



HAL
open science

Dual Transcriptome of Post-Germinating Mutant Lines of *Arabidopsis thaliana* Infected by *Alternaria brassicicola*

Mailen Ortega-Cuadros, Laurine Chir, Sophie Aligon, Nubia Velasquez, Tatiana Arias, Jerome Verdier, Philippe Grappin

► **To cite this version:**

Mailen Ortega-Cuadros, Laurine Chir, Sophie Aligon, Nubia Velasquez, Tatiana Arias, et al.. Dual Transcriptome of Post-Germinating Mutant Lines of *Arabidopsis thaliana* Infected by *Alternaria brassicicola*. *Data*, 2024, 9 (11), pp.137. 10.3390/data9110137 . hal-04799033

HAL Id: hal-04799033

<https://univ-angers.hal.science/hal-04799033v1>

Submitted on 22 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Dual Transcriptome of Post-Germinating Mutant Lines of *Arabidopsis thaliana* Infected by *Alternaria brassicicola*

Mailen Ortega-Cuadros ^{1,2,†}, Laurine Chir ^{1,†}, Sophie Aligon ¹, Nubia Velasquez ¹, Tatiana Arias ³, Jerome Verdier ¹ and Philippe Grappin ^{1,*}

¹ Institut Agro Rennes-Angers, University Angers, INRAE, IRHS, SFR 4207 QuaSaV, F-49000 Angers, France; mailen.ortega@udea.edu.co (M.O.-C.); laurine.chir@agrocampus-ouest.fr (L.C.); sophie.aligon@agrocampus-ouest.fr (S.A.); nubiayin@gmail.com (N.V.); jerome.verdier@inrae.fr (J.V.)

² Instituto de Biología, Facultad de Ciencias Exactas y Naturales, Universidad Antioquia, Calle 67 N° 53-108, Medellín 050010, Colombia

³ Fundación Orquídeas para la Paz, Sabaneta 055450, Colombia; tatiana.tatianaarias@gmail.com

* Correspondence: philippe.grappin@institut-agro.fr

† These authors contributed equally to this work.

Abstract: *Alternaria brassicicola* is a seed-borne pathogen that causes black spot disease in Brassica crops, yet the seed defense mechanisms against this fungus remain poorly understood. Building upon recent reports that highlighted the involvement of indole pathways in seeds infected by *Alternaria*, this study provides transcriptomic resources to further elucidate the role of these metabolic pathways during the interaction between seeds and fungal pathogens. Using RNA sequencing, we examined the gene expression of glucosinolate-deficient mutant lines (*cyp79B2/cyp79B3* and *qko*) and a camalexin-deficient line (*pad3*), generating a dataset from 14 samples. These samples were inoculated with *Alternaria* or water, and collected at 3, 6, and 10 days after sowing to extract total RNA. Sequencing was performed using DNBSeq™ technology, followed by bioinformatics analyses with tools such as FastQC (version 0.11.9), multiQC (version 1.13), Venny (version 2.0), Salmon software (version 0.14.1), and R packages DESeq2 (version 1.36.0), ClusterProfiler (version 4.12.6) and ggplot2 (version 3.4.0). By providing this valuable dataset, we aim to contribute to a deeper understanding of seed defense mechanisms against *Alternaria*, leveraging RNA-seq for various analyses, including differential gene expression and co-expression correlation. This work serves as a foundation for a more comprehensive grasp of the interactions during seed infection and highlights potential targets for enhancing crop protection and management.

Dataset: The RNA-seq data were submitted to the NCBI. This dataset is publicly available through the GEO database with accession number GSE214602 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE214602>, accessed on 25 August 2024) and all the supplementary tables can be accessed via Mendeley data (<https://data.mendeley.com/datasets/c9d453jtwr/2>, accessed on 25 August 2024).

Dataset License: license under which the dataset is made available (CC0, CC-BY, CC-BY-SA, CC-BY-NC, etc.).

Keywords: glucosinolates; camalexin; necrotrophic fungi; RNA-seq; germinating seeds; plant–pathogen interaction



Citation: Ortega-Cuadros, M.; Chir, L.; Aligon, S.; Velasquez, N.; Arias, T.; Verdier, J.; Grappin, P. Dual Transcriptome of Post-Germinating Mutant Lines of *Arabidopsis thaliana* Infected by *Alternaria brassicicola*. *Data* **2024**, *9*, 137. <https://doi.org/10.3390/data9110137>

Academic Editor: Flavio Licciulli

Received: 26 August 2024

Revised: 6 November 2024

Accepted: 14 November 2024

Published: 18 November 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Summary

Secondary metabolites such as indole glucosinolates and camalexin [1] both originate from tryptophan (Trp) and play a crucial role in the defense mechanisms of Brassicaceae plants against certain phytopathogens [1–4]. One such seed-borne pathogen is *Alternaria brassicicola* (referred to as *Alternaria* hereinafter), a necrotrophic fungus responsible for inducing black spot disease in Brassica crops [5,6]. In plants, the antifungal activity from

these metabolites has been extensively documented [1,3,7]; however, their role in seed defense remains poorly studied.

Recently, our research group has been studying the seed defense mechanisms against fungal pathogens using the *Arabidopsis thaliana*/*Alternaria brassicicola* pathosystem [6,8]. We focused on developmental stages closely related to seed germination, specifically germinating seeds, seedling emergence (stages just before and after radicle protrusion through seed covering layers), and establishment (autotrophic stage), corresponding to 3, 6, and 10 days after sowing (DAS), respectively [8].

First, a transcriptomics experiment was conducted on *Arabidopsis* Col0 genotype (wild-type—WT) infected by *Alternaria* [9]; subsequently, phenotyping analyses were performed to assess colonization rates and necrosis development in defense-defective mutant seeds (WT was used as the control) [8]. These studies enabled the identification of a specific immune response in seeds against *Alternaria*. We found that the necrotrophic fungus might modulate the seed transcriptome to facilitate colonization and induce necrosis development through the glucosinolate pathway. Additionally, the camalexin-deficient line displayed a necrosis pattern like the WT strain [8]. To enhance our comprehension of the involvement of glucosinolate pathways in the seed immune response, we investigated the transcriptomic profiles of the following mutant *Arabidopsis* seeds: a *cyp79B2 cyp79B3* double mutant, which is defective in indole glucosinolates (*cyp79B2/B3*) [10]; a *cyp79B2 cyp79B3 myb28 myb29* quadruple mutant, which is defective in both indole and aliphatic glucosinolates (*qko*) [11]; and a camalexin-deficient mutant (*pad3*) [12]. Seeds were subjected to infection with an *Alternaria* inoculum (10^4 conidia/mL) or imbibed in water as a control. At 3, 6, and 10 DAS, RNA isolation was conducted, and samples with optimal quality were sent to the Beijing Genomics Institute for sequencing using DNBSTM technology. Our dataset contains 84 clean reads from 14 samples. A sequenced reads quality filter was performed using FastQC version 0.11.9 [13] and MultiQC version 1.13 [14] was performed in all files. High-quality reads were mapped on the *Arabidopsis* [15] and the *Alternaria* [16] reference genomes using Salmon version 0.14.1 [17]. The expression change was calculated by DESeq2 packages version 1.36.0 [18]. Gene expression changes were inferred by comparing infected and healthy WT or mutant seed expression values, as well as performing pairwise comparisons of expression values between mutant and WT genotypes, both under infected conditions. Functional annotation was obtained using the package ClusterProfiler version 4.12 [19]. This study provides RNA-seq data as resources that can significantly contribute to elucidating the seed defense mechanism against fungal necrotrophs. By utilizing glucosinolate mutants, these expression data enable a deeper exploration of the role of the indole glucosinolate pathway in this intricate defense mechanism.

2. Data Description

2.1. Identification of Dual Transcriptome

After sequencing, the reads were filtered, and high-quality reads were aligned and mapped to the *Arabidopsis* [15] and *Alternaria* [16] reference genomes. The completed RNA-seq data were deposited in the NCBI Sequence Read Archive (SRA) database under the repository name NCBI GEO with the data identification number GSE214602 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE214602>, accessed on 25 August 2024).

Table 1 provides a summary of the mapped reads to their respective reference genomes for both the plant and the necrotrophic fungus, presenting the percentage of reads mapped for each biological replicate of each one. Notably, the samples from the inoculation group exhibit variations in mapped reads between *Arabidopsis* and *Alternaria* across different post-infection time points. Mapped reads to *Arabidopsis* remain relatively high, indicating successful mapping to the plant genome. In contrast, the lower percentage of reads mapped to the *Alternaria* genome may be due to the preliminary state and ongoing curation of the *Alternaria* Abra43 genome [16], which could have influenced mapping accuracy.

Table 1. Summary of mapped reads to reference genomes of both *Arabidopsis* and *Alternaria*. Mutant lines: *cyp79B2/B3*, a double mutant defective in indole glucosinolate [10]; *qko*, a quadruple mutant defective in indole and aliphatic glucosinolates [11]; and *pad3*, a camalexin-deficient mutant [12]. Inoc: seed inoculated with Abra43; water: seed without fungal inoculum; developmental stages: 3, 6, and 10 days after sowing (DAS); REP: biological replicate. % Aligned: % mapped reads; M Aligned: mapped reads (millions); M Seqs: total sequences (millions).

Sample Name	M Seqs	Mapped Reads Percentages to <i>Arabidopsis</i> Genome		Mapped Reads Percentages to <i>Alternaria</i> Genome	
		% Aligned	M Aligned	% Aligned	M Aligned
cyp_inoc_3d_REP1	25.2	95.1%	24.0	1.0%	0.3
cyp_inoc_3d_REP2	50.9	85.5%	43.5	8.2%	4.2
cyp_inoc_3d_REP3	51.2	89.8%	46.0	4.9%	2.5
cyp_inoc_6d_REP1	51.0	72.7%	37.1	17.4%	8.9
cyp_inoc_6d_REP2	52.5	44.7%	23.5	37.6%	19.7
cyp_inoc_6d_REP3	50.2	62.8%	31.5	24.5%	12.3
cyp_inoc_10d_REP1	50.8	46.4%	23.6	35.8%	18.2
cyp_inoc_10d_REP2	51.8	45.5%	23.6	36.2%	18.8
cyp_inoc_10d_REP3	51.9	27.2%	14.1	48.4%	25.1
qko_inoc_3d_REP1	52.1	30.7%	16.0	47.5%	24.8
qko_inoc_3d_REP2	51.9	38.5%	20.0	41.2%	21.4
qko_inoc_3d_REP3	51.9	24.4%	12.7	52.2%	27.1
qko_inoc_6d_REP1	52.1	16.9%	8.8	55.6%	29.0
qko_inoc_6d_REP2	52.3	28.4%	14.9	48.0%	25.1
qko_inoc_6d_REP3	52.1	9.1%	4.7	58.7%	30.6
pad3_inoc_3d_REP1	50.9	65.6%	33.3	20.3%	10.3
pad3_inoc_3d_REP2	50.7	34.1%	17.3	40.4%	20.5
pad3_inoc_3d_REP3	51.0	76.0%	38.7	13.3%	6.8
pad3_inoc_6d_REP1	50.4	83.3%	41.9	9.0%	4.5
pad3_inoc_6d_REP2	50.1	41.1%	20.6	38.3%	19.2
pad3_inoc_6d_REP3	51.3	65.6%	33.6	20.8%	10.7
cyp_water_3d_REP1	25.2	96.4%	24.3	0.0%	0.0
cyp_water_3d_REP2	25.2	95.4%	24.1	0.0%	0.0
cyp_water_3d_REP3	25.2	96.7%	24.4	0.0%	0.0
cyp_water_6d_REP1	25.2	95.4%	24.0	0.0%	0.0
cyp_water_6d_REP2	25.2	95.0%	23.9	0.0%	0.0
cyp_water_6d_REP3	25.2	96.1%	24.2	0.0%	0.0
cyp_water_10d_REP1	25.3	96.2%	24.3	0.0%	0.0
cyp_water_10d_REP2	25.2	96.4%	24.3	0.0%	0.0
cyp_water_10d_REP3	25.3	96.2%	24.3	0.0%	0.0
qko_water_3d_REP1	25.2	96.4%	24.3	0.0%	0.0
qko_water_3d_REP2	25.2	96.5%	24.3	0.0%	0.0
qko_water_3d_REP3	26.4	96.7%	25.6	0.0%	0.0
qko_water_6d_REP1	25.8	94.2%	24.3	0.0%	0.0
qko_water_6d_REP2	26.5	95.8%	25.4	0.0%	0.0
qko_water_6d_REP3	26.5	95.3%	25.2	0.0%	0.0
pad3_water_3d_REP1	26.0	94.8%	24.7	0.0%	0.0
pad3_water_3d_REP2	25.9	94.2%	24.4	0.0%	0.0
pad3_water_3d_REP3	26.0	95.7%	24.9	0.0%	0.0
pad3_water_6d_REP1	25.9	95.5%	24.7	0.0%	0.0
pad3_water_6d_REP2	26.0	95.8%	24.9	0.0%	0.0
pad3_water_6d_REP3	25.8	95.3%	24.6	0.0%	0.0

Additionally, control samples exposed to water consistently exhibit a high number of mapped reads for the *Arabidopsis* reference genome, confirming the absence of infection. Also, no reads were aligned to the *Alternaria* genome in the control samples, supporting the specificity of the alignment process and validating the absence of fungal contamination in the control conditions.

2.2. Differentially Expressed Genes from Infected and Healthy *Arabidopsis* Mutant Seeds at 3 and 6 DAS

After the quality control of reads and reference base assemblies, differentially expressed genes (DEGs) were obtained by comparing between infected and healthy mutant seeds at 3 and 6 DAS using the DESeq2 tool [18]. Genes with $\log_2FC > 1$ or < -1 and Benjamani–Hochberg score < 0.05 were considered as DEGs. A total of 256, 2954, and 1942 DEGs were identified, respectively, for *cyp79B2/B3*, *qko*, and *pad3* at 3 DAS and 5345, 3949, and 6768 at 6 DAS. The lists of these DEGs can be found in Table S1, including information related to gene’s foldchange, “Gene Symbol” and “Gene TAIR Computational Description” from The *Arabidopsis* information Resource—TAIR [20]—among others. The total number DEGs, classified as both up- and downregulated, for each mutant line at 3 and 6 DAS is shown in Table 2.

Table 2. Total number of differentially expressed genes (DEGs) in *cyp79B2/B3*, *qko*, and *pad3* *Arabidopsis* mutant lines. DEGs from comparison between infected and healthy mutant seeds ($\log_2FC > 1$ or < -1 and Benjamani–Hochberg adjusted p -value < 0.05).

Mutant Lines	DEGs	3 DAS	6 DAS
<i>cyp79B2/B3</i>	Upregulated	237	3362
	Downregulated	19	1983
<i>qko</i>	Upregulated	1817	2092
	Downregulated	1137	1857
<i>pad3</i>	Upregulated	797	3703
	Downregulated	1145	3065

Previously, we analyzed sequences from WT seeds infected with *Alternaria* [9]. Sampling, sequencing, and bioinformatic procedures were close to those of the current study; we overlapped the DEGs identified in the WT with those in the *Arabidopsis* mutants *cyp79B2/B3*, *qko*, and *pad3* using Venn diagrams [21]. The number of DEGs within the intersections, both up- and downregulated, varies based on the plant’s developmental stage and the presence of seed mutations. Notably, our observations revealed that the WT shares several common genes with the *pad3* mutant at both 3 and 6 DAS (Figure 1).

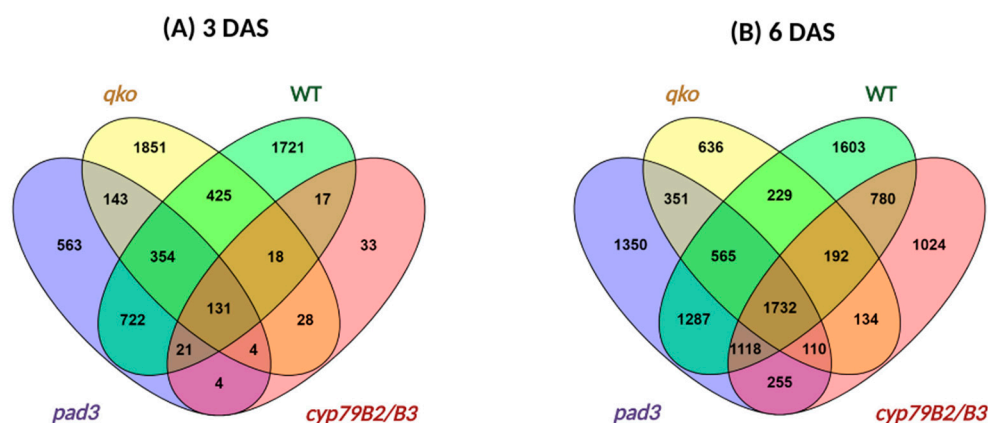


Figure 1. Venn diagrams showing comparisons of differentially expressed genes (DEGs) induced by *Alternaria* on *Arabidopsis* mutant lines (*cyp79B2/B3*, *qko*, and *pad3*) and the WT [9] during pre-germinative stage (3 DAS) and early seedling establishment (6 DAS): (A) DEGs at 3 DAS; (B) DEGs at 6 DAS. Genes with $\log_2FC > 1$ or < -1 and Benjamani–Hochberg adjusted p -value < 0.05 were considered as DEGs.

The functional annotation of DEGs obtained by the comparison between healthy and infected seeds allowed us to compare the enrichment of GO terms in the WT genotype

and in the different mutants *cyp79B2/B3*, *qko*, and *pad3* (Figure 2). The observed differences highlight that the mutated genes participate in the response to *Alternaria* infection in a wild genetic background of the Col0 ecotype. This observation is in agreement with the differences in infection symptoms previously observed in young seedlings [8]. A previous transcriptomic study carried out on the WT Col0 genotype on rosette leaves infected with *Alternaria* identified 505 genes involved in the response to infection [22]. Among these genes, a number of 1, 382, and 400 genes were found to be induced, respectively, in our samples at 3, 6, and 10 DAS [8]. Clearly, the response to *Alternaria* at the germination stage (3 DAS) differs radically from that observed after germination (6 DAS) in the established seedling (10 DAS). These last two stages present a response similar to the response observed in infected rosette leaves [8,22].

Moreover, Narusaka et al. [23] illustrate that the transcriptome of *Alternaria*-infected leaves in the *pad3* mutant, compared to the WT Col0 genotype, shows a defect in the induction of genes in the salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) defense pathways. Both genotypes exhibit very similar hypersensitive response (HR) reactions at the infected leaf level; however, the *pad3* mutant shows increased susceptibility to *Alternaria* colonization. Narusaka et al. [23] hypothesize that *pad3* not only affects camalexin production but also delays the induction of *Alternaria*-responsive genes mediated by the HR reaction. Other studies at the post-germination stage of the seed (6 DAS) found no differences in symptoms between the WT and *pad3* genotype [8]. The RNA-seq data described here (Table 3) indicate that the genes involved in the SA, JA, and ET defense pathways are indeed induced in greater numbers in the WT genotype compared to the *pad3* mutant at both the pre-germination stage (3 DAS) and at the post-germinative stage. At the post-germinative stage (6 DAS), the number of induced defense genes increases and the difference in the number of induced genes between the WT and *pad3* is also observed, but to a lesser extent. This suggests that, from the onset of development during germination, the *pad3* mutation results in a reduced *Alternaria*-mediated induction of SA, JA, and ethylene defense pathways. At the stage of seedling emergence, the difference between the two genotypes becomes smaller.

Table 3. Defense response genes induced by *Alternaria*. A list of defense-related genes was constructed using defense response terms from Gene Ontology. These genes were then identified among the upregulated genes in the WT and *pad3* at 3 and 6 DAS and compared between both genotypes. JA: jasmonic acid; ET: ethylene; SA: salicylic acid; ROS: reactive oxygen species; GSL: glucosinolates.

Defense Pathways and Metabolites	No. of Genes at 3DAS		No. of Genes at 6DAS	
	WT	<i>pad3</i>	WT	<i>pad3</i>
JA	1	1	22	15
ET	41	22	118	104
SA	92	71	273	252
ROS	21	13	61	55
Indol	57	48	113	99
Phytoalexin	11	11	20	19
GSL	11	5	21	17

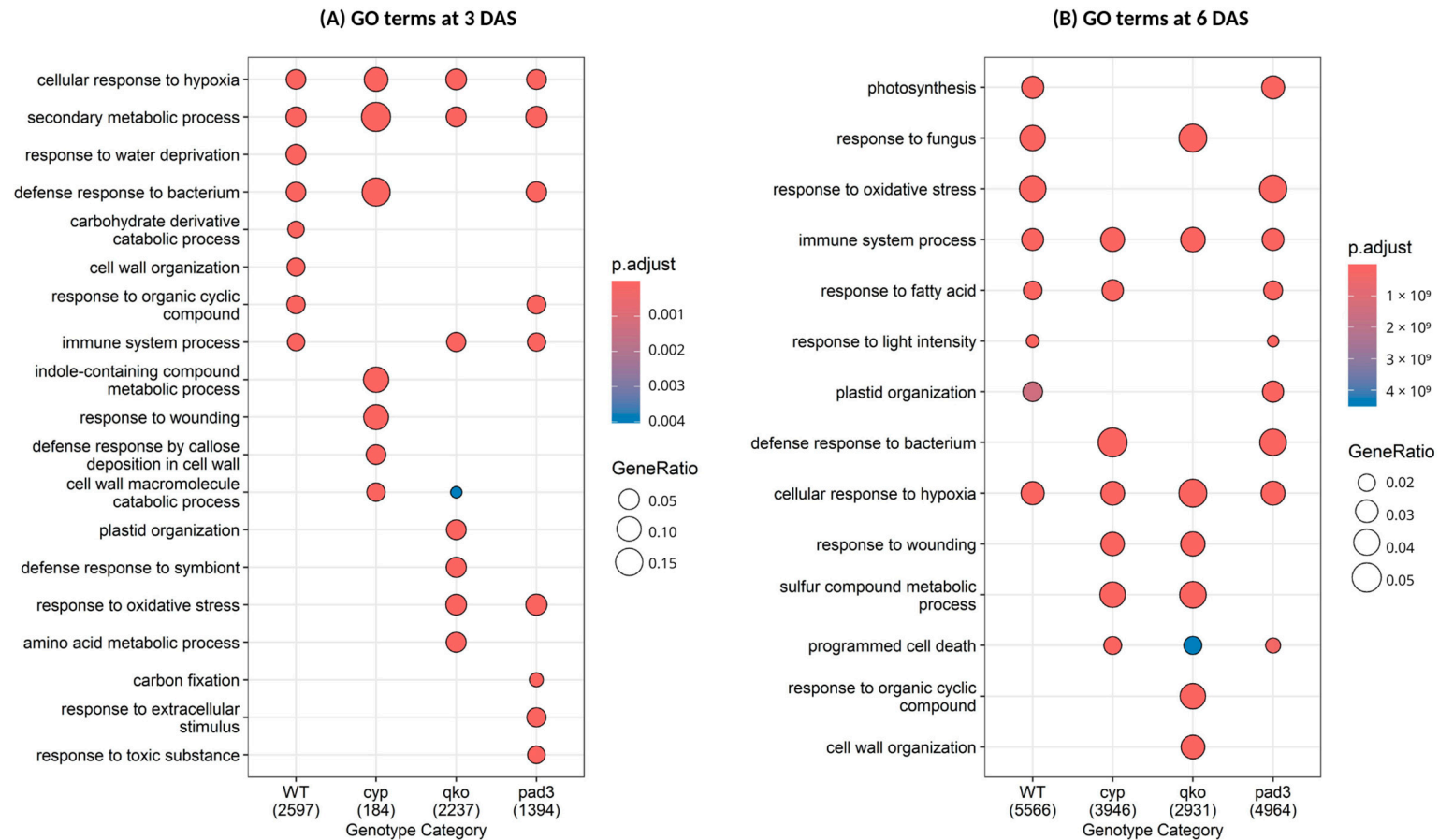


Figure 2. Major overrepresented biological functions of DEGs (shown in Figure 1) in the WT and mutant *Arabidopsis* seeds in response to *Alternaria* infection at 3 and 6 DAS. **(A)** Top 7 enriched GO terms at 3 DAS. **(B)** Top 7 enriched GO terms at 6 DAS. Dot size represents the gene ratio (ratio of input genes annotated in a term). The color scale indicates adjusted p -values, calculated with the ClusterProfiler algorithm using a hypergeometric test and corrected with the Benjamini–Hochberg method [19].

2.3. Comparative Analysis of Gene Expression Changes in *Alternaria*-Infected Seeds of *Arabidopsis* Mutants and Wild-Type at 3 and 6 Days After Sowing

Gene expression changes for *Arabidopsis* (Table S2) and *Alternaria* (Table S3) are provided as separate datasets. All expression changes correspond to pairwise comparisons between the mutants and the WT *Arabidopsis* seeds infected by *Alternaria*. Figure 3 illustrates the number of genes categorized as downregulated, non-significant, and upregulated at 3 and 6 DAS.

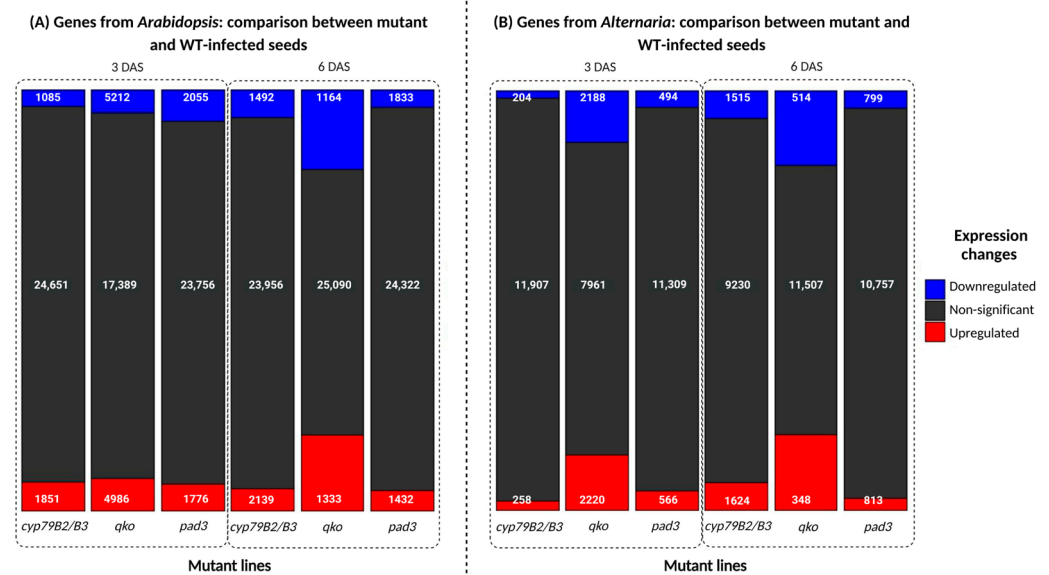


Figure 3. Bar charts showing expression changes analyses from both *Arabidopsis* and *Alternaria* at 3 and 6 days after sowing (DAS); (A) corresponds to expression change from *Arabidopsis* mutant lines, and (B) to expression changes from *Alternaria* on *Arabidopsis* mutant lines. The log₂ (fold change) corresponds to gene expression changes from the comparison between the infected mutant and infected WT genotypes [9] ($\log_2FC > 1$ or < -1 and Benjamani–Hochberg adjusted p -value < 0.05). Colors scale: blue represents downregulated genes, red upregulated genes, and black insignificant expression change.

2.4. Gene Expression Changes in *cyp79B2/B3* *Arabidopsis* Mutant at 10 DAS in Both Plant and Fungi Transcriptomes

Gene expression changes at 10 DAS were only obtained for the plant mutant *cyp79B2/B3* (Table S4), and these results are illustrated through volcano plots (Figure 4). Figure 4A,B depict genes from *Arabidopsis* while Figure 4C corresponds to genes from *Alternaria*, both in the mutant *cyp79B2/B3*. Gene expression changes for Figure 4A,C were obtained by comparing between infected and healthy *cyp79B2/B3* seeds, while Figure 4B values were calculated through a pairwise comparison between *cyp79B2/B3* and the WT-infected seeds.

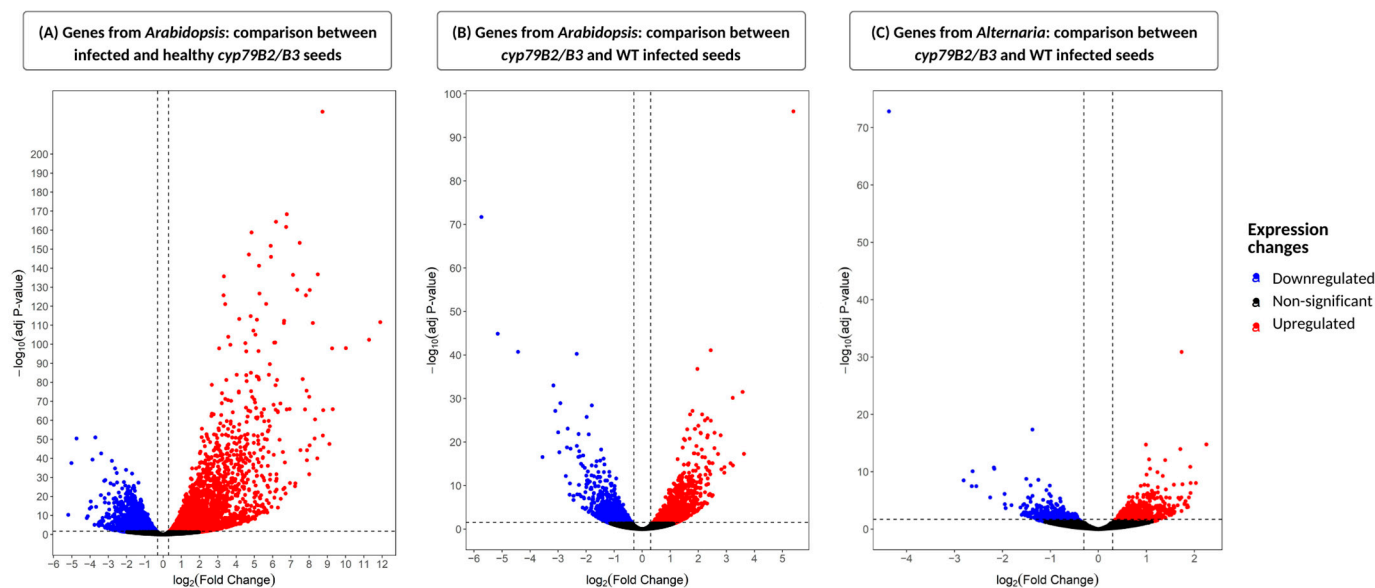


Figure 4. Volcano plots showing the changes in gene expression analyses in *cyp79B2/B3* at 10 days after sowing (DAS) for both *Arabidopsis* and *Alternaria*. Genes with $\log_2\text{FC} > 1$ or < -1 and Benjamini–Hochberg adjusted p -value < 0.05 were considered differentially expressed. Colors scale: blue represents downregulated genes, red upregulated genes and black insignificant expression changes.

3. Methods

3.1. Plant Material and Treatments

Seeds from three different mutant lines were used in this study: *cyp79B2/B3*, an indole glucosinolate mutant (*cyp79B2 cyp79B3*) [10], *qko*, an indole and aliphatic glucosinolate mutant (*cyp79B2 cyp79B3 myb28 myb29*) [11], and *pad3*, a camalexin-deficient mutant [12]. Mutant seeds were derived from the Columbia—Col0—genetic background, and the WT *Arabidopsis* accession Col-0 was used as the control [9]. All seeds were obtained from plants grown under controlled conditions (20 °C, 16 h light photoperiod, and 70% relative humidity) at the Institut de Recherche en Horticulture et Semences (IRHS), Beaucouzé, France [9]. In brief, the seeds were surface-sterilized and infected with an inoculum of *Alternaria*, strain Abra43, at a concentration of 10^4 conidias/mL or imbibed in water as controls. All samples were incubated in a controlled growth chamber for 3, 6, and 10 days, following the protocol described by Ortega-Cuadros et al. [9]. Three biological replicates were collected for each condition and preserved at -80 °C until further use.

3.2. RNA Extraction and Sequencing

RNA isolation was performed for each sample using 20 mg of seeds. Samples from the *cyp79B2/B3* mutants were collected at 3, 6, and 10 DAS, while samples from *qko* and *pad3* mutants were collected only at 3 and 6 DAS. Total RNA was extracted using the NucleoSpin® RNA Plus kit (Macherey-Nagel, Düren, Germany) with previous modifications [8]. The quantity and integrity (Ribosomal Integrity Number—RIN) for RNA were measured with a NanoDrop ND-100 (NanoDrop Technologies, DE, USA) and 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), respectively. Samples with optimal parameters were sent to Beijing Genomics Institute—BGI (<https://www.bgi.com>, accessed on 25 August 2024)—Hong Kong, for cDNA library preparation, and paired-end sequencing (PE100, 40 M) using the DNBseq™ technology.

3.3. Data Analysis

After sequencing, BGI filtered the raw reads, removing adaptor sequences, contamination, and low-quality reads. Furthermore, the quality control of the raw reads was checked and summarized with the FastQC version 0.11.9 [13] and MultiQC tools version

1.13 [14]. Reads with Phred scores ≥ 35 were selected as high-quality reads. The quasi-mapping alignment method from Salmon version 0.14.1 [17] was employed for mapping the reads corresponding to *Arabidopsis* on the reference genome of *Arabidopsis* Araport 11 [15], while the reads from *Alternaria* were mapped on the *Alternaria* Abra43 reference genome [16]. Transcript abundance was also measured as transcripts per million (TPM) using Salmon [17].

DESeq2 algorithms (package version 1.36.0) [18] were used to obtain genes expression changes for both the plants and fungi. Pairwise comparisons for gene expression values were made between healthy and infected mutant seed (Table S1), as well as between the mutants and the WT infected by *Alternaria* (Tables S2 and S3). Genes with $\log_2FC > 1$ or < -1 and Benjamani–Hochberg adjusted p -values < 0.05 were considered as differentially expressed. Functional enrichment analysis was conducted using the ClusterProfiler 4.0 package version 4.12.6 [19]. To identify significantly enriched GO terms associated with biological processes, an adjusted p -value threshold of < 0.05 was set following the Benjamani–Hochberg multiple testing correction [19].

To illustrate gene expression changes and DEGs, Venn diagrams [21] (Figure 1), stacked bar charts (Figure 3), and volcano plots (Figure 4) were employed. Some of the plots were generated using the ggplot2 (version 3.4.0) [24] package in R studio version 4.2.0.

4. User Notes

Previously, we provided an RNA dataset for the WT genotype, which exhibited specific expression patterns in response to *Alternaria* [9]. The transcriptomic analysis revealed a significant upregulation of indole metabolism in seeds infected by *Alternaria* [8]. These findings were further supported by a phenotyping study using defense-deficient mutants. Surprisingly, seeds from glucosinolate mutants infected by *Alternaria* showed a lower infection rate compared to the WT strain.

In line with our previous advancements, this dataset represents a valuable addition, providing detailed genetic expression information from glucosinolate mutant seeds exposed to *Alternaria*. It establishes a solid foundation for future research aimed at unraveling the synergistic function of glucosinolate pathways in seed defense and exploring how these interactions can be manipulated to enhance pathogen resistance in crops. Furthermore, this study encourages the analysis of transcriptomic data from both the host and the seed-borne pathogen perspectives, highlighting the significance of model pathosystem. These data can not only be leveraged in future research but can also form the basis for developing crop protection strategies and innovative disease management in sustainable agriculture.

Supplementary Materials: The following supporting information can be downloaded at <https://data.mendeley.com/datasets/c9d453jtwr/2>: Table S1: Differential expression genes from the comparison between infected and healthy *Arabidopsis* mutant seeds; Table S2: Expression changes from the comparison between *Arabidopsis* mutant and WT seeds, both infected by *Alternaria*. Table S3: Expression changes in *Alternaria* on *Arabidopsis* mutant and WT seeds. Table S4: Expression change in *cyp79B2B3* at 10 days after sowing (DAS) for both plant and fungi.

Author Contributions: Conceptualization, P.G., T.A., M.O.-C. and N.V.; methodology, P.G., N.V., S.A. and L.C.; validation, P.G., N.V. and J.V.; formal analysis, P.G., M.O.-C. and J.V.; investigation, P.G., M.O.-C. and L.C.; resources, P.G.; data curation, M.O.-C. and J.V.; writing—original draft preparation, P.G. and M.O.-C.; writing—review and editing, P.G., T.A., M.O.-C. and J.V.; visualization, P.G.; supervision, P.G., S.A. and J.V.; project administration, P.G.; funding acquisition, P.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was conducted in the framework of the regional program “Objectif Végétal, Research, Education and Innovation in Pays de la Loire”, supported by the French Region Pays de la Loire, Angers Loire Métropole, and the European Regional Development Fund.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The RNA-seq datasets are publicly available in the repository NCBI GEO, number GSE214602: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE214602>, accessed on 25 August 2024 and supplementary tables were deposited in Mendeley data (<https://data.mendeley.com/datasets/c9d453jtwr/2>, accessed on 25 August 2024).

Acknowledgments: We would like to thank Barbara Ann Halkier from DynaMo Center (Copenhagen, Denmark) for providing glucosinolate-defective mutants. We also give thanks to the FUNGISEM team for their support in this investigation.

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

References

- Kliebenstein, D.J. Secondary Metabolites and Plant/Environment Interactions: A View through *Arabidopsis thaliana* Tinged Glasses. *Plant Cell Environ.* **2004**, *27*, 675–684. [CrossRef]
- Evivie, E.R.; Ogwu, M.C.; Cang, W.; Xu, R.; Li, J. Progress and Prospects of Glucosinolate Pathogen Resistance in Some Brassica Plants. *J. Appl. Nat. Sci.* **2019**, *11*, 556–567. [CrossRef]
- Kosaka, A.; Pastorczyk, M.; Piślewska-Bednarek, M.; Nishiuchi, T.; Ono, E.; Suemoto, H.; Ishikawa, A.; Frerigmann, H.; Kaido, M.; Mise, K.; et al. Tryptophan-Derived Metabolites and BAK1 Separately Contribute to *Arabidopsis* Postinvasive Immunity against *Alternaria brassicicola*. *Sci. Rep.* **2021**, *11*, 1488. [CrossRef] [PubMed]
- Clay, N.K.; Adio, A.M.; Denoux, C.; Jander, G.; Ausubel, F.M. Glucosinolate Metabolites Required for an *Arabidopsis* Innate Immune Response. *Science* **2009**, *323*, 95–101. [CrossRef] [PubMed]
- Dharmendra, K.; Neelam, M.; Yashwant, K.B.; Ajay, K.; Kamlesh, K.; Kalpana, S.; Gireesh, C.; Chanda, K.; Sushil, K.S.; Raj, K.M.; et al. *Alternaria* Blight of Oilseed Brassicas: A Comprehensive Review. *Afr. J. Microbiol. Res.* **2014**, *8*, 2816–2829. [CrossRef]
- Pochon, S.; Terrasson, E.; Guillemette, T.; Iacomi-Vasilescu, B.; Georgeault, S.; Juchaux, M.; Berruyer, R.; Debeaujon, I.; Simoneau, P.; Champion, C. The *Arabidopsis thaliana*-*Alternaria brassicicola* Pathosystem: A Model Interaction for Investigating Seed Transmission of Necrotrophic Fungi. *Plant Methods* **2012**, *8*, 16. [CrossRef] [PubMed]
- Sellam, A.; Iacomi-Vasilescu, B.; Hudhomme, P.; Simoneau, P. In Vitro Antifungal Activity of Brassinin, Camalexin and Two Isothiocyanates against the Crucifer Pathogens *Alternaria brassicicola* and *Alternaria brassicae*. *Plant Pathol.* **2007**, *56*, 296–301. [CrossRef]
- Ortega-Cuadros, M.; De Souza, T.L.; Berruyer, R.; Aligon, S.; Pelletier, S.; Renou, J.-P.; Arias, T.; Champion, C.; Guillemette, T.; Verdier, J.; et al. Seed Transmission of Pathogens: Non-Canonical Immune Response in *Arabidopsis* Germinating Seeds Compared to Early Seedlings against the Necrotrophic Fungus *Alternaria brassicicola*. *Plants* **2022**, *11*, 1708. [CrossRef] [PubMed]
- Ortega-Cuadros, M.; Chir, L.; Aligon, S.; Arias, T.; Verdier, J.; Grappin, P. Dual-Transcriptomic Datasets Evaluating the Effect of the Necrotrophic Fungus *Alternaria brassicicola* on *Arabidopsis* Germinating Seeds. *Data Brief* **2022**, *44*, 108530. [CrossRef] [PubMed]
- Zhao, Y.; Hull, A.K.; Gupta, N.R.; Goss, K.A.; Alonso, J.; Ecker, J.R.; Normanly, J.; Chory, J.; Celenza, J.L. Trp-Dependent Auxin Biosynthesis in *Arabidopsis*: Involvement of Cytochrome P450s CYP79B2 and CYP79B3. *Genes Dev.* **2002**, *16*, 3100–3112. [CrossRef] [PubMed]
- Sun, J.Y.; Sønderby, I.E.; Halkier, B.A.; Jander, G.; de Vos, M. Non-Volatile Intact Indole Glucosinolates Are Host Recognition Cues for Ovipositing *Plutella xylostella*. *J. Chem. Ecol.* **2009**, *35*, 1427–1436. [CrossRef] [PubMed]
- Glazebrook, J.; Ausubel, F.M. Isolation of Phytoalexin-Deficient Mutants of *Arabidopsis thaliana* and Characterization of Their Interactions with Bacterial Pathogens. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 8955–8959. [CrossRef] [PubMed]
- Andrews, S. Babraham Bioinformatics—FastQC A Quality Control Tool for High Throughput Sequence Data. Available online: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/> (accessed on 22 March 2022).
- Ewels, P.; Magnusson, M.; Lundin, S.; Käller, M. MultiQC: Summarize Analysis Results for Multiple Tools and Samples in a Single Report. *Bioinformatics* **2016**, *32*, 3047–3048. [CrossRef] [PubMed]
- Cheng, C.; Krishnakumar, V.; Chan, A.P.; Thibaud-Nissen, F.; Schobel, S.; Town, C.D. Araport11: A Complete Reannotation of the *Arabidopsis thaliana* Reference Genome. *Plant J.* **2017**, *89*, 789–804. [CrossRef]
- Belmas, E.; Briand, M.; Kwasiborski, A.; Colou, J.; N’Guyen, G.; Iacomi, B.; Grappin, P.; Champion, C.; Simoneau, P.; Barret, M.; et al. Genome Sequence of the Necrotrophic Plant Pathogen *Alternaria brassicicola* Abra43. *Genome Announc.* **2018**, *6*, e01559-17. [CrossRef] [PubMed]
- Patro, R.; Duggal, G.; Love, M.I.; Irizarry, R.A.; Kingsford, C. Salmon Provides Fast and Bias-Aware Quantification of Transcript Expression. *Nat. Methods* **2017**, *14*, 417–419. [CrossRef] [PubMed]
- Love, M.I.; Huber, W.; Anders, S. Moderated Estimation of Fold Change and Dispersion for RNA-Seq Data with DESeq2. *Genome Biol.* **2014**, *15*, 550. [CrossRef] [PubMed]
- Wu, T.; Hu, E.; Xu, S.; Chen, M.; Guo, P.; Dai, Z.; Feng, T.; Zhou, L.; Tang, W.; Zhan, L.; et al. ClusterProfiler 4.0: A Universal Enrichment Tool for Interpreting Omics Data. *Innovation* **2021**, *2*, 100141. [CrossRef] [PubMed]
- TAIR The *Arabidopsis* Information Resource (TAIR). Available online: <https://www.arabidopsis.org> (accessed on 25 August 2024).
- Oliveros, J.C.; Venny. An Interactive Tool for Comparing Lists with Venn’s Diagrams 2007–2015. Available online: <https://bioinfo.cnb.csic.es/tools/venny/index.html> (accessed on 3 April 2024).

22. van Wees, S.C.M.; Chang, H.-S.; Zhu, T.; Glazebrook, J. Characterization of the Early Response of *Arabidopsis* to *Alternaria brassicicola* Infection Using Expression Profiling. *Plant Physiol.* **2003**, *132*, 606–617. [[CrossRef](#)] [[PubMed](#)]
23. Narusaka, Y.; Narusaka, M.; Seki, M.; Ishida, J.; Nakashima, M.; Kamiya, A.; Enju, A.; Sakurai, T.; Satoh, M.; Kobayashi, M.; et al. The CDNA Microarray Analysis Using an *Arabidopsis* Pad3 Mutant Reveals the Expression Profiles and Classification of Genes Induced by *Alternaria brassicicola* Attack. *Plant Cell Physiol.* **2003**, *44*, 377–387. [[CrossRef](#)] [[PubMed](#)]
24. Wickham, H. *Ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2016; ISBN 978-3-319-24277-4.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.