Transcriptome Analysis of Tryptophan-Induced Resistance against Potato Common Scab

Pan Zhao, Lu Liu, Jingjing Cao, Zhiqin Wang, Yonglong Zhao and Naiqin Zhong

Abstract: Potato common scab (CS) is a worldwide soil-borne disease that severely reduces tuber quality and market value. We observed that foliar application of tryptophan (Trp) could induce resistance against CS. However, the mechanism of Trp as an inducer to trigger host immune responses is still unclear. To facilitate dissecting the molecular mechanisms, the transcriptome of foliar application of Trp and water (control, C) was compared under Streptomyces scabies (S) inoculation and uninoculation. Results showed that 4867 differentially expressed genes (DEGs) were identified under S. scabies uninoculation (C-vs-Trp) and 2069 DEGs were identified under S. scabies inoculation (S-vs-S+Trp). Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses indicated that Trp induced resistance related to the metabolic process, response to stimulus, and biological regulation. As phytohormone metabolic pathways related to inducing resistance, the expression patterns of candidate genes involved in salicylic acid (SA) and jasmonic acid/ethylene (JA/ET) pathways were analyzed using qRT-PCR. Their expression patterns showed that the systemic acquired resistance (SAR) and induced systemic resistance (ISR) pathways could be co-induced by Trp under S. scabies uninoculation. However, the SAR pathway was induced by Trp under S. scabies inoculation. This study will provide insights into Trp-induced resistance mechanisms of potato for controlling CS, and extend the application methods of Trp as a plant resistance inducer in a way that is cheap, safe, and environmentally friendly.

Keywords: Solanum tuberosum; common scab; transcriptome analysis; tryptophan; induced resistance

1. Introduction

The potato (Solanum tuberosum) is the fourth most important food crop in China after rice, wheat, and maize. In 2017, the potato-cultivated area was over 5.67 million hectares, and its production exceeded 99.15 million metric tons [1]. However, numerous diseases threaten the growth of potato, especially common scab (CS) disease [2]. CS in potato tubers results in skin lesions, leading to the production of unmarketable potatoes for fresh consumption, seeds and others [3–5]. In recent years, CS has caused a substantial economic loss worldwide and has become one of the most severe concerns for potato farmers [6–8].

CS is caused by the soil-borne Gram-positive, filamentous bacteria genus Streptomyces [9]. At least 12 species in Streptomyces have been reported to be able to produce scab symptoms [9–11], among which S. scabies is the first and best-characterized species [9,12,13]. Thaxtomin is a plant phytotoxin secreted by Streptomyces and are supposed to induce CS skin lesion symptoms [3,9,14]. As the predominant form of thaxtomin produced by
S. scabies, thaxtomin A inhibits plant cellulose biosynthesis, especially cellobiose and celloatriose biosynthesis [15–17]. The secretion of thaxtomin A in potato tubers alters plant physiologies, such as Ca\(^{2+}\), H\(^+\) inflowing, and polysaccharide deposition, resulting in tissue necrosis and reduced potato quality [10,18]. Genetic evidence has shown that eliminating critical genes of thaxtomin A synthesis, including txtA, txtB and txtC, negatively affects the pathogenicity of S. scabies. It is well-known that txtA and txtB encode two nonribosomal peptide synthetases, and txtC encodes cytochrome P450-type monooxygenase in thaxtomin A biosynthesis [9,19–21].

Although the causal agent of CS is well-diagnosed, it is still challenging to control CS effectively. The applications of individual strategies, such as crop rotation, chemical fumigation, or fungicides, have all proven insufficient [10,22]. Meanwhile, chemical methods are expensive and environmentally unfriendly. Therefore, new nontoxic and cheap strategies need to be searched for. Plant immunity inducers, which are green biological agents able to control diseases, have attracted much attention around the world. Immunity inducers are a class of immune-active compounds that can induce resistance in plants and promote healthy plant growth. Induced resistance in plants is triggered by biological or chemical inducers that protects the plant’s nonexposed parts against future attack by pathogenic microbes [23–25]. This resistance can be divided into induced systemic resistance (ISR), and systemic acquired resistance (SAR), induced by nonpathogenic microbes and pathogenic microbes, or based on the nature of the elicitor and the regulatory pathways involved [25]. SAR is dependent on the accumulation of phytohormone salicylic acid (SA) and pathogenesis-related (PR) proteins [26–29]. However, ISR is dependent of the phytohormone jasmonic acid/ethylene (JA/ET) [30,31]. Induced resistance is expressed not only locally at the site but also systemically in other parts of the plant that are separated from the inducer, enhancing the level of protection against a broad spectrum of attackers [32].

Plant-induced resistance provides a new strategy for controlling potato scab disease. In practice, foliar sprays of auxins and related molecules have been used for controlling CS [33–35]. Some chemicals act as elicitors or inducers that can trigger host immune responses [23,24]. For example, 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA) [36], benzo[b]thiadiazole (BTH), β-aminobutyric acid (BABA), and acetylsalicylic acid (ASA) [33–35,37–41] have been used to suppress the development of CS. It has been reported that tryptophan (Trp), a precursor of auxin and secondary metabolites, could protect plants against some fungal, bacterial, and insect attacks [42–46]. Here, we found that the application of Trp could induce potato resistance to control CS. Considering that Trp is a relatively cheap chemical, this observation expanded the range of inducers that could be used to protect potato in the field. There are few reports stating that Trp application as an inducer can enhance the resistance of potato to CS. Thus, it is valuable to investigate the mechanisms of Trp-induced resistance against potato CS.

Transcriptome analysis is an important method for studying the expression of a large number of genes in a given tissue [47]. RNA transcript profiling can rapidly and effectively provide information for genome-wide transcript characterization, differential gene expression analysis, variant detection, and gene-specific expression. This technology is used to analyze the transcriptome in response to different biotic or abiotic stresses, including low-nitrogen stress [48], high-light stress [49], drought response to different soybean cultivars [50], salt stress [51,52], Cd stress [53], and sweet orange response to Citrus tristeza virus [54].

In this study, we analyzed transcriptome datasets from the leaves of potato plantlets. Leaves sprayed with Trp or water were examined from two groups (C-vs-Trp and S-vs-S+Trp) of potato plants. The differentially expressed genes (DEGs) of the two groups were identified to deepen the understanding of Trp-induced resistance to potato CS. These data will be critical to identifying defense-related genes that are regulated by exogenous Trp and to prompting our understanding of potato–S. scabies interactions.
2. Results

2.1. Foliar Treatment of Trp Enhances Potato Resistance against CS

Although Trp has been reported to protect plants against some fungi and bacteria, foliar application of Trp to defend against the soil disease CS has not been reported. It was hypothesized that Trp may act as an inducer to enhance resistance to CS in potato. Therefore, a field trial was designed to test the effectiveness of Trp. The results of disease incidence and yield indicated that 100 mg/L Trp was the best concentration (Figure S1).

Thus, 100 mg/L Trp was sprayed on potato plants in pots during tuber initiation. At this time, tubers are easily infected by the bacteria. The potato tubers were harvested from pots and assessed for CS. Tubers > 2 g were washed under running water and scored for disease incidence, disease index, total tuber mass and control efficacy. Tubers inoculated with S. scabies (S) showed strong susceptibility to disease compared to the control (C), showing that the inoculation was very successful. As shown in Figure 1, the scabs on the surface of the tubers treated with S. scabies and Trp (S + Trp) were reduced compared to tubers treated with only S. scabies. Then, we scored the total tuber mass, disease incidence, disease index and control efficacy. As shown in Table 1, the tuber mass of Trp was the best. After inoculation with S. scabies, foliar application of Trp decreased the disease incidence and disease index. Compared with S, the disease incidence of S+Trp was decreased from 90% to 59.09% and the disease index of S+Trp tubers was decreased from 53 to 27.27. The control efficacy of S+Trp compared with S was 48.54%. These results suggest that foliar treatment with Trp could enhance potato resistance against CS.

![Figure 1. Disease symptoms of potatoes in pots. C: S. scabies-uninoculated plants treated with water; Trp: S. scabies-uninoculated plants treated with Trp; S: S. scabies-inoculated plants treated with water; S + Trp: S. scabies-inoculated plants treated with Trp.](image)

| Treatment | Total Tuber Mass (g) | Disease Incidence (%) | Disease Index | Control Efficacy (%) |
|-----------|----------------------|-----------------------|---------------|----------------------|
| C         | 323.40 ± 7.82        | 0                     | 0             | –                    |
| Trp       | 370.51 ± 1.70        | 0                     | 0             | –                    |
| S         | 271.13 ± 5.85        | 90.00 ± 7.08          | 53.00 ± 6.95  | –                    |
| S + Trp   | 342.67 ± 10.89       | 59.09 ± 2.53 **       | 27.27 ± 1.18 ** | 48.54 ± 8.27         |

The asterisks denote statistically significant differences, as determined by Student’s t-test, ** p < 0.01. Three biological repetitions were performed.

2.2. Analyses of the Transcriptome Datasets

The quality of the total RNA was assessed using the Agilent Bioanalyser 2100 (Agilent Technologies, Santa Clara, CA, USA) prior to subsequent experiments. A total of 12 RNA libraries were sequenced in depth, ranging from 102.19 to 105.84 million raw reads (Table S1). After filtering (removal of low-quality, joint contamination, and excessive unknown bases), 122.11 GB of data (fq.gz) was generated in total. More than 97.78% and 89.49% of the clean reads reached the Q20 and Q30 levels, respectively. More than 78.64% of the clean reads were mapped to the reference genome, including more than 58.61% unique reads (Table S2). The assessment of the filtered data indicated that the filtered sequences were high-quality.
The transcriptional levels were normalized using the fragments per kilobase per million reads (FPKM) method. Based on the criteria (|fold change, FC| ≥ 2 and Q-value ≤ 0.001), DEGs were defined. The number of DEGs was depicted as volcano plots (Figure 2a,b). Under *S. scabies* uninoculation (C-vs-Trp), compared with C plants, 3,158 DEGs were upregulated and 1,709 were downregulated (Figure 2a). Under *S. scabies* inoculation (S-vs-S+Trp), compared with the S-treated plants, we found that 1,297 DEGs were upregulated and 772 were downregulated (Figure 2b). The C-vs-Trp group had more DEGs than the S-vs-S+Trp group. A Venn diagram analysis showed the unique and shared DEGs in the two groups (Figure 2c). In total, 810 DEGs were common between the two groups (Table S3). Among these 810 DEGs, 592 DEG showed the same trends in gene expression, including 387 DEGs being upregulated and 205 downregulated, and the remaining 218 DEGs had different expression trends between the two groups. These DEGs were involved in the starch and sucrose metabolism pathway, metabolic pathway, and plant hormone signal transduction pathway. There were 486 upregulated DEGs and 324 downregulated DEGs in the C-vs-Trp group. These genes were mainly categorized as catalytic activity and metabolic process, and were mainly involved in metabolic pathways, biosynthesis of secondary metabolites pathways and plant hormone signal biosynthesis. There were 506 upregulated and 304 downregulated DEGs in the S-vs-S+Trp group. Most of the genes were categorized as catalytic activity, metabolic process, transporter activity, and so on. The pathways or biological processes included biological regulation, binding, RNA transport, and cutin biosynthesis. Furthermore, 4,057 DEGs, including 2,627 upregulated DEGs and 1,385 downregulated DEGs, were specific to the C-vs-Trp group (Table S3). These DEGs were involved in plant hormone signal transduction, plant-pathogen interaction, glycan degradation, and wax biosynthesis. 1,259 DEGs, including 807 upregulated DEGs and 452 downregulated DEGs, were specific to the S-vs-S+Trp group (Table S3). These DEGs were involved in the mRNA surveillance pathway, base excision repair, and non-homologous end-joining pathway. These results showed that more DEGs were upregulated when treated with Trp without pathogens.

2.3. GO and KEGG Enrichment Analyses of DEGs

Gene Ontology (GO) enrichment analysis was applied to categorize the DEGs (Figure 3). In the two groups, DEGs were classified into three GO categories: biological process (BP), cellular component (CC), and molecular function (MF). In the C-vs-Trp group, the BP category was mainly enriched in cellular process (721 DEGs), metabolic process (679 DEGs), response to stimulus (318 DEGs), biological regulation (274 DEGs), and regulation of biological process (242 DEGs); in the CC category, DEGs were mainly enriched in membrane (979 DEGs), membrane part (930 DEGs), cell (710 DEGs), cell part (679 DEGs) and organelle (509 DEGs); in the MF category, the top five items were catalytic activity (1,435 DEGs), binding (1,358 DEGs), transporter activity (177 DEGs), transcription regulator activity (110 DEGs) and molecular function regulator (84 DEGs). In the S-vs-S+Trp group, the DEGs enriched were similar to those in the C-vs-Trp group. In the BP category, the top five items were cellular process (368 DEGs), metabolic process (348 DEGs), biological regulation (100 DEGs), response to stimulus (100 DEGs), and regulation of biological process (94 DEGs); in the CC category, the top five items were membrane (342 DEGs), membrane part (330 DEGs), cell (315 DEGs), cell part (306 DEGs) and organelle (245 DEGs); in the MF category, the top five items were binding (568 DEGs), catalytic activity (559 DEGs), transporter activity (75 DEGs), transcription regulator activity (37 DEGs) and molecular function regulator (37 DEGs). The results showed the same categorization of BP and MF in the two groups. It was also clearly visible that more genes were upregulated than downregulated for each group (Figure S2). There was a slight difference between the two groups. For example, in the “response to stimulus” process, there were more upregulated genes than downregulated ones in C-vs-Trp group. However, there were more downregulated genes than upregulated ones in S-vs-S+Trp group. The genes related
hormones, stimulus, transcription regulator activity, and metabolic process should be given more attention in further research.

Figure 2. Volcano plot (a, b) and Venn diagram (c) of DEGs in the C-vs-Trp group and S-vs-S+Trp group.

Figure 3. Gene Ontology (GO) enrichment analysis of DEGs in the C-vs-Trp (a) and S-vs-S+Trp (b) groups.
Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed to further understand the DEGs. In the diagrams, the significant results were based on the Rich factor (RF) and Q-value (the smaller the Q-value is, the more significant the results; the larger RF is, the more significant the results). In the C-vs-Trp group, the major pathways were plant hormone signal transduction (268 DEGs, 0.21 RF), sesquiterpenoid and triterpenoid biosynthesis (55 DEGs, 0.28 RF), other glycan degradation (50 DEGs, 0.27 RF) and cutin, suberine and wax biosynthesis (38 DEGs, 0.25 RF); in the S-vs-S+Trp group, the major pathways were sesquiterpenoid and triterpenoid biosynthesis (17 DEGs, 0.09 RF), mRNA surveillance pathway (34 DEGs, 0.07 RF), fatty acid metabolism (19 DEGs, 0.08 RF) and RNA degradation (39 DEGs, 0.07 RF) (Figure 4).

Figure 4. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs in the C-vs-Trp group (a) and S-vs-S+Trp (b) group. The X-axis represents the enrichment factor, and the Y-axis represents the pathway name. The depth of color represents the Q-value, and the size of the dot represents the number of DEGs.

2.4. Validation of RNA-seq Data by qRT–PCR

To validate the transcriptome sequencing results, qRT–PCR was performed to assess the expression levels of genes. A total of 18 genes were selected, of which 7 were differentially expressed in the two groups, 3 were only differentially expressed in the S-vs-S+Trp group, 6 were only differentially expressed in the C-vs-Trp group, and 2 were not DEGs in both groups. As shown in Figure 5, the qRT–PCR results were consistent with the transcriptome sequencing results. The results indicated that the obtained RNA-seq data are reliable.

2.5. Candidate Trp-Induced DEGs in Phytohormone Pathways

The genes in the phytohormone pathways were correlated with induced resistance. Based on functional annotation and pathway analysis, several candidate DEGs related to phytohormone pathways were found to be notable in both groups. These were SA-, JA- and ET-related genes, such as SA biosynthesis-related genes ICS (isochorismate synthase), PAL (phenylalanine ammonia-lyase), SA-responsive genes PR1 (pathogenesis-related protein 1) and NPR1 (nonexpressor of pathogenesis related genes 1), NPR1 transcription factor gene WRKY, SA glucosylation genes SAGT (salicylic acid glucosyltransferase), JA biosynthesis-related genes LOX (lipoxygenase) and OPR (12-oxophytodienoate reductase), and ET biosynthesis-related genes ACO (1-aminocyclopropane-1-carboxylate oxidase) and ACS (1-aminocyclopropane-1-carboxylate synthase).
Figure 5. qRT-PCR validation of 18 DEGs. The asterisks denote statistically significant differences, as determined by Student’s t-test, * $p < 0.05$, ** $p < 0.01$. Three biological repetitions were performed.

In the C-vs-Trp group, the expression of SA biosynthesis-related genes ICS and PAL was significantly induced by Trp. All the SAGT genes were downregulated. Furthermore, three NPR1 genes, one PRI gene and six WRKY genes were upregulated. Foliar treatment of Trp also induced the expression of JA biosynthesis-related genes. Seven LOX genes and two OPR genes were upregulated. The same results were seen in the ET-related genes. The expression of four ACO genes and seven ACS genes was induced (Table S4). Moreover, we selected some candidate genes with high FC values from the group and detected their relative expression levels by qRT-PCR (Figure 6a).

In the S-vs-S+Trp group, there were similar results in the expression of the SA biosynthesis-related genes. Two ICS genes and one PAL gene were upregulated. Three of the SAGT genes were downregulated. One NPR1 gene, three PRI genes and two WRKY genes were upregulated. However, in the JA/ET biosynthesis-related genes, Trp downregulated genes expression. Four LOX genes were downregulated. The same results were
seen in the ET pathway-related genes. The four ACO genes and one ACS gene were all downregulated (Table S4). Some genes with high FC values were selected to verify the differences in expression using qRT–PCR (Figure 6b).

![Graphs showing gene expression](image)

**Figure 6.** The relative expression of some candidate genes belonging to the C-vs-Trp group (a) and S-vs-S+Trp group (b) was detected by qRT–PCR. The asterisks denote statistically significant differences, as determined by Student’s *t*-test, *p* < 0.05, **p** < 0.01. Three biological repetitions were performed.

3. Discussion

As a globally serious potato disease, CS is difficult to control via single management. Chemical methods are frequently used but expensive and environmentally unfriendly. The overuse or inappropriate use of chemical agents results in serious problems, especially environmental pollution and food safety, in agriculture [55,56]. Techniques to induce plant resistance represent a new and rapidly developing field of research and development [56]. To reduce the usage of chemical agents, we need to search for nontoxic and effective methods. Previous studies have reported that Trp could protect plants against some fungal, bacterial, and insect attacks [42–46]. In particular, Trp-derived secondary metabolites play important roles in defense responses, such as producing serotonin [57] and increasing the accumulation of camalexin, indole-carboxylic acid (ICA), and IAA [58,59]. Exogenous Trp can strongly inhibit the production of thaxtomin A by pathogenic *S. scabies* in a liquid thaxtomin-inducing growth medium [60,61]. Thus, the foliar application of Trp induces a broad-spectrum resistance exhibiting a great potential to avoid plant diseases.
We found that foliar treatment with Trp enhances potato resistance to CS and can increase its yield (Figures 1 and S1). There are no reports that foliar application of Trp can control potato CS. Thus, it is of value to investigate the mechanisms of Trp-induced resistance against CS. We used transcriptome sequencing technology to analyze the mechanisms of the induced resistance of potato by foliar application of Trp against CS. A total of 4867 and 2069 DEGs were identified in the C-vs-Trp and S-vs-S+Trp groups, respectively. Based on GO and KEGG analyses, DEGs induced by Trp were found to be involved in the metabolic process, response to stimulus and biological regulation (Figures 3 and 4).

In view of the phytohormone pathways related to induced resistance, we analyzed key genes in hormone signaling pathways, such as SA, JA and ET. Induced resistance is an important mechanism by which plants enhance their defense ability via inducers in response to a broad range of pathogen attacks [30]. At present, the two forms of induced resistance, SAR and ISR, have been used in conventional agriculture against pathogens [62]. Via comparative transcriptome analysis, we found that SAR and ISR can be co-induced by Trp without pathogen treatment. Under *S. scabies* treatment, SAR is the pathway induced by Trp treatment.

SAR is required for the accumulation of PR proteins (and transcripts) and SA [26–29]. In this study, we analyzed the hormones of SA-related DEGs. In the C-vs-Trp and S-vs-S+Trp groups, the expression of SA-related genes was induced by Trp. *ICS* and *PAL* expression was upregulated, suggesting that SA biosynthesis may be increased. The *SAGT* genes were also downregulated. SAGT enzymes convert most of the produced SA to SAG, which is stored in the vacuole. Knockout mutants of the Arabidopsis *SAGT* genes showed increased disease resistance and free SA levels [63]. *AtSGT1* gene overexpression results in a reduction in SA content and reduced plant resistance [64]. SAR is typically characterized by augmentation of SA and PR genes’ activation. SAR-induced plants showed increased expression of SA-dependent *PR1* [29]. In our study, the expression of *PR1* genes in the two groups was upregulated by Trp. In fact, exogenous application of SA can activate PR gene expression and resistance in plants without pathogen inoculation [65,66]. These results imply that the SA signaling pathway is required to induce resistance by Trp.

ISR is mediated by a JA/ET-sensitive pathway and does not involve the accumulation of PR proteins or SA [30,31]. It is generally believed that this antagonism occurs between SA and JA, and we also analyzed the expression of SA. JA biosynthesis-related genes, including *LOX* and *OPR*, and ET synthesis-related genes, including *ACO* and *ACS*, were found. In the C-vs-Trp group, the expression of *LOX*, *OPR*, *ACO*, and *ACS* was shown to increase after treatment with Trp. Surprisingly, the expression of these responsive genes was downregulated by foliar treatment with Trp. There were significant differences in the expression of these genes between the two groups. Both synergistic and antagonistic interactions between SA and JA have been reported [67]. The interaction between SA and JA is either concentration-dependent or tissue-specific and dynamic [68]. In our results, we presumed that Trp-mediated resistance is different with or without pathogen attack. Without pathogens, foliar treatment with Trp induced SA and JA increases at low levels. Therefore, the resistance induced by Trp overlaps with ISR and SAR. This was also found in a recent study showing that the SA- and JA-biosynthesis pathways can be co-induced [69]. In contrast, in the S-vs-S+Trp group, SA biosynthesis-related genes were induced, and JA-related genes were downregulated. This showed that SAR was induced by Trp rather than ISR when the pathogen was inoculated. NPR1 expression is consistent with *ICS* and *PAL*, which are SA biosynthesis genes. NPR1 is a common regulator of ISR and SAR [66]. The SAR and ISR pathways are independent but have an overlapping requirement for NPR1 [70]. In both groups, the expression of NPR1 was increased. These results showed that Trp-mediated resistance required NPR1 to regulate the SAR and ISR pathways in pathogen inoculation. This is different from Si-mediated LB resistance in potato that occurs through ET/JA- and NPR1-dependent signaling pathways [71]. In a study of SA- and JA-mediated gene expression, the WRKY70 transcription factor was likely to be involved in mediating SA-JA crosstalk [72]. However, in our data, no WRKY70 genes
were found among the DEGs. Whether the other transcription factors play a role is unclear; we require further analysis of our data in future works.

There are many elicitors that can initiate the plant defense response [62]. These include many chemical compounds, such as SA, BTH, BABA, or PBZ, or biological compounds including metabolites, oligosaccharides, glycoproteins, glycopeptides, proteins, polypeptides, lipids, and other cellular components [56]. Amino acids, such as methionine, can activate the ROS defense pathway and induce defense-related genes [73]. Lower concentrations of JA and BTH enable the simultaneous expression of both SAR and ISR pathways [74]. The combination of ISR and SAR can increase protection against pathogens that are resisted through both pathways in addition to extending protection to a broader spectrum of pathogens than ISR/SAR alone [29]. Trp-induced resistance is unlike these elicitors. In the S-vs-S+Trp/C-vs-Trp groups, there are different signaling pathways involved in Trp-induced resistance. Further research, such as protein assays, plant hormone measurements, and cell assays, is needed to validate the functions of interesting genes.

In this study, we revealed that foliar treatment with Trp can induce resistance of potato against CS. Our results showed that Trp induced resistance though different pathways under different conditions. Under pathogen inoculation, SAR is the pathway induced by Trp treatment. Without pathogens inoculation, SAR and ISR can be co-induced by Trp. This study provides useful information for research into Trp-induced resistance mechanisms and extends the application of Trp as an alternative agent to control CS, especially considering the field conditions.

4. Materials and Methods

4.1. Determine the Proper Concentration of Trp

To determine the proper concentration of Trp, a series of concentrations solutions (50 mg/L, 100 mg/L, 200 mg/L, and 400 mg/L) were prepared in water. The field trial was arranged in a plot in which CS occurred year by year. The plot consisted of five subplots of the same area (4.2 m × 4 m) that sowed the same number of seeds at the same time. The different concentrations of Trp were sprayed on field potato plants 3 weeks after seed germination. Water was sprayed as the control. Trp solution or water was sprayed onto the leaves of different potato groups until runoff. Field tubers were harvested at 120 days after seeding and assessed for disease incidence and yield.

4.2. Plant Materials

The CS-susceptible potato cultivar, Shepody, was used. The potato plantlets were multiplied through plant tissue culture technology and grown in pot trials. Briefly, stems of potato plantlets were cut into node explants of similar length. These single-node explants were cultured on solid Murashige and Skoog (M&S) medium (pH 5.8) at 24 °C in an 8 h/16 h light cycle. Two weeks later, five established non-embryogenic callus lines of similar size were selected and transferred into pots filled with the same amount of autoclaved vermiculite. The selected potato lines were maintained under the same growth conditions. In brief, the pots were cultured at 24 °C with a 16 h light/8 h dark photoperiod and 70 μmol m−2 s−1 photon flux density provided by fluorescent lamps. For further trials, there were 3 plots (diameter 25 cm) that 5 seedlings per pot to replicate each treatment.

4.3. Pathogen Inoculation and Tryptophan Treatments

The S. scabies strain 4.1765, a highly pathogenic isolate from the China General Microbiological Culture Collection (CGMCC), was used in our experiments. The S. scabies strain was cultured on an oatmeal medium (OM) plate for 15 days at 28 °C. Then, the pathogenic colonies were inoculated into tryptic soy broth (TSB) and incubated at 28 °C in a rotary shaker (200 rpm) for three days. The cell pellets were harvested by centrifugation (10 min, 5000 × g) and washed with sterile water. The bacterial cells were resuspended in fresh water to a final concentration of 1 × 10⁶ conidia/mL for inoculation. This concentration of the suspension was determined based on the relationship between amount of pathogenic
S. scabies and the incidence of CS on tubers [75,76]. Then, 10 mL inoculation buffer was poured near the roots of seedlings. Therefore, a total of 50 mL inoculation buffer was inoculated into each pot.

The potato plants were divided into two groups and maintained under the same growth conditions. One was subjected to S. scabies infection, and another group was S. scabies uninoculation. Twenty-one days post-inoculation, at which time the tubers initiate and bacteria are highly infectious [77,78], Trp solution or water was sprayed onto the leaves of different potato groups until runoff. The concentration of the tryptophan solution was set at 100 mg/L, because this relatively low concentration could effectively inhibit the spread of CS disease. For the S. scabies-uninoculated plants, leaves were treated with tryptophan (Trp) and water (C). The same treatment of the leaves was performed in the S. scabies-inoculated plants (S+Trp and S).

After 4 days treatment, two top leaves were collected from each plant. Leaves collected from 5 different plants in one pot were pooled together as one sample for RNA extraction. Three replicates were repeated for each sample.

4.4. Disease Assessment

Tubers harvested 90 days after seedlings were planted in pots (3 pots, 5 plants per plot) were graded and assessed for CS. Tubers > 2 g were washed under running water and scored for the percentage of tuber area covered by scab.

The disease index was calculated by the following equation:

\[
\text{Disease index} = \left[ \frac{\sum (n \times 1 + n \times 2 + n \times 3 + n \times 4 + n \times 5)}{(N \times 5)} \right] \times 100. \quad (n = \text{number of tubers corresponding to the numerical grade. } N = \text{total number of potato tubers assessed.} \quad 5 = \text{high score on the severity of scale).}
\]

The percentage of tuber area covered: 0. No symptom of scab; 1. 0–12.5%; 2. 12.6–25%; 3. 26–50%; 4. 51–75%; 5. 76–100%.

Control efficacy = (disease index of control − disease index of treated)/disease index of control × 100%.

4.5. RNA Extraction and RNA Sequencing

In total, twelve samples of four conditions were used for total RNA extraction and library construction. Total RNA was isolated using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and library construction and sequencing were conducted by The Beijing Genomics Institute (BGI, Beijing, China, \url{http://www.genomics.cn/index.html}; accessed on 6 May 2022). The BGISEQ-500 platform was used for RNA sequencing and generated raw data [79,80]. The raw reads of the transcriptome data were filtered with SOAPnuke software to remove unsatisfactory reads with low quality, joint contamination, and excessive unknown bases.

The clean RNA-Seq data have been deposited in the SRA database under NCBI (Accession No. PRJNA611872).

4.6. Data Mapping, Analysis and Functional Annotation

The filtered clean reads were saved as FASTQ data and aligned to the potato reference genome S. tuberosum group phureja DM1-3 v6.1 (\url{http://spuddb.uga.edu/}; accessed on 20 April 2022) by HISAT [81–83].

The updated reference genome was generated by adding the newly identified transcripts into the original potato reference sequences. The clean reads were mapped to the updated reference sequences by Bowtie 2 [82–84]. The abundance of transcripts was estimated using the FPKM [85] method.

DEGs were analyzed by DEGseq [86,87] with the parameters “FC ≥ 2” and “Q-value ≤ 0.001”. GO functional enrichment of DEGs was performed based on the GO database (\url{http://www.geneontology.org/}; accessed on 22 April 2022), and pathway enrichment of DEGs was performed based on the KEGG database (\url{http://www.genome.jp/kegg/}; accessed on 23 April 2022). Meanwhile, GO term enrichment and KEGG path-
way enrichment analyses were conducted using the “phyper” function of the R package. FDR < 0.05 was defined as the threshold for significant enrichment.

4.7. qRT–PCR Analysis

Total RNA was extracted using the Plant Total RNA Extraction kit (Tiangen, Beijing, China) according to the manufacturer’s instructions. cDNA was synthesized from 1 µg total RNA using the TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgene, Beijing, China). qRT–PCR was performed using the SYBR® Green Realtime PCR Master Mix (Toyobo, Osaka, Japan). All qRT–PCRs were analyzed using CFX96 Touch (Bio-Rad, Hercules, CA, USA) with three technical replicates and three biological replicates. The relative expression levels of selected genes were identified using the $2^{-\Delta\Delta CT}$ method [88]. StActin (Soltu.DM.04G007480) was used as an internal control. The primers for qRT–PCR are shown in Table S5.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23158420/s1.

Author Contributions: Conceptualization, P.Z. and N.Z.; methodology, L.L., J.C. and P.Z.; validation, L.L. and J.C.; data analysis, Z.W. and P.Z.; investigation, Y.Z., P.Z. and N.Z.; writing, L.L. and P.Z.; visualization, Z.W. and Y.Z.; supervision, P.Z. and N.Z.; project administration, P.Z. and N.Z.; funding acquisition, P.Z. and N.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Key Area Research and Development Program of Guangdong Province in China (2020B0202010005), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA24020104, XDA28030202), the National Natural Science Foundation of China (31601622), Science and Technology Poverty Alleviation Project of The Chinese Academy of Sciences (KJF-FP-201905, KJF-FP-202001), and Key Technologies R & D Program of Inner Mongolia (2021GG0300).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The clean data presented in this study are available in the Sequence Read Archive (SRA) database in NCBI (Accession No. PRJNA611872).

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

References
1. FAOSTAT. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 6 May 2019).
2. Kirk, W.W. Introduction to 2013 symposium on bacterial diseases of potatoes. Am. J. Potato Res. 2015, 92, 215–217. [CrossRef]
3. Loria, R.; Bukhalid, R.A.; Fry, B.A.; King, R.R. Plant pathogenicity in the genus Streptomyces. Plant Dis. 1997, 81, 836–846. [CrossRef] [PubMed]
4. Wanner, L.A.; Kirk, W.W. Streptomyces—from basic microbiology to role as a plant pathogen. Am. J. Potato Res. 2015, 92, 236–242. [CrossRef]
5. Santos-Cervantes, M.E.; Felix-Gastelum, R.; Herrera-Rodriguez, G.; Espinoza-Mancillas, M.G.; Mora-Romero, A.G.; Leyva-López, N.E. Characterization, pathogenicity and chemical control of Streptomyces acidiscabies associated to potato common scab. Am. J. Potato Res. 2017, 94, 14–25. [CrossRef]
6. Hiltunen, L.H.; Weckman, A.; Ylhainen, A.; Rita, H.; Richter, E.; Valkonen, J.P.T. Responses of potato cultivars to the common scab pathogens, Streptomyces scabies and S. turigiscabies. Ann. Appl. Biol. 2005, 146, 395–403. [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. Wilson, C.R. A summary of common scab disease of potato research from Australia. In Proceedings of the International Potato Scab Symposium, Sapporo, Japan, 6–7 September 2004.
9. Loria, R.; Kers, J.; Joshi, M. Evolution of plant pathogenicity in Streptomyces. Annu. Rev. Phytopathol. 2006, 44, 469–487. [CrossRef]
10. Dees, M.W.; Wanner, L.A. In search of better management of potato common scab. Potato Res. 2012, 55, 249–268. [CrossRef]
11. Sarwar, A.; Latif, Z.; Zhang, S.; Zhu, J.; Zechel, D.L.; Bechthold, A. Biological control of potato common scab with rare isatropolone C compound produced by plant growth promoting Streptomyces A1RT. Front. Microbiol. 2018, 9, 1126. [CrossRef] [PubMed]
12. Lambert, D.H.; Loria, R.; Labeda, D.P.; Saddler, G.S. Recommendation for the conservation of the name Streptomyces scabies. Request for an Opinion. Int. J. Syst. Evol. Microbiol. 2007, 57, 2447–2448. [CrossRef]
13. Lambert, D.H.; Loria, R. Streptomyces scabies sp. nov., nom. rev. Int. J. Syst. Evol. Microbiol. 1989, 39, 387–392. [CrossRef]
14. King, R.R.; Lawrence, C.H.; Calhoun, L.A. Chemistry of phytotoxins associated with Streptomyces scabies the causal organism of potato common scab. J. Agric. Food Chem. 1992, 40, 834–837. [CrossRef]
15. King, R.R.; Lawrence, C.H.; Clark, M.C.; Calhoun, L.A. Isolation and characterization of phytotoxins associated with Streptomyces scabies. J. Chem. Soc. Chem. Commun. 1989, 13, 849–850. [CrossRef]
16. Johnson, E.G.; Joshi, M.V.; Gibson, D.M.; Loria, R. Cello-oligosaccharides released from host plants induce pathogenicity in scab-causing Streptomyces species. Physiol. Mol. Plant Pathol. 2007, 71, 18–25. [CrossRef]
17. Bischoff, V.; Cookson, S.J.; Wu, S.; Scheible, W.R. Thaxtomin A affects CES-A-complex density, expression of cell wall genes, cell wall composition, and causes ectopic lignification in Arabidopsis thaliana seedlings. J. Exp. Bot. 2009, 60, 955–965. [CrossRef]
18. Errahki, R.; Dauphin, A.; Meimoun, P.; Lehner, A.; Reboutier, D.; Vatsa, P.; Briand, J.; Madlona, K.; Rona, J.P.; Baakate, M.; et al. An early Ca^{2+} influx is a prerequisite to thaxtomin A-induced cell death in Arabidopsis thaliana cells. J. Exp. Bot. 2008, 59, 4259–4270. [CrossRef]
19. Healy, F.G.; Wach, M.; Krasnoff, S.B.; Gibson, D.M.; Loria, R. Involvement of a cytochrome P450 monooxygenase in thaxtomin A biosynthesis by Streptomyces acidiscabies. J. Bacteriol. 2002, 184, 2019–2029. [CrossRef]
20. Barry, S.M.; Kers, J.A.; Johnson, E.G.; Song, L.; 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]
21. 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]
22. Hosny, M.; Abo-Elyoura, K.A.M.; Asran, M.R.; Saed, F.A. Chemical control of potato common scab disease under field conditions. Arch. Phytopathol. Plant Prot. 2014, 47, 2193–2199. [CrossRef]
23. Kuc, J. Induced immunity to plant disease. BioScience 1982, 32, 854–860.
24. Klopper, J.W.; Tuzun, S.; Kuc, J.A. Proposed definition related to induced disease resistance. Biocontrol Sci. Technol. 1992, 2, 349–351. [CrossRef]
25. Pieterse, C.M.J.; Zamioudis, C.; Berendsen, R.L.; Weller, D.M.; Van Wees, S.C.M.; Bakker, P.A.H.M. Induced systemic resistance by beneficial microbes. Annu. Rev. Phytopathol. 2014, 52, 347–375. [CrossRef][PubMed]
26. Cameron, R.K.; Dixon, R.A.; Lamb, C.J. Biologically induced systemic acquired resistance in Arabidopsis thaliana. Plant J. 1994, 5, 715–725. [CrossRef]
27. Uknes, S.; Mauch-Mani, B.; Moyer, M.; Potter, S.; Williams, S.; Dincher, S.; Chandler, D.; Slusarenko, A.; Ward, E.; Ryals, J. Acquired resistance in Arabidopsis. Plant Cell 1992, 4, 645–656. [PubMed]
28. Ward, E.R.; Uknes, S.J.; Williams, S.C.; Dincher, S.S.; Zender, D.L.; Alexander, D.C.; Ahl-Goy, P.; Métraux, J.P.; Ryals, J.A. Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell 1991, 3, 1085–1094. [CrossRef][PubMed]
29. Choudhary, D.K.; Prakash, A.; Johri, B.N. Induced systemic resistance (ISR) in plants: Mechanism of action. Indian J. Microbiol. 2007, 47, 289–297. [CrossRef][PubMed]
30. Van Loon, L.C.; Bakker, P.A.H.M.; Pieterse, C.M.J. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 1998, 36, 453–483. [CrossRef][PubMed]
31. Pieterse, C.M.J.; van Wees, S.C.M.; van Pelt, J.A.; Knoester, M.; Laan, R.; Gerrits, H.; Weisbeek, P.J.; van Loon, L.C. A novel signaling pathway controlling induced systemic resistance in Arabidopsis. Plant Cell 1998, 10, 1571–1580. [CrossRef][PubMed]
32. Walters, D.R.; Ratsep, J.; Havis, N.D. Controlling crop diseases using induced resistance: Challenges for the future. J. Exp. Bot. 2013, 64, 1263–1280. [CrossRef]
33. McIntosh, A.H.; Bateman, G.L.; Chamberlain, K.; Dawson, G.W.; Burrell, M.M. Decreased severity of potato common scab after foliar sprays of 3,5-dichlorophenoxyacetic acid, a possible antipathogenic agent. Annu. Appl. Biol. 1981, 99, 275–281. [CrossRef]
34. McIntosh, A.H.; Bateman, G.L.; Chamberlain, K. Substituted benzoic and picolinic acids as foliar sprays against potato common scab. Annu. Appl. Biol. 1988, 112, 397–401. [CrossRef]
35. Tegg, R.S.; Gill, W.M.; Thompson, H.K.; Davies, N.W.; Ross, J.J.; Wilson, C.R. Auxin-induced resistance to common scab disease of potato linked to inhibition of thaxtomin A toxicity. Plant Dis. 2008, 92, 1321–1328. [CrossRef][PubMed]
36. Meyer, R.; Slater, V.; Dubery, I.A. A phytotoxic protein-lipopolysaccharide complex produced by Verticillium dahliae. Phytochemistry 1994, 35, 1449–1453. [CrossRef]
37. Thompson, H.K.; Tegg, R.S.; Corkrey, R.; Wilson, C.R. Optimal rates of 2,4-dichlorophenoxyacetic acid foliar application for control of common scab in potato. Ann. Appl. Biol. 2014, 165, 293–302. [CrossRef]
38. Bokshi, A.I.; Morris, S.C.; Deverall, B.J. Effects of benzothiadiazole and acetylalicylic acid on ß-1,3-glucanase activity and disease resistance in potato. Plant Pathol. 2003, 52, 22–27. [CrossRef]
39. Bengtsson, T.; Weighill, D.; Proux-Wéra, E.; Levander, F.; Resjo, S.; Burra, D.D.; Moushib, L.I.; Hedley, P.E.; Lijeröth, E.; Jacobson, D.; et al. Proteomics and transcriptomics of the BABA-induced resistance response in potato using a novel functional annotation approach. BMC Genomics 2014, 15, 315. [CrossRef][PubMed]
40. Zimmerli, L.; Métraux, J.-P.; Mauch-Mani, B. β-Aminobutyric acid-induced protection of Arabidopsis against the necrotrophic fungus Botrytis cinerea. Plant Physiol. 2001, 126, 517–523. [CrossRef]

41. Conrath, U.; Beckers, G.J.M.; Langenbach, C.J.G.; Jaskiewicz, M.R. Priming for enhanced defense. Annu. Rev. Phytopathol. 2015, 53, 97–119. [CrossRef]

42. Barth, C.; Jander, G. Arabidopsis myrosinases TGG1 and TGG2 have redundant function in glucosinolate breakdown and insect defense. Plant J. 2006, 46, 549–562. [CrossRef]

43. Bednarek, P.; Osbourn, A. Plant-microbe interactions: Chemical diversity in plant defense. Science 2009, 324, 746–748. [CrossRef] [PubMed]

44. Lu, H.P.; Luo, T.; Fu, H.W.; Wang, L.; Tan, Y.Y.; Huang, J.Z.; Wang, Q.; Ye, G.Y.; Gatehouse, A.M.R.; Lou, Y.G.; et al. Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. Nat. Plants 2018, 4, 338–344. [CrossRef] [PubMed]

45. Miao, Y.; Xu, L.; He, X.; Zhang, L.; Shaban, M.; Zhang, X.; Zhu. Suppression of tryptophan synthase activates cotton immunity by triggering cell death via promoting SA synthesis. Plant J. 2019, 2, 329–345. [CrossRef] [PubMed]

46. Legault, G.S.; Lerat, S.; Nicolas, P.; Beaulieu, C. Tryptophan regulates thaxtomin A and indole-3-acetic acid production in Streptomyces scabiei and modifies its interactions with radish seedlings. Phytopathology 2011, 101, 1045–1051. [CrossRef] [PubMed]

47. Wilhelm, B.T.; Marguerat, S.; Watt, S.; Schubert, F.; Wood, V.; Goodhead, I.; Penkett, C.J.; Rogers, J.; Bähler, J. Dynamic repertoire of a eukaryotic transcriptome surveyed at single-nucleotide resolution. Nature 2008, 453, 1239–1243. [CrossRef]

48. Yan, H.; Shi, H.; Hu, C.; Luo, M.; Xu, C.; Wang, S.; Li, N.; Tang, W.; Zhou, Y.; Wang, C.; et al. Transcriptome differences in response mechanisms to low-nitrogen stress in two wheat varieties. Int. J. Mol. Sci. 2021, 22, 12278. [CrossRef] [PubMed]

49. Luo, Y.; Teng, S.; Yin, H.; Zhang, H.; Tuo, X.; Tran, L.S.P. Transcriptome analysis reveals roles of anthocyanin- and Jasmonic acid-biosynthetic pathways in rapseed in response to high light stress. Int. J. Mol. Sci. 2021, 22, 13027. [CrossRef] [PubMed]

50. Xuan, H.; Huang, Y.; Zhou, L.; Deng, S.; Wang, C.; Xu, J.; Wang, H.; Zhao, J.; Guo, N.; Xing, H. Key soybean seedlings drought-responsive genes and pathways revealed by comparative transcriptome analysis of two cultivars. Int. J. Mol. Sci. 2022, 23, 2893. [CrossRef] [PubMed]

51. Yu, Y.; Yu, M.; Zhang, S.; Song, T.; Zhang, M.; Zhou, H.; Wang, Y.; Xiang, J.; Zhang, X. Transcriptomic identification of wheat AP2/ERF transcription factors and functional characterization of TaERF-6-3A in response to drought and salinity stresses. Int. J. Mol. Sci. 2022, 23, 3272. [CrossRef]

52. Ouertani, R.N.; Arasappan, D.; Abid, G.; Chikha, M.B.; Jardak, R.; Mahmoudi, H.; Mejri, S.; Ghorbel, A.; Ruhlman, T.A.; Jansen, R.K. Transcriptomic analysis of salt-stress-responsive genes in barley roots and leaves. Int. J. Mol. Sci. 2011, 22, 8155. [CrossRef]

53. Yang, J.; Li, L.; Zhang, X.; Wu, S.; Han, X.; Li, X.; Xu, J. Comparative transcriptomics analysis of roots and leaves under Cd stress in Calotropis gigantea L. Int. J. Mol. Sci. 2022, 23, 3329. [CrossRef]

54. Ramírez-Pool, J.A.; Xoconostle-Cázares, B.; Calderón-Pérez, B.; Ibarra-Laclette, E.; Villafrán, E.; Lira-Carmona, R.; Ruiz-Medrano, R. Transcriptomic analysis of the host response to mild and severe CTV strains in naturally infected Citrus sinensis orchards. Int. J. Mol. Sci. 2022, 23, 2435. [CrossRef] [PubMed]

55. Dai, W.B. Research on prevention and control of chinese agricultural ecological environment pollution to ensure food safety. Adv. Mater. Res. 2013, 616–618, 2247–2250.

56. Qiu, D.; Dong, Y.; Zhang, Y.; Li, S.; Shi, F. Plant immunity inducer development and application. Mol. Plant-Microbe. Interact. 2017, 30, 355–360.

57. Ishihara, A.; Hashimoto, Y.; Tanaka, C.; Dubouzet, J.G.; Nakao, T.; Matsuda, F.; Nishioka, T.; Miyagawa, H.; Wakasa, K. The tryptophan pathway is involved in the defense responses of rice against pathogenic infection via serotonin production. Plant J. 2007, 48, 481–495. [CrossRef]

58. Glaowischnig, E. Camalexin. Phytochemistry 2007, 68, 401–406. [CrossRef]

59. Frerrigman, H.; Pišlewski-Bednarek, M.; Sánchez-Vallet, A.; Molina, A.; Glaowischnig, E.; Gigolashvili, T.; Bednarek, P. Regulation of pathogen-triggered tryptophan metabolism in Arabidopsis thaliana by MYB transcription factors and indole glucosinolate conversion products. Mol. Plant 2016, 9, 682–695. [CrossRef]

60. Babcock, M.J.; Eckwall, E.C.; Schottel, J.L. Production and regulation of potato-scab-inducing phytotoxins by Streptomyces scabies. J. Gen. Microbiol. 1993, 139, 1579–1586. [CrossRef]

61. Lauzier, A.; Goyer, C.; Ruest, L.; Brezezinski, R.; Crawford, D.L.; Beaulieu, C. Effect of amino acids on thaxtomin A biosynthesis by Streptomyces scabies. Can. J. Microbiol. 2002, 48, 359–364. [CrossRef] [PubMed]

62. Vallad, G.E.; Goodman, R.M. Systemic acquired resistance and induced systemic resistance in conventional agriculture. Crop Sci. 2004, 44, 1920–1934. [CrossRef]

63. Noutoshi, Y.; Okazaki, M.; Shirasu, K. Imprimatins A and B: Novel plant activators targeting salicylic acid metabolism in Arabidopsis thaliana. Plant Signal. Behav. 2012, 7, 1715–1717. [CrossRef] [PubMed]

64. Song, J.T.; Koo, Y.J.; Seo, H.S.; Kim, M.C.; Choi, Y.D.; Kim, J.H.; Choi, Y.D.; Kim, J.H. Overexpression of AtSGT1, an Arabidopsis salicylic acid glucosyltransferase, leads to increased susceptibility to Pseudomonas syringae. Phytochemistry 2008, 69, 1128–1134. [CrossRef] [PubMed]

65. Friedlich, L.; Lawton, K.; Reuss, W.; Masner, P.; Specker, N.; Rella, M.G.; Meier, B.; Dincher, S.; Staub, T.; Uknes, S.; et al. A benzo[b]thiadiazole derivative induces systemic acquired resistance in tobacco. Plant J. 1996, 10, 61–70. [CrossRef]

66. Dong, X. NPR1, all things considered. Curr. Opin. Plant Biol. 2004, 7, 547–552. [CrossRef]
67. Kunkel, B.N.; Brooks, D.M. Cross-talk between signaling pathways in pathogen defense. *Curr. Opin. Plant Biol.* 2002, 5, 325–331. [CrossRef]

68. Mur, L.A.J.; Kenton, P.; Atzorn, R.; Miersch, O.; Wasternack, C. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiol.* 2006, 140, 249–262. [CrossRef]

69. Sudheeran, P.K.; Sela, N.; Carmeli-Weissberg, M.; Ovadia, R.; Panda, S.; Feygenberg, O.; Maurer, D.; Oren-Shamir, M.; Aharoni, A.; Alkan, N. Induced defense response in red mango fruit against *Colletotrichum gloeosporioides*. *Hortic. Res.* 2021, 8, 17. [CrossRef] [PubMed]

70. van Wees, S.C.M.; de Swart, E.A.M.; van Pelt, J.A.; van Loon, L.C.; Pieterse, C.M.J. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 2000, 97, 8711–8716. [CrossRef] [PubMed]

71. Xue, X.; Geng, T.; Liu, H.; Yang, W.; Zhong, Z.; Zhu, C.; Chu, Z. Foliar application of silicon enhances resistance to *Physphtothora infestans* through the ET/ JA- and NPR1- dependent signaling pathways in potato. *Front. Plant Sci.* 2021, 12, 609870. [CrossRef] [PubMed]

72. Li, J.; Brader, G.; Palva, E.T. The WRKY70 transcription factor: A node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. *Plant Cell* 2004, 16, 319–331. [CrossRef]

73. Boubakri, H.; Wahab, M.A.; Chong, J.; Gertz, C.; Gandonoua, S.; Mliki, A.; Bertsch, C.; Soustre-Gacougnolle, I. Methionine elicits *H*2*O*2 generation and defense gene expression in grapevine and reduces *Plasmodura viticola* infection. *J. Plant Physiol.* 2013, 170, 1561–1568. [CrossRef]

74. Walling, L.L. Induced resistance: From the basic to the applied. *Trends Plant Sci.* 2001, 6, 445–447. [CrossRef]

75. Manome, A.; Kageyama, A.; Kurata, S.; Yokomaku, T.; Koyama, O.; Kanagawa, T.; Tamaki, H.; Tagawa, M.; Kamagata, Y. Quantification of potato common scab pathogens in soil by quantitative competitive PCR with fluorescent quenching-based probes. *Plant Pathol.* 2008, 57, 887–896. [CrossRef]

76. Qu, X.; Wanner, L.A.; Christ, B.J. Using the txtAB operon to quantify pathogenic *Streptomyces* in potato tubers and soil. *Phytopathology* 2008, 98, 405–412. [CrossRef] [PubMed]

77. Adams, M.J.; Lapwood, D.H. Studies on the lenticel development, surface microflora and infection by common scab (*Streptomyces scabiei*) of potato tubers growing in wet and dry soils. *Ann. Appl. Biol.* 1978, 90, 335–343. [CrossRef]

78. Khatri, B.B.; Tegg, R.S.; Brown, P.H.; Wilson, C.R. Temporal association of potato tuber development with susceptibility to common scab and *Streptomyces scabiei*-induced responses in the potato periderm. *Plant Pathol.* 2011, 60, 776–786. [CrossRef]

79. Patch, A.M.; Nones, K.; Kazakov, S.H.; Newell, F.; Wood, S.; Leonard, C.; Holmes, O.; Xu, Q.; Addala, V.; Creaney, J.; et al. Germline and somatic variant identification using BGISEQ-500 and HiSeq X Ten whole genome sequencing. *PLoS ONE* 2018, 13, e0190264. [CrossRef] [PubMed]

80. Zhu, F.Y.; Chen, M.X.; Ye, N.H.; Qiao, W.M.; Gao, B.; Law, W.K.; Tian, Y.; Zhang, D.; Zhang, D.; Liu, T.Y.; et al. Comparative performance of the BGISEQ-500 and Illumina HiSeq4000 sequencing platforms for transcriptome analysis in plants. *Plant Methods* 2018, 14, 69. [CrossRef] [PubMed]

81. Kim, D.; Langmead, B.; Salzberg, S.L. HISAT: A fast spliced aligner with low memory requirements. *Nat. Methods* 2015, 12, 357–360. [CrossRef] [PubMed]

82. Li, S.; Tian, Y.; Wu, K.; Ye, Y.; Yu, J.; Zhang, J.; Liu, Q.; Hu, M.; Li, H.; Tong, Y.; et al. Modulating plant growth–metabolism coordination for sustainable agriculture. *Nature* 2018, 560, 595–600. [CrossRef]

83. Tian, Y.; Fan, M.; Qin, Z.; Lv, H.; Wang, M.; Zhang, Z.; Zhou, W.; Zhao, N.; Li, X.; Han, C.; et al. Hydrogen peroxide positively regulates brassinosteroid signaling through oxidation of the BRASSINAZOLE-RESISTANT1 transcription factor. *Nat. Commun.* 2018, 9, 1063. [CrossRef]

84. Langmead, B.; Salzberg, S.L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 2012, 9, 357–359. [CrossRef] [PubMed]

85. Catalanotto, C.; Pallotta, M.; ReFalo, P.; Sachs, M.S.; Vassylie, L.; Macino, G.; Cognoni, C. Redundancy of the two dicer genes in transgene-induced posttranscriptional gene silencing in *Neurospora crassa*. *Mol. Cell. Biol.* 2004, 24, 2536–2545. [CrossRef]

86. Wang, L.; Feng, Z.; Wang, X.; Wang, X.; Zhang, X. DEGseq: An R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* 2010, 26, 136–138. [CrossRef] [PubMed]

87. Yang, Y.H.; Dudoit, S.; Luu, P.; Lin, D.M.; Peng, V.; Ngai, J.; Speed, T.P. Normalization for cDNA microarray data: A robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Res.* 2002, 30, e15. [CrossRef] [PubMed]

88. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods* 2001, 25, 402–408. [CrossRef]