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Revealing Key Genes and Pathways in Potato Scab Disease Resistance through Transcriptome Analysis

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Abstract: Potato scab, a global soil-borne disease caused by *Streptomyces*, is pivotal in developing resistant cultivars due to its complex resistance mechanisms. This study investigates the transcriptomic responses in potato to common scab using resistant variety CS10 and susceptible CS11 post *S.scabie* inoculation (0 d and 10 d, 12 cDNA libraries). Differential expression analysis identified 147 key DEGs (Differentially Expressed Genes) essential in disease recognition, signal transduction, and defense. GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analyses revealed several significant metabolic pathways, such as ADP binding, heme binding, chloroplast thylakoid membrane, photosynthesis, glutathione metabolism, and homologous recombination, among others. Notably, the correlation between chloroplast pathways (GO:0019745) and photosynthesis (map00195) highlights photosynthesis's role in potato scab response, while the oxygen transport (GO:0031408)-related glutathione metabolism pathway (map00480) emphasizes antioxidant defenses. Furthermore, three potential resistance genes were validated: Ethylene Response Factor ERF010 (*LOC102589042*), Disease Resistance Protein RPP13 (*LOC102605863*), and Cytochrome P450 83B1 (*LOC102604056*), demonstrating the linkage between metabolic pathways and pathogen response. These findings offer insights into potato's molecular resistance mechanisms against potato scab, supporting the breeding of resistant varieties and comprehensive disease management, thus advancing sustainable agriculture.

Keywords: potato scab resistance; transcriptomics; DEGs; qRT-PCR; plant pathogen interaction

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1. Introduction

The potato (*Solanum tuberosum* L.) stands as a vital global food crop; its healthy growth is crucial for global food security [1,2]. However, in recent years, with the increasing area and duration of potato cultivation, their healthy growth is increasingly threatened by various phytopathological conditions [3,4], particularly by the soil-borne disease potato scab, caused by *Streptomyces* species [5,6]. This disease not only leads to significant losses in yield and quality but also adversely affects market value and storage processing [7]. Globally, potato yield is significantly impacted by pests and diseases, with estimated losses ranging from 8% to 21% [8]. According to the Food and Agriculture Organization (FAO), annual potato production in China in recent years has been approximately 79 million tons. Considering the current wholesale market price of potatoes at RMB 2.3 per kilogram, the economic loss attributed to potato scab disease is estimated to be in the billions of RMB. This estimation not only reveals the substantial economic impact of potato scab disease on agricultural economy but also highlights the urgency and importance of intensified

research and control measures for this disease (, Food and Agriculture Organization (FAO). Rome, Italy, 2023).

The pathogenesis of potato scab and plant defense strategies have been a focal point of research. Currently, the thaxtomin family of phytotoxins is recognized as a major factor in pathogenicity, with its biosynthetic genes located on a mobile pathogenicity island in the pathogen's genome [9,10]. This consists of two main sections: the toxin synthesis zone, housing a cluster of biosynthesis genes, and the colonization zone, assisting in pathogen virulence [11]. Among these, the *txtA* gene is especially crucial as it not only inhibits cell wall synthesis, leading to cell death, but also exacerbates cell damage through its impact on transmembrane transport [12,13]. It, along with the *txtB* gene, is responsible for condensing phenylalanine and nitrated tryptophan into dipeptides [14]. Additionally, the *txtD* gene encoding nitric oxide synthase and the *txtE* gene encoding a novel cytochrome P450 monooxygenase participate in the initial stages of toxin synthesis [15], while *txtC* finalizes the synthesis by hydroxylating the dipeptide to form the toxin [16]. On the other hand, genes like *nec1* and *tomA* in the colonization phase weaken the plant's defense and enhance the pathogen's virulence [17,18]. While the functions of these genes have been partially unraveled, their interaction with plant defense mechanisms at the molecular and transcriptomic levels requires further investigation.

Recent studies have revealed genes and pathways involved in plant defense mechanisms. Zhang's research demonstrated that chitosan enhances potato resistance against *Phytophthora infestans*, activating defense pathways and inducing defense gene expression [2]. Another study compared the transcriptomes of potato cultivars resistant to *Ralstonia solanacearum*, discovering unique defense mechanisms in each cultivar, such as chitin interactions and specific metabolic pathways [3]. Bengtsson's research on BABA-induced resistance in potatoes unveiled various defense responses, emphasizing the variability in response among cultivars [4]. These studies collectively deepen our understanding of the genetic and biochemical pathways in potato defense mechanisms. However, a comprehensive understanding of the metabolic pathways in potatoes under pathogen stress remains to be explored.

This study uses potato cultivars identified under various pathogenic infections to characterize key genes and potential metabolic pathways in scab resistance using high-throughput transcriptomic sequencing and quantitative PCR. The findings aim to provide potential markers for breeding resistant varieties and scientific bases for developing effective control strategies against potato scab disease.

2. Materials and Methods

2.1. Experimental Materials and Pathogen Inoculation

This study involved ten potato varieties with varying levels of scab resistance: Chunshu 10 (CS10), Eugene (EG), Atlantic (AL), Kexin 1 (KX1), Xisen 6 (XS6), Chunshu 11 (CS11), Zhongshu 18 (ZS18), N3, Zhongshu 19 (ZS19), and Feiwuruita (FW). These varieties were specifically selected for their widespread cultivation in Jilin Province, reflecting the practical agricultural landscape of the region. High-quality seed potatoes, free from scab lesions, were provided by the Institute of Economic Plants, Jilin Academy of Agricultural Sciences. Cultivation took place in Fanjiatunzhen, Gongzhuling City, Jilin Province (43.46° N, 125.07° E), characterized by a temperate, humid continental monsoon climate in 2021, which provides an ideal setting for the development of potato scab. Pre-planting procedures included sprouting and tuber disinfection. Employing a randomized block design, each potato variety was planted in ridges measuring 8 m × 65 cm, spaced 65 cm × 30 cm apart. To ensure experimental reliability, we replicated each variety three times.

Pathogens for the study included *S. aureofaciens* (*SA*), *S. turgidisabies* (*SG*), and *S. scabie* (*SS*), obtained from the Institute of Agricultural Biotechnology, Jilin Academy of Agricultural Sciences. These three pathogens represent the main physiological races that predominantly infect potatoes in Jilin Province, causing potato scab diseases. For cultivating these pathogens, ISP-2 medium was prepared by adding 4.0 g of yeast extract, 10.0 g of

malt extract, and 4.0 g of glucose to 1 L of distilled water [19]. The pH was adjusted to 7.3, followed by sterilization at 121 °C for 15 min. Pathogens cultured on ISP-2 solid medium were transferred to sterilized ISP-2 liquid medium and grown at 25 °C and 150 rpm for 24–72 h, until reaching a concentration of 2×10^7 spores/mL (Figure 1A–C). Four weeks post planting, the potatoes were inoculated using a combination of hole application and root watering, injecting 150 mL of the spore suspension into each hole [20]. Throughout the growth period, manual weeding, avoidance of chemical pesticides, and provision of adequate water and fertilizer were meticulously maintained.

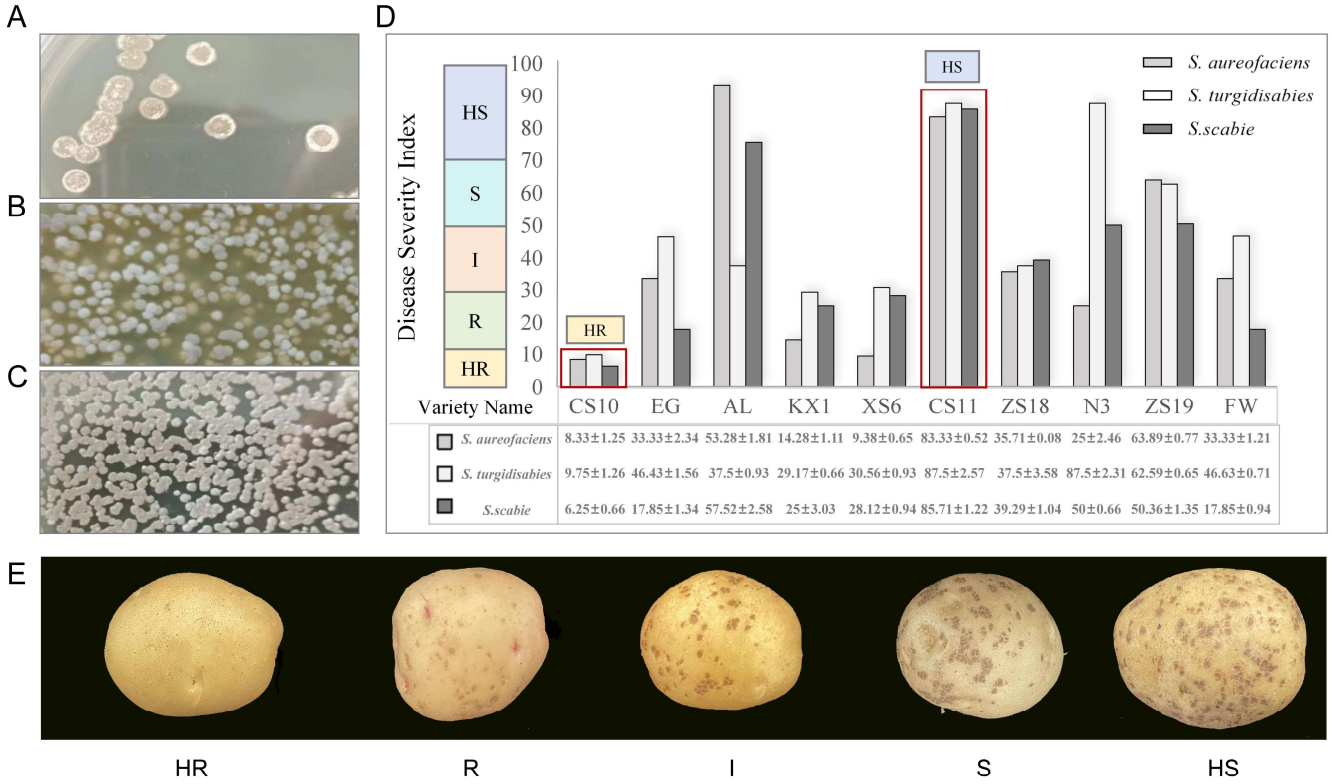

Figure 1. Assessment of resistance to potato scab and morphological evolution of pathogen colonies. Microscopic morphological changes of single colonies of the potato scab pathogen during spore development: initial sporulation stage (**A**), intermediate transition from white to grey spores (**B**), and the late stage where spores are almost entirely grey, indicating maturity (**C**). (**D**) Assessment of disease resistance levels to potato scab in different potato varieties. The horizontal axis represents the ten distinct potato varieties, while the vertical axis quantifies DSI and corresponding RL against potato scab. The inset table provides specific DSI values following challenge with the three distinct scab pathogens. Values are presented as mean ± standard deviation. (**E**) Resistance performance of potato varieties after inoculation with *S. scabie* pathogen.

2.2. Disease Detection and Resistance Evaluation

Upon reaching maturity, the potato plants from each variety were harvested in three independent replicates. The harvested tubers were then carefully washed, drained, and categorized based on the severity of scab symptoms (Supplementary Table S1). The evaluation of resistance involved three primary metrics: Incidence Rate (IR), calculated as the percentage of diseased tubers relative to the total number of harvested tubers, using the formula IR = (number of diseased tubers/total harvested tubers) × 100%; Disease Severity Index (DSI), determined by assigning a weighted value to each disease severity level and calculating the average, as per the formula DSI = Σ (number of tubers at each disease level × corresponding level)/(total tubers × highest disease level) × 100; Resistance Level (RL), which, based on the DSI, was categorized into five levels: highly resistant (HR,

$0 \leq$ DSI ≤ 10), resistant (R, $10 <$ DSI ≤ 30), intermediate (I, $30 <$ DSI ≤ 50), susceptible (S, $50 <$ DSI ≤ 70), and highly susceptible (HS, DSI > 70) [21,22].

2.3. Transcriptome Sequencing and Analysis

In 2022, a subset of potato varieties, representing high resistance and susceptibility profiles, were selected for pot cultivation and inoculation. During the sprouting period, these were irrigated with a 2×10^7 spores/mL spore suspension. Sampling occurred at two critical time points: immediately pre-inoculation (0 days) and post inoculation (10 days). The collected samples were promptly frozen in liquid nitrogen and stored at -80 °C for further analysis.

RNA extraction followed standard protocols, with the assessment of RNA purity, concentration, and integrity performed using a NanoPhotometer (Implen GmbH, Munich, Germany), Qubit 3.0 Fluorometer, and Agilent 2100 RNA Nano 6000 Assay Kit, respectively [23]. For transcriptome library construction, 3 µg of high-quality RNA per sample underwent several processing steps including mRNA enrichment, fragmentation, cDNA synthesis and purification, end repair, A-tailing, adapter ligation, and 150 bp paired-end sequencing using the HiSeq platform. The resulting sequences underwent rigorous quality control checks. Alignment of these sequences was performed against the *Solanum tuberosum* v3 genome, using Bowtie for indexing and HISAT2 software (version 2.2.1) for efficient and precise alignment, leveraging the Burrows-Wheeler Transform (BWT) algorithm [24–26].

2.4. Differential Gene Expression, Functional Annotation, and Enrichment Analysis

Gene expression levels were quantified using the fragments per kilobase of exon per million fragments mapped (FPKM) method, which accounts for gene length and sequencing depth. The calculation formula is as follows: $\text{FPKM} = \frac{10^3 \times \text{F}}{\text{NL}/10^6}$. This approach facilitates a more accurate representation of gene expression [27,28]. Differential expression analysis identified genes that were significantly upregulated or downregulated in comparison to control samples, focusing on fold changes and adjusted *p*-values [29,30].

Enrichment analysis was conducted using the clusterProfiler package in R, targeting GO and KEGG databases [31–33]. This analysis aimed to identify significant functional categories or pathways, with a threshold for significance set at p-value ≤ 0.05.

The functional annotation of DEGs was achieved through BLAST comparisons against various databases, including the Nucleotide Database (NT), Non-Redundant Protein Database (NR), Universal Protein Resource (Uniprot), Clusters of Orthologous Groups of proteins (COG), GO, and KEGG. This comprehensive approach allowed for a detailed understanding of gene and protein functions, including structural, functional, and biological roles [34].

2.5. Quantitative Real-Time PCR (qRT−PCR) Analysis of DEGs

Selected DEGs, based on their functional annotations and enrichment analysis results, were further investigated using qRT-PCR. The primer sequences for these genes were designed using Primer 3 (https://primer3.ut.ee/, (accessed on 5 October 2023). Total RNA was extracted from potato samples using the MiniBEST Plant RNA Extraction Kit (TaKaRa, 9769) and reverse transcribed into cDNA with PrimeScript™ RT Master Mix (TaKaRa, RR036A). qRT-PCR analyses were performed on an Applied Biosystems QuantStudio 6 Real-Time PCR System, using StActin97 as the reference gene. Each gene's expression was quantified in three biological replicates, and relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method [35,36].

2.6. Statistical Analysis

All statistical analyses were performed using Microsoft Excel 2013 and SPSS 19.0. Data visualization, including graphical representations of gene expression levels, was conducted using GraphPad Prism 9 [37]. Group comparisons were carried out using Duncan's multiple range tests, ensuring the robustness of the statistical evaluations. Data are presented as

the mean ± standard deviation (SD), with a *p*-value of less than 0.05 deemed statistically significant [38].

3. Results

3.1. Resistance Identification of Potato Varieties

Following inoculation with the pathogens *SS*, *SA*, and *SG*, significant variation was observed in three primary metrics—IR, DSI, and RL—across the ten studied potato varieties (Supplementary Table S2). Notably, CS10 and CS11 exhibited contrasting DSIs in three pathogens. CS10 presented a lower DSI, indicative of a robust resistance profile, categorizing it as a highly resistant variety (Figure 1D). In contrast, CS11 demonstrated a lower incidence rate and DSI, which qualifies it as a highly susceptible variety, suggesting a pronounced vulnerability to the pathogens (Figure 1E). The selection of CS10 and CS11 for detailed analysis in this study was a strategic decision aimed at capturing the contrasting responses to scab infection between these two varieties. This juxtaposition allows for a more nuanced exploration of the genetic and molecular bases of scab resistance and susceptibility in potatoes.

3.2. Transcriptome Sequencing and Mapping

In an effort to understand the transcriptional underpinnings of potato resistance to scab, we profiled the transcriptomes of Chunshu 10 (CS10) and Chunshu 11 (CS11), which demonstrate markedly different responses to the disease. Total RNA was extracted from samples harvested 0 and 10 days post inoculation with the scab pathogen *S. scabie*. This resulted in a comprehensive dataset comprising twelve samples (Supplementary Table S3). High-throughput sequencing, conducted on the Illumina NovaSeq 6000 platform, yielded a substantial 63.89 gigabases (Gb) of data. The sequencing output averaged 42,009,542 clean reads per sample, achieving a high-quality score (Q30) of 94.11% alongside a mean GC content of 42.76%. A significant proportion of the reads, ranging from 80.49% to 87.33%, aligned with the reference genome *Solanum tuberosum* v3, suggesting an exhaustive capture of the potato transcriptome during pathogenic challenge. These high-quality sequence data set the stage for in-depth differential gene expression analysis and subsequent unraveling of the genetic basis of resistance.

3.3. Transcriptomic Analysis Reveals Overall Trends in Differential Gene Expression

Transcriptomic profiling of the potato varieties CS10 and CS11 revealed a high degree of consistency in the distribution of gene expression levels. The density plot (Figure S1A) demonstrates that the majority of gene expression values across all samples are concentrated in a specific range, indicating that despite individual variances, the overall pattern of gene expression is similar. The box plot (Figure S1B) further elucidates this similarity, showing comparable medians and interquartile ranges across samples, while the correlation heatmap (Figure S1C) displays a high correlation in gene expression levels among samples, reflecting a unified trend in expression. These results suggest that although differential genes are present, the overall gene expression patterns remain stable across samples, providing a reliable foundation for further investigation into the mechanisms of resistance.

3.4. Comparative Analysis of Shared Differential Genes across Different Time Points in Potato Varieties

In an effort to dissect the dynamic expression of genes in response to potato scab, this study categorized samples from CS10 and CS11 potato varieties into four groups based on the inoculation timeline: CS10 0 days post inoculation (Group C), CS10 10 days post inoculation (Group T), CS11 0 days post inoculation (Group C1), and CS11 10 days post inoculation (Group T1). Following stringent selection criteria (fold change ≥ 2, p-value < 0.05, q-value < 0.05), the number of DEGs within each group comparison was ascertained (Supplementary Table S4). The gene expression differential statistics chart (Figure 2A) succinctly illustrates the upregulated, downregulated, and total DEG counts for

each group comparison. For instance, the comparison between Groups C1 and T1 revealed 2181 upregulated and 1526 downregulated genes, summing to a total of 3707 DEGs. The comparison between Groups C and T revealed 1580 upregulated and 1833 downregulated genes, summing to a total of 3413 DEGs. Volcano plots (Figure 2C–F) delineate the fold change in gene expression and the statistical significance of expression variation across these four comparison groups. Despite the identification of a considerable number of DEGs, the majority did not exhibit significant expression changes.

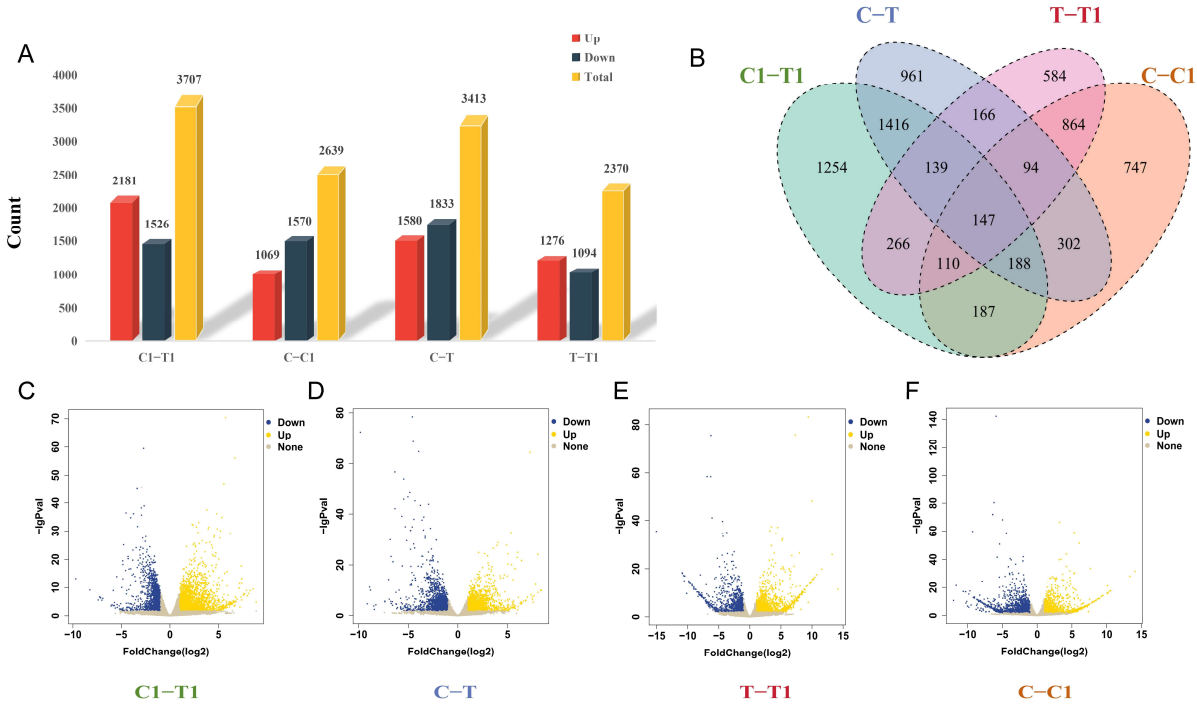

Figure 2. Analysis of DEGs across four temporal groups in potato varieties. (**A**) Statistical bar chart of DEGs. "Up" indicates the number of genes upregulated in the treatment group compared to the control group, "Down" represents the number of downregulated genes, and "Total" denotes the overall number of genes with significant differential expression between the two groups. (**B**) Venn diagram of DEGs at different time points. (**C**–**F**) Volcano plots of DEGs between four groups. Blue points represent significantly downregulated genes, yellow points indicate significantly upregulated genes, and gray points correspond to genes with non-significant differential expression, visually reflecting the magnitude and significance of gene expression changes.

We employed Venn diagrams to analyze the DEGs in potato varieties CS10 and CS11 before and after inoculation with potato scab (Figure 2B). The analysis revealed that CS10 exhibited 961 differential genes (C−T) 10 days post inoculation, reflecting gene activation post-infection, particularly in disease resistance. Similarly, CS11 demonstrated 1254 differential genes (C1−T1), likely linked to mechanisms of disease resistance, stress response, cellular repair, and metabolism, indicating a broad defensive and adaptive response upon infection. Further comparison between CS10 and CS11 showed 747 differential genes (C−C1) in the uninfected state, possibly related to innate disease resistance, involving basic immunity and genetic diversity. Additionally, 584 differential genes (T−T1) post infection highlighted the differences in gene expression related to pathogen resistance and variety-specific defense mechanisms. Overall, 147 core DEGs were identified, suggesting their central role in combating common scab, involving disease recognition, signal transduction, and defense mechanisms. These findings provide significant insights into the molecular response mechanisms of potatoes to common scab, crucial for future variety improvement and disease management strategies.

3.5. Functional Enrichment Analysis of Shared DEGs

In our quest to elucidate the molecular determinants of resistance to potato common scab, a targeted functional enrichment analysis was performed on 147 core differential genes identified. First, we conducted GO enrichment analysis to delineate their roles in biological processes (BP), cellular components (CC), and molecular functions (MF). The analysis spanned 127 GO pathways, encompassing 53 BPs, 22 CCs, and 51 MFs (Supplementary Table S5). With a *p*-value threshold set below 0.05, bar charts of enrichment levels were created. Six BPs were significantly enriched, including carbohydrate transport (GO:0008643), pentacyclic triterpenoid biosynthetic process (GO:0019745), defense response (GO:0006952), oxylipin biosynthetic process (GO:0031408), hydrogen peroxide catabolic process (GO:0042744), and response to biotic stimulus (GO:0009607) (Figure 3A). The CC category showed notable enrichment in the extracellular region (GO:0005576) (Figure 3B), and the MF category highlighted cysteine-type endopeptidase inhibitor activity (GO:0004869) and flavonoid 3′,5′-hydroxylase activity (GO:0033772) (Figure 3C). Integrating all categories, the bar chart depicted the top 10 pathways with the highest enrichment (Figure 3D), with the ADP binding pathway (GO:0008643) having the highest number of DEGs at 110, followed by the heme binding pathway (GO:0005576) with 120 DEGs, and the chloroplast thylakoid membrane (GO:0019745) with 63 DEGs. These findings suggest a pivotal role for these pathways and associated genes in pathogen recognition, signal transmission, and initiating defense responses during scab infection.

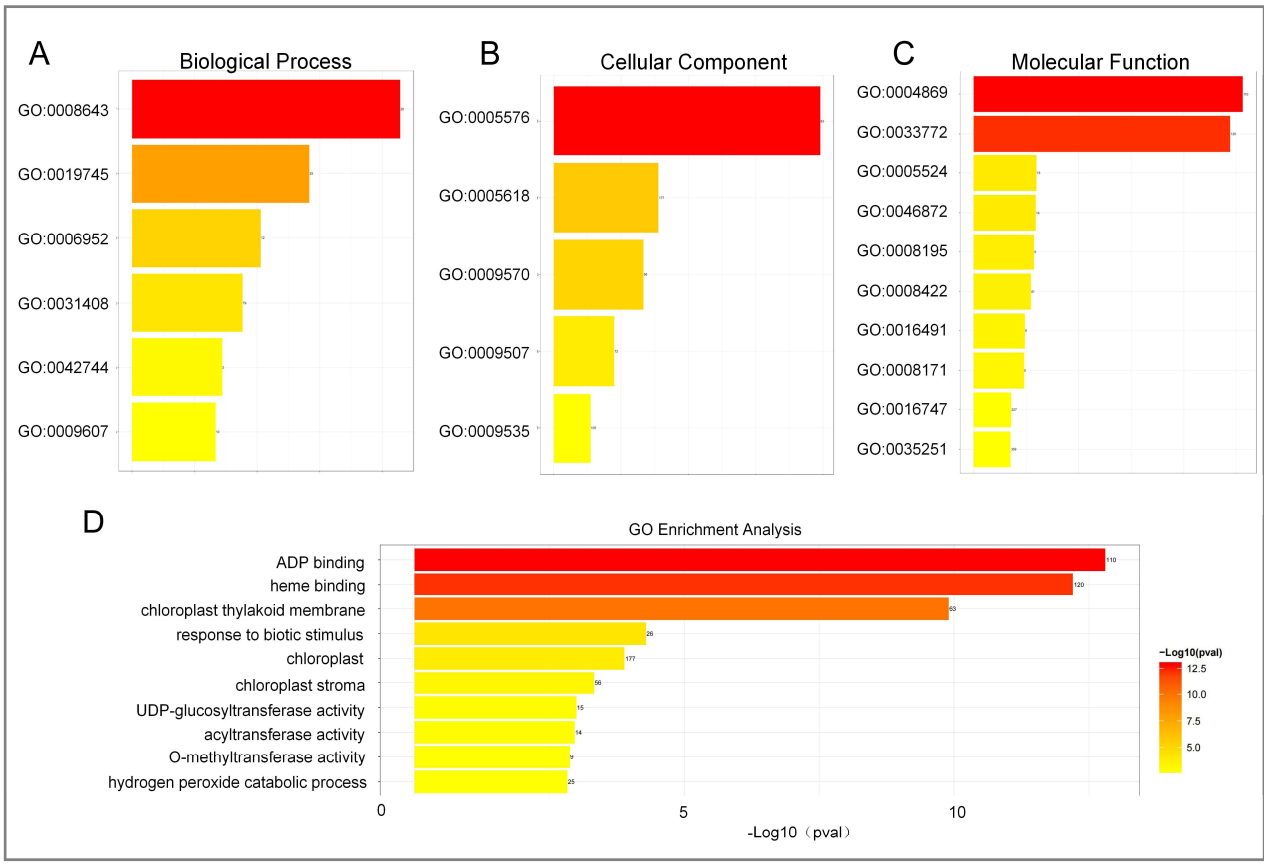

Figure 3. Bar chart of GO enrichment pathways for 147 DEGs. (**A**–**C**) display the enrichment results categorized into BP, CC, and MF, respectively. (**D**) shows the top 10 pathways with the most significant overall enrichment. The *x*-axis represents the −Log10 transformed *p*-value, and the *y*-axis lists the enriched GO pathways. Numbers within the chart denote the quantity of DEGs enriched in each pathway. The depth of color correlates with the degree of enrichment, with darker colors indicating higher enrichment levels.

Setting a *p*-value threshold of less than 0.05 for significance, a KEGG pathway enrichment analysis was conducted to identify the key biological functions of 147 DEGs. The analysis revealed six significantly enriched metabolic pathways (Figure 4A, Supplementary Table S6), with photosynthesis (map00195) and glutathione metabolism (map00480) showing the highest enrichment with gene ratio of 4.60% and 3.68%, respectively. Figure 4B indicates these pathways were significantly enriched in the CS10 and CS11 potato varieties at the initial point of scab inoculation (C-C1), suggesting their potential role in the early response to the disease. Ten days post inoculation (T-T1), pathways like homologous recombination (map03440), phenylalanine metabolism (map00360), mismatch repair (map03430), and isoquinoline alkaloid biosynthesis (map00950) were notably enriched between the two varieties, implicating their relevance to the progression and pathological response of scab in potatoes. Six DEGs were detailed within the photosynthesis pathway (Supplementary Figure S2), with five upregulated and one downregulated, reflecting the regulatory role of photosynthesis in the potato's response to scab. Additionally, five DEGs in the glutathione metabolism pathway (Supplementary Figure S3), including two upregulated and three downregulated, might play a crucial role in maintaining intracellular redox balance and defending against pathogenic invasion, underscoring the significance of these metabolic pathways in the resistance mechanism against scab disease.

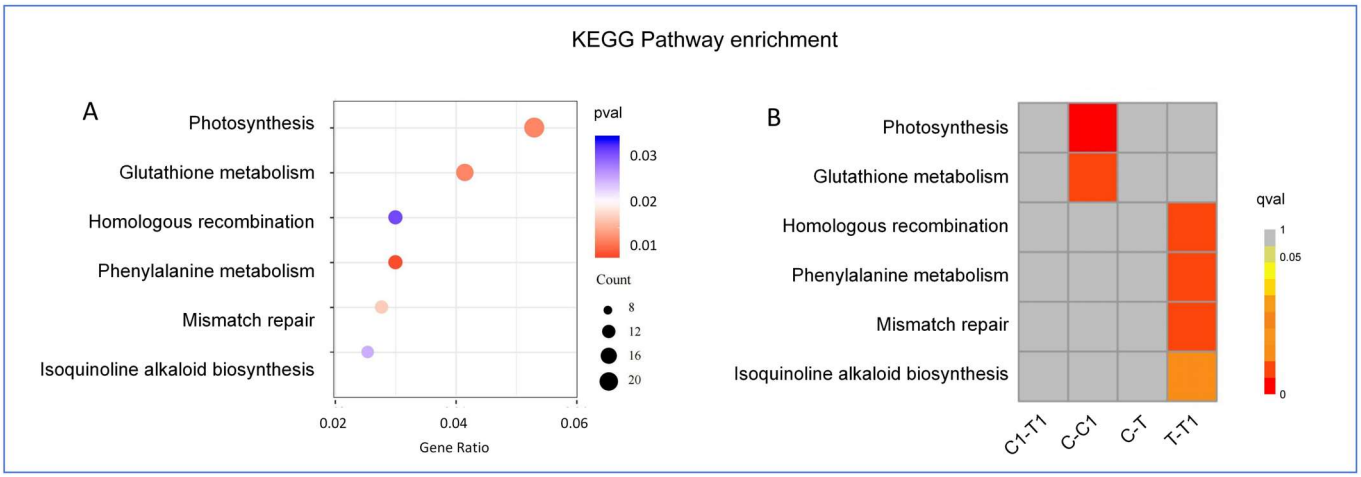

Figure 4. KEGG pathway enrichment analysis of 147 DEGs. (**A**) depicts a bubble chart for KEGG pathway enrichment, determined by a *p*-value threshold of less than 0.05. The *y*-axis lists the pathway names, while the *x*-axis shows the gene ratio, and the size of the bubble indicates the number of genes enriched in each pathway. (**B**) presents a distribution map of enriched pathways across four comparative groups, according to the q-value of sample enrichment within each pathway (Supplementary Table S7). Different color intensities represent varying levels of enrichment significance.

3.6. Gene Function Annotation Analysis of Shared DEGs

In this study, comprehensive functional annotation was performed on 147 core DEGs across six major bioinformatics databases: NR, NT, COG, GO, KEGG, and SwissProt. The annotation coverage was high, with NR and NT databases achieving rates of 99.32% and 100%, respectively (Supplementary Table S8). Subsequent integrated analysis of these annotations highlighted ten genes strongly associated with potato common scab (Table 1), implicated in pathogen defense, stress response, and metabolic regulation. Notably, genes such as the Ethylene-responsive transcription factor ERF010 (*LOC102589042*), Abscisic acid-insensitive protein (*LOC102597983*), chloroplastic Allene oxide synthase (*LOC102577479*), and the Disease resistance protein RPP13 (*LOC102605863*) were identified as playing pivotal roles in enhancing pathogen defense and stress response modulation. Particularly, RPP13 is underscored as a key gene in the investigation of potato common scab resistance mechanisms. Additional genes like Cytochrome P450 83B1 (*LOC102604056*), Snakin-2 (*LOC102588973*),

and enzymes related to cell wall fortification (*LOC102590869* and *LOC102588686*) were also noted for their critical functions in potato scab resistance. Moreover, the enzyme involved in flavonoid synthesis (*LOC102582164*) was indicated for its role in metabolic regulation within the potato scab defense response. These annotated genes provide invaluable insights into the biological functions during the potato scab response, contributing to a deeper understanding of the molecular mechanisms of the disease.

Table 1. Ten key candidate genes derived from the combined results of enrichment and functional annotation analyses.

NO.	Gene Name	Annotation Description	Potential Relevance to PCS
1	*LOC102589042*	Ethylene-responsive transcription factor ERF010	May enhance pathogen defense.
2	*LOC102597983*	Abscisic acid-insensitive 5-like protein 5	Could participate in stress response.
3	*LOC102577479*	Allene oxide synthase, chloroplastic	May contribute to jasmonic acid-related defense.
4	*LOC102605863*	Disease resistance protein RPP13	Likely involved in direct disease resistance.
5	*LOC102604056*	Cytochrome P450 83B1	Could be part of defense compound metabolism.
6	*LOC102588973*	Snakin-2	Might produce antimicrobial peptides against PCS.
7	*LOC102590869*	Caffeic acid 3-O-methyltransferase	May strengthen plant cell walls.
8	*LOC102588686*	Beta-glucosidase 18	Could activate antimicrobial agents.
9	*LOC102589603*	Feruloyl CoA ortho-hydroxylase 2	May alter cell wall to prevent pathogen entry.
10	*LOC102582164*	Flavonoid 3′,5′-hydroxylase	Involved in synthesizing defense-related flavonoids.

3.7. Cross-Validation of Crucial DEGs between qRT-PCR and Transcriptome Profiles

Under four distinct treatment conditions, we harvested potato leaf samples and designed specific primers for 10 key DEGs (Supplementary Table S9). We analyzed the relative expression levels of these genes using qRT-PCR. By correlating with transcriptome data, we identified three genes that exhibited consistent expression trends across all conditions, suggesting a stable regulatory role in the plant's defense response to scab disease (Figure 5).

Specifically, the gene *LOC102589042* encodes the Ethylene-responsive transcription factor ERF010, which consistently showed downregulation across all treatment groups. This aligns with the critical role of ethylene in plant stress responses, particularly in pathogen defense and stress signaling. The downregulation of ERF010 may reflect the plant's adjustment of its ethylene signaling pathways in response to scab infection. The gene *LOC102605863*, which encodes the disease resistance protein RPP13, exhibited a consistent upregulation under various conditions. This pattern strongly suggests its direct involvement in the plant's defense mechanism against scab disease. RPP13 plays a crucial role in the plant's innate immune system by recognizing specific pathogen effectors and initiating defensive responses. Consequently, *LOC102605863* emerges as a prime candidate gene potentially pivotal in conferring resistance to potato scab disease. Meanwhile, *LOC102604056*, which encodes Cytochrome P450 83B1, was downregulated in all treatment combinations, suggesting that the plant may be modulating various biosynthetic reactions in response to scab disease attack, including the synthesis of plant hormones, detoxification processes, and the production of secondary metabolites involved in defense responses.

The consistent expression patterns of these three genes reinforce their potential roles in the defense mechanism against potato scab. These findings provide significant insights into the molecular mechanisms of potato defense against scab disease and identify potential candidates for resistance breeding programs.

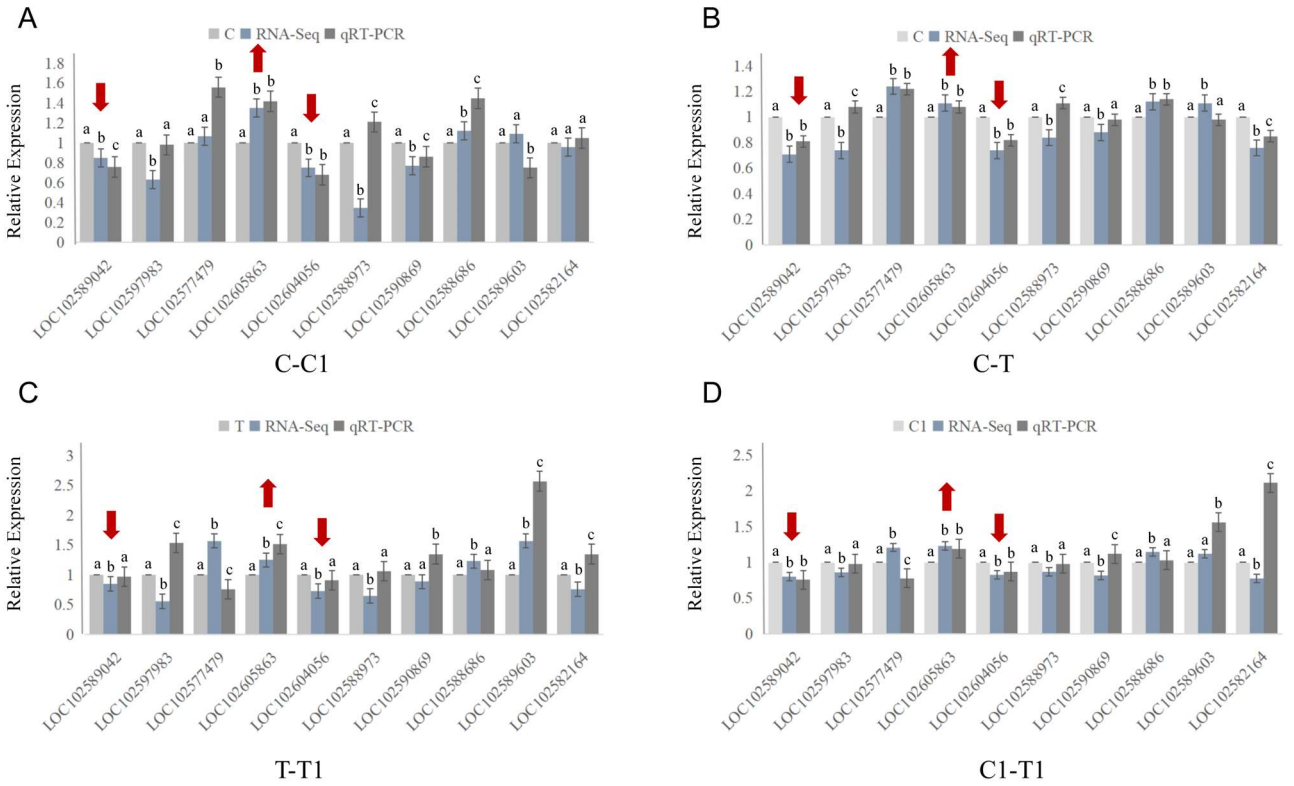

Figure 5. Comparative relative expression levels of 10 DEGs across four treatment combinations. (**A**–**D**) display the relative expression levels of selected key DEGs across C-C1, C-T, T-T1, and C1-T1 treatment combinations. Light grey represents the selected controls in each combination, blue represents the results of the transcriptome analysis, and black represents the results of the qRT-PCR experiments. Red arrows highlight the three genes that exhibit consistent expression trends across all treatment conditions. Error bars denote the means ± SDs from three independent replicates. Different letters indicate significant differences at the 0.05 level according to Student's *t*-test.

4. Discussion

Potato scab disease, as a significant soil-borne disease globally, holds crucial importance for agriculture worldwide. Breeding resistant varieties is considered one of the most effective control strategies [39,40]; hence, comprehensively understanding the pathogenic mechanism of scab disease and the plant's defense mechanisms is key to disease management. While the pathogenic mechanism of potato scab has been partially elucidated, further studies are needed to explore its interaction with plant defense mechanisms at the molecular and transcriptional levels. This study aims to reveal biological pathways and resistance genes related to potato scab resistance through transcriptomic analysis, which not only enhances our understanding of the genetic mechanisms underlying potato scab disease resistance but also aims to offer practical implications for breeding strategies.

Transcriptomic analysis, an effective tool for identifying plant disease resistance genes, has been widely applied and proven practical [41,42]. For instance, Gong used this approach to explore the response mechanism of potatoes to drought stress, revealing expression differences of specific genes under varying conditions [43]. Similarly, Tiwari studied the impact of nitrogen stress on the potato transcriptome, successfully identifying nitrogen-deficiency sensitive genes [44]. Tang's study on the impact of heat stress on potato leaves highlighted the crucial roles of heat shock proteins and transcription factors [45]. These studies have enriched our understanding of plant stress resistance mechanisms, demonstrating the value of transcriptomic analysis. While our transcriptomic analysis has provided valuable insights into plant disease resistance genes, it is important to note the limitations of this approach. Specifically, transcriptomic data do not encompass post-transcriptional modifications and

protein-level interactions, which are critical components of gene expression regulation and protein function. Future research integrating proteomic analysis would be beneficial to gain a more comprehensive understanding of the plant stress response.

In our study, we focused on analyzing the transcriptomic data of potato scab-resistant variety CS10 and disease-susceptible variety CS11. Sampling at different time points after *Streptomyces* infection, we identified 147 core DEGs and their potential biological pathways. The integrated analysis of GO and KEGG enrichment results revealed significant interconnections and functional overlaps. Notably, we highlighted the correlation between the chloroplast thylakoid membrane pathway in GO and the photosynthesis pathway in KEGG, emphasizing the central role of photosynthesis in responding to scab disease stress [46]. Furthermore, the close relationship between the oxygen transport and cellular respiration pathways in GO and the glutathione metabolism pathway in KEGG highlights the importance of antioxidant defense mechanisms in combating scab disease [47]. Additionally, we provided detailed insights into the expression patterns of three potential disease resistance genes: Ethylene Response Factor ERF010 (*LOC102589042*), disease resistance protein RPP13 (*LOC102605863*), and Cytochrome P450 83B1 (*LOC102604056*). Their expression patterns underscore a tight linkage between metabolic pathways and pathogen response. The downregulation of ERF010 suggests a potential modulation of the ethylene signaling pathway in response to scab infection, prompting further exploration of its interplay with other hormonal pathways in plant immunity [48,49]. Baharudin's research highlighted ethylene's role in regulating plant responses post infection, including defensive actions and repair processes [50]. The downregulation of ERF010 suggests a potential modulation of the ethylene signaling pathway in response to scab infection, necessitating further exploration of its interplay with other hormonal pathways in plant immunity.

The upregulation of the RPP13 gene emphasizes its central role in the plant's innate immune system, particularly in pathogen recognition and defense mechanism activation. Bittner's research highlighted the significant amino acid diversity within the LRR domain of RPP13 in response to various virulence factors of *Peronospora parasitica*, underscoring the importance of these amino acid sequences in plant–pathogen interactions [51]. A study by Rose revealed that such diversity is maintained through reciprocal selection between the plant and the pathogen, indicating functional differences of RPP13 in combating various pathogen strains [52]. Additionally, studies in barley identified RPP13-like genes and analyzed their potential roles in disease resistance, illustrating their importance in the plant defense mechanism [53]. These studies collectively emphasize the diversity and pivotal role of the RPP13 gene family in pathogen recognition and resistance in plants. In our study, the upregulation of RPP13 in response to scab infection suggests the active engagement of the plant's immune system, potentially triggering localized or systemic defense responses, highlighting the significance of R gene-mediated resistance in plant defense.

The consistent downregulation of Cytochrome P450 83B1 indicates metabolic adjustments in plants under pathogenic stress, highlighting the diverse and crucial role of P450 enzymes in plant defense. Research on basidiomycete biotrophic plant pathogens suggests that unique patterns in the P450 enzyme family significantly influence their interactions with host plants [54]. Studies on the *CaCYP1* gene in chili pepper demonstrate its role in salicylic acid and gibberellin signaling pathways in plant defense against bacterial pathogens [55]. Additionally, research on the *CaCYP450A* gene emphasizes the importance of P450 enzymes in plant defense responses to microbial pathogens [56]. The observed downregulation of Cytochrome P450 83B1 in our study may reflect a strategic reallocation of metabolic resources by the plant in response to scab attack, providing key insights into the complex defense mechanisms plants deploy against pathogenic attacks. This analysis pinpointed three key DEGs as the most likely candidates associated with resistance to potato scab. Each gene plays a unique and integral role in the plant's sophisticated defense mechanisms, contributing to a complex network of plant–pathogen interactions. This discovery provides pivotal insights into the multifaceted resistance mechanisms against pathogenic assaults.

Through a comprehensive analysis of GO, KEGG enrichment, and key disease resistance genes, this study unravels a myriad of metabolic pathways and signaling processes essential in plant responses to scab disease. This foundational understanding sheds light on the genetic mechanisms of potato scab disease resistance, with significant implications for potato breeding. The implications of our findings for potato breeding are substantial, and we recognize the importance of translating these insights into practical breeding strategies. Future research aims to deepen our understanding by developing mutant lines for precise validation of gene functions. Additionally, expanding studies to include proteomic and metabolomic analyses will enhance our comprehension of molecular interactions under pathogenic stress, advancing genetic engineering and crop resistance strategies.

While identifying key pathways and potential genes, it is crucial to address potential off-target effects, particularly when manipulating genes related to chloroplast pathways, photosynthesis, oxygen transport, and glutathione metabolism. Recognizing the broader implications on potato growth, future studies will not only focus on functional validation but also comprehensively assess off-target effects. Advanced techniques will be employed to minimize non-specific alterations, ensuring responsible advancements in potato scab resistance studies and practical applications for agricultural use. We aim to explore the integration of identified genes into breeding programs, taking into consideration cost-effectiveness and scalability.

In contemplating the broader applications of our findings, it is paramount to consider the environmental implications, especially within the framework of sustainable agriculture practices. While our study lays the foundation for understanding genetic mechanisms and breeding implications, we recognize the importance of further investigations into the ecological impact of implementing genetic or molecular strategies for disease resistance in potato crops. Future research endeavors will aim to provide a more holistic view, considering factors such as biodiversity, soil health, and overall sustainability in the context of deploying these strategies in real-world agricultural scenarios.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14020291/s1, Figure S1: Analysis of gene expression quantifies consistency and variability between CS10 and CS11; Figure S2: The photosynthesis pathway with differential gene expressions highlighted: red for upregulated genes and green for downregulated genes; Figure S3: The glutathione metabolism pathway with differential gene expressions highlighted: red for upregulated genes and green for downregulated genes. Table S1: Grading criteria for potato scab disease severity; Table S2: Disease response of different potato varieties to three pathogens; Table S3: Summary of transcriptome sequencing analysis results for samples; Table S4: Number of DEGs obtained from inter-group comparisons; Table S5: GO enrichment analysis of 147 DEGs; Table S6: KEGG pathway enrichment analysis of 147 DEGs; Table S7: Q-values corresponding to enriched pathways across four comparative groups; Table S8: Summary of annotation results for 147 DEGs across six different databases; Table S9: The primers used for qRT-PCR of ten important DEGs.

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References

1. Ugent, D. The Potato: What is the botanical origin of this important crop plant, and how did it first become domesticated? *Science* **1970**, *170*, 1161–1166. [CrossRef] [PubMed]
2. Zaheer, K.; Akhtar, M.H. Potato production, usage, and nutrition—A review. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 711–721. [CrossRef] [PubMed]
3. Zhang, H.; Fen, X.U.; Yu, W.U.; H, H.U.; Dai, X. Progress of potato staple food research and industry development in China. *J. Integr. Agric.* **2017**, *16*, 2924–2932. [CrossRef]
4. Visser RG, F.; Bachem CW, B.; de Boer, J.M.; Bryan, G.J.; Chakrabati, S.K.; Feingold, S.; Gromadka, R.; van Ham, R.C.; Huang, S.W.; Jacobs, J.M.E.; et al. Sequencing the potato genome: Outline and first results to come from the elucidation of the sequence of the world's third most important food crop. *Am. J. Potato Res.* **2009**, *86*, 417–429. [CrossRef]
5. Dees, M.W.; Wanner, L.A. In search of better management of potato common scab. *Potato Res.* **2012**, *55*, 249–268. [CrossRef]
6. Lambert, D.H.; Loria, R. *Streptomyces acidiscabies* sp. nov. *Int. J. Syst. Evol. Microbiol.* **1989**, *39*, 393–396. [CrossRef]
7. Hill, J.; Lazarovits, G. A mail survey of growers to estimate potato common scab prevalence and economic loss in Canada. *Can. J. Plant Pathol.* **2005**, *27*, 46–52. [CrossRef]
8. Tscharntke, T.; Clough, Y.; Wanger, T.C.; Jackson, L.; Motzke, I.; Perfecto, I.; Vandermeer, J.; Whitbread, A. Global food security, biodiversity conservation and the future of agricultural intensification. *Biol. Conserv.* **2012**, *151*, 53–59. [CrossRef]
9. Healy, F.G.; Wach, M.; Krasnoff, S.B.; Gibson, D.M.; Loria, R. The txtAB genes of the plant pathogen *Streptomyces* acidiscabies encode a peptide synthetase required for phytotoxin thaxtomin A production and pathogenicity. *Mol. Microbiol.* **2000**, *38*, 794–804. [CrossRef]
10. Kers, J.A.; Cameron, K.D.; Joshi, M.V.; Bukhalid, R.A.; Morello, J.E.; Wach, M.J.; Gison, D.M.; Loria, R. A large, mobile pathogenicity island confers plant pathogenicity on *Streptomyces* species. *Mol. Microbiol.* **2005**, *55*, 1025–1033. [CrossRef]
11. Loria, R.; Kers, J.; Joshi, M. Evolution of plant pathogenicity in *Streptomyces*. *Annu. Rev. Phytopathol.* **2006**, *44*, 469–487. [CrossRef] [PubMed]
12. Bignell, D.R.D.; Fyans, J.K.; Cheng, Z. Phytotoxins produced by plant pathogenic *Streptomyces* species. *J. Appl. Microbiol.* **2014**, *116*, 223–235. [CrossRef] [PubMed]
13. Lerat, S.; Babana, A.H.; El Oirdi, M.; El Hadrami, A.; Daayf, F.; Beaudoin, N.; Bouarab, K.; Beaulieu, C. *Streptomyces scabiei* and its toxin thaxtomin A induce scopoletin biosynthesis in tobacco and *Arabidopsis thaliana*. *Plant Cell Rep.* **2009**, *28*, 1895–1903. [CrossRef] [PubMed]
14. Joshi, M.; Rong, X.; Moll, S.; Kers, J.; Franco, C.; Loria, R. *Streptomyces turgidiscabies* secretes a novel virulence protein, Nec1, which facilitates infection. *Mol. Plant-Microbe Interact.* **2007**, *20*, 599–608. [CrossRef] [PubMed]
15. Barry, S.M.; Kers, J.A.; Johnson, E.G.; Song, L.J.; Aston, P.R.; Patel, B.; Krasnoff, S.B.; Crane, B.R.; Gibson, D.M.; Loria, R.; et al. Cytochrome P450–catalyzed L-tryptophan nitration in thaxtomin phytotoxin biosynthesis. *Nat. Chem. Biol.* **2012**, *8*, 814–816. [CrossRef] [PubMed]
16. Braun, S.; Gevens, A.; Charkowski, A.; Allen, C.; Jansky, S. Potato common scab: A review of the causal pathogens, management practices, varietal resistance screening methods, and host resistance. *Am. J. Potato Res.* **2017**, *94*, 283–296. [CrossRef]
17. Bukhalid, R.A.; Loria, R. Cloning and expression of a gene from *Streptomyces scabies* encoding a putative pathogenicity factor. *J. Bacteriol.* **1997**, *179*, 7776–7783. [CrossRef]
18. Hiltunen, L.H.; Weckman, A.; Ylhäinen, A.; Rita, H.; Richter, E.; Valkonen, J.P.T. Responses of potato cultivars to the common scab pathogens, *Streptomyces scabies* and *S. turgidiscabies*. *Ann. Appl. Biol.* **2005**, *146*, 395–403. [CrossRef]
19. Lapwood, D.H.; Hering, T.F. Soil moisture and the infection of young potato tubers by *Streptomyces scabies* (common scab). *Potato Res.* **1970**, *13*, 296–304. [CrossRef]
20. Wanner, L.A. A new strain of *Streptomyces* causing common scab in potato. *Plant Dis.* **2007**, *91*, 352–359. [CrossRef]
21. Driscoll, J.; Coombs, J.; Hammerschmidt, R.; Kirk, W.; Wanner, L.; Douches, D. Greenhouse and field nursery evaluation for potato common scab tolerance in a tetraploid population. *Am. J. Potato Res.* **2009**, *86*, 96–101. [CrossRef]
22. Goth, R.W.; Haynes, K.G.; Young, R.J.; Wilson, D.R.; Lauer, F.I. Relative resistance of the potato cultivar Krantz to common scab caused by *Streptomyces scabies* as determined by cluster analysis. *Am. Potato J.* **1995**, *72*, 505–511. [CrossRef]
23. Wagner, G.P.; Kin, K.; Lynch, V.J. Measurement of mRNA abundance using RNA-seq data: RPKM measure is inconsistent among samples. *Theory Biosci.* **2012**, *131*, 281–285. [CrossRef] [PubMed]
24. Kim, D.; Langmead, B.; Salzberg, S.L. HISAT: A fast spliced aligner with low memory requirements. *Nat. Methods* **2015**, *12*, 357–360. [CrossRef] [PubMed]
25. Langmead, B.; Trapnell, C.; Pop, M.; Salzberg, S.L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **2009**, *10*, R25. [CrossRef] [PubMed]

26. Thorvaldsdóttir, H.; Robinson, J.T.; Mesirov, J.P. Integrative Genomics Viewer (IGV): High-performance genomics data visualization and exploration. *Brief. Bioinform.* **2013**, *14*, 178–192. [CrossRef]
27. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R.; 1000 Genome Project Data Processing Subgroup. The sequence alignment/map format and SAMtools. *Bioinformatics* **2009**, *25*, 2078–2079. [CrossRef] [PubMed]
28. Trapnell, C.; Roberts, A.; Goff, L.; Pertra, G.; Kim, D.; Kelley, D.R.; Pimentel, H.; Salzberg, S.L.; Rinn, J.L.; Pachter, L. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat. Protoc.* **2012**, *7*, 562–578. [CrossRef]
29. Wang, L.; Feng, Z.; Wang, X.; Wang, X.W.; Zhang, X.G. DEGseq: An R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* **2010**, *26*, 136–138. [CrossRef]
30. 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]
31. Young, M.D.; Wakefield, M.J.; Smyth, G.K.; Oshlack, A. Gene ontology analysis for RNA-seq: Accounting for selection bias. *Genome Biol.* **2010**, *11*, R14. [CrossRef]
32. Mao, X.; Cai, T.; Olyarchuk, J.G.; Wei, L. Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. *Bioinformatics* **2005**, *21*, 3787–3793. [CrossRef]
33. Kanehisa, M.; Araki, M.; Goto, S.; Hattori, M.; Hirakawa, M.; Itoh, M.; Katayama, T.; Kawashima, S.; Okuda, S.; Tokimatsu, T.; et al. KEGG for linking genomes to life and the environment. *Nucleic Acids Res.* **2007**, *36* (Suppl. S1), D480–D484. [CrossRef]
34. Li, B.; Dewey, C.N. RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinform.* **2011**, *12*, 323. [CrossRef] [PubMed]
35. Ye, M.; Peng, Z.; Tang, D.; Yang, Z.M.; Li, D.W.; Xu, Y.M.; Zhang, C.Z.; Huang, S.W. Generation of self-compatible diploid potato by knockout of *S-RNase*. *Nat. Plants* **2018**, *4*, 651–654. [CrossRef]
36. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [CrossRef] [PubMed]
37. Mavrevski, R.; Traykov, M.; Trenchev, I.; Trencheva, M. Approaches to modeling of biological experimental data with GraphPad Prism software. *WSEAS Trans. Syst. Control* **2018**, *13*, 242–247.
38. Tallarida, R.J.; Murray, R.B.; Tallarida, R.J.; Murray, R.B. Duncan multiple range test. In *Manual of Pharmacologic Calculations: With Computer Programs*; Springer: New York, NY, USA, 1987; pp. 125–127.
39. Jansky, S.H.; Rouse, D.I. Multiple disease resistance in interspecific hybrids of potato. *Plant Dis.* **2003**, *87*, 266–272. [CrossRef]
40. Wanner, L.A.; Haynes, K.G. Aggressiveness of *Streptomyces* on four potato cultivars and implications for common scab resistance breeding. *Am. J. Potato Res.* **2009**, *86*, 335–346. [CrossRef]
41. Ward, J.A.; Ponnala, L.; Weber, C.A. Strategies for transcriptome analysis in nonmodel plants. *Am. J. Bot.* **2012**, *99*, 267–276. [CrossRef]
42. Hrdlickova, R.; Toloue, M.; Tian, B. RNA-Seq methods for transcriptome analysis. *Wiley Interdiscip. Rev. RNA* **2017**, *8*, e1364. [CrossRef]
43. Gong, L.; Zhang, H.; Gan, X.; Zhang, L.; Chen, Y.; Nie, F.; Shi, L.; Li, M.; Guo, Z.; Zhang, G.; et al. Transcriptome profiling of the potato (*Solanum tuberosum* L.) plant under drought stress and water-stimulus conditions. *PLoS ONE* **2015**, *10*, e0128041. [CrossRef] [PubMed]
44. Tiwari, J.K.; Buckseth, T.; Zinta, R.; Saraswati, A.; Singh, R.K.; Rawat, S.; Dua, V.K.; Chakrabati, K.S. Transcriptome analysis of potato shoots, roots and stolons under nitrogen stress. *Sci. Rep.* **2020**, *10*, 1152. [CrossRef] [PubMed]
45. Tang, R.; Gupta, S.K.; Niu, S.; Li, X.Q.; Yang, Q.; Zhu, W.; Haroon, M. Transcriptome analysis of heat stress response genes in potato leaves. *Mol. Biol. Rep.* **2020**, *47*, 4311–4321. [CrossRef]
46. Voss, I.; Sunil, B.; Scheibe, R.; Raghavendra, A.S. Emerging concept for the role of photorespiration as an important part of abiotic stress response. *Plant Biol.* **2013**, *15*, 713–722. [CrossRef] [PubMed]
47. Fernandez-Checa, J.C.; Garcia-Ruiz, C.; Colell, A.; Morales, A.; Mari, M.; Miranda, M.; Ardite, E. Oxidative stress: Role of mitochondria and protection by glutathione. *Biofactors* **1998**, *8*, 7–11. [CrossRef] [PubMed]
48. Dal'Sasso, T.C.S.; Rocha, V.D.; Rody, H.V.S.; Costa, M.D.L.; Oliveira, L.O.D. The necrosis-and ethylene-inducing peptide 1-like protein (NLP) gene family of the plant pathogen *Corynespora cassiicola*. *Curr. Genet.* **2022**, *68*, 645–659. [CrossRef]
49. Lynch, J.; Brown, K.M. Ethylene and plant responses to nutritional stress. *Physiol. Plant.* **1997**, *100*, 613–619. [CrossRef]
50. Baharudin, N.F.; Osman, N.I. Plant development, stress responses and secondary metabolism under ethylene regulation. *Plant Stress* **2023**, *7*, 100146. [CrossRef]
51. Bittner-Eddy, P.D.; Crute, I.R.; Holub, E.B.; Beynon, J.L. RPP13 is a simple locus in Arabidopsis thaliana for alleles that specify downy mildew resistance to different avirulence determinants in Peronospora parasitica. *Plant J.* **2000**, *21*, 177–188. [CrossRef]
52. Rose, L.E.; Bittner-Eddy, P.D.; Langley, C.H.; Michelmore, R.W.; Beynon, J.L. The maintenance of extreme amino acid diversity at the disease resistance gene, RPP13, in *Arabidopsis thaliana*. *Genetics* **2004**, *166*, 1517–1527. [CrossRef] [PubMed]
53. Cheng, J.; Fan, H.; Li, L.; Hu, B.; Liu, H.; Liu, Z. Genome-wide identification and expression analyses of *Rpp13*-like genes in barley. *BioChip J.* **2018**, *12*, 102–113. [CrossRef]

54. Qhanya, L.B.; Matowane, G.; Chen, W.; Sun, Y.X.; Letsimo, E.M.; Parvez, M.; Yu, J.H.; Mashele, S.S.; Syed, K. Genome-wide annotation and comparative analysis of cytochrome P450 monooxygenases in Basidiomycete biotrophic plant pathogens. *PLoS ONE* **2015**, *10*, e0142100. [CrossRef] [PubMed]
55. Kim, Y.C.; Kim, S.Y.; Paek, K.H.; Choi, D.; Park, J.M. Suppression of *CaCYP1*, a novel cytochrome P450 gene, compromises the basal pathogen defense response of pepper plants. *Biochem. Biophys. Res. Commun.* **2006**, *345*, 638–645. [CrossRef] [PubMed]
56. Hwang, I.S.; Hwang, B.K. Role of the pepper cytochrome P450 gene *CaCYP450A* in defense responses against microbial pathogens. *Planta* **2010**, *232*, 1409–1421. [CrossRef]