Functional analysis of SlERF01 gene in the disease resistance to S.lycopersici

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Abstract

Background: Tomato gray leaf spot disease caused by *Stemphylium lycopersici* (*S. lycopersici*) is a serious disease that can severely affect tomato production. So far, only resistance gene *Sm* has been reported and the molecular mechanism of tomato resistance to the disease remains unclear. To better understand this mechanism of tomato resistance to *S. lycopersici*, qRT-PCR analysis, physiological index determination, microscopic observation and transgenic technology were used in this study.

Results: Our results showed that *SlERF01* could be strongly induced by *S. lycopersici* and the exogenous hormones salicylic acid (SA) and jasmonic acid (JA). Furthermore, overexpression of *SlERF01* enhanced the hypersensitive response to *S. lycopersici* and elevated the expression of defense genes in tomato. Furthermore, the accumulation of lignin, callose and H_2O_2 was increased in transgenic lines after inoculation with *S. lycopersici.* Here, our results showed that *SlERF01* played an indispensable role in among multiple SA, JA and ROS signaling pathways to confer resistance to *S. lycopersici* invasion. Our findings also indicated that *SlERF01* could activate *PR1* gene expression and enhance resistance to *S. lycopersici*.

Conclusions: We identified *SlERF01*, a novel tomato AP2/ERF transcription factor. The Functional verification demonstrated *SlERF01* positively regulated tomato resistance to *S. lycopersici*. Our findings indicate that *SlERF01* plays a key role in the multiple SA, JA and ROS signaling pathways to confer resistance to invasion by *S. lycopersici.* The findings of this study not only can help to better understanding of mechanisms of response to pathogens, but also will enable targeted breeding strategies for tomato resistance to *S. lycopersici*.

Background

In the long-term competitive relationship between plants and pathogens, plants have developed a series of defense mechanisms to resist the threat of pathogens, including bacteria, viruses, fungi and insects [1-2]. Two defense systems, PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI), have been established to prevent pathogenic invasion [3]. Many early signaling components of PTI and ETI activate a series of downstream integrated defense responses to prevent further damage[4]. In fact, substantial overlap of defense responses exists between PTI and ETI [5].

These defensive signaling responses include reactive oxygen species (ROS) bursts and callose and lignin accumulation and lead to localized cell and tissue death [6-7], termed the hypersensitive response (HR), at the site of pathogenic invasion to limit pathogen growth [8-10]. Therefore, HR is associated with R gene-triggered resistance, leading to localized cell and tissue death with corresponding downstream defense responses [11-13]. H_2O_2 is a type of ROS that can induce HR [14], and lignin and callose accumulation is associated with HR, limiting pathogen growth by strengthening the cell walls.

If plant defense responses are induced at the site of infection, the systemic defense response is activated in other plant tissues to prevent further invasion by the pathogen. Systemic acquired resistance (SAR) is

characterized by long-lasting and broad-spectrum effects [15], which can be triggered by PTI- and ETI-mediated pathogen recognition and are related to the levels of salicylic acid (SA) in localized cells and distant tissues. Previous studies have shown that the defense hormone SA plays an essential role in the SAR signaling pathway by inducing SAR-related gene expression via the regulatory protein *NPR1* and a transcriptional coactivator [16].

Gray leaf spot disease, a destructive fungal disease in plants such as pepper, cotton, spinach and eggplant caused by *S. lycopersici*, is considered a major factor limiting the yield and quality of cultivated tomatoes worldwide [17]. However, effective methods to control this disease remain unavailable. Hence, the development of resistant cultivars is the most efficient strategy to control the disease, in contrast to chemical control. *Sm* is thus far the only dominant gene that has been proven to be highly resistant to *S. lycopersici*. Identification of other disease resistance genes and further application of these genes are urgently needed. In addition, the mechanism underlying the resistance of tomato to *S. lycopersici* remains poorly understood. Therefore, identification of the molecular mechanism underlying the *Sm*-mediated resistance to *S. lycopersici* and other resistance genes is urgently required for breeding resistant tomato cultivars.

AP2/ERF-like transcription factors (TFs) have been identified to play an important role in disease resistance to various pathogens [18-19]. To date, a total of 137 ERF domain-containing proteins have been identified in the tomato genome, most of which have been reported to be involved in the response to biotic and abiotic stress or hormonal responses; however, only a few members have been characterized [20]. In this study, to better understand this mechanism of tomato resistance to *S. lycopersici*, a novel tomato AP2/ERF TF, *SlERF01*, was identified. Our results showed that *SlERF01* was directly or indirectly involved in the defensive response to *S. lycopersici* in tomato via multiple signaling regulatory networks. This study not only preliminarily identifies the function of *SlERF01* but also provides a new resistance gene resource for cultivating resistant tomato varieties.

Methods

Plant materials and *S. lycopersici* inoculation

Tomato cultivars (resistant cv. Motelle, susceptible cv. Moneymaker) were provided by the Chinese Academy of Agricultural Sciences. Seedlings of the transgenic line cv. Micro-Tom and *Nicotiana benthamiana* were available in our laboratory. Tomato and tobacco plants were grown in a greenhouse at 25-28°C and 60% relative humidity under a 14 h/10 h light/dark cycle.

S. lycopersici was isolated from tomato plants and plated on potato dextrose agar (PDA) in Petri dishes at 25-28°C for 10 days with a 12-h photoperiod. Then, 4-week-old tomato seedlings of Motelle, Moneymaker and Micro-Tom were inoculated with a conidial suspension (1×10^4 conidia/ml), while control plants were sprayed with sterilized water. Plants were maintained in a greenhouse (25-28°C) with

a relative humidity >80%. The disease index was evaluated post inoculation, and leaves were harvested at 0 and 3 days post inoculation (dpi) for further analysis.

Gene cloning and bioinformatics analysis

The 5'- and 3'-end cDNA sequences were cloned by homologous recombination using the NovoRec® PCR Cloning Kit. Specific primers for the target sequence were designed using Primer 6.0 software, and the target gene *SlERF01* was cloned by PCR with the following reaction protocol: 94°C for 3 min; 35 cycles of 30 s at 94°C, 45 s at 60°C, and 30 s/kb at 72°C; and 72°C for 10 min. The part-CAM-SLERF01 vector was constructed for the identification of positive clones. All the primers used in the study are shown in Table S1.

The *SlERF01* sequence was examined using the NCBI CDD database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), and the identified sequences were analyzed using DNAMAN5.0 (Data S2). A phylogenetic tree was constructed by MEGA5.2 using the AP2/ERF family proteins of tomato.

Subcellular localization

The full-length *SLERF01* ORF without the termination codon was amplified by PCR using a high-fidelity polymerase with the specific primers GFP-*SLERF01*-F and GFP-*SLERF01*-R. The pCAM35:: *SlERF01*-GFP fusion gene was constructed by inserting the PCR products into the pCAM35:: GFP vector between the KpnI and XbaI sites. The pCAM35:: GFP (control) and pCAM35:: *SLERF01*-GFP vectors were transformed into *Agrobacterium tumefaciens* GV3101. Single clones were selected and cultured in liquid LB containing the corresponding antibiotics. Transformed *Agrobacterium* cells were concentrated by centrifugation, harvested, diluted to OD_{600} = 0.4, and injected into *N. benthamiana* leaves with a syringe. Two days after agroinfiltration, green fluorescent protein (GFP) was imaged using a laser scanning confocal microscope (FV10-ASW, OLYMPUS).

Transformation of tomato

The full-length coding sequence (CDS) of *SlERF01* was amplified by PCR and cloned into a part-CAM vector harboring XhoI and XbaI sites. The pCAM-*SLERF01* overexpression vector was constructed, and the pCAM-*SlERF01* recombinant plasmid and the pCAM plasmid were transferred into *A. tumefaciens* strain GV3101 (BioVector NTCC Inc., Beijing, China). pCAM-*SlERF01* (overexpression vector) and pCAM (empty vector) were transferred into the susceptible cultivar Micro-Tom using tomato genetic transformation technology.

The target sequence of *SlERF01* was amplified by PCR with specific primers. After digestion with EcoRI and BamHI, the TRV vector was ligated to the PCR product. The virus-induced gene silencing (VIGS) vectors TRV::*SlERF01* TRV::00 and TRV::*PDS* were constructed and propagated in LB medium containing 50 mg/mL kanamycin for sequencing verification. Then, the recombinant plasmids were transferred into

A. tumefaciens strain GV3101, and the cells were cultured in induction medium (10 mM MES, 10 mM MgCl, 2.50 μg/mL kanamycin, 100 μg/mL rifampicin and 200 μM acetosyringone) to OD_{600} = 0.3. Finally, TRV1 was mixed with TRV2 at a volumetric ratio of 1:1 and incubated for 3 h; 3-4-leaf cv. Motelle plants were then infiltrated with each mixture using a 1 ml syringe containing approximately 0.5-1 mL of *Agrobacterium* cells. The treated plants were sampled at the indicated time points for further analysis, and 3 biological replicates were used for this test.

qRT-PCR analysis and physiological index determination

Expression analysis of the overexpressing and VIGS plants was performed by qRT-PCR. Total RNA was extracted from tomato leaves using TRIzol [21]. cDNA synthesis was performed with a reverse transcription kit (TaKaRa) according to the manufacturer's instructions. The qRT-PCR system contained 10 μL of 2× TransStart Top Green qPCR SuperMix (TransGen, China), 0.5 μL of upstream/downstream primers, 2 μL of the cDNA template, and ddH_2O to a total volume of 20 μL. The qRT-PCR program was as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 5 s, 62°C for 15 s and 72°C for 30 s. The $2^{-\Delta\Delta CT}$ method [22] was used to analyze the qRT-PCR data with EFα1 as a reference gene [23]. The qRT-PCR primers are listed in Table S1.

For exogenous hormone treatment, 0.2 mM SA and 0.4 mM JA were sprayed on tomato plants (control plants were sprayed with water) for different amounts of time (SA: 0, 12, 24, 48 and 72 h; JA: 0, 24, 48, 72 and 96 h). The levels of the endogenous hormones SA and JA were determined by high-performance liquid chromatography (HPLC). SA and JA were extracted from leaves by a modified method described by Llugany et al. [24], and their concentrations were then measured on an AB Sciex QTRAP 5500 instrument (USA) according to the manufacturer's instructions. Samples were collected from three individual plants for analyses of SA, JA content and gene expression. Data from three independent experiments were statistically analyzed according to the Student's t-test and the probability of $p < 0.05$ was considered as significant difference.

Microscopic observation

Trypan blue staining [25], 3,3-diaminobenzidine (DAB) staining, toluidine blue (TB) staining and aniline blue (AB) staining were used to observe the progression of *S. lycopersici* infection and production of H_2O_2, lignin and callose in *SlERF01*-overexpressing and *SlERF01*-VIGS plants.

The leaves were collected at 0 and 3 d after inoculation. Cell death was observed using TB staining, with destaining in Farmer's solution (95% ethanol, chloroform and acetic acid at a volumetric ratio of 6:3:1) for 3 h and boiling in 0.1% trypan blue solution at 65°C for 2 h, followed by transfer to a saturated chloral hydrate solution for 4 h. Finally, the leaves were observed under a light microscope.

Production of H_2O_2 was detected by DAB staining [26]. The infected tomato leaves were incubated in 0.1% DAB solution at room temperature in the dark for 12 h and boiled in a 96% ethanol solution for 10 min. Finally, the leaves were observed under a light microscope. Lignin was observed using the TB

staining method [27]. The infected tomato leaves were placed in formaldehyde/acetic acid/ethanol (FAA) solution for 24 h and then stained with 0.05% TB solution. The leaves were observed under a light microscope. Callose was detected using the AB staining method [28]. The infected tomato leaves were placed in FAA solution, cleared with 100% ethanol solution and then stained with 0.07 M K_2HPO_4 in 0.01% AB solution for 24 h. Finally, the leaves were observed under a fluorescence microscope. Leaf Samples were collected from three individual plants for analyses of HR, H_2O_2, lignin and callose accumulation.

Results

Cloning and phylogenetic analysis of *SlERF01*

The full-length CDS of *SlERF01* was cloned by PCR using cDNA derived from tomato (Table S1 for PCR primers). The CDS of *SlERF01* encodes a 240 amino acid protein that has one AP2/ERF domain and belongs to the ERF TF B-3 family (Fig. 1a). Database analysis of conserved protein sequences showed that *SlERF01* contains an ERF domain, a putative nuclear localization signal (NLS) and a putative activation domain (AD), among which only the ERF domain is conserved between *SlERF01* and other ERF proteins (Fig. 1b). Further analysis showed that *SlERF01* shares low similarity with other ERF proteins in terms of their whole putative protein sequences; however, sequence alignment showed a high degree of homology in the ERF domain regions. Thus, the phylogenetic analysis results showed that *SlERF01* may encode a novel ERF protein that participates in the disease resistance response.

Subcellular localization of *SlERF01*

A *SlERF01*-GFP fusion was constructed. The *SlERF01*::GFP fusion gene was transformed into the *A. tumefaciens* GV3101 strain with the empty GFP vector as a negative control, and *N. benthamiana* leaves were then infected. The results showed that *SlERF01* was localized in the nucleus (Fig. 2).

SlERF01 improves disease resistance against *S. lycopersici* in tomato

To identify the function of *SlERF01* in *S. lycopersici* resistance in tomato, overexpression (OE) and TRV-mediated VIGS vectors were constructed for further analysis. Finally, 3 *SlERF01*-overexpressing tomato lines with the highest expression (lines 5, 11 and 15) and 3 TRV lines with the lowest expression (lines 3, 7 and 8) were generated for further analysis (Fig. 3). Overexpression of *SlERF01* resulted in a typical HR phenotype at 3 dpi with *S. lycopersici*, and the susceptibility symptoms of SlERF01-overexpressing transgenic plants (OE) were significantly less severe than those of susceptible plants. The transgenic line exhibited enhanced resistance to *S. lycopersici* infection compared with that of the empty vector control (35s::00).

Furthermore, the HR was found to be weaker and slower in *SlERF01*-silenced plants (TRV) than in empty vector plants (TRV::00). Typical disease lesions were observed on *SlERF01*-silenced plants at 3 dpi, and no obvious susceptible symptoms were observed on the leaves from the TRV::00 plant (Fig. 3a).

Furthermore, necrotic lesions and perforated center symptoms were evident on the leaves of susceptible plants. These results indicated that *SlERF01* promoted *S. lycopersici* resistance in tomato.

The effects of disease resistance in tomato were also evaluated by examining HR-related cell death and accumulation of H_2O_2, lignin, and callose by staining with trypan blue, DAB, TB and AB, respectively. For trypan blue staining, a strong HR at 3 dpi with *S. lycopersici* was observed in *SlERF01*-overexpressing (35s::*SlERF01*) plants. In contrast, no visible HR was observed in the empty vector (35s::*00*) plants at 3 dpi; the hyphae gradually grew, and the lesions were aggravated and transparent. In contrast to the overexpression plants, the leaves from the *SlERF01*-silenced plants were sensitive to *S. lycopersici* infection. HR was impaired in TRV::*SlERF01* plants compared to TRV::00 plants at 3 dpi with *S. lycopersici*; hyphal spreading was observed, and the lesions were aggravated and perforated. However, a strong HR was observed on the leaves from TRV::00 plants (Fig. 4). These results showed that *SlERF01* can trigger HR in tomato leaves

In addition, H_2O_2 production was observed in tomato leaves from 35s::*SlERF01* overexpression plants by DAB staining (Fig. 4). At 3 dpi, H_2O_2 accumulation was not detectable in TRV::*SlERF01* plants compared to the TRV::00 empty vector plants. This result indicated that *SlERF01* can induce H_2O_2 generation as a defensive response to *S. lycopersici* infection.

To further explore the potential mechanism, lignin and callose production was analyzed in the 35s::*SlERF01* overexpression plants, TRV::*SlERF01* plants and empty vector (35s::00 and TRV::00) plants at 3 dpi. The accumulation of lignin and callose in the leaves of 35s::*SlERF01* overexpression plants was higher than that in the leaves of 35s::00 empty vector plants at 3 dpi (Fig. 4). However, the intensities and areas of fluorescence in the leaves of TRV:: *SlERF01*-silenced plants were weaker than those in the leaves of TRV::00 plants. Based on all of the above results, we conclude that *SlERF01* overexpression enhances the resistance of tomato to *S. lycopersici* compared to that of control plants.

Silencing of *SlERF01* decreased the expression levels of the defense-related gene *PR1* after infection with *S. lycopersici*

In previous transcriptome sequencing experiments, we found that the differentially expressed genes *SlERF01* and *PR1* were significantly upregulated in the pathway “Plant hormone signal transduction”. Here, qRT-PCR was used to identify the regulatory relationship among *SlERF01* and *PR* in the “Plant hormone signal transduction” pathway. As shown in Fig. 7, once *SlERF01* was silenced, the expression level of *PR1* was significantly suppressed compared to that of TRV::00. Therefore, we proposed *SlERF01* enhance disease resistance to *S. lycopersici* by regulating the expression of the PR1 gene in tomato.

SlERF01 may require the SA and JA signaling pathways to enhance disease resistance in tomato

The above results show that overexpression of *SlERF01* can improve disease resistance against *S. lycopersici* in tomato. In addition, our previous study showed that *SlERF01* is involved in the significantly enriched KEGG pathway “Plant hormone signal transduction”. qRT-PCR was used to determine whether

the transcript levels of *SlERF01* were associated with SA- and JA-induced resistance in resistant plants during *SlERF01* infection. Compared with the control (water-sprayed plants), plants treated with 0.2 mM exogenous SA exhibited an approximately 34-fold increase in *SlERF01* transcript levels at 24 h (Fig. 5). After SA treatment, *SlERF01* was significantly up-regulated and reached its peak expression at 24h, showed the gene expression pattern in response to SA induction in resistant plants MO. In the control material MT, *SlERF01* was up-regulated in 12h and 48h after treatment with SA, the rapid decline in 24h, showing the irregular change. So in control material MT, *SlERF01* was up-regulated at the different time points but did not show the gene expression pattern in response to SA induction.

Treatment with JA also significantly enhanced the expression of *SlERF01*, with the peak expression level being 28-fold higher than that of the control. These results showed that *SlERF01* could be significantly upregulated by SA and JA treatment. In resistant material MO, SlERF01 showed the gene expression pattern in response to JA induction. However, *SlERF01* was not significantly up-regulated at different time points in MT and did not response to JA induction.

It is well established that SA and JA play important roles in the plant defense response to pathogens. To analyze the hormonal response to *S. lycopersici* infection, LC-MS was performed to measure the JA and SA contents in T1-generation *SlERF01*-overexpressing plants. The SA and JA levels of T1-generation *SlERF01*-overexpressing tomato plants were significantly higher than those of the control plants after inoculation with *S. lycopersici* (Fig. 6). After inoculation with *S. lycopersici*, the SA levels in the *SlERF01*-overexpressing plants were 5-fold higher than those in the empty vector plant, and the JA levels were approximately 3-fold higher than those in the empty vector plant (Fig. 6). Thus, overexpression of *SlERF01* could significantly enhance the production of SA and JA, once again indicating that *SlERF01* probably participates in both the SA and JA signaling pathways to improve the disease resistance to *S. lycopersici* in tomato.

Discussion

SlERF01 is a novel tomato AP2/ERF transcription factor that is localized in the nucleus

To date, approximately 137 genes that encode conserved protein AP2/ERF domains have been identified in the tomato genome, and AP2/ERF proteins play an important role in the transcriptional regulation of a variety of abiotic and biotic stress responses. Previous studies have shown that A-subgroup TFs are involved in the regulation of abiotic stress responses. However, almost all the AP2 genes of the B subgroup have important functions in biotic stress responses. Furthermore, an increasing number of B-subfamily genes have been identified as being involved in the resistance to bacterial, fungal and viral diseases [29].

In the present study, *SlERF01* was isolated from tomato and shown to be upregulated after *S. lycopersici* treatment. In addition, phylogenetic analysis showed that *SlERF01* belonged to the B-3 subfamily of ERF protein families, and a few B-3 subfamily members have been shown to regulate plant disease resistance [30]. Analysis of conserved protein sequences in *SlERF01* showed a low similarity to ERF1; however, the

sequence homology was very high in the ERF domain regions (Fig. 1B). Here, our results showed that the cDNA of *SlERF01* probably encodes a novel ERF protein that is involved in the disease resistance response. Subcellular localization analysis showed that *SlERF01* is a nuclear-localized protein, consistent with previous studies on most ERF proteins.

SlERF01 enhances tomato resistance to *S. lycopersici*

It is well established that the over-expression of ERFs could enhance plant disease resistance to fungi, bacteria, and viruses. Previous studies have identified that the over-expression of AaERF1 could positively regulated *Artemisia annua* resistance to *Botrytis cinerea* [31]. Furthermore, studies have showed that once the tobacco OPBP1 gene was introduced into rice, it could enhance the resistance to Magnaporthe grisea and Rhizoctonia solani [32].

Our present study showed that the overexpression of *SlERF01*could significantly enhance resistance to *S. lycopersici* infection compared with controls. Typical disease lesions were observed on *SlERF01*-silenced plants with no obvious susceptible symptoms in the TRV::00 plants. Moreover, studies have indicated that HR and the accumulation of H_2O_2, lignin and callose are stronger in resistant cultivars than in susceptible cultivars, leading to improved disease resistance [33-34]. Consistent with these previous studies, our studies showed that the overexpression of *SlERF01* not only led to HR-induced cell death but also increased the accumulation of H_2O_2, lignin and callose in transgenic tomatoes compared with the controls. These results indicated that *SlERF01* may also participate in resistance against *S. lycopersici* via ROS signaling (Fig. 8).

SlERF01 positively regulates the expression of PR1 and enhances tomato disease resistance

Recently, some ERF TFs, such as OsERF1, Pti4 and AtERF1, have been suggested to play a role in disease resistance responses. As discussed in the introduction, overexpression of ERFs in plants could enhance plant disease resistance by regulating PR gene expression [35]. The regulation of PR gene expression by ERF TFs via binding to GCC boxes or DRE/CRT cis-acting elements in gene promoter regions has been extensively studied [35-38]. Nevertheless, studies have shown that sequences flanking the GCC box affect binding efficiency [39], suggesting that multiple ERFs probably regulate various gene sets.

SlERF01 may require the SA and JA signaling pathways to enhance disease resistance in tomato

In previous transcriptome sequencing experiments, we found that *SlERF01* expression could be induced by *S. lycopersici* in both resistant and susceptible materials and highly upregulated in the resistant material after inoculation with *S. lycopersici. SlERF01* could be induced by exogenous SA and JA, suggesting that *SlERF01* is probably the responsive component of the JA and SA signaling pathways. Furthermore, SA and JA are important signaling molecules that are involved in the disease resistance response to biotic and abiotic stress [40-41]. Previous studies also showed that exogenous application of SA could induce the expression of PR genes and enhance the resistance to multiple pathogens [42]. The SA and JA/ethylene (ET) signaling pathways were identified as being antagonistic or synergistic in

response to disease resistance [43-45]. Studies have shown that OsERF1 integrates the SA and JA signaling pathways in the defense response to pathogens [46]. Previous studies have shown that ROS and SA pathways have parallel functions to ensure optimal induction of SAR [47]. Combined with the above studies, our results showed that *SlERF01* not only responded to SA and JA but also increased the accumulation of H_2O_2, lignin and callose in transgenic tomato. Here, we propose that *SlERF01* plays a critical role in the crosstalk among SA, JA and ROS, conferring resistance to invasion by *S. lycopersici* (Fig. 8).

Conclusions

In this study, we found that *SlERF01* was a novel tomato AP2/ERF transcription factor that was localized in the nucleus. The *SlERF01* positively regulated tomato resistance to *S. lycopersici* combined the analysis of overexpression and gene silence. Interestingly, *SlERF01* plays a key role in the multiple SA, JA and ROS signaling pathways to confer resistance to invasion by *S. lycopersici.* The results of preliminary functional verification demonstrated that *SlERF01* induces disease resistance by up regulating the expression of PR1gene. Ultimately, this study provides valuable resources for further studies of the molecular mechanisms involved in the disease resistance and breeding strategies for tomato varieties.

Abbreviations

Stemphylium lycopersici: *S. lycopersici*; PTI: PAMP-triggered immunity; ETI: Effector-triggered immunity; ROS: reactive oxygen species; HR: hypersensitive response; SAR: Systemic acquired resistance; PR1: Pathogenesis-related protein 1-like; R gene: resistance gene; qRT-PCR: Real-time quantitative reverse transcription-polymerase chain reaction; VIGS: virus-induced gene silencing; SA: Salicylic acid; JA: Jasmonate;

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of the supporting data

The datasets supporting the results of this study are included within the article and its additional files.

The materials are available upon request by contacting the corresponding author.

Data regarding the phylogenetic tree and sequence alignment of *SlERF0* is shown in Fig. 1.

Data regarding the subcellular localization of *SlERF01* is shown in Fig. 2.

Data regarding the overexpression of *SlERF01* in tomato is shown in Fig. 3.

Data regarding the histopathological observation of HR-related cell death and accumulation of H_2O_2, lignin and callose is shown in Fig. 4.

Data regarding the resistance induced by exogenous SA and JA against *S. lycopersici* infection in tomato is shown in Fig. 5.

Data regarding the hormone level analysis of control and transgenic lines is shown in Fig. 6.

Data regarding the expression levels between *SlERF01* and PR1 is shown in Fig. 7.

Data regarding the hypothetical model for the tomato defense response to *S. lycopersici* is shown in Fig.8.

Competing interests

The authors have no competing interests to declare.

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Author contributions

JL conceived and designed the experiments. HY performed the experiments and data analysis and wrote the manuscript. All authors reviewed and approved the final manuscript.

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Figures

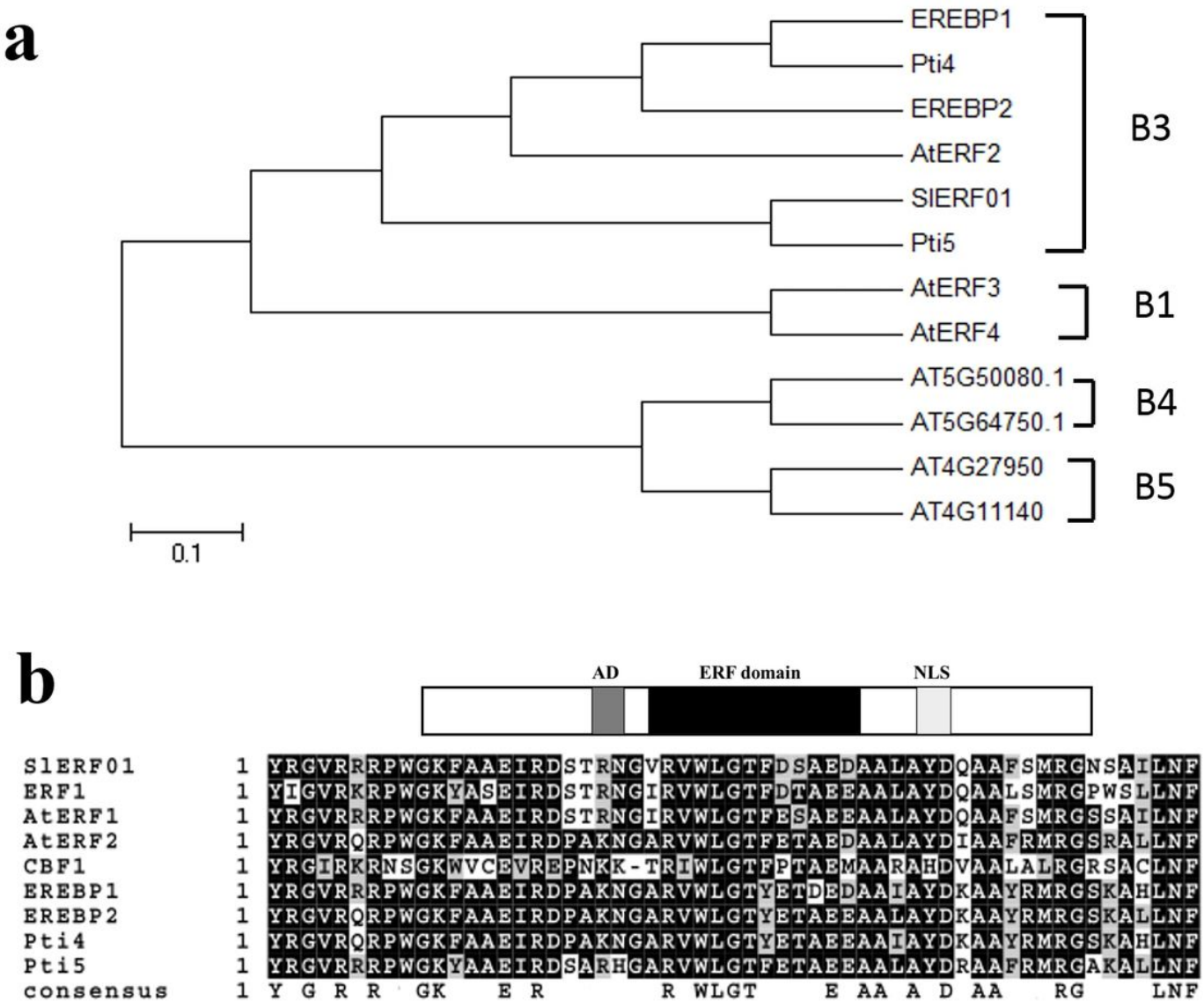

Figure 1

Phylogenetic tree and sequence alignment of SlERF01. a Phylogenetic tree of SlERF01 and other ERF proteins; the phylogenetic tree was constructed by ClustalW using amino acid sequences of the AP2/ERF domain. Subfamilies of ERF proteins are divided by the broken line. The classification was described by Sakuma et al. (2002). b Alignment of SlERF01 with other ERF proteins. SlERF01 is composed of an ERF domain, a putative nuclear localization signal (NLS) and a putative activation domain (AD), as shown in Fig. 1b. The black and light gray represent identical and conserved amino acids, respectively, and darker colors are correlated with higher percentages of the same amino acid.

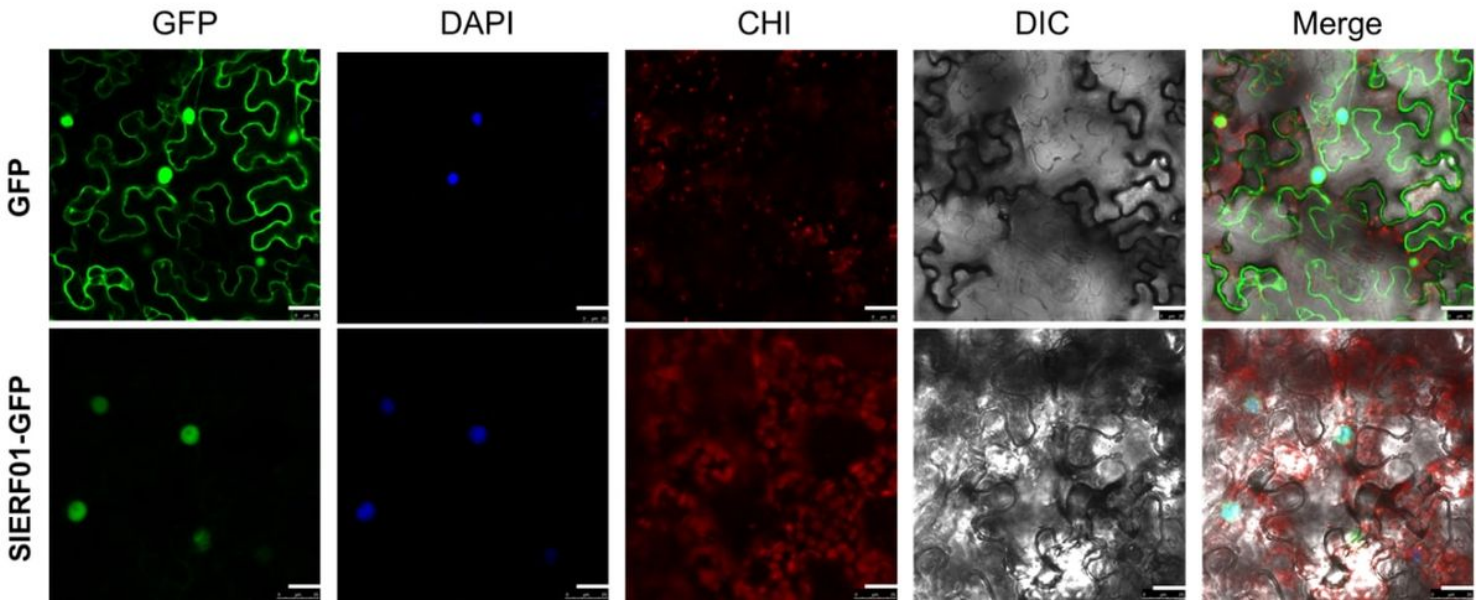

Figure 2

Subcellular localization of SlERF01. SlERF01-GFP was localized in the nucleus, and GFP was localized throughout the cells. GFP: green fluorescence field, DAPI: DAPI field (nuclear staining), CHI: chloroplast spontaneous fluorescence field, DIC: open field, Merge: superposition field. Excitation light wavelengths: GFP field: 488 nm, DAPI field: 358 nm, CHI field: 488 nm. Merged images were obtained 2 days after agroinfiltration. Bars = 25 μM.

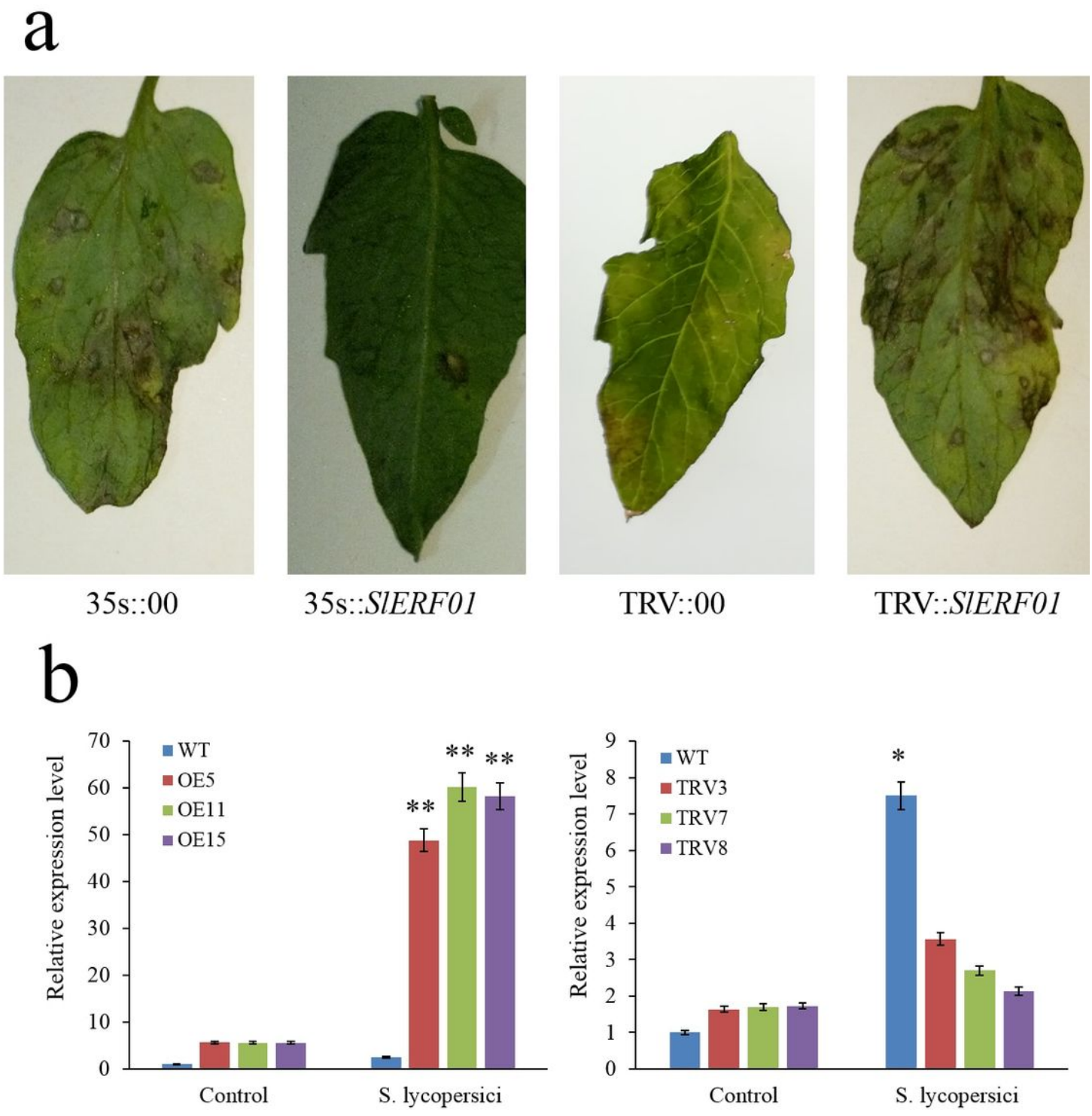

Figure 3

Overexpression of SlERF01 enhances disease resistance in tomato. a Disease symptoms in the wild-type plants, SlERF01-overexpressing transgenic plants and silenced plants post inoculation with S. lycopersici. Transgenic plants (35s::SlERF01) showed a highly resistant phenotype. Silenced plants (TRV::SlERF01) exhibited severe symptoms of disease. Empty vector in the overexpressing Micro-Tom plant (35s::00); empty vector in the silenced plant of the resistant cultivar Motelle (TRV::00). b Expression levels of

SlERF01 in wild-type plants, overexpression plants and VIGS lines. Three overexpression (OE) lines (OE5, OE11 and OE15) and three VIGS (TRV) lines (TRV3, TRV7 and TRV8) were analyzed by qRT-PCR. Three biological replicates were designed for each sample. The asterisks indicate significant differences in expression levels between transgenic lines and control lines (**P < 0.01; *P < 0.05, Student's t-test).

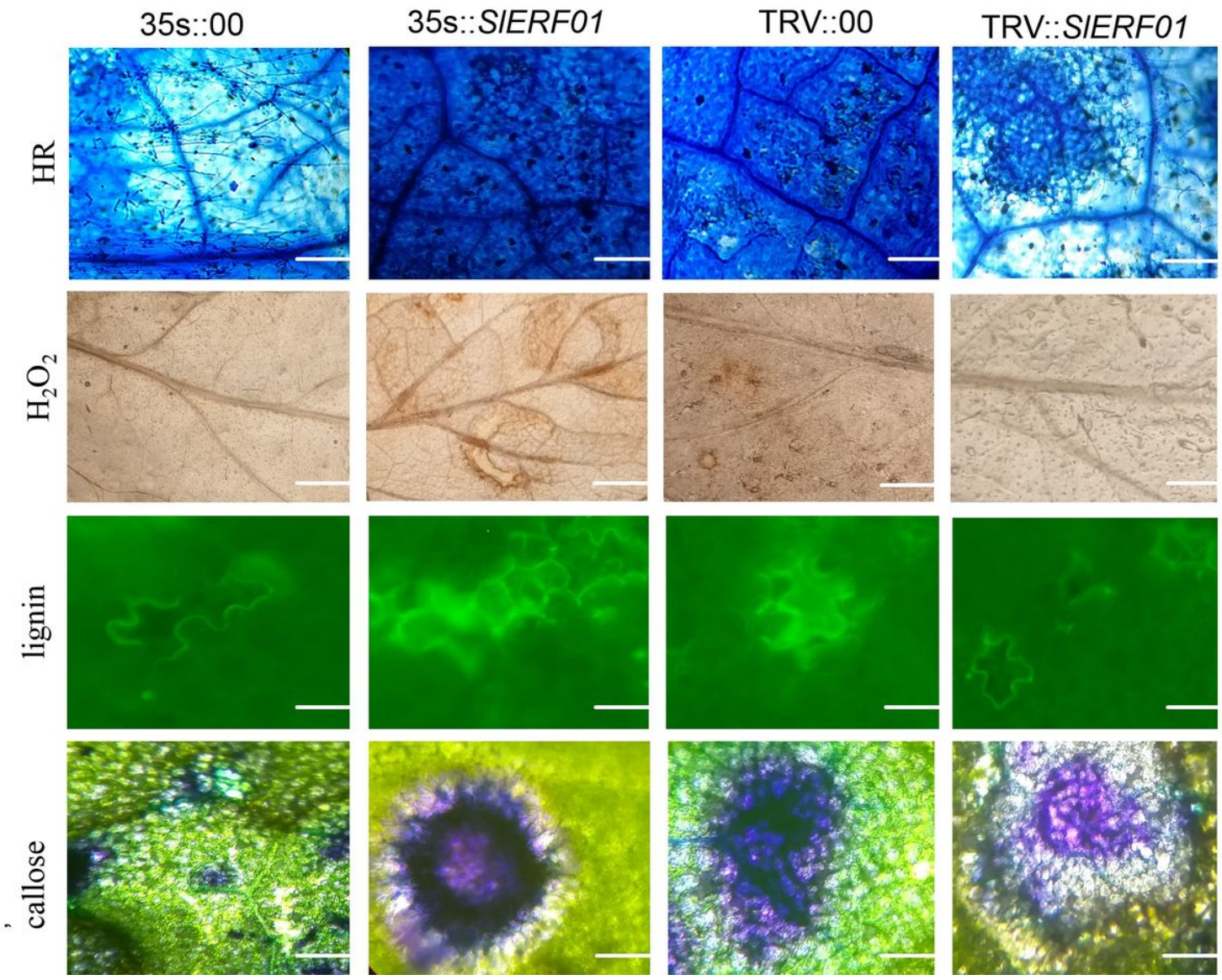

Figure 4

Histopathological observation of HR-related cell death and accumulation of H2O2, lignin and callose. Similar results were obtained in three independent experiments. Bars= 25 μM.

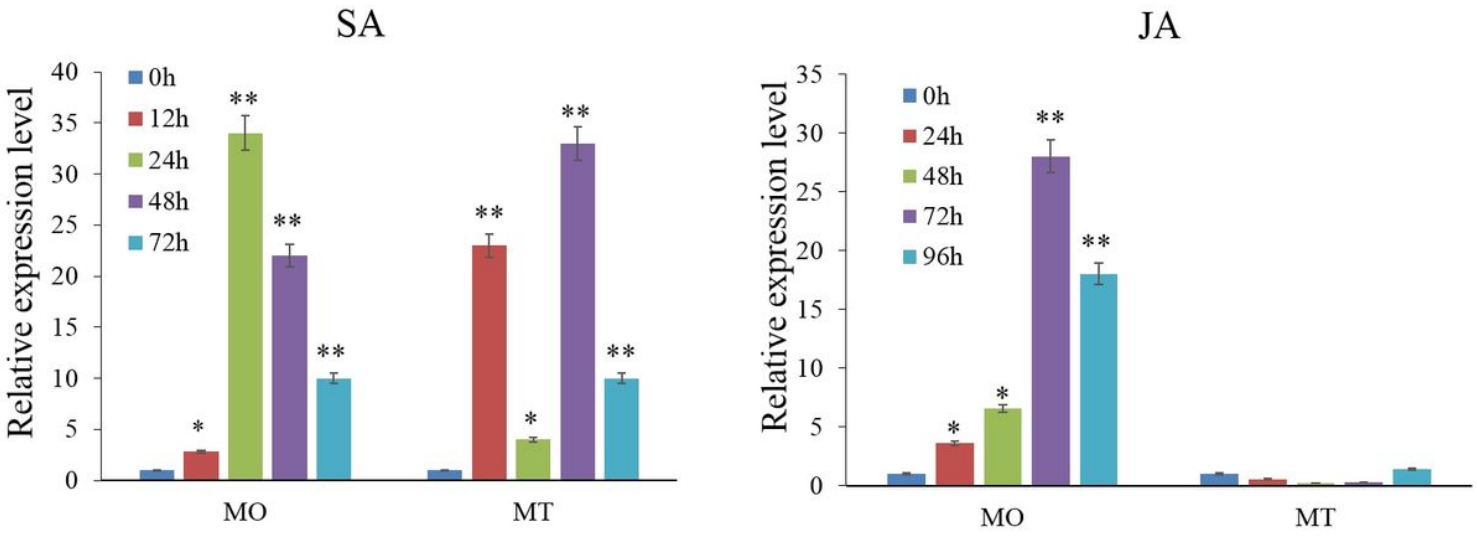

Figure 5

Resistance induced by exogenous SA and JA against S. lycopersici infection in tomato. MO: resistant cv. Motelle, MT: control cv. Micro-Tom. The asterisks indicate significant differences in expression levels between hormone treatments and controls (water-sprayed plants). Similar results were obtained in three independent experiments (**P < 0.01; *P < 0.05, Student's t-test).

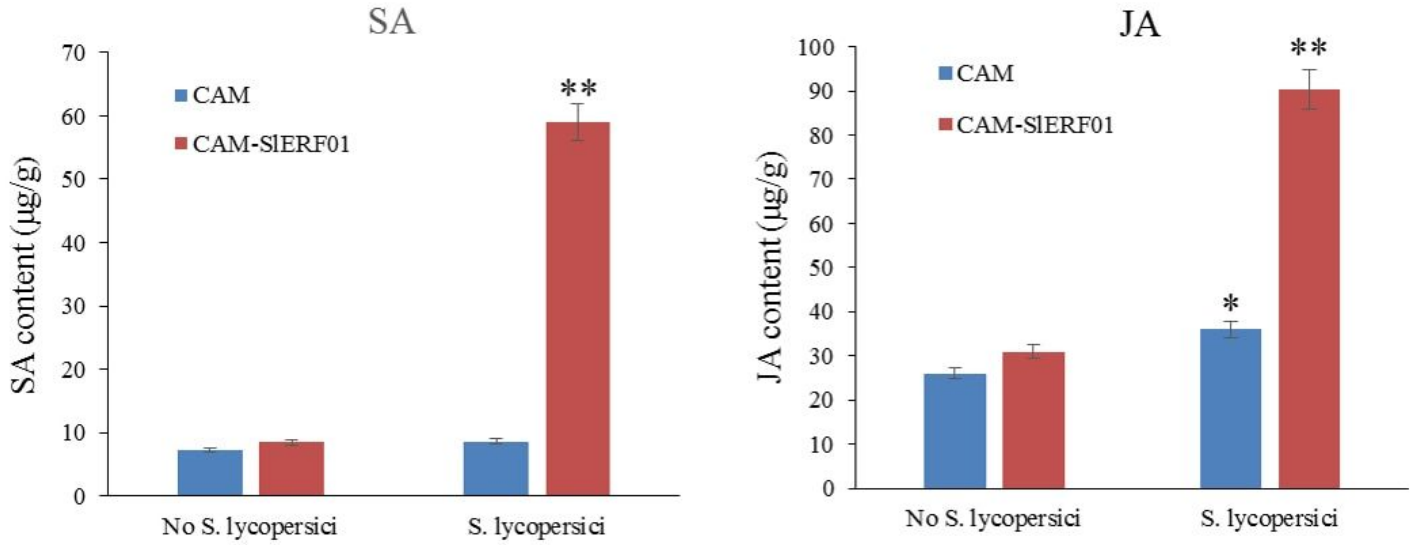

Figure 6

SA and JA hormone levels in SlERF01-overexpressing lines. The asterisks indicate significant differences in the expression levels between transgenic lines and control lines. Data presented from three independent experiments (**P < 0.01; *P < 0.05, Student's t-test).

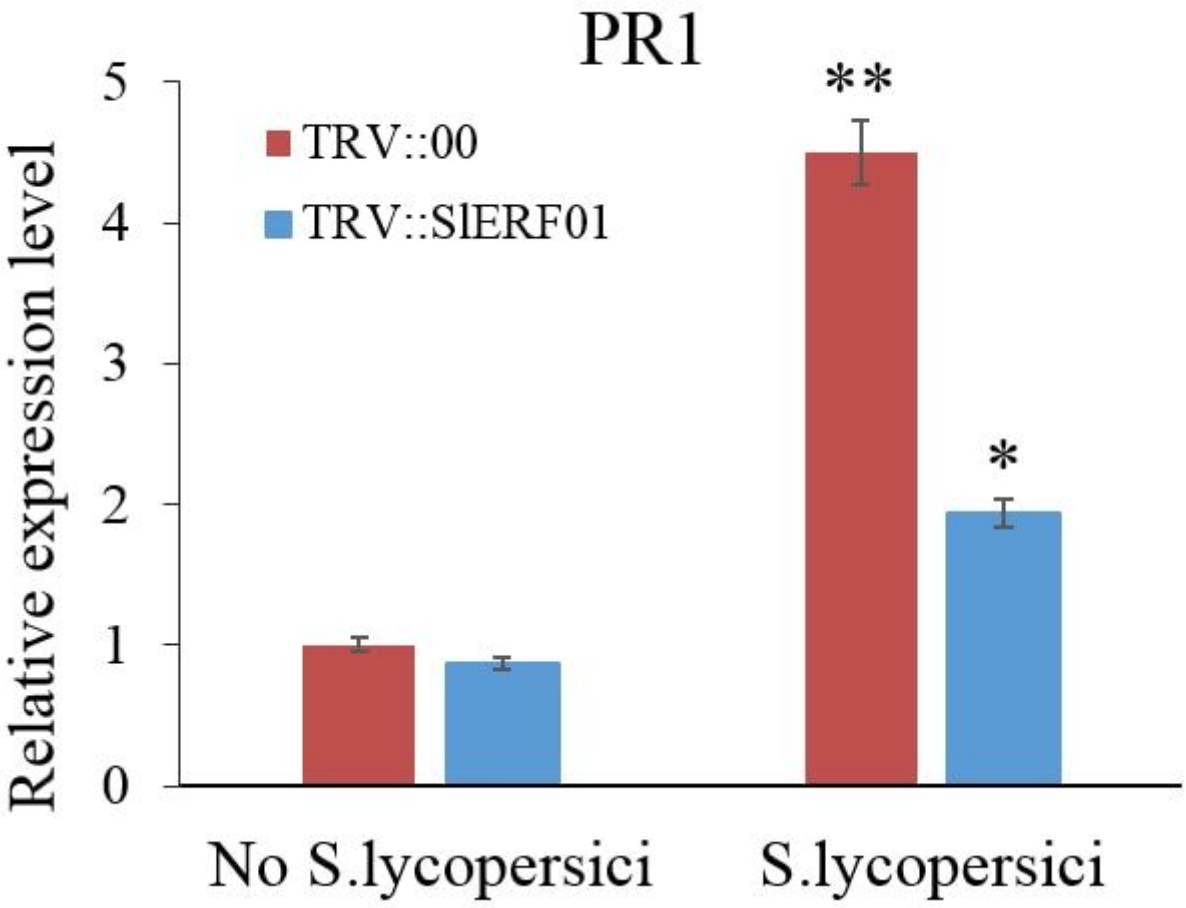

Figure 7

Silencing SlERF01 decreased the expression level of the defense-related gene PR1 after infection with S. lycopersici. TRV::00, empty vector plant; TRV::SlERF01, SlERF01-silenced plants; The asterisks indicate significant differences in the expression levels between silenced lines and control lines. Similar results were obtained in independent experiments (**P < 0.01; *P < 0.05, Student's t-test).

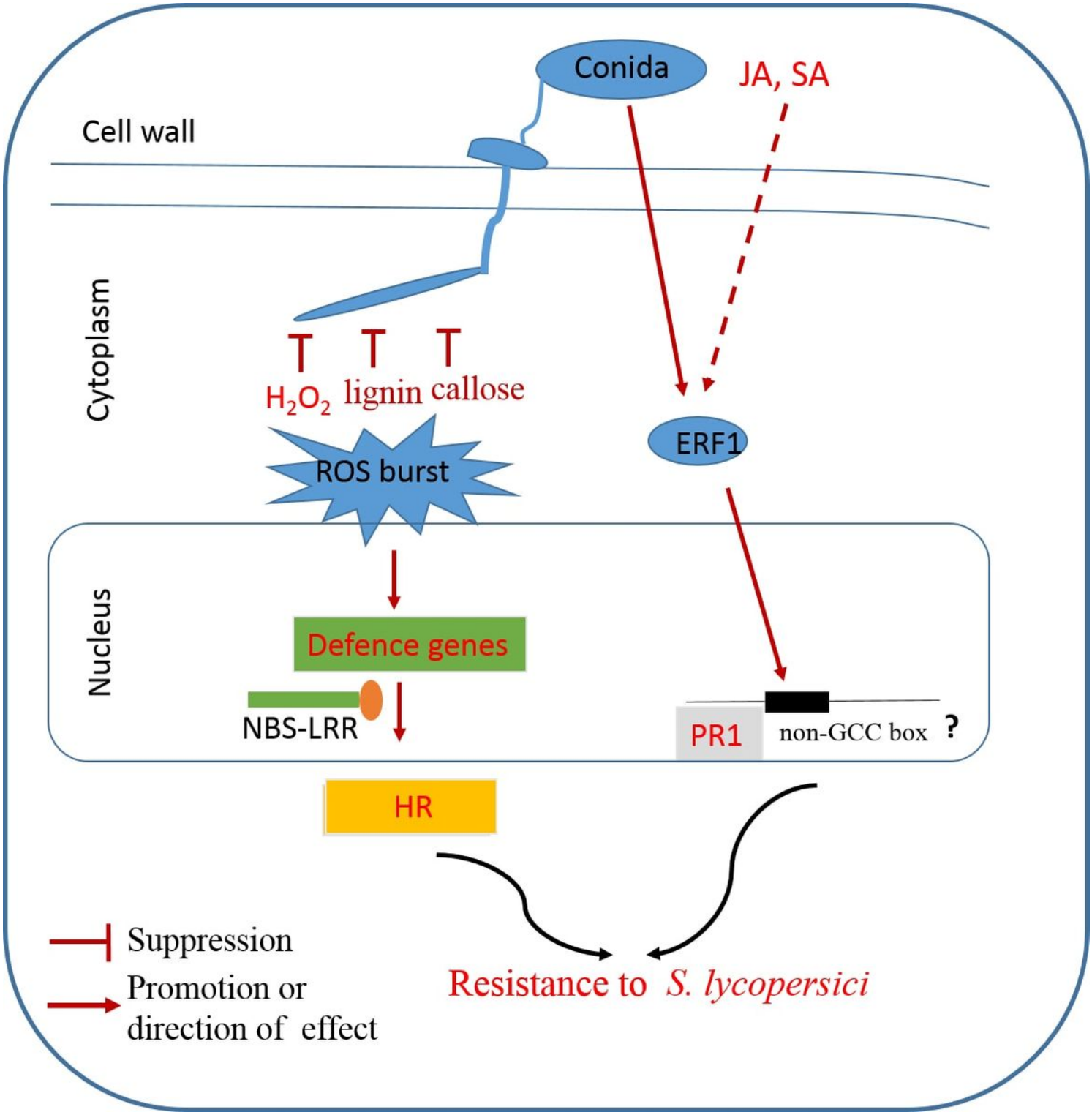

Figure 8

Hypothetical model for the tomato defense response to S. lycopersici based on the results of this study.

Supplementary Files

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- TableS1.docx