

Article

Proteomic Insights into *Solanum torvum* Responses to *Verticillium dahliae* Infection and Functional Validation of *StoAOS1*

Yu Zhang ¹, Lei Shen ², Xu Yang ^{2,*}  and Liangjun Li ^{2,*}

¹ College of Plant Protection, Yangzhou University, 88 University South Road, Yangzhou 225009, China; d160074@yzu.edu.cn

² College of Horticulture and Landscape Architecture, Yangzhou University, 88 University South Road, Yangzhou 225009, China; shenlei07@yzu.edu.cn

* Correspondence: yangxu@yzu.edu.cn (X.Y.); ljli@yzu.edu.cn (L.L.)

Highlights

What are the main findings?

- Through iTRAQ-based quantitative proteomic screening, a critical gene associated with Verticillium wilt resistance was identified in *Solanum torvum*.
- Its resistance function was subsequently verified using virus-induced gene silencing (VIGS) and transgenic overexpression assays.

What are the implications of the main findings?

- These findings suggest that *StoAOS1* positively regulates Verticillium wilt resistance in *Solanum torvum*, likely through a JA-dependent defense pathway.
- This study provides a candidate gene resource for improving Verticillium wilt resistance in eggplant.

Abstract

Eggplant (*Solanum melongena* L.) is an important solanaceous vegetable crop cultivated worldwide. Verticillium wilt, caused by *Verticillium dahliae*, severely restricts eggplant growth and yield, while most cultivated eggplant varieties show only limited resistance to this disease. In contrast, *Solanum torvum*, a wild relative of eggplant, exhibits strong natural resistance to Verticillium wilt. The molecular mechanisms underlying the contrasting responses of cultivated eggplant and *S. torvum* to *V. dahliae* infection remain poorly understood. In this study, high-throughput iTRAQ-based quantitative proteomics was used to analyze root protein profiles of *S. torvum* after *V. dahliae* inoculation. A differentially expressed protein, *StoAOS1*, was identified in *S. torvum* and was strongly induced by *V. dahliae* infection. *StoAOS1* encodes a key enzyme in the jasmonic acid (JA) biosynthetic pathway. Further analysis showed that exogenous methyl jasmonate (MeJA) treatment markedly induced *StoAOS1* expression. Virus-induced gene silencing of *StoAOS1* significantly compromised Verticillium wilt resistance in *S. torvum*, accompanied by reduced acid-insoluble lignin accumulation and decreased transcript levels of the defense-related marker genes *StoPDF1.2*, *StoVSP2*, and *StoThi2.1*. Conversely, transient overexpression of *StoAOS1* in *Nicotiana benthamiana* enhanced resistance to *V. dahliae*. Together, these findings suggest that *StoAOS1* positively regulates Verticillium wilt resistance in *S. torvum*, likely through a JA-dependent defense pathway.



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1. Introduction

Eggplant (*Solanum melongena* L.) is a solanaceous vegetable crop of considerable economic significance, and its cultivation now extends to diverse regions across the globe. One of the most serious biotic constraints affecting eggplant production is Verticillium wilt, a disease caused predominantly by the soil-inhabiting vascular pathogen *Verticillium dahliae* Kleb. [1,2]. Upon entering the root system, the fungus colonizes the xylem vessels and disperses systemically via the transpiration stream, eventually giving rise to vascular discoloration, foliar chlorosis, wilting, defoliation, and, under conditions of severe infection, eventual plant death [1,2]. Because *V. dahliae* produces microsclerotia that can persist in soil for extended periods and maintains an unusually broad host range, the pathogen often remains viable within infested fields for many years, and once it becomes established, Verticillium wilt proves exceptionally difficult to manage through conventional control measures [1,2]. Relative to cultivated eggplants, which generally exhibit only limited resistance to this disease, its wild relative *Solanum torvum* displays strong innate resistance and has consequently been employed widely as a resistant rootstock or as donor germplasm in eggplant improvement programs [3,4]. A detailed understanding of the molecular basis underlying this resistance in *S. torvum* is therefore of substantial importance for the eventual development of resistant eggplant cultivars through breeding.

At present, the precise mechanisms by which eggplant mounts resistance against Verticillium wilt remain incompletely resolved. Accumulated evidence suggests that the resistant phenotype is underpinned by both preformed physical or chemical barriers and inducible defense responses that are activated following pathogen challenge. Constitutively present antimicrobial metabolites, including alkaloids and various phenolic compounds, may serve to restrict fungal proliferation during the earliest stages of root invasion, whereas induced reactions—among them cell-wall reinforcement, lignin deposition, maintenance of reactive oxygen species (ROS) homeostasis, accumulation of pathogenesis-related proteins, and hormone-mediated signaling cascades—collectively act to contain the pathogen within the vascular tissue and limit its systemic spread [4,5]. Several genes derived from *S. torvum* have previously been implicated in resistance to *V. dahliae*, including *StoL13a* [6], *StoNPR1* [7], *StoCYP77A2* [8], *StoWRKY6* [9], *StoMYB41* [10], and *StoERF109* [11]. Nevertheless, these genetic determinants account for only a fraction of the overall resistance phenotype, and the key regulatory proteins, along with the downstream defense networks they orchestrate, remain to be more fully characterized if we are to understand how *S. torvum* achieves its notably high level of resistance.

Phytohormone signaling plays a central regulatory role in plant immunity, and the pathways governed by salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are recognized as major nodes through which defense responses are modulated [12,13]. Although SA has traditionally been linked to defense against biotrophic pathogens, whereas JA and ET are more commonly associated with resistance to necrotrophic pathogens and herbivorous insects, the response to vascular pathogens such as *V. dahliae* tends to be considerably more complex and may well require the coordinated engagement of multiple hormonal pathways [5,12,13]. In this context, JA signaling has increasingly drawn attention as a critical component of the plant's defensive arsenal against *V. dahliae*, and its contribution is now the subject of growing investigative interest. In cotton, for example, modulation of JA biosyn-

thesis and signaling affects host resistance to Verticillium wilt, suggesting that JA-associated pathways may contribute to the regulation of vascular disease resistance [14–16].

Proteome-level analysis provides a powerful approach for identifying defense-associated proteins that may not be fully predicted from transcript abundance alone. Isobaric tags for relative and absolute quantification (iTRAQ)-based quantitative proteomics enables comparative analysis of protein accumulation across multiple biological samples with high sensitivity and reproducibility, and has been widely used to investigate plant responses to biotic stress [17–19]. In plant–pathogen interaction studies, iTRAQ-based proteomics has been useful for identifying proteins involved in hormone signaling, cell-wall modification, secondary metabolism, oxidative stress responses, and disease resistance. In *Gossypium thurberi* roots challenged with *V. dahliae*, iTRAQ-based proteomic profiling has previously uncovered a substantial number of differentially accumulated proteins that map to SA signaling, phenylpropanoid metabolism, lignin biosynthetic routes, and general defense responses [19]. By contrast, comparable proteomic data for eggplant and its wild relatives in response to this vascular pathogen remain scarce. Given this gap, quantitative proteomic analysis using the iTRAQ platform may offer a productive avenue for pinpointing candidate regulatory factors that contribute to the pronounced Verticillium wilt resistance observed in *S. torvum*.

Within the suite of hormone pathways implicated in defense, the JA biosynthetic route merits particular attention in the context of stress-triggered immune activation. A key enzymatic component of this route is allene oxide synthase (AOS), which belongs to the CYP74 branch of the cytochrome P450 superfamily and catalyzes a critical step in the octadecanoid pathway leading to JA production [20,21]. Its biologically active derivatives. AOS-encoding genes are broadly conserved across the plant kingdom and are often upregulated in response to mechanical wounding, insect herbivory, exogenous JA application, and pathogen attack [22,23]. Loss-of-function studies have demonstrated that disrupting AOS compromises wound-triggered JA accumulation in *Arabidopsis* [24], whereas inducible overexpression of the rice (*Oryza sativa*) *OsAOS2* gene leads to elevated endogenous JA levels, upregulation of pathogenesis-related transcripts, and improved resistance against fungal infection [25]. In the same species, both *OsAOS1* and *OsAOS2* have been shown to participate in herbivore-induced JA biosynthesis and associated defensive responses [26]. Despite these findings, whether AOS genes play a comparable role in *S. torvum* resistance to Verticillium wilt has not yet been experimentally addressed.

Current studies on disease resistance in eggplant have rarely investigated hormone responses to *V. dahliae* infection, and experimental molecular evidence remains limited. To explore the molecular determinants of Verticillium wilt resistance in *S. torvum*, we established an infection system using this highly resistant wild relative and applied iTRAQ-based quantitative proteomic profiling to root tissues collected following *V. dahliae* inoculation. The resulting dataset of differentially accumulated proteins was then subjected to a series of downstream analyses, including functional categorization, pathway overrepresentation testing, RT-qPCR-based transcript validation, and construction of defense-related interaction networks. From the proteomic screening, proteins involved in jasmonic acid biosynthesis and signal transduction pathways were significantly enriched. Among these proteins, AOS exhibited the most pronounced expression change. We therefore hypothesize that this pathway may mediate the resistance response of *Solanum torvum* against Verticillium wilt, and the AOS gene may act as a key regulatory gene. Finally, a *V. dahliae*-responsive AOS homolog, designated *StoAOS1*, was identified in *S. torvum*. The expression behavior of this gene was subsequently examined under both pathogen challenge and phytohormone treatments, and its physiological relevance was assessed through virus-induced gene silencing in *S. torvum* alongside transient heterologous overexpression in *Nicotiana*

benthamiana. Collectively, our findings indicate that *StoAOS1* acts as a positive modulator of Verticillium wilt resistance, most probably via a JA-linked defense pathway, and thus represents a promising candidate gene for future efforts aimed at enhancing Verticillium wilt tolerance in cultivated eggplant.

2. Materials and Methods

2.1. Plant Cultivation and Growth Condition

Seeds of *S. torvum*, eggplant, and *N. benthamiana* were germinated and grown following protocols described in earlier reports [10,11,27]. Seedlings of *S. torvum* and *N. benthamiana* were maintained in a growth chamber under controlled conditions set to 25 °C, 60% relative humidity, and a 16 h light/8 h dark photoperiod.

2.2. *V. dahliae* Cultivation and Inoculation

The procedures for culturing *V. dahliae* and preparing inoculum were adopted from previously established methods [11,28]. Fungal stocks were first revived on solid potato dextrose agar (PDA) at 25 °C. Agar plugs containing mycelia were then transferred to liquid Czapek Dox Broth Medium (Cat# MM1010-250G) and incubated on a rotary shaker at 23 °C and 200 rpm for 5 days. Spores were harvested by passing the liquid culture through eight layers of sterile gauze, and the resulting suspension was adjusted to a final concentration of 1×10^7 spores/mL.

For inoculation, *S. torvum* seedlings at the four-true-leaf stage, selected for uniformity of growth, were carefully uprooted from the substrate and their roots were rinsed under running tap water. The cleaned root systems were then submerged in the *V. dahliae* spore suspension for 1 h, after which the seedlings were transferred to Hoagland nutrient solution for a 2-day incubation period. Root tissues from inoculated plants were harvested and immediately frozen in liquid nitrogen for subsequent RNA extraction. For phenotype evaluation, the root systems of *S. torvum* seedlings were gently wounded before being irrigated with 15 mL of the spore suspension. Following inoculation, the treated plants were returned to the growth chamber maintained at 25 °C, 60% relative humidity, and a 16 h light/8 h dark cycle.

2.3. Protein Extraction

Total protein isolation from *S. torvum* tissues was carried out using a modified phenol extraction protocol established in a previous study [29]. Fresh frozen tissue samples were thoroughly ground into fine powder with liquid nitrogen, and crude proteins were precipitated using pre-chilled 10% trichloroacetic acid (TCA)-acetone solution at −20 °C for 60 min. After centrifugation, the resulting pellets were rinsed with ice-cold acetone and incubated again at low temperature to eliminate soluble impurities. Dried protein precipitates were re-suspended in phenol extraction buffer and fully homogenized, followed by incubation with pH 7.5 Tris-HCl-balanced phenol solution. After stratification via centrifugation, the upper phenolic phase containing target proteins was collected. Proteins were further precipitated with pre-cooled 0.1 M ammonium acetate-methanol mixture at −20 °C for 1 h. Residual organic solvents were removed through sequential washing with ice-cold methanol and acetone. Purified protein powder was dissolved in lysis buffer, and high-speed centrifugation was applied to obtain clear supernatant. The final total protein samples were stored at −80 °C for subsequent quantification and quantitative proteomic analysis.

2.4. Protein Quality Validation

Protein concentration was quantified via the Bradford colorimetric assay [30]. This method relies on the specific binding interaction between Coomassie Brilliant Blue dye

and protein molecules. The free dye exhibits maximum absorbance at 488 nm, while the protein–dye complex produces a stable absorption peak at 595 nm, with absorbance values positively correlated with protein concentration. The binding reaction reaches equilibrium rapidly within 2 min and remains stable for 1 h at room temperature. For protein integrity assessment, 10 µg of protein from each sample was separated by 12% SDS-PAGE. Gel fixation, staining and decolorization procedures were performed following the optimized protocol reported by [31]. After thorough rinsing to remove background staining, gels were scanned with an ImageScanner (Uppsala, Sweden) at 300 dpi grayscale resolution to evaluate protein integrity, purity and sample consistency.

2.5. Protein Reduction, Alkylation and Enzymatic Digestion

Protein digestion was performed using the filter-aided sample preparation (FASP) approach to improve digestion efficiency. A total of 100 µg quantified protein per sample was precipitated with pre-chilled acetone and lyophilized under vacuum conditions. The lyophilized protein pellets were fully dissolved in iTRAQ dissolution buffer, subjected to reduction treatment with specific reagents at 60 °C for 1 h, and then alkylated with cysteine-blocking reagents at room temperature for 10 min to prevent disulfide bond reformation. The treated protein solution was transferred to a 10 kDa ultrafiltration tube for repeated desalting and buffer replacement via high-speed centrifugation. After complete buffer exchange, sequencing-grade trypsin was added for overnight enzymatic hydrolysis at 37 °C. Digested peptide fragments were harvested by centrifugation, and the ultrafiltration membrane was eluted repeatedly to recover residual peptides. All collected eluates were combined uniformly for subsequent labeling procedures.

2.6. iTRAQ Peptide Labeling

Peptide labeling was conducted in strict accordance with the official specifications of the iTRAQ reagent kit. Frozen reagents were restored to room temperature and centrifuged briefly for complete collection before being fully dissolved in isopropanol. A total of 50 µL peptide solution containing 100 µg digested products was mixed with corresponding iTRAQ labeling reagents and incubated at ambient temperature for 2 h to complete stable covalent modification. Ultrapure water was added to terminate the labeling reaction. All individually labeled peptide samples were pooled, vortexed thoroughly and centrifuged. The mixed sample was finally vacuum-lyophilized to prepare for chromatographic separation.

2.7. Strong Cation Exchange (SCX) Chromatography Fractionation

Lyophilized peptide mixtures were redissolved in SCX Buffer A and fractionated using an Agilent 1200 HPLC (Santa Clara, CA, USA) system equipped with a Michrom chromatographic column. Dual ultraviolet detection wavelengths of 215 nm and 280 nm were adopted, with a constant flow rate of 0.3 mL/min throughout elution. Peptide fractions were collected based on programmed elution time: one fraction was collected within the first 5 min, one fraction was acquired every 4 min from 6 min to 44 min, and the final eluent from 45 min to 50 min was collected as a single fraction, generating 12 independent fractions in total. All fractionated samples were fully lyophilized for subsequent LC-MS/MS detection.

2.8. LC-MS/MS Analysis Based on TripleTOF Mass Spectrometry

Lyophilized SCX fraction samples were redissolved in Nano-RPLC Buffer A for mass spectrometry detection. Peptide separation was implemented on an Eksigent nanoLC-Ultra™ 2D system coupled with a TripleTOF 5600 mass spectrometer (AB SCIEX, Framingham, MA, USA). Prepared samples were loaded onto a C18 pre-column at a flow rate of 2 µL/min and desalted continuously for 10 min. A linear gradient elution program was

applied on the C18 analytical column, where the proportion of mobile phase B was elevated from 5% to 35% within 70 min to achieve effective peptide separation.

Mass spectral data were acquired in information-dependent analysis (IDA) mode with a NanoSpray III ion source. Key instrumental parameters were set as follows: spray voltage at 2.5 kV, curtain gas pressure at 30 PSI, nebulizer gas pressure at 5 PSI, and ion source heating temperature at 150 °C. The full-scan TOF-MS acquisition time was set to 250 ms per spectrum. In each fixed 2.5 s scanning cycle, up to 35 secondary spectra were automatically collected from precursor ions with 2+ to 5+ charge states and signal intensity over 150 counts per second. A dynamic exclusion duration of 18 s was configured to avoid repeated scanning of identical peptide ions, which matched the chromatographic peak characteristics and optimized overall detection coverage.

2.9. Database Search and Protein Identification

All raw MS/MS spectra were processed and analyzed using Protein Pilot v5.0 (AB SCIEX, Framingham, MA, USA) equipped with the Paragon algorithm. Spectral matching was performed against a custom *Solanum torvum* protein database constructed from self-built transcriptome data. A decoy database combined with the PSPEP module was utilized to calculate the false discovery rate (FDR) and eliminate false positive matches. Protein identification and relative quantification were accomplished by comparing experimental tandem mass spectra with theoretical spectral profiles predicted from the transcriptome-derived database.

2.10. Exogenous MeJA Treatment

For exogenous MeJA treatment, *S. torvum* seedlings at the 4–6 leaf stage were sprayed with 100 µM MeJA every 6 h. Treated leaves were harvested at 0, 1, 3, 6, and 12 h after treatment.

2.11. Plant Total RNA Extraction, Synthesis of cDNA, and RT-qPCR

Total RNA extraction, cDNA synthesis, and RT-qPCR were carried out according to protocols detailed in previous work [10,11]. Tissue samples were pulverized to a fine powder using a high-throughput plant tissue grinder in the presence of liquid nitrogen. The ground material was lysed with TRIpure Reagent lysis buffer (RN0102, Aidlab Biotechnologies Co., Ltd., Beijing, China), and phase separation was achieved by adding chloroform. RNA was precipitated with isopropanol, washed with 75% ethanol, and then collected by centrifugation. Messenger RNA from the total RNA pool was reverse-transcribed into cDNA using the HiScript III RT SuperMix for qPCR (+gDNA wiper) (R323-01, Vazyme, Nanjing, China).

For quantification of target gene transcripts, RT-qPCR was performed with ChamQ Universal SYBR qPCR Master Mix (Q711-02, Vazyme, Nanjing, China) in a reaction mixture containing 1 µL cDNA, 0.2 µL each of forward and reverse primers, 5 µL SYBR mix, and 3.6 µL ddH₂O. The reaction procedure was as follows: pre-denaturation at 95 °C for 30 s; cyclic reaction at 95 °C for 10 s and 60 °C for 30 s, for 40 cycles; and melting curve analysis at 95 °C for 15 s, 60 °C for 60 s, and 95 °C for 15 s. *StoActin* was used as the reference gene to normalize the transcript levels of target genes. Three biological replicates were used. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression levels of target genes [29]. The primer sequences are listed in Table S4.

2.12. Vector Construction

For the VIGS assay, a 300 bp specific DNA fragment of *StoAOS1* was amplified by PCR using specific primer pairs and then cloned into the *EcoR* I site of the destination vector TRV2, which was digested with the DNA restriction endonuclease QuickCut™ *EcoR*

I (1611, Takara, Kyoto, Japan), to generate the recombinant vector TRV2:*StoAOS1*. For overexpression of *StoAOS1* in *N. benthamiana*, the full-length coding sequence of *StoAOS1* without the stop codon was amplified by PCR and cloned into the *Kpn* I and *Xba* I sites of the pCambia1300-GFP vector digested with the DNA restriction endonucleases QuickCut™ *Kpn* I (1618, Takara, Kyoto, Japan) and QuickCut™ *Xba* I (1634, Takara, Kyoto, Japan), generating the 35S:*StoAOS1*-GFP vector.

2.13. *Agrobacterium Tumefaciens* Cultivation and VIGS Assay

TRV1, TRV2:00, TRV2:*StoPDS*, or TRV2:*StoAOS1* was transformed into *A. tumefaciens* strain GV3101 competent cells and then cultured in liquid Luria–Bertani medium containing 50 µg/mL kanamycin and 50 µg/mL rifampicin at 28 °C and 200 rpm for 8 h on a shaker. GV3101 cells were harvested by centrifugation at 25 °C and 8000 rpm for 10 min and then resuspended in infiltration buffer (10 mM MES, 10 mM MgCl₂, and 200 µM acetosyringone, pH 5.4) to an optical density at 600 nm (OD₆₀₀) of 0.8. Cells carrying the TRV1 plasmid were mixed with cells harboring the TRV2:00, TRV2:*StoPDS*, or TRV2:*StoAOS1* construct at a 1:1 ratio. The mixtures were gently incubated on a shaker at 28 °C and 60 rpm for 3 h and then infiltrated into the cotyledons of *S. torvum* seedlings. The infiltrated seedlings were placed in an illuminated incubator at 20 °C in the dark for 48 h. Following dark treatment, seedlings were placed back into the growth chamber under the same environmental conditions (25 °C, 60% relative humidity, 16 h light/8 h dark) and allowed to grow for approximately 3 weeks.

2.14. Measurement of Fungal Biomass

Fungal biomass in plant tissues was assessed using a previously described method [28]. *StoAOS1*-silenced and control seedlings that had been inoculated with *V. dahliae* were sampled at 0, 1, 3, and 7 days post-inoculation, and total DNA was extracted from the harvested tissues. The relative abundance of the *V. dahliae* ITS gene, serving as an indicator of fungal colonization, was measured by RT-qPCR and normalized against the *S. torvum StoActin* reference gene.

2.15. Fungal Recovery Assay

A fungal re-isolation assay was performed following the protocol of a prior study [10]. At 7 days after inoculation, stem segments from both *StoAOS1*-silenced and control plants were collected, surface-sterilized, and placed onto the surface of solid PDA medium. The plates were then incubated in a biochemical incubator at 25 °C for 5 days to allow fungal outgrowth.

2.16. Detection of Acid-Insoluble Lignin Content

Acid-insoluble lignin content was determined according to procedures described in earlier work [30,31].

2.17. Genetic Transformation of *N. benthamiana*

Genetic transformation of *N. benthamiana* was carried out as described previously [32].

2.18. Data Analysis

All statistical analyses were performed using SPSS 26.0 software (IBM, Armonk, NY, USA). Prior to statistical comparison, the Shapiro–Wilk test was used to verify the normal distribution of experimental data, and Levene’s test was applied to evaluate the homogeneity of variances. Student’s *t*-test was adopted for pairwise comparisons between two groups only when the data satisfied both normality and homogeneity of variance. All

quantitative data are presented as the mean $\pm$ standard deviation (SD) from at least three independent biological replicates.

3. Results

3.1. Proteomic Profiling of *S. torvum* in Response to *V. dahliae* Infection

To characterize the resistance phenotype of *S. torvum* against *V. dahliae*, seedlings of this wild relative and the susceptible cultivar YZ were inoculated with a preparation containing both fungal spores and crude toxin. By 7 days post-inoculation (dpi), the YZ plants had developed pronounced Verticillium wilt symptoms, including chlorosis and wilting of leaves, whereas the *S. torvum* plants remained largely symptom-free. At 14 dpi, the YZ plants had undergone complete wilting and mortality, with visible mycelial growth on the stem surface; in marked contrast, the *S. torvum* plants not only survived but also continued to produce newly emerging leaves (Figure 1a). Correspondingly, the disease index recorded for YZ at 7 dpi was substantially higher than that for *S. torvum*, with values of 49.2 and 16.1, respectively. By 14 dpi, this index had risen in both genotypes, reaching 98.6 in YZ and 43.5 in *S. torvum* (Figure 1b). These observations confirm that *S. torvum* possesses markedly stronger Verticillium wilt resistance than the susceptible YZ line.

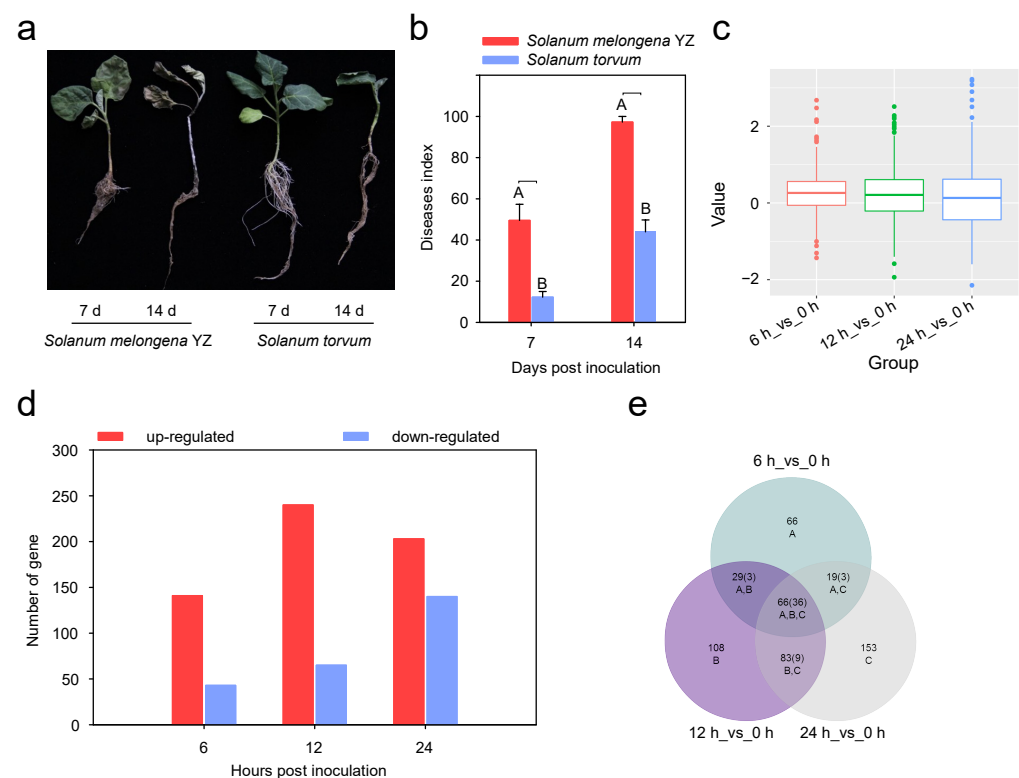


Figure 1. Phenotypic identification and proteomic analysis of *S. torvum* roots. (a) Phenotypic assessment of *S. melongena* YZ and *S. torvum* inoculated with *V. dahliae* at 7 and 14 days. (b) Disease index of *S. melongena* and *S. torvum* inoculated with *V. dahliae* at 7 and 14 days. All experiments were performed with three biological replicates, and the results are presented as means $\pm$ standard deviation. Statistical analyses were conducted using Student's *t*-test, and different uppercase letters indicate extremely significant differences between groups ($p < 0.01$). (c) Principal component analysis of proteome sequencing data. (d) Analysis of the number of DAPs in roots of *S. torvum* inoculated with *V. dahliae* at 6, 12, and 24 h post-inoculation. (e) Venn analysis of DAPs in roots of *S. torvum* inoculated with *V. dahliae* at 6, 12, and 24 h post-inoculation.

To explore the molecular events underlying this resistance at the protein level, iTRAQ-based quantitative proteomic profiling was conducted on root tissues harvested from

S. torvum following *V. dahliae* inoculation. Principal component analysis (PCA) of the proteomic dataset revealed distinct clustering patterns among samples, indicating that the data were reproducible and of high quality (Figure 1c). Applying selection criteria of an Unused score greater than 1.3 and at least two matched peptides per protein, we identified a total of 2374 high-confidence proteins. Using a fold-change threshold of ≥ 1.5 to define differential abundance, we detected 832 differentially abundant proteins (DAPs) across the three post-inoculation time points examined. At 6 h post-inoculation (hpi), 184 DAPs were identified, of which 141 showed up-regulation and 43 showed down-regulation. At 12 hpi, the number of DAPs increased to 305, with 240 up-regulated and 65 down-regulated. At 24 hpi, 343 DAPs were detected, comprising 203 up-regulated and 140 down-regulated proteins (Figure 1d). The number of DAPs increased progressively with infection time, and up-regulated proteins accounted for the majority of DAPs at each time point, suggesting that *V. dahliae* infection induced broad activation of protein accumulation in *S. torvum* roots.

A Venn diagram was used to compare the DAPs identified at different time points after inoculation. In total, 95 DAPs were shared between 6 and 12 hpi, 85 were shared between 6 and 24 hpi, and 149 were shared between 12 and 24 hpi. In addition, 66 DAPs were common to all three time points (Figure 1e). The largest overlap was observed between 12 and 24 hpi, suggesting that partially shared defense-related metabolic pathways may be activated during the middle and later stages of the early infection response in *S. torvum*.

3.2. GO and KEGG Enrichment Analyses of DAPs

Functional annotation of the DAPs identified in *S. torvum* roots after *V. dahliae* inoculation was pursued through Gene Ontology (GO) enrichment analysis. The significantly enriched GO terms fell into the three standard categories of biological process, cellular component, and molecular function. Across the three time points, the numbers of significantly enriched GO terms were 489 at 6 hpi, 814 at 12 hpi, and 924 at 24 hpi, indicating that an expanding array of functional categories was recruited into the defense response as infection progressed. Nevertheless, the specific categories that showed significant enrichment, as well as the number of terms within each category, varied considerably among the three time points. Nine categories were consistently enriched across all three time points, including “catabolic process,” “cellular catabolic process,” “response to stress,” and “small molecule metabolic process.” In contrast, other categories—such as “epoxygenase P450 pathway,” “ATP metabolic process,” and “single-organism catabolic process”—were significantly enriched only at specific stages of infection. The top 20 significantly enriched GO terms at 6, 12, and 24 hpi were further analyzed (Figure 2a, Table S2). These enriched terms suggested that *V. dahliae* infection induced broad changes in defense-related biological processes in *S. torvum* roots.

A large number of DAPs were associated with phytohormone biosynthesis and signal transduction. These included 18 proteins related to salicylic acid biosynthesis and signaling, 27 proteins associated with JA biosynthesis and signaling, and 15 proteins involved in ethylene biosynthesis and signaling. In addition, two proteins related to brassinosteroid biosynthesis were identified. These results indicate that multiple hormone pathways may participate in the defense response of *S. torvum* against *V. dahliae*. Proteins associated with structural defense and secondary metabolism were also highly represented among the DAPs. In particular, two proteins involved in chitin biosynthesis, 11 proteins related to lignin biosynthesis, one protein associated with phytoalexin biosynthesis, and 11 proteins involved in cell-wall biosynthesis were identified. Several DAPs were also annotated as being involved in responses to fungal stimulus, regulation of programmed cell death, and ROS metabolism (Table S1). These findings suggest that cell-wall reinforcement, secondary metabolite production, oxidative stress regulation, and hormone-mediated signaling may act together to support Verticillium wilt resistance in *S. torvum*.

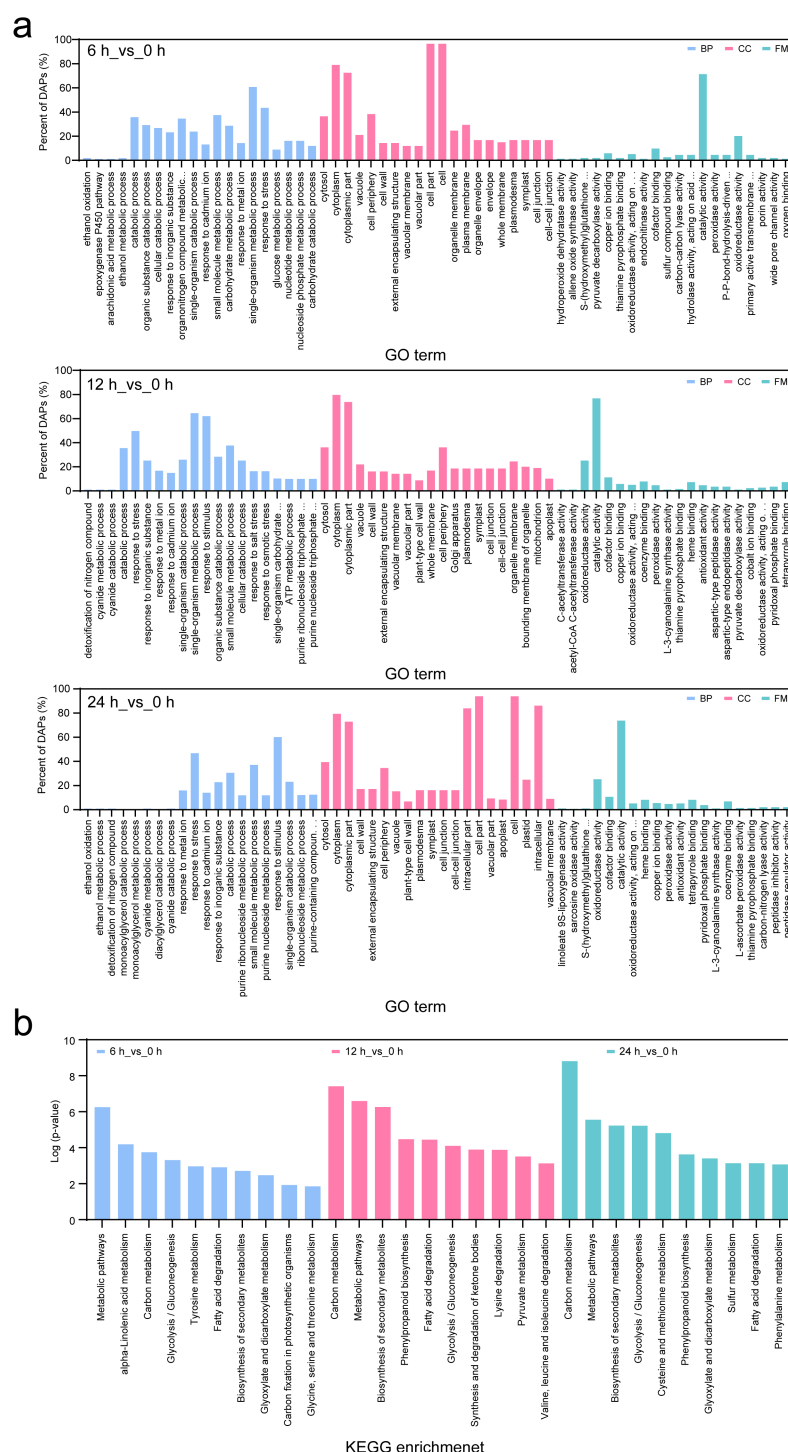


Figure 2. GO and KEGG enrichment analyses of DAPs. **(a)** GO analysis of DAPs in roots of *S. torvum* inoculated with *V. dahliae* at 6, 12, and 24 h post-inoculation. **(b)** KEGG enrichment analysis of DAPs in roots of *S. torvum* inoculated with *V. dahliae* at 6, 12, and 24 h post-inoculation.

KEGG pathway enrichment analysis was subsequently performed to gain further insight into the metabolic and signaling routes represented by the DAPs. At 6 hpi, the 184 DAPs were distributed across 73 KEGG pathways, with eight of these showing significant enrichment. At 12 hpi, 305 DAPs mapped to 88 pathways, among which 14 were significantly enriched. At 24 hpi, 343 DAPs were assigned to 87 pathways, with 19 pathways reaching statistical significance (Figure 2b, Table S3). The progressive increase in the number of significantly enriched pathways over the course of infection suggests that the

proteomic response in *S. torvum* roots became increasingly multifaceted during the early phase of *V. dahliae* colonization. Among the pathways identified, phenylpropanoid biosynthesis stood out as being strongly activated following pathogen inoculation (Figure 2b). This particular pathway is intimately connected with the deposition of physical and chemical barriers that impede pathogen ingress and also contributes to both local and systemic signaling components of the defense response. A total of 22 DAPs related to phenylpropanoid metabolism showed significant changes in abundance. Among them, PER12 was up-regulated at multiple time points after *V. dahliae* inoculation, suggesting an enhanced peroxidase-mediated oxidative response. Lignin biosynthesis-related proteins, such as CYP73A5, were also differentially accumulated. These results suggest that peroxidase activity and lignin deposition may contribute to the resistance response of *S. torvum* to *V. dahliae* infection.

3.3. Identification and Expression Validation of Candidate P450 Family Genes

Further analysis of the DAP dataset identified 10 cytochrome P450 family proteins that were significantly differentially accumulated after *V. dahliae* inoculation. Functional annotation indicated that these P450 proteins may be involved in multiple defense-related processes. Among them, StoCYP82C4 and StoCYP19-2 were associated with plant disease resistance; StoCYP72A7, StoCYP72A9, and StoCYP74A/StoAOS1 were related to hormone responses; StoCYP84A1, StoCYP73A5, and StoCYP707A3 were associated with lignin biosynthesis; StoCYP71B34 was linked to stress responses; and StoCYP51G1 was related to sterol biosynthesis. To validate the proteomic results, the transcript levels of the 10 candidate P450 genes were examined by RT-qPCR after *V. dahliae* inoculation. Among these genes, eight showed expression patterns that were consistent between transcript abundance and protein accumulation, and the highest relative expression was observed for StoCYP74A/StoAOS1 (Figure S1), supporting the reliability of the proteomic data. One gene could not be directly compared because it was specifically detected in eggplant, whereas another gene showed an inconsistent pattern between transcript and protein levels. This discrepancy may reflect post-transcriptional regulation, differences in protein stability, or a temporal delay between mRNA accumulation and protein abundance. Overall, these results suggest that P450 gene family members are broadly involved in the response of *S. torvum* to *V. dahliae* infection and may contribute to Verticillium wilt resistance through hormone regulation, lignin biosynthesis, stress responses, and secondary metabolism.

3.4. Sequence Analysis of StoAOS1

The coding sequence of StoAOS1 was determined to be 1533 bp in length, encoding a predicted protein of 510 amino acids. To examine the domain architecture of this protein, the deduced amino acid sequence was submitted to SMART analysis (<https://smart.embl.de/>, accessed on 12 May 2026). The results revealed the presence of a canonical cytochrome P450 conserved domain within StoAOS1 (Figure 3a,b), placing this protein within the CYP74A/AOS subfamily of the cytochrome P450 superfamily. To further assess the sequence characteristics of StoAOS1, multiple sequence alignment was carried out using AOS protein sequences derived from a range of plant species. This alignment showed that StoAOS1 exhibits the highest degree of sequence identity with StAOS1 from potato (*Solanum tuberosum*) (Figure 3b). A phylogenetic tree was then constructed to evaluate the evolutionary placement of StoAOS1 relative to its homologs from other selected species. The resulting phylogeny indicated that StoAOS1 is most closely related to potato StAOS1 and tomato (*Solanum lycopersicum*) SLAOS3 (Figure 3c), pointing to a close evolutionary relationship among AOS proteins from solanaceous taxa.

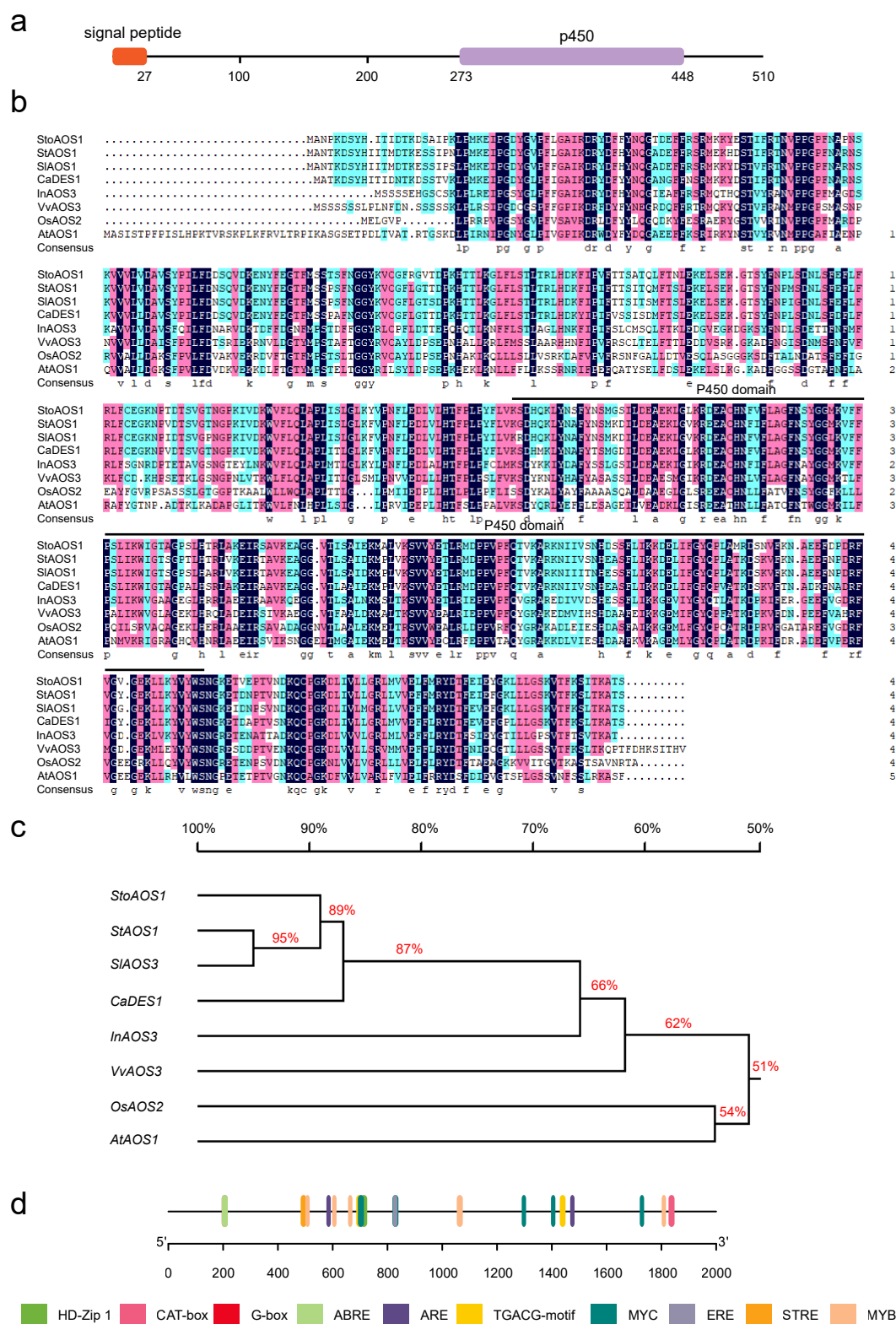


Figure 3. Sequence analysis of *StoAOS1*. (a) Conserved domain analysis of *StoAOS1*. (b) Multiple alignment of the amino acid sequence of *StoAOS1* with homologous proteins, including *StAOS1* in *S. tuberosum*, *SIAOS1* in *S. lycopersicum*, *CaDES1* in pepper, *InAOS3* in *Ipomoea nil*, *VvAOS3* in *Vitis vinifera*, *OsAOS2* in rice, and *AtAOS1* in *A. thaliana*. For identity shading, green corresponds to 50–75%, red to 75–100%, and black to 100% identity. (c) Phylogenetic tree analysis. (d) Prediction of *cis*-elements in the *StoAOS1* promoter. The main *cis*-elements in the *StoAOS1* promoter included HD-Zip 1, CAT-box, G-box, ABRE, ARE, TGACG-motif, MYC, ERE, STRE, and MYB.

In addition, the 2000 bp promoter region upstream of the *StoAOS1* coding sequence was subjected to *cis*-acting element prediction using the PlantCARE database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>, accessed on 15 June 2026). In addition to elements responsive to light, the promoter was found to harbor multiple *cis*-elements associated with phytohormone signaling, transcription factor binding, stress responses, and various aspects of plant growth and development. Among the hormone-responsive elements identified were ABRE, ERE, and TGACG-motif elements. Transcription factor-binding motifs included G-box, MYB, and MYC elements, while stress-responsive motifs such as ARE and STRE were also detected. Growth- and development-related elements, such as HD-Zip 1 and CAT-box, were present in the promoter region (Figure 3d). These results suggest that *StoAOS1* may be regulated by multiple endogenous and environmental signals, particularly hormone- and stress-related pathways.

3.5. MeJA Induces the Expression of *StoAOS1* in *S. torvum*

Previous studies have shown that AOS-mediated JA biosynthesis contributes to plant defense against fungal pathogens [25]. To determine whether *StoAOS1* is involved in the JA-mediated signaling pathway in *S. torvum*, RT-qPCR was performed to examine the transcript levels of *StoAOS1* in leaves after exogenous MeJA treatment. Samples were collected at 0, 1, 3, 6, and 12 h after treatment. The results showed that MeJA treatment significantly induced *StoAOS1* expression as early as 1 h after application. The transcript level of *StoAOS1* continued to increase and reached its highest level at 6 h, followed by a decline at 12 h. *StoAOS1* expression at 12 h nevertheless remained significantly higher than that in the ddH₂O-treated control plants (Figure S2). Overall, *StoAOS1* expression showed a transient induction pattern, increasing during the early response to MeJA and then decreasing at the later stage. These results indicate that *StoAOS1* is responsive to exogenous MeJA and may participate in JA-mediated signaling in *S. torvum*.

3.6. Silencing of *StoAOS1* Decreases Resistance of *S. torvum* to *Verticillium* Wilt

To further investigate the role of *StoAOS1* in *Verticillium* wilt resistance, virus-induced gene silencing (VIGS) was used to suppress *StoAOS1* expression in *S. torvum*. The silencing efficiency was first examined by RT-qPCR. Compared with the control plants (TRV:00), *StoAOS1*-silenced plants (TRV:*StoAOS1*) showed a significantly lower transcript level of *StoAOS1* in the roots, indicating that *StoAOS1* was successfully silenced (Figure 4a). The *StoAOS1*-silenced and control plants were then subjected to root wounding followed by *V. dahliae* inoculation through root irrigation. At 9 dpi, the leaves of *StoAOS1*-silenced plants showed obvious chlorosis, whereas no visible symptoms were observed in the control plants (Figure 4b). Consistently, the disease index of *StoAOS1*-silenced plants was significantly higher than that of the controls (Figure 4c). These results indicate that silencing *StoAOS1* reduced the resistance of *S. torvum* to *Verticillium* wilt.

To further evaluate pathogen colonization, fungal biomass in the stems of *StoAOS1*-silenced and control plants was quantified by RT-qPCR at 0, 1, 3, and 7 dpi. Fungal biomass increased gradually with infection time. At 3 dpi, *StoAOS1*-silenced plants accumulated significantly higher fungal biomass than control plants. This difference became more pronounced at 7 dpi, when fungal biomass in *StoAOS1*-silenced plants was significantly higher than that in the controls (Figure 4d). These results suggest that silencing *StoAOS1* promoted the proliferation of *V. dahliae* in *S. torvum*. Pathogen recovery assays further supported this result. At 14 dpi, stem segments from *StoAOS1*-silenced and control plants were placed on potato dextrose agar (PDA) medium. Rapid hyphal growth was observed from stem segments of *StoAOS1*-silenced plants, whereas no regenerated hyphae were detected from control stem segments (Figure 4e). This result was consistent with the fungal

biomass analysis. Lignin content was also measured in roots, stems, and leaves. The lignin levels in all three tissues were lower in *StoAOS1*-silenced plants than in control plants, with significant reductions observed in roots and stems (Figure 4f). These results suggest that *StoAOS1* may contribute to lignin accumulation in *S. torvum*, thereby strengthening structural defense against pathogen infection.

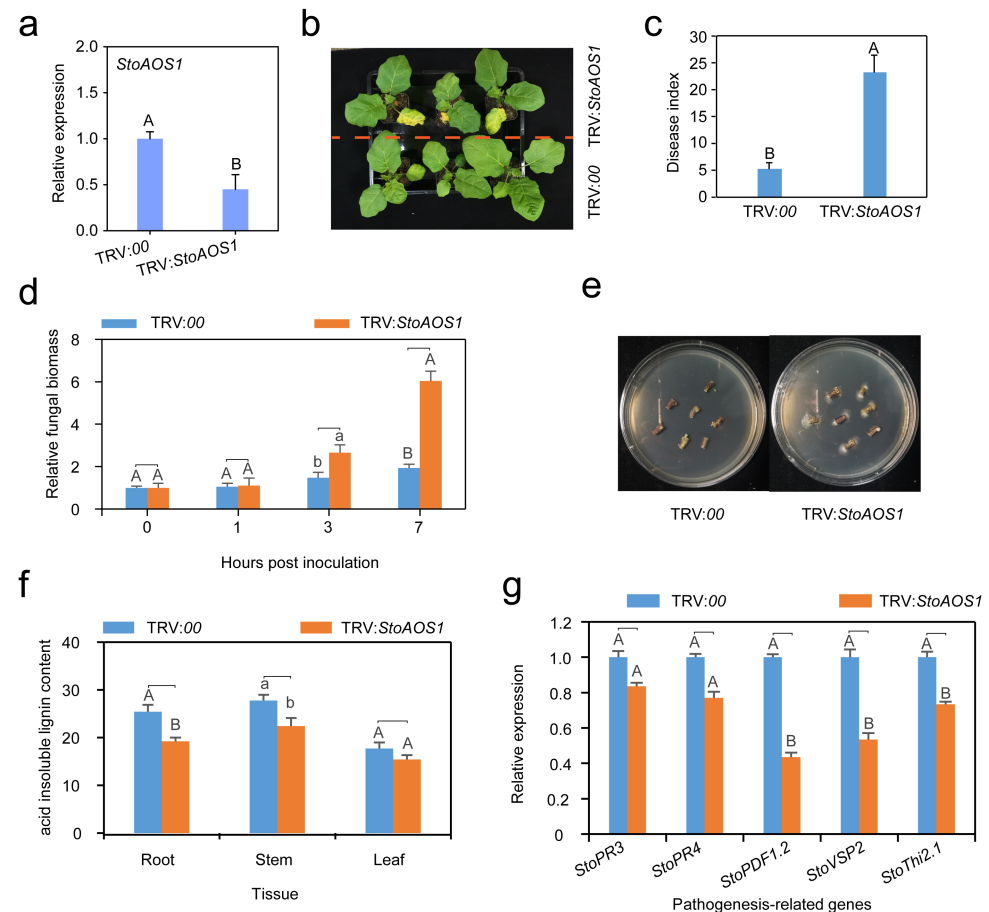


Figure 4. Silencing of *StoAOS1* decreases the tolerance of *S. torvum* to *Verticillium* wilt. (a) Detection of *StoAOS1* silencing efficiency. (b) Phenotypic assessment of *StoAOS1*-silenced and control plants inoculated with *V. dahliae* at 9 days. (c) Disease index analysis of *StoAOS1*-silenced and control plants inoculated with *V. dahliae* at 9 days. (d) Measurement of fungal biomass. Fungal biomass in *StoAOS1*-silenced and control plants was measured at 0, 1, 3, and 7 dpi by RT-qPCR. (e) Fungal recovery assay. Stems collected from *StoAOS1*-silenced and control plants inoculated with *V. dahliae* at 14 dpi were placed on solid PDA medium. (f) Detection of acid-insoluble lignin content in roots, stems, and leaves of *S. torvum* inoculated with *V. dahliae*. (g) Analysis of relative transcript levels of defense-related genes. The relative transcript levels of defense-related genes, including *StoPR3*, *StoPR4*, *StoPDF1.2*, *StoVSP2*, and *StoThi2.1*, were detected in roots of *StoAOS1*-silenced and control *S. torvum* inoculated with *V. dahliae* at 24 h post-inoculation. In (a,c,d,f,g), all experiments were performed with three biological replicates, and the results are presented as means $\pm$ standard deviation. Statistical analyses were conducted using Student's *t*-test, and different uppercase letters indicate extremely significant differences between groups ($p < 0.01$), and different lowercase letters indicate significant differences between groups ($p < 0.05$).

At 12 h after *V. dahliae* inoculation, transcript levels of several defense-related marker genes were assessed by RT-qPCR in both control and *StoAOS1*-silenced plants. Compared with the control group, the silenced lines exhibited significantly reduced transcript accumulation for *StoPDF1.2*, *StoVSP2*, and *StoThi2.1*, whereas the expression levels of *StoPR3* and *StoPR4* remained largely unchanged (Figure 4g). These findings imply that the defense

pathway modulated by *StoAOS1* may operate through *StoPDF1.2*, *StoVSP2*, and *StoThi2.1* and may be distinct from the route involving *StoPR3* and *StoPR4*. Taken together, the data support a model in which *StoAOS1* functions as a positive regulator of Verticillium wilt resistance in *S. torvum*.

3.7. Overexpression of *StoAOS1* Enhances Resistance to *V. dahliae* in *N. benthamiana*

To provide additional functional validation of *StoAOS1*, we heterologously overexpressed this gene in *Nicotiana benthamiana*. RT-qPCR analysis confirmed that the transcript level of *StoAOS1* was markedly elevated in the OE-1 and OE-5 overexpression lines relative to wild-type (WT) plants, indicating successful transgene expression (Figure 5a). WT plants and the two overexpression lines were subsequently inoculated with *V. dahliae*. At 6 dpi, the OE-1 and OE-5 plants displayed milder leaf wilting symptoms compared with the WT (Figure 5b). Consistent with this visual assessment, the disease index recorded for the overexpression lines was significantly lower than that for the WT (Figure 5c). These results collectively indicate that ectopic expression of *StoAOS1* confers enhanced resistance to *V. dahliae* in *N. benthamiana*.

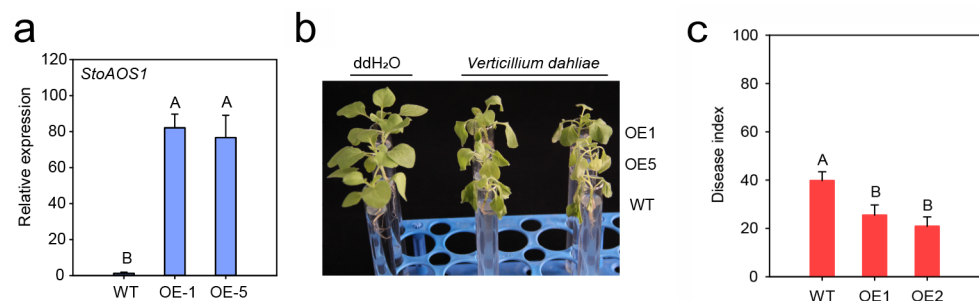


Figure 5. Overexpression of *StoAOS1* enhances tolerance of *N. benthamiana* to Verticillium wilt. (a) Analysis of relative expression levels of *StoAOS1* in WT, OE-1, and OE-5 plants. (b) Phenotypic assessment of WT, OE-1, and OE-5 plants inoculated with *V. dahliae* at 6 dpi. (c) Disease index analysis of WT, OE-1, and OE-5 plants inoculated with *V. dahliae* at 6 dpi. In (b,c), all experiments were performed with three biological replicates, and the results are presented as means $\pm$ standard deviation. Statistical analyses were conducted using Student's *t*-test, and different uppercase letters indicate extremely significant differences between groups ($p < 0.01$).

4. Discussion

Verticillium wilt is classified as a typical vascular disease, and achieving effective resistance against it depends on both rapid detection of the invading pathogen and the subsequent orchestrated induction of multiple layers of host defense [33,34]. In the work reported here, application of iTRAQ-based quantitative proteomic profiling to *S. torvum* roots following *V. dahliae* challenge revealed widespread alterations in protein abundance. A substantial subset of the DAPs identified could be assigned to phytohormone biosynthetic routes and associated signal transduction cascades, encompassing proteins that function in the SA, JA, ET, and brassinosteroid pathways. These observations point to the conclusion that resistance in *S. torvum* against *V. dahliae* is not governed by any single signaling module; rather, it appears to be under the control of a coordinated hormone-dependent defense network. SA, JA, and ET are established as central regulators of plant immunity, and their respective signaling pathways are known to engage in context-dependent crosstalk during the course of plant–pathogen interactions [12,13]. Canonical models often link SA primarily with resistance to biotrophic pathogens and with systemic acquired resistance [35,36], while JA and ET are more frequently associated with defense against necrotrophic pathogens and with induced systemic resistance. Nevertheless, the response to vascular pathogens like *V. dahliae* tends to be considerably more intricate and may well entail the integrated action of

several hormonal routes [37]. In the present context, the observed induction of *StoAOS1*—a protein involved in JA biosynthesis—suggests that JA-associated signaling likely makes a significant contribution to the early defensive response mounted by *S. torvum* against *V. dahliae*.

Beyond hormone-related proteins, the DAP dataset also included a number of candidates implicated in cell-wall biosynthesis, lignin deposition, chitin biosynthetic processes, phytoalexin accumulation, ROS homeostasis, regulation of programmed cell death, and responses to fungal elicitation. Taken together, these results indicate that structural reinforcement of cell walls and the activation of secondary metabolic routes constitute important facets of the overall defense strategy employed by *S. torvum* in response to pathogen challenge. The phenylpropanoid pathway was strongly activated after pathogen inoculation [38]. This pathway provides precursors for lignin biosynthesis and contributes to the formation of physical and chemical barriers that restrict pathogen invasion and systemic spread [39,40]. In this study, phenylpropanoid-related proteins, including PER12 and CYP73A5, were differentially accumulated after *V. dahliae* inoculation, suggesting that peroxidase activity and lignin deposition may contribute to the restriction of fungal colonization. Similar roles for lignin biosynthesis and cell-wall reinforcement have been reported in other plant—*V. dahliae* interaction systems, particularly in cotton, where lignin-related genes enhance resistance by strengthening cell walls and limiting pathogen expansion [41,42]. Among the candidate proteins identified in the proteomic analysis, *StoAOS1* attracted particular attention because of its association with JA biosynthesis. As a key enzyme operating within the octadecanoid route of JA biosynthesis, AOS is assigned to the CYP74 branch of the cytochrome P450 family [43]. The reaction catalyzed by this enzyme converts fatty acid hydroperoxides—themselves products of lipoxygenase activity—into allene oxide intermediates that are inherently unstable; these are then transformed through subsequent enzymatic steps into 12-oxo-phytodienoic acid and eventually into JA and its biologically active derivatives [21,43]. Given the well-established involvement of JA in plant defensive responses, AOS-encoding genes have been subjected to functional characterization across a range of plant species. In rice, for instance, overexpression of *OsAOS2* leads to elevated endogenous JA pools, upregulation of pathogenesis-related transcripts, and improved resistance to fungal pathogens [25]. Such observations lend support to the notion that AOS-mediated JA biosynthesis constitutes a significant component of plant immunity against fungal invaders.

In the current work, *StoAOS1* emerged from the proteomic screening as a *V. dahliae*-inducible gene belonging to the P450 family in *S. torvum*. Sequence analysis revealed that *StoAOS1* carries a conserved P450 domain and displays close phylogenetic affinity with AOS proteins from other solanaceous species, thereby supporting its assignment to the CYP74A/AOS subfamily. Promoter prediction further uncovered a range of *cis*-acting regulatory motifs associated with hormonal signaling, transcription factor binding, stress responses, and developmental processes—specifically, ABRE, ERE, TGACG-motif, MYB, MYC, ARE, and STRE elements. These promoter features are in good agreement with the expression behavior observed for *StoAOS1*, which proved responsive to both *V. dahliae* challenge and exogenous MeJA treatment. Upon MeJA application, transcript accumulation of *StoAOS1* rose rapidly, reached a peak at 6 h, and subsequently declined by 12 h while still remaining above the control level. This transient induction profile is suggestive of a role for *StoAOS1* in JA signaling, possibly involving feedback regulation of the JA biosynthetic pathway. Furthermore, following *V. dahliae* inoculation, *StoAOS1* was markedly upregulated in *S. torvum*, a pattern consistent with earlier observations that AOS genes can be activated by both pathogen attack and JA treatment. In rice, *OsAOS2* is known to respond to *Magnaporthe grisea* infection [25], and exogenous MeJA has been shown to

trigger expression of JA-biosynthetic genes, including various AOS homologs [44]. The earlier engagement of JA-associated defenses may, at least in part, account for the superior resistance of *S. torvum*, as it could serve to restrict pathogen proliferation during the critical early phases of infection.

Functional assays provided further evidence that *StoAOS1* acts as a positive regulator of Verticillium wilt resistance. Suppression of *StoAOS1* expression through virus-induced gene silencing in *S. torvum* resulted in a marked attenuation of resistance to *V. dahliae*, as manifested by more pronounced leaf chlorosis, an elevated disease index, increased fungal biomass within host tissues, and enhanced recovery of the pathogen from infected stem segments. These observations indicate that *StoAOS1* is necessary for the full expression of resistance in *S. torvum* against Verticillium wilt. Measurements of acid-insoluble lignin content revealed reductions in roots, stems, and leaves of *StoAOS1*-silenced plants, with statistically significant decreases recorded in roots and stems. Given that *V. dahliae* enters through the root system and disseminates via the vascular network, lignin deposition in these particular tissues is likely to play a critical role in impeding pathogen movement [1,42]. Diminished lignification in *StoAOS1*-silenced plants may therefore compromise cell-wall-based physical barriers and permit more extensive fungal colonization. This interpretation finds support from previous work in cotton, where knockdown of lignin biosynthetic genes such as *GhLAC4* and *GhCADs* attenuated defense-induced lignification and compromised resistance to *V. dahliae* [42,45]. *StoAOS1* may thus contribute to Verticillium wilt resistance not solely through JA signaling per se, but also via downstream defensive outputs including lignin accumulation. The expression profiles of defense-related marker genes lend additional weight to this conclusion. Following *V. dahliae* inoculation, transcript levels of *StoPDF1.2*, *StoVSP2*, and *StoThi2.1* were significantly reduced in *StoAOS1*-silenced plants, whereas those of *StoPR3* and *StoPR4* remained largely unaltered. Given that *PDF1.2* and *VSP2* are commonly regarded as markers of JA-dependent defense responses [46], these data suggest that *StoAOS1* may govern a specific subset of JA-responsive genes in *S. torvum*. The lack of substantial changes in *StoPR3* and *StoPR4* further implies that not all defense-related routes are equally reliant upon *StoAOS1* function. Hence, *StoAOS1* appears to operate through a particular JA-associated branch of the defense network rather than functioning as a global regulator of all pathogenesis-related transcripts.

The positive regulatory role of *StoAOS1* received additional confirmation from heterologous overexpression experiments in *N. benthamiana*. Relative to wild-type controls, transgenic lines overexpressing *StoAOS1* exhibited milder wilting symptoms and lower disease indices upon *V. dahliae* challenge. Collectively, these data support a positive role for *StoAOS1* in the defensive response of *S. torvum* against Verticillium wilt. In the present study, we characterized the function of *StoAOS1* via heterologous overexpression in *Nicotiana benthamiana*. It should be noted that further verification using stable transgenic materials of eggplant or *Solanum torvum* is still required in subsequent research to consolidate our conclusions.

5. Conclusions

In the present investigation, iTRAQ-based quantitative proteomic profiling was employed to characterize the response of *S. torvum* to *V. dahliae* infection. The proteomic data revealed activation of multiple defense-related pathways during the early stages of infection, encompassing phytohormone signaling, phenylpropanoid metabolism, lignin biosynthetic routes, cell-wall modification, and oxidative stress responses. Among the DAPs identified, *StoAOS1*—a CYP74A/AOS subfamily member involved in JA biosynthesis—emerged as a candidate positive regulator of Verticillium wilt resistance. The strong induction of *StoAOS1* by both *V. dahliae* and MeJA treatment points to its participation in JA-associated

defense signaling. Functional analyses demonstrated that silencing *StoAOS1* in *S. torvum* compromised Verticillium wilt resistance, promoted fungal colonization, diminished acid-insoluble lignin accumulation, and suppressed expression of several defense-associated genes. Conversely, heterologous overexpression of *StoAOS1* in *N. benthamiana* conferred enhanced resistance to *V. dahliae*. Taken together, these results suggest that *StoAOS1* positively regulates Verticillium wilt resistance, likely by modulating JA-mediated defense responses and lignin-associated structural defense. This study provides candidate gene resources for improving Verticillium wilt resistance in eggplant. Our findings offer valid evidence to unravel the molecular mechanism governing Verticillium wilt resistance in eggplant and lay a solid molecular foundation for molecular breeding and the development of disease-resistant eggplant varieties.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae12080949/s1>, Table S1. Statistics of pathogen defense-related proteins in *S. torvum* roots after inoculation. Table S2. GO analysis of DAPs in roots of *S. torvum* inoculated with *V. dahliae* at 6, 12, and 24 h post-inoculation. Table S3. KEGG enrichment analysis of DAPs in roots of *S. torvum* inoculated with *V. dahliae* at 6, 12, and 24 h post-inoculation. Table S4. Primer pair sequences used in this study. Figure S1. Transcript expression analysis of candidate *P450* genes. The transcript levels of candidate *P450* genes, including *StoCYP82C4*, *StoCYP19-2*, *StoCYP72A7*, *StoCYP72A9*, *StoCYP74A/StoAOS1*, *StoCYP84A1*, *StoCYP73A5*, *StoCYP707A3*, *StoCYP71B34*, and *StoCYP51G1*, were detected in roots of *S. torvum* inoculated with *V. dahliae* at 0, 6, 12, and 24 h post-inoculation by RT-qPCR. All experiments were performed with three biological replicates, and the results are presented as means $\pm$ standard deviation. Statistical analyses were conducted using Student's *t*-test, and different uppercase letters indicate extremely significant differences between groups ($p < 0.01$). Figure S2. Transcript expression analysis of *StoAOS1* after exogenous MeJA treatment. Transcript levels of *StoAOS1* were detected by RT-qPCR in leaves of *S. torvum* sprayed with exogenous 100 μ M MeJA at 0, 1, 3, 6, and 12 h after treatment. All experiments were performed with three biological replicates, and the results are presented as means $\pm$ standard deviation. Statistical analyses were conducted using Student's *t*-test, and different uppercase letters indicate extremely significant differences between groups ($p < 0.01$).

Author Contributions: Conceptualization, Y.Z., L.L. and X.Y.; methodology, Y.Z.; software, Y.Z.; validation, Y.Z.; formal analysis, Y.Z.; investigation, Y.Z.; resources, Y.Z.; data curation, X.Y.; writing—original draft preparation, Y.Z.; writing—review and editing, Y.Z. and L.S.; visualization, Y.Z.; supervision, Y.Z.; project administration, L.L. and X.Y.; funding acquisition, X.Y. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

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

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