes could potentially influence the outcome of the interaction between a specific pathogen isolate and host genotype.

The DEGs detected involve a broad spectrum of biological activities related to pathogenicity factors in *P. nicotianae*. The PHIB blast showed a much higher percentage (68.75%) of up-regulated DEGs involved in pathogenicity of the *Wz-Wz* isolate compared to the *Wz*-H isolate (31.03%), indicating that the *Wz-Wz* isolate more efficiently recruited pathogenicity-associated genes when infecting partially resistant K 326 *Wz/Wz*. Particularly, some genes with essential roles in pathogenicity were only found up-regulated in the *Wz-Wz* isolate. These genes included PPTG_10595 that encodes a protein belonging to the ABC transporter superfamily, and PPTG_12158 that encodes ULK/ULK, a protein kinase, which has an important role in autophagy.

ABC transporters, also known as ATP Binding Cassette transporters, are of significant importance in regulating ion transport, chromosome condensation and DNA repair, mRNA processing in eukaryotes [35]. Studies have demonstrated ABC transporters are also involved in virulence [36] and toxicant efflux [37,38] in plant pathogenic fungi. It was speculated that ABC transporters export toxic phytoalexins in pathogens, therefore, contributing to pathogenicity. Comparing to *P. infestans*, ABC transporter gene family in *P. nicotianae* was significantly expanded, which suggested their crucial roles in evolutionary host adaptation [39]. The specific function of PPTG_10595 remains unclear, and given its versatility in biological processes, it is likely a higher expression of this gene could contribute to a higher aggressiveness in *P. nicotianae*.

ULK/ULK protein kinase (autophagy related protein 1, Atg1) is localized at the autophagy initiation site and initiates autophagy, which is critical in cell differentiation, secondary metabolism, and programmed cell death in eukaryotes [40]. In plant pathogens, autophagy has a vital role in pathogenicity. Silencing of *Atg1* highly reduced conidiation and led to a reduction or loss of pathogenicity in *Magnaporthe oryzae* [41], *Botrytis cinerea* [42], *Fusarium graminearum* [43]. In *P. sojae*, expression of multiple autophagy related protein genes was increased during infection, and autophagy was highly induced during sporangium formation and cyst germination. Silencing autophagy related genes in *P. sojae* significantly reduced sporulation and pathogenicity, and in some cases led to defective haustorium formation, suggesting a central role of autophagy in both the development and pathogenicity in *P. sojae* [44]. Little is known of *Atg1* in *P. nicotianae*, but it is possible

that the up-regulated *Atg1* expression in the *Wz-Wz* isolate could be a major contributor to aggressiveness on the resistant host K 326 *Wz/Wz*.

Three significantly enriched GO terms were assigned to down-regulated genes and one to up-regulated genes in the *Wz-Wz* isolate. Sixteen enriched GO terms were attributed to down-regulated genes in *Wz*-H isolate, however, no enriched GO terms were linked to up-regulated genes. These observations indicate that a broader spectrum of biological functions were affected because of the down-regulation of the genes in the *Wz*-H isolate.

The three enriched GO terms, "sulfate reduction" (GO: 0019419), "sulfate assimilation, phosphoadenylyl sulfate reduction by phosphoadenylyl-sulfate reductase (thioredoxin)" (GO: 0019379), and "sulfate assimilation" (GO: 0000103) that were assigned to down-regulated genes in the *Wz-Wz* isolate, were also enriched for the down-regulated genes in *Wz*-H isolate. Sulfate reduction and sulfate assimilation are two important biological processes changing sulfate into sulfide, which is then used for the synthesis of methionine, cysteine, and other metabolites [45]. The biological significance of methionine is predominantly because the methionine codon AUG is the most common start codon that initiates protein synthesis [46]. Cysteine is a strong antioxidant with the potential to trap reactive oxygen species (ROS), stabilizes the high-order structures of proteins, and serves as an active center for the bioactivity of proteins [47]. When genes involved in sulfate assimilation and sulfate reduction processes are down-regulated, their effects on the synthesis and bioactivity of proteins can be profound. The fact that these 3 GO terms were enriched for the down-regulated genes in both *Wz-Wz* and *Wz*-H isolates seems to suggest that *Wz* resistance in tobacco was disrupting the protein synthesis in *P. nicotianae* as a defense mechanism.

In addition to the GO terms mentioned above, another 13 enriched GO terms were assigned to the down-regulated genes in *Wz*-H isolate, including "oxidoreductase activity" (GO: 0016651, GO: 0000103, GO: 0009071; GO: 0016671). The "oxidation reduction process" catalyzed by oxidoreductases was found to be one of the two enriched GO terms distinguished the fungal pathogen *Colletotrichum kahawae* on coffee compared to its non-pathogenic sibling species [48], suggesting a substantial contribution of oxidoreductases in general pathogenicity in plant pathogens. Prospectively, a vital role of oxidoreductases in *P. nicotianae* aggressiveness is speculated. Down-regulation of genes annotated as oxidoreductases in the *Wz*-H isolate could potentially hamper its performance on K 326 *Wz/Wz* compared to *Wz-Wz* isolate.

Genes with differential transcript usage (DTU) also was investigated in the two isolates of *P. nicotianae*. Differential transcript usage is primarily the result of alternative splicing events that regulate translational processes. This allows for the formation of protein variants (isoforms) with different cellular functions or properties originating from a single gene, tremendously diversifying proteins encoded by genomes. Detection of genes with DTU has been widely used in RNAseq data analysis in human research [49]. The importance of DTU in genes in plant pathogens is largely unexplored, but a study with *Pseudoperonospora cubensis* showed that a functional effector protein *PscRXLR1* was generated from the alternative splicing of the *Psc_781.4* gene that encodes a putative multi-drug transporter [50]. This finding prompted our interest in genes with DTU in *P. nicotianae*. A total of 27 genes in *Wz-Wz* and 60 genes in *Wz*-H with DTU were identified with predicted functional consequences. Among those genes, 33.3% in *Wz-Wz* and 23.3% in *Wz*-H were pathogenicity-associated genes found in PHIB. How DTU in the genes could alter the aggressiveness in *P. nicotianae* needs to be further researched, but DTU in genes such as PPTG_00215 encoding eukaryotic translation initiation factor 1A, PPTG_06129 encoding pre-mRNA 3′ end processing protein WDR33, and PPTG_17135 encoding CCR4-NOT transcription complex subunit 1 signifies its role in translational biological processes in response to *Wz* resistance. Evidence of the importance of transcription factors in pathogen aggressiveness was confirmed in the genome-wide-association study (GWAS) in *C. kahawae* [48]. A slight change in pathogen genes or transcription factors associated in gene regulatory networks has been recognized to have a profound evolutionary impact [51].

Eight SNPs were identified between *Wz*-*Wz* and *Wz*-H. One SNP was located in the genetic region encoding a crinkler effector. Crinklers and RXLRs are two major classes of effectors secreted by oomycetes to facilitate pathogen infection [52]. The Crinkler protein family was first identified to cause leaf crinkling and necrosis when expressed in plants [53]. However, several Crinklers in *P. infestans* and *P. capsici* target the host nucleus during infection [54,55]. Effector proteins are coded by fast-evolving genes to overcome the immune system of the plants [56]. The SNP identified in the Crinkler effector coding region in our study may have critical roles in the interaction between *Wz*-*Wz* isolate and tobacco genotype K 326 *Wz/Wz.* Three SNPs occurred in gene *PPTG_03590*, with two of them occurring within 10 nucleobases in the gene. *PPTG_03590* encodes a hypothetical protein concerved in *Phytophthora* species with unknown function. The high frequencey of SNPs occurring in this gene highlighted the importance of its encoding protein and the need for functional identification of the protein.

To have a comprehensive understanding of the *P. nicotianae*—tobacco interaction, the transcriptomic changes in tobacco were investigated. Given the different genetic backgrounds of K 326 *Wz/Wz* and Hicks, the DEGs detected when comparing K 326 *Wz/Wz* to Hicks inoculated with a given isolate of *P. nicotianae* could have resulted from the constitutive differences in gene expression in the two genotypes. To compensate for this, the 94 up-regulated and 163 down-regulated genes commonly found in K 326 *Wz/Wz* compared to Hicks regardless of the isolate used for inoculation were considered as the background difference for the two tobacco genotypes. Among the 94 up-regulated genes in K 326 *Wz/Wz*, a number of defense related genes were detected, including Nitab4.5_0007488g0040.1 and Nitab4.5_0001477g0080.1 that encodes pathogenesis-related (PR) protein 1a and five genes (Nitab4.5_0003154g0030.1, Nitab4.5_0000754g0140.1, Nitab4.5_0003324g0100.1, Nitab4.5_0014015g0010.1, Nitab4.5_0013087g0020.1) that encode proteinase inhibitors important in inhibiting pathogen proteases. The PR-protein 1a is required to initiate systemic acquired resistance (SAR), a defense response effective against a broad spectrum of plant pathogens [57]. Proteinase inhibitors play a fundamental role in plant basal defense by inhibiting pathogen proteases or by regulating endogenous plant proteases [58]. The functions of these genes were confirmed by GO enrichment analysis. GOs were enriched for up-regulated genes in the functional categories "serine-type endopeptidase inhibitor activity" (GO: 0004867), "peptidase inhibitor activity" (GO: 0030414), "peptidase regulator activity" (GO: 0061134), "endopeptidase inhibitor activity" (GO: 0004866), "endopeptidase regulator activity" (GO: 0061135). The elevated expression of these genes gives K 326 *Wz/Wz* an advantage in response to pathogen attack. Interestingly, compared to Hicks, a number of genes encoding glutathione S-transferase or glutathione S-transferase like protein were down regulated in K 326 *Wz/Wz*. Glutathione S-transferase are multifunctional enzymes ubiquitous in plants. They are highly inducible by a wide range of stresses including pathogen infection [59]. In a previous study on black shank resistance in tobacco, plants that had silenced glutathione S-transferase had increased resistance to *P. nicotianae* infection, suggesting that glutathione S-transferase was able to act as a negative regulator of defense responses in tobacco [60]. The connection between the down-regulated expression of glutathione S-transferase genes and up-regulated defense-associated genes in K 326 *Wz/Wz* remains to be explored.

For the down-regulated genes identified exclusively in K 326 *Wz/Wz* inoculated with the *Wz*-*Wz* isolate, enriched GO terms included "nucleosome organization" (GO: 0034728), "nucleosome assembly" (GO: 0006334), "chromosome organization" (GO: 0051276), "chromatin assembly or disassembly" (GO: 0006333), "DNA packaging complex" (GO: 0044815), "protein complex assembly" (GO: 0006461), suggesting the nuclear biosynthesis processes were impeded in K 326 *Wz/Wz* compared to Hicks when inoculated with the *Wz*-*Wz* isolate. This phenomenon was not observed when K 326 *Wz/Wz* was inoculated with the *Wz*-H isolate. Along with previous studies where *Phytophthora* targeted host nuclei to suppress defense [54,61], our observations suggested that isolates of *P. nicotianae* adapted

to partial resistance in tobacco were able to interrupt biological processes in host nuclei to facilitate infection.

5. Conclusions

Few molecular studies have been completed to identify factors that might determine the overall aggressiveness of plant pathogens. Quantitative traits such as aggressiveness are challenging to dissect. A GWAS study on aggressiveness in *C. kahawae* strongly suggested that aggressiveness is associated with some small effect SNPs and is not regulated by causal mutations, which would indicate that aggressiveness might be a variable and very complex trait regulated by differential gene expression and corresponding regulatory mechanisms [48]. Our study was designed to enhance our understanding of the genetic mechanisms underlying *P. nicotianae* adaptation to partial resistance in tobacco using dual RNAseq. Overall, results from this study suggest that isolates of *P. nicotianae* adapted to partial resistance are able to recruit a high percentage of pathogenicity-associated genes when infecting a partially resistant genotype, and are more tolerant to the defenses expressed by *Wz* resistance. Finally, isolates adapted to the source of partial resistance used were able to severely hinder nuclear synthesis processes in K 326 *Wz/Wz*.

Wz resistance in K 326 *Wz/Wz* potentially disrupts protein synthesis in *P. nicotianae* as a defense mechanism. A broad spectrum and a high level of expression of defense related genes were recruited by K 326 *Wz/Wz* to inhibit non-adapted isolates of *P. nicotianae* compared to the adapted isolate. This was confirmed by the observation of a wide range of biological activities were affected by the down-regulated DEGs in the non-adapted isolate on K 326 *Wz/Wz*.

It would be beneficial if additional studies involving more isolates with distinct aggressiveness levels could be used to confirm our findings. Notwithstanding, sets of differentially expressed genes and genes with differential transcript usage were generated and can be researched via additional functional analyses to substantiate their roles in *P. nicotianae* aggressiveness. These findings provide a foundation for further investigation of the molecular mechanisms underlying pathogen adaptation to partial resistance in host plants.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11040656/s1, Table S1: PHIB blast results for DEGs identified in the *Wz*-*Wz* and *Wz*-H isolates of *Phytophthora nicotianae*, Table S2: DEGs identified in the *Wz*-*Wz* and *Wz*-H isolates of *Phytophthora nicotianae* with differential transcript usage (DTU), Table S3: PHIB blast results for DEGs identified in *Wz*-*Wz* and *Wz*-H isolates of *Phytophthora nicotianae* with differential transcript usage (DTU), Table S4: DEGs identified in K326 *Wz/Wz* inoculated with either *Wz*-*Wz* or *Wz*-H isolate of *Phytophthora nicotianae*.

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