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Thiol peroxidase CaTPX1 of pepper enhances resistance against *Phytophthora capsici* by stabilizing CaCDC48

Ruixue Qi^{1†}, Yuanyuan Gao^{1†}, Wenju Tian¹, Qian Yao¹ and Yu Du^{1*}

Abstract

Identification of key defense-related genes in pepper (*Capsicum annuum* L.) against *Phytophthora capsici* is critical for developing effective disease-resistant breeding strategies. In this study, a cDNA virus-induced gene silencing (VIGS) library in *Nicotiana benthamiana* was screened, leading to the identification of a thiol peroxidase (TPX1) as a positive regulator of plant immunity against *P. capsici*. Silencing of the TPX1 homolog in *N. benthamiana*, pepper, and tomato resulted in decreased resistance, while overexpression of TPX1 homologs in pepper and tomato significantly enhanced resistance to *P. capsici*. To explore whether TPX1's function in disease resistance depends on its perception of endogenous H₂O₂, the peroxidatic/catalytic cysteine (CaTPX1^{C51S}) was mutated to interrupt the oxidation signaling. The results showed that CaTPX1^{C51S} lost its ability to positively regulate immunity. To investigate the disease resistance mechanism of CaTPX1, immunoprecipitation-mass spectrometry (IP-MS) was performed, identifying a cell division cycle 48 (CDC48) protein as an interacting partner of CaTPX1. Co-immunoprecipitation (Co-IP) and luciferase complementation imaging (LCI) assays confirmed the interaction between CaCDC48 and CaTPX1, with the CaTPX1^{C51S} mutant exhibiting significantly reduced binding affinity for CaCDC48. Further analysis revealed that CaCDC48 is stabilized by CaTPX1, but not by CaTPX1^{C51S}, indicating that CaTPX1 interacts with CaCDC48 via Cys51, potentially facilitating the transmission of the H₂O₂ signal to CaCDC48. In addition, CaCDC48 was found to positively regulate pepper defense against *P. capsici*. In conclusion, our findings suggest that thiol peroxidase CaTPX1 targets and stabilizes CaCDC48 to enhance plant immunity against *P. capsici*.

Keywords Pepper, *Phytophthora capsici*, Virus-induced gene silencing, Plant resistance, Thiol peroxidase

Introduction

Pepper (*Capsicum annuum* L.) is a globally cultivated vegetable of significant economic value, primarily utilized as a spice (Kim et al. 2014; Ou et al. 2018). Its production is severely impacted by *Phytophthora* blight, a devastating soilborne disease caused by the oomycete pathogen *Phytophthora capsici*, resulting in considerable yield losses (Quesada-Ocampo et al. 2023; Shi et al.

2025). As a highly virulent pathogen, *P. capsici* has a broad host range, infecting crops such as peppers, tomatoes, eggplants, and cucurbits (Lamour et al. 2012). Current disease management heavily relies on chemical fungicides, although their use raises significant environmental concerns (Long et al. 2025). Therefore, genetic modification and genome editing have emerged as promising sustainable strategies for pathogen control (van Esse et al. 2020; Zhang et al. 2021). However, the molecular mechanisms underlying pepper resistance to *P. capsici* remain inadequately understood. Identifying and functionally characterizing resistance genes is essential for developing *P. capsici*-resistant cultivars. Thus, the discovery of key resistance genes and the subsequent breeding

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of resistant varieties offer both economically viable and environmentally sustainable solutions for managing *Phytophthora* blight in pepper.

The study of gene function in many crops has been constrained by the limited availability of efficient genetic transformation methods. Virus-induced gene silencing (VIGS) provides a solution by using plant viral vectors to selectively silence host genes (Lu et al. 2003a; Burch-Smith et al. 2004; Fernandez-Calvino et al. 2016). VIGS does not require transgenesis and has been extensively used for gene function analysis in plants. For example, a tobacco rattle virus (TRV)-mediated VIGS screen of 4,532 random clones from a *Nicotiana benthamiana* cDNA library identified five clones conferring resistance to tomato spotted wilt virus (TSWV), revealing that the *ribosomal protein S6* (*RPS6*) gene family is critical for TSWV susceptibility (Helderman et al. 2022). Additionally, Lu et al. (2003b) screened a normalized cDNA library of 4,992 clones and identified 79 suppressors of the hypersensitive response (HR) to *Pseudomonas syringae*. Given the pivotal role of model organisms in advancing plant biology research (Gasparini et al. 2025), *N. benthamiana* was selected as the model plant for screening target genes.

Plants defend against pathogens through their innate immune system (Wu et al. 2023). Upon pathogen detection by immune receptors, a cascade of immune responses is triggered, starting with pattern-triggered immunity (PTI) followed by effector-triggered immunity (ETI) (Adachi et al. 2015; Ngou et al. 2022). PTI responses involve various defense mechanisms, including reactive oxygen species (ROS) production, calcium influx, mitogen-activated protein kinase (MAPK) activation, and induction of defense-related genes (Yuan et al. 2021; Osborne 2024). ROS act as critical secondary messengers, mediating downstream immune responses and playing essential roles in plant growth, development, and responses to biotic and abiotic stresses (Mittler et al. 2022; Wu et al. 2023). Plant peroxidases are recruited to transmit H_2O_2 signals and maintain cellular redox homeostasis. Among these, *thiol peroxidases* (TPXs) function as sensors and transducers of H_2O_2 (Calvo et al. 2013). Specific TPX genes, including *peroxiredoxin* (*PRXII*B), *peroxiredoxin Tpx1*, and *Arabidopsis glutathione peroxidase* (*AtGPX3*), relay H_2O_2 signals to downstream target proteins through oxidative post-translational modifications, facilitating redox signaling and enhancing plant resistance (Miao et al. 2006; Calvo et al. 2013; Bi et al. 2022).

Moreover, disease resistance genes often interact with downstream target proteins to regulate plant immune responses. For example, StMPK7 interacts with and stabilizes the potato RNA-binding proteins StUBA2a/b,

enhancing defense mechanisms (Li et al. 2022a). Similarly, PopbZIP2 binds to the promoters of its target genes, *PopGRF3* and *PopAPA1*, increasing cellular proanthocyanidin levels to strengthen pathogen resistance (Tan et al. 2025). Based on these observations, *TPX1* may act as a novel disease-resistance module, mediating immune responses through interactions with downstream target proteins.

Capsicum annuum Cell Division Cycle 48 (CDC48) is a highly conserved ATPase chaperone-like protein present in plants, microorganisms, and mammals (Ines et al. 2025). While its biochemical properties have been extensively characterized in microorganisms and animals, its role in plant immunity remains less understood (Li et al. 2022b). Emerging evidence suggests CDC48's involvement in plant immunity (Ao et al. 2021; Ines et al. 2025). For instance, overexpression of CDC48 restricts tobacco mosaic virus (TMV) infection in *Nicotiana tabacum* (Nicolas-Frances et al. 2024). In *Arabidopsis*, CDC48 is upregulated during infection, interacts with viral movement proteins, and promotes their degradation (Niehl et al. 2012). Additionally, *AtCDC48* negatively regulates NLR-mediated immunity (Copeland et al. 2016), while *NtCDC48* participates in cryptogin signaling and immune responses (Rosnoblet et al. 2017). Beyond immunity, CDC48 is involved in cell division and plant growth, as the CDC48 complex modulates the levels of chloroplast-ubiquitinated proteins (Li et al. 2022b). However, the specific role of CDC48 in pepper remains unexplored.

In this study, VIGS coupled with detached leaf assays was employed to screen a *N. benthamiana* cDNA-VIGS library, leading to the identification of *TPX1* as a positive regulator conferring significant resistance to *P. capsici*. Further characterization revealed that CaTPX1 interacts with its downstream partner CaCDC48, enhancing plant immunity by binding to and stabilizing CaCDC48 via oxidative signal transduction at the Cys51 residue. These findings provide valuable genetic resources for mechanistic studies of pepper disease resistance.

Materials and Methods

Plant growth conditions and pathogen inoculation assays

N. benthamiana and pepper plants were grown in a growth chamber under a 16-h light/8-h dark photoperiod at 25°C. Detached plant leaves were inoculated with a *P. capsici* zoospore suspension, with 500 zoospores per infection site on pepper leaves and 200 zoospores per infection site on *N. benthamiana* leaves. After inoculation, the leaves were incubated at 25°C with 100% relative humidity for 2–3 days, after which the lesion diameters were measured. Whole-plant infection assays were performed as described by Yang et al. (2025). The zoospore

suspension was sprayed onto tomato plants until runoff, with the zoospore concentration adjusted to approximately 1×10^5 zoospores/mL. At 3 day post infiltration (dpi), DNA was extracted from infected leaves, and RT-qPCR was conducted to assess *P. capsici* biomass.

Pathogen strains and growth conditions

The *P. capsici* isolates BS11-1 and BYA5 were cultured as previously described (Li et al. 2025a). Agar pieces (0.5×0.5 cm) containing mycelia were transferred to liquid CA medium and incubated for 3–5 days under standard conditions. The liquid medium was replaced daily with sterile distilled water to induce sporangia formation, followed by a cold shock at 4°C for 30 min, then a 30-min equilibration at 25°C to release zoospores.

Plasmid construction

The coding regions of *CaTPX1* (CA01g23340) and *CaCDC48* (CA09g02170) were PCR-amplified from the cDNA of the pepper cultivar 'Qimen' and subsequently inserted into various vectors for functional assays. For VIGS assays, fragments of 200–300 bp were cloned into the pTRV2 vector using the VIGS tool. For transient expression assays, *CaTPX1* and its mutant *CaTPX1^{CS1S}* were inserted into the pART27-CGFP vector. Point mutants, *CaTPX1^{CS1S}-GFP*, were generated by overlap extension PCR using *CaTPX1-GFP* as a template. *CaCDC48* was cloned into both pART27-C4myc and pART27-CGFP vectors. For luciferase complementation imaging (LCI) assays, *CaTPX1* and *CaCDC48* were cloned into the pCAMBIA1300-CLuc and pCAMBIA1300-NLuc vectors, respectively, to generate cluc-*CaTPX1* and *CaCDC48*-nluc constructs. All cloning fragments and point mutations were generated by PCR using primers specified in Table S1. PCR amplification was carried out using the *TransStart FastPfu* DNA Polymerase Kit.

Agroinfiltration and VIGS

The *N. benthamiana* cDNA-VIGS library was constructed following the method described by Helderma et al. (2022). *Agrobacterium tumefaciens* strain C58C1, harboring TRV2 plasmids containing random cDNA inserts, was preserved in 96-well plates at –80°C. For primary screening, 16 groups were prepared by mixing 5–6 *Agrobacterium* strains per group, which were then co-infiltrated with TRV1 (1:1 ratio, $OD_{600}=0.3$) into *N. benthamiana* plants. Groups displaying altered susceptibility to *P. capsici* were subjected to secondary screening with individual TRV2 clones combined with TRV1. The full screening process involved 96 TRV2-containing *Agrobacterium* clones from the cDNA-VIGS library, with five replicates of *N. benthamiana* plants per mixture,

followed by pathogen inoculation assays conducted three weeks post-infiltration.

Agrobacterium suspensions were adjusted to an OD_{600} of 0.3 for Co-IP, LCI, and infection assays. For co-expression experiments, agrobacteria containing different plasmids were mixed in a 1:1 ratio prior to agroinfiltration. To investigate the roles of *CaTPX1* and *CaCDC48* in pepper resistance responses, *A. tumefaciens* cultures containing TRV2 (either TRV-*CaTPX1* or TRV-*CaCDC48*) and TRV1 were mixed in a 1:1 ratio ($OD_{600}=0.8$) and infiltrated into the first two fully expanded leaves of 3-week-old pepper plants. After a 3-week growth period to establish VIGS, plants were either inoculated with *P. capsici* or analyzed for silencing efficiency. For transient expression assays, *A. tumefaciens* strain GV3101 harboring the vectors was cultured in liquid LB medium at 28°C for 24 h, harvested by centrifugation, and resuspended in agroinfiltration buffer.

RNA Isolation and RT-qPCR Analysis

Plant total RNA was extracted using the Plant RNA Isolation Mini Kit, and first-strand cDNA was synthesized from the total RNA using the Evo M-MLV RT Mix Kit. For quantitative PCR analysis, cDNA was used as the template in reactions containing SYBR Green Supermix, performed on the iQ7 Real-Time Cycler (Life Technologies, USA). Silencing efficiency detection primer pairs were designed outside the VIGS-targeting sequence.

Mass spectrometry analysis of CaTPX1-interacting proteins

Total proteins were extracted from pepper plants transiently expressing *CaTPX1-GFP* after 2 days and subsequently incubated with GFP-trap_A beads at 4°C for 2 h. After incubation, the beads were washed with dilution buffer. The bead-enriched protein complexes were pre-treated as previously described (Li et al. 2022a) and then submitted to high-sensitivity LC-MS/MS analysis (ThermoFisher, QExactive HF-X, Waltham, USA).

Co-immunoprecipitation and Western blot analysis

For Co-IP assays, 8 µL of GFP-Trap beads was incubated with 1.5 mL of protein extract at 4°C for 2 h, followed by multiple washes with GTEN buffer (Du et al. 2021). Immunoprecipitated protein complexes were then denatured in loading buffer. Western blot analysis was performed as described by Du et al. (Du et al. 2021), using primary antibodies anti-GFP and anti-myc in blocking buffer, followed by incubation with HRP-conjugated secondary antibodies. Protein signals were detected using an ECL Western blot detection kit.

Luciferase complementation imaging (LCI) assay

LCI assays were conducted as previously described (Chen et al. 2008). *Agrobacterium* cultures harboring cLUC and nLUC plasmids were co-infiltrated into *N. benthamiana* leaves for 2–3 days. Prior to imaging, infiltrated leaves were treated with luciferin substrate and dark-adapted for 5 min, after which luminescent signals were captured using a CCD camera system.

Tomato transformation

A. tumefaciens GV3101 harboring *CaTPX1-GFP* was transformed into tomato cultivar Ailsa Craig (AC) through an optimized tissue culture protocol. Fully expanded tomato cotyledons were cut into segments and pre-cultured on MS0 medium (MS, 1.5 mg/L Zeatin, 0.5 mg/L IAA). *Agrobacterium*, cultured in LB liquid medium, was collected and resuspended in liquid MS0 medium to OD₆₀₀=0.6 for explant inoculation. After a 2-day co-cultivation period in darkness, the explants were sequentially transferred to selective media: MS1 (MS, 50 mg/L kanamycin, 2 mg/L Zeatin, 300 mg/L Timentin) for callus induction, MS2 (MS, 50 mg/L kanamycin, 0.2 mg/L Zeatin, 300 mg/L Timentin) for shoot regeneration, and MS3 (MS, 50 mg/L kanamycin, 0.2 mg/L IAA, 300 mg/L Timentin) for root development. The acclimatized transformants were then transferred to sterile soil in a growth chamber. One week after transplanting into pots, PCR was performed to detect the transformants using primers for the 35S promoter and reverse primers for the full-length *CaTPX1* gene (pKAN-F and *CaTPX1-GFP*-HindIIIIR).

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Differences in lesion areas between groups were assessed using Student's *t*-tests. All statistical analyses were performed with GraphPad Prism software (version 10). Each experiment was independently replicated at least three times to ensure reproducibility.

Results

NbTPX1 acts as a positive regulator of immunity against *P. capsici*

This study utilized an *N. benthamiana* cDNA-VIGS library to conduct a random screen for disease resistance or susceptibility genes to *P. capsici*. The screening method followed that of Helderma et al. (2022). Detached leaf assays were conducted using *P. capsici* isolate BS11-1. The results indicated that the initial 10 groups displayed symptoms of either disease resistance or susceptibility (Fig. 1a). Specifically, 13 genes in *N. benthamiana* leaves resulted in smaller lesions compared to the control TRV-*GUS* plants 2 days after inoculation with *P. capsici*,

suggesting that silencing 13.5% of these genes increased susceptibility to *P. capsici*. Conversely, 15 genes in *N. benthamiana* leaves produced larger lesions compared to the control, indicating that 15.6% of these genes conferred resistance to *P. capsici* (Fig. 1a, b). The remaining 70.9% of the clones showed no significant difference in lesion size compared to the control (Fig. 1b). Among these, clone 11-93 was found to promote *N. benthamiana* resistance to *P. capsici* (Fig. 1c, d; Table S2). Sequencing revealed that this clone encodes a thiol peroxidase gene, which was named *NbTPX1* (Niben101Scf03711g01005). To identify the *Solanaceae* orthologs of 11-93, homologous protein sequences from pepper, tomato, potato, *N. benthamiana*, and *Arabidopsis thaliana* were retrieved via BLAST searches (Sol Genomics Network and TAIR), and several homologous proteins were identified. Phylogenetic analysis revealed that *CaTPX1* (CA01g23340) is the ortholog of *NbTPX1* in *N. benthamiana* (Fig. 1e).

TPX1 positively regulates plant immunity to *P. capsici*

To further investigate the role of *TPX1* in plant immunity, *CaTPX1* (CA01g23340) was silenced in pepper, and the plants were inoculated with *P. capsici*. RT-qPCR results indicated a significant downregulation of *CaTPX1* expression in TRV-*CaTPX1* plants compared to TRV-*GUS* plants (Fig. S1a, b). Detached leaf assays showed that silencing *CaTPX1* in pepper (TRV-*CaTPX1*) increased susceptibility to *P. capsici* infection (Fig. 2a, b). To confirm this, *CaTPX1-GFP* was transiently expressed in pepper leaves, which were then inoculated with *P. capsici*. The results demonstrated that transient overexpression of *CaTPX1-GFP* significantly enhanced disease resistance in pepper leaves compared to *GUS-GFP* (Fig. 2c, d), further supporting the role of *CaTPX1* as a positive regulator of plant immunity. Additionally, *CaTPX1* expression was upregulated following inoculation of detached leaves with *P. capsici* zoospores, peaking at 6 h post-inoculation (Fig. S1e).

To further investigate the role of *TPX1* in plant defense, the homologous gene of *TPX* in tomato (*SITPX1*) was silenced. RT-qPCR analysis confirmed that *SITPX1* expression was significantly downregulated in TRV-*SITPX1* tomato plants (Fig. S2a, b). Detached leaf assays revealed that TRV-*SITPX1* plants developed significantly larger lesions compared to control TRV-*GUS* plants (Fig. 3a, b). Additionally, *CaTPX1-GFP* was transformed into tomato cultivar AC via *Agrobacterium*-mediated stable transformation. Genomic DNA extracted from the transformed tomato plants was subjected to PCR detection, with untransformed wild-type AC used as a control. PCR results confirmed the presence of the target size bands in OE-6, OE-9, and OE-11 lines, which were preliminarily identified as

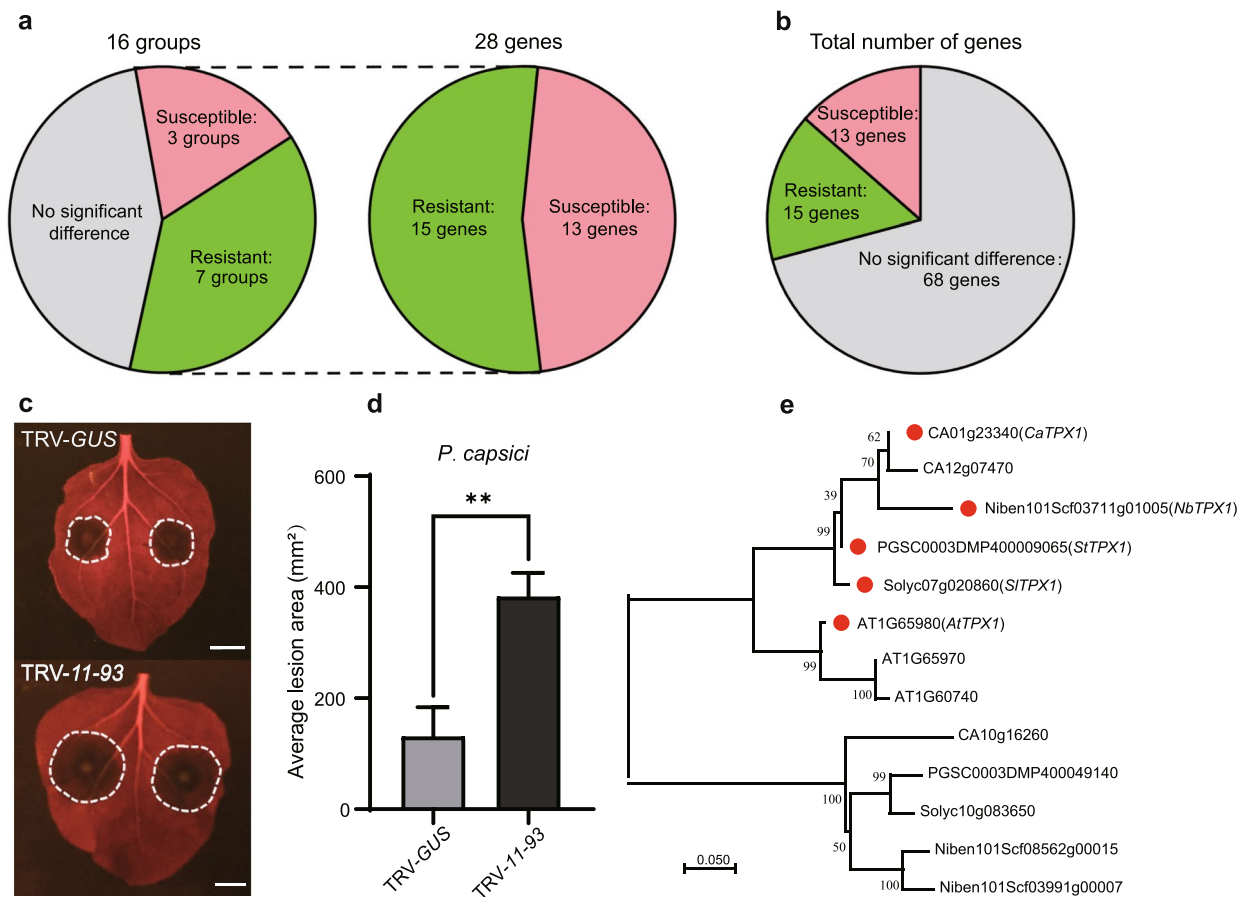


Fig. 1 A virus-induced gene silencing (VIGS)-based random screen to identify positive regulators of immunity in *Nicotiana benthamiana* against *Phytophthora capsici*. **a** The number of random gene groups screened in the first round (left) and the number of confirmed tobacco rattle virus (TRV) genes associated with disease symptoms (**b**) in detached leaf assays. **b** Total number of random genes screened. **c** Silencing of the 11-93 clone increased *P. capsici* lesion areas on *N. benthamiana* leaves at 2 days after inoculation. Scale bar, 1 cm. **d** Average lesion areas on leaves expressing TRV-GUS and TRV-11-93 ($n \geq 8$; one-sided Student's *t*-test; **, $P \leq 0.01$). **e** Phylogenetic analysis of Thiol Peroxidase 1 (TPX1) proteins from *Arabidopsis* and *Solanaceae* plants. The red dots represent the homologous protein sequence of TPX1

positive transformants (Fig. S2c, d). Detached leaves from 8-week-old transgenic tomato plants were used for detached leaf assays. Statistical analysis showed that, compared to wild-type AC plants, lesions on *CaTPX1*-GFP-OE leaves were smaller (Fig. 3c, d). Seeds from the OE-11 (T_0) line were harvested and planted. PCR testing of the T_1 generation seedlings confirmed the presence of two positive transformants, OE-1 and OE-3 (Fig. S2e, f). Whole-plant infection assays revealed that the *CaTPX1*-GFP overexpression lines exhibited milder disease symptoms 3 days post-inoculation with *P. capsici* compared to wild-type AC plants (Fig. 3e; Fig. S2g). Pathogen biomass accumulation assays showed lower pathogen colonization levels in the *CaTPX1*-GFP overexpression lines than in wild-type plants (Fig. 3f). These results confirmed that overexpression of *CaTPX1* in tomato enhances plant immunity against *P. capsici*.

The Cys51 of CaTPX1 is crucial for plant immunity

Previous studies have demonstrated that sulfenylation of thiol peroxidases like *PRXIIB* at the peroxidatic/catalytic cysteine is essential for stomatal immunity, with cysteine serving as a critical site for enzyme activity (Rouhier and Jacquot 2005; Bi et al. 2022). To determine whether oxidative signal transduction is also involved in *CaTPX1*, the orthologous protein sequences of *AtTPX1*, *CaTPX1*, *SlTPX1*, *StTPX1*, and *NbTPX1* were aligned. The results indicated that the Cys51 residue is conserved across these species (Fig. S3). To investigate the importance of *CaTPX1*^{Cys51} oxidation in activating immunity against *P. capsici*, the key cysteine (C) residue was substituted with a serine (S) residue (*CaTPX1*^{C51S}), thereby disrupting the oxidation signal. To functionally characterize the redox-sensitive Cys51 residue of *CaTPX1*, *GUS*-GFP and *CaTPX1*^{C51S}-GFP were transiently expressed in

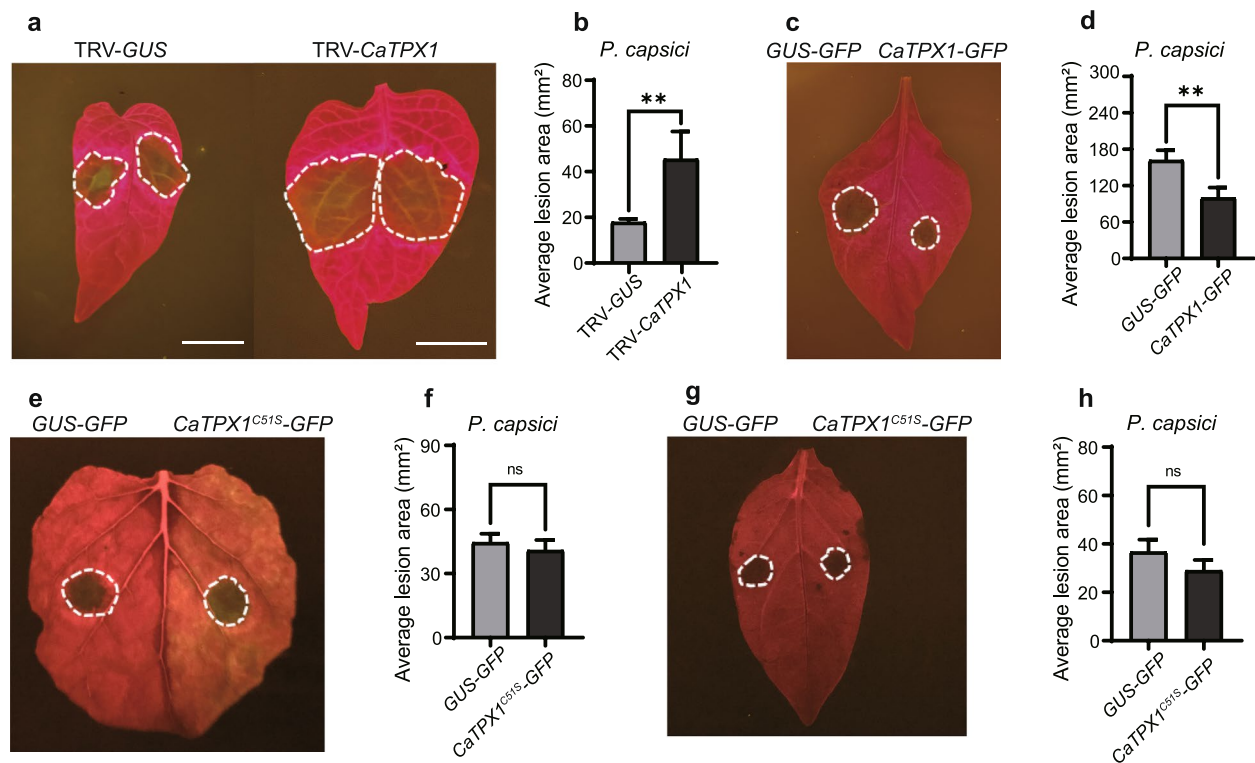


Fig. 2 *Capsicum annuum* Thiol Peroxidase 1 (CaTPX1) positively regulates pepper immunity to *Phytophthora capsici*. **a** Symptoms of *P. capsici* lesion in detached pepper leaves from plants expressing tobacco rattle virus (TRV)-GUS and TRV-CaTPX1 at 3 days after inoculation. Scale bar, 1 cm. **b** Average lesion areas on leaves expressing TRV-GUS and TRV-CaTPX1. Asterisks indicate significant differences ($n \geq 8$; one-sided Student's *t*-test; **, $P \leq 0.01$). **c** A representative leaf expressing CaTPX1-GFP and GUS-GFP (control) and inoculated with *P. capsici* isolate BYA5 for 3 days. **d** Average lesion areas on pepper leaves expressing GUS-GFP and CaTPX1-GFP. **e** A detached *N. benthamiana* leaf expressing peroxidase/catalytic cysteine (CaTPX1^{C51S})-GFP and GUS-GFP that was inoculated with *P. capsici* isolate BYA5 for 2 days after inoculation. **f** Average lesion areas on a *N. benthamiana* leaf expressing CaTPX1^{C51S}-GFP and GUS-GFP. **g** A detached pepper leaf expressing CaTPX1^{C51S}-GFP and GUS-GFP after 3 days of inoculation. **h** Average lesion areas on a pepper leaf expressing CaTPX1^{C51S}-GFP and GUS-GFP

N. benthamiana and pepper leaves. Detached leaf assays conducted 1 dpi showed no significant difference in lesion size between CaTPX1^{C51S}-GFP-expressing leaves and the control, indicating that Cys51 is essential for CaTPX1-mediated immunity against *P. capsici* (Fig. 2e–h).

CaCDC48 interacts with CaTPX1

To identify the target proteins of CaTPX1 in plants, this study transiently expressed CaTPX1-GFP in pepper leaves and performed Co-IP to isolate interacting protein complexes. LC-MS/MS analysis was then conducted to examine the candidate interacting proteins of CaTPX1. The results revealed that 677 target proteins exhibited increased expression levels following CaTPX1 overexpression. Among these, several candidate proteins interacting with CaTPX1 were identified based on genetic annotations, with the top 20 listed in Table S3. After confirming the interactions and conducting functional analysis, CaCDC48 was selected as a potential target. To

verify the interaction between CaTPX1 and CaCDC48, CaCDC48-myc was transiently co-expressed with either CaTPX1-GFP or GFP in *N. benthamiana* leaves, followed by Co-IP. The results showed that CaCDC48-myc specifically co-immunoprecipitated with CaTPX1-GFP, but not with the negative control GFP (Fig. 4a). LCI assays were further performed to confirm the interaction. The results demonstrated that the combination of cluc-CaTPX1 with CaCDC48-nluc restored luciferase catalytic activity, similar to the positive control *Solanum tuberosum* Mitogen-activated Protein Kinase Kinase 1 (StMKK1)-nluc and cluc-20303 (Du et al. 2021), whereas co-expression of CaCDC48-nluc and cluc-20303 did not (Fig. 4b). These findings confirmed that CaCDC48 interacts strongly with CaTPX1.

Furthermore, to assess whether the mutation of the CaTPX1 Cys51 residue affects its interaction with CaCDC48, Co-IP and LCI assays were conducted. The results showed that CaTPX1^{C51S}-GFP exhibited significantly reduced binding affinity for CaCDC48-myc

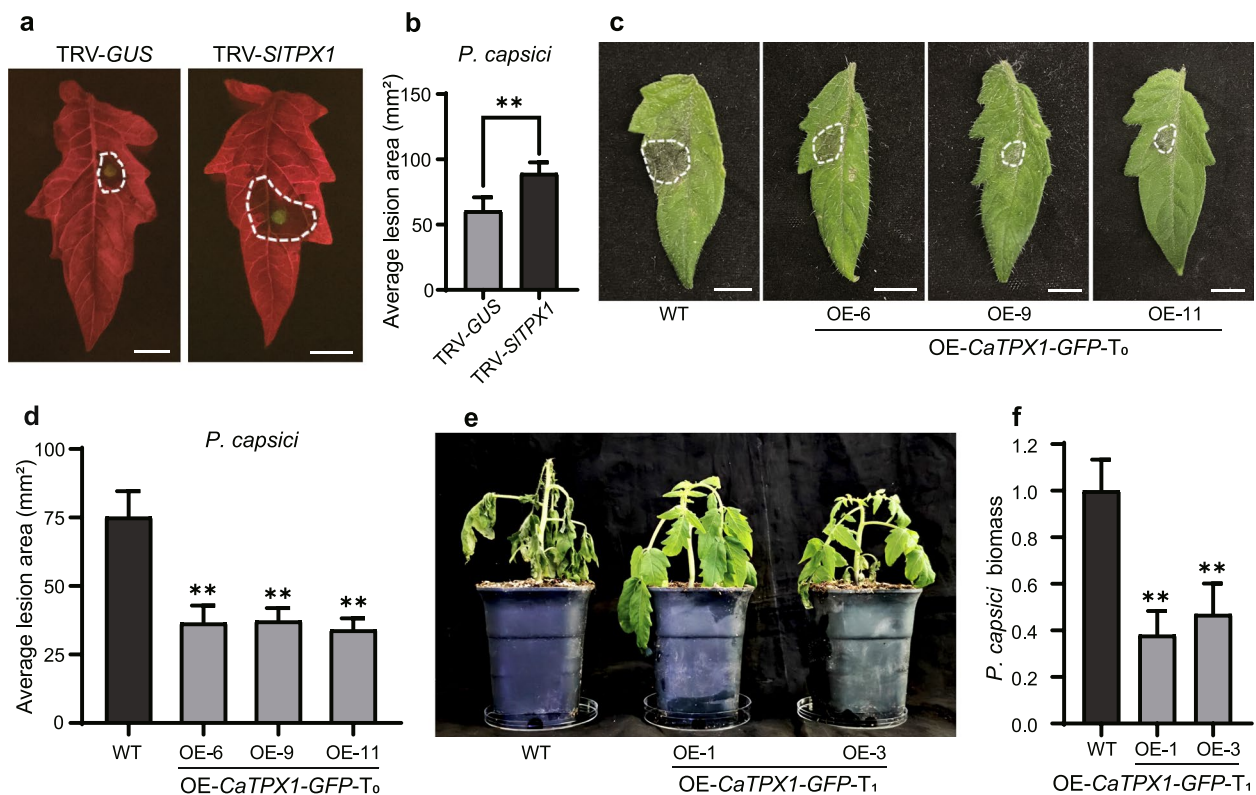


Fig. 3 *Capsicum annuum* Thiol peroxidase 1 (CaTPX1) and *Solanum lycopersicum* TPX1 (SITPX1) positively regulate tomato defense against *Phytophthora capsici*. **a** Symptom of *P. capsici*-inoculated detached tomato leaf expressing tobacco rattle virus (TRV)-GUS and TRV-SITPX1 2 days after inoculation. Scale bar, 1 cm. **b** Average lesion areas on inoculated tomato leaves expressing TRV-GUS and TRV-SITPX1. **c–d** Symptom (**c**) and average lesion areas (**d**) of *P. capsici*-inoculated detached tomato leaves from wild-type Ailsa Craig (WT) and transgenic overexpressing (OE) lines (OE-CaTPX1-GFP-T₀) at 2 days after inoculation. Scale bar, 1 cm. **e** Plant phenotype of WT and transgenic plants (OE-CaTPX1-GFP) after inoculation with *P. capsici* for 2 days. **f** *P. capsici* biomass in the WT and two transgenic tomato lines (OE-1 and OE-3, T₁ generation) overexpressing CaTPX1-GFP after inoculation for 2 days. Asterisks indicate significant differences ($n \geq 8$; one-sided Student's *t*-test; **, $P \leq 0.01$)

(Fig. 4a, b). These results provide strong evidence that the oxidative signal transduction of CaTPX1, mediated by its conserved Cys51 residue, is crucial for its interaction with CaCDC48.

CaCDC48 is stabilized by CaTPX1

Interestingly, co-expression of CaTPX1-GFP with CaCDC48-myc resulted in enhanced protein expression of CaCDC48-myc compared to co-expression with the negative control GFP (Fig. 4a). To determine if CaCDC48 is stabilized by CaTPX1, CaCDC48-myc was transiently co-expressed with either CaTPX1-GFP or CaTPX1^{C51S}-GFP in *N. benthamiana* leaves, with CaCDC48-myc and GFP co-expressed as a negative control. Total proteins were extracted at 2 dpi and subjected to western blot analysis. The results indicated that co-expression of CaTPX1-GFP and CaCDC48-myc likely increased the protein accumulation of CaCDC48 compared to the negative control (Fig. 4c).

CaCDC48 is a positive regulator of plant immunity against *P. capsici*

To investigate the role of CaCDC48 in plant immunity against *P. capsici*, CaCDC48-GFP and control GUS-GFP were transiently expressed in pepper leaves, followed by detached leaf assays and *Pathogenesis-Related* (PR) gene expression analysis. The results revealed that CaCDC48-GFP expression significantly reduced lesion size compared to the control GUS-GFP (Fig. 5a, b). PR gene analysis indicated that overexpression of CaCDC48-GFP induced the expression of CaPR1 and CaPR10 (Fig. S4a, b). To further confirm the function of CaCDC48, VIGS was used to silence CaCDC48 in pepper plants. The results showed that TRV-CaCDC48 plants exhibited significantly larger lesions compared to the control TRV-GUS plants (Fig. 5c, d), supporting the positive regulatory role of CaCDC48 in plant defense against *P. capsici*. The expression of CaCDC48 was significantly downregulated in TRV-CaCDC48

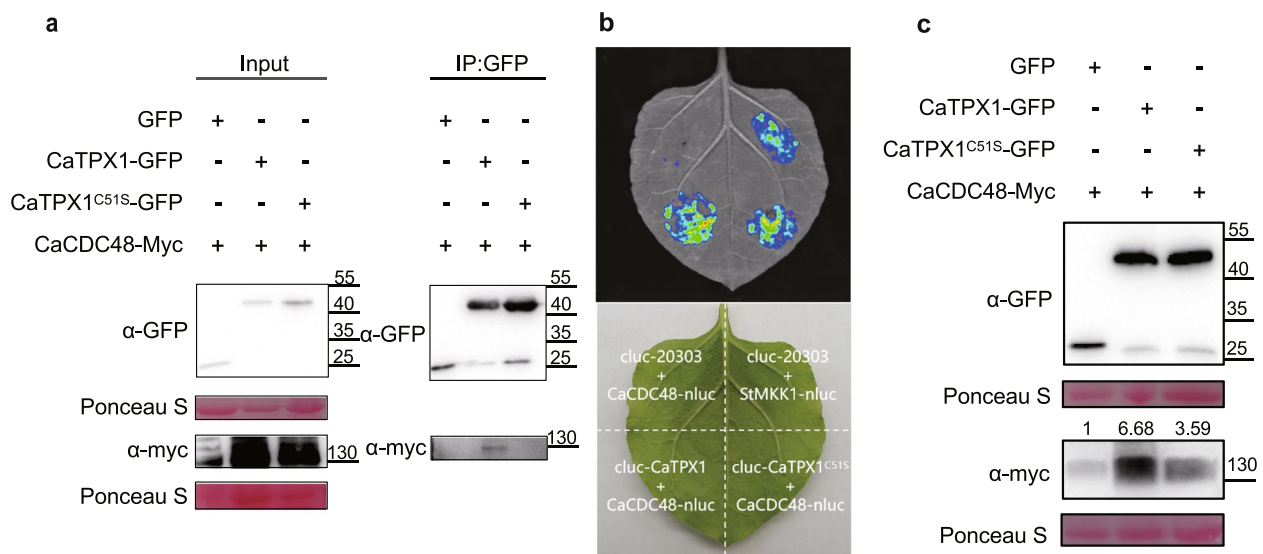


Fig. 4 *Capsicum annuum* Thiol peroxidase 1 (CaTPX1) interacts with and stabilizes *Capsicum annuum* Cell Division Cycle 48 (CaCDC48). **a** Coimmunoprecipitation (Co-IP) assays demonstrates that CaTPX1-GFP interacts with CaCDC48-myc, while peroxidatic/catalytic cysteine (CaTPX1^{C51S})-GFP exhibited significantly weakened binding affinity to CaCDC48-myc. Protein loading is indicated by Ponceau S stain. Proteins were extracted 2 days post infiltration (dpi). GFP-Trap beads (IP:GFP) were used for immunoprecipitation. **b** Firefly luciferase complementation imaging (LCI) assays for interaction between CaTPX1 and CaCDC48. The CaTPX1^{C51S} mutant exhibited significantly reduced binding affinity to CaCDC48. Fluorescence imaging was taken at 2 dpi. Co-expression between CaCDC48-nluc and cluc-20303 was used as a negative control, while the combination of *Solanum tuberosum* Mitogen-activated Protein Kinases Kinase 1 (StMKK1)-nluc with cluc-20303 acts as the positive control. The luminescence signals (upper) and the corresponding bright-field image (bottom) of the same leaf infiltrated with the designated vectors are shown. **c** Detection of levels of proteins extracted at 2 dpi from the *N. benthamiana* leaves co-expressing CaCDC48-myc and CaTPX1-GFP, CaTPX1^{C51S}-GFP, or GFP. Protein loading is indicated by Ponceau S staining. Values indicate the relative intensity of myc normalized to Rubisco

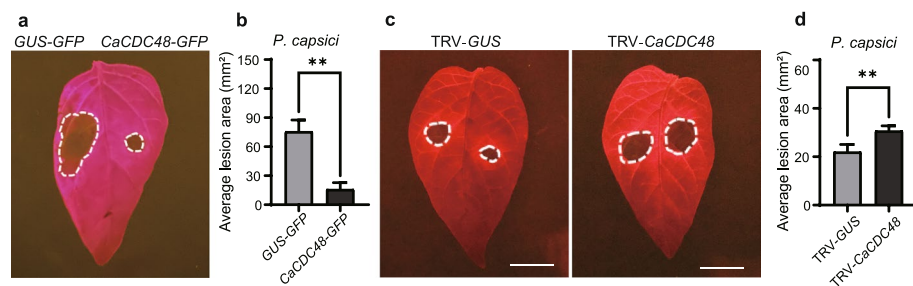


Fig. 5 *Capsicum annuum* Cell Division Cycle 48 (CaCDC48) positively regulates pepper resistance to *Phytophthora capsici*. **a–b** Symptom (**a**) and average lesion areas (**b**) in the pepper leaves expressing GUS-GFP and CaCDC48-GFP at 3 days after inoculation of *P. capsici*. **c–d** Symptom (**c**) and average lesion areas (**d**) in the pepper leaves from tobacco rattle virus (TRV)-GUS and TRV-CaCDC48 plants at 3 days after inoculation. Scale bar, 1 cm. Asterisks indicate significant differences ($n \geq 8$; one-sided Student's *t*-test; **, $P \leq 0.01$)

plants compared to the TRV-GUS control (Fig. S1c, d). Additionally, CaCDC48 expression increased following inoculation of detached leaves with *P. capsici* zoospores, peaking at 6 h post-inoculation (Fig. S1f).

Discussion

Thiol peroxidase genes are evolutionarily conserved across a wide range of organisms, highlighting their essential functional roles (Fomenko et al. 2011). The present study identified a thiol peroxidase gene,

NbTPX1 (11-93), which enhances *N. benthamiana* resistance to the oomycete pathogen *P. capsici*. Phylogenetic analysis revealed close homology between *NbTPX1*, *CaTPX1*, and *SlTPX1*. To determine whether the role of *TPX1* in plant defense is conserved across species, *CaTPX1* and *SlTPX1* were silenced in pepper and tomato using VIGS, followed by infection assays. The results demonstrated that both *CaTPX1* and *SlTPX1* positively regulate plant resistance to *P. capsici*. Supporting these findings, heterologous expression

of *CaTPX1-GFP* in tomato and transient overexpression in pepper, followed by pathogen infection assays, further confirmed *TPX1*'s conserved role in enhancing *P. capsici* resistance across *Solanaceous* species. Integrating infection assay results from *N. benthamiana*, pepper, and tomato with multiple sequence alignment analyses, these findings highlight the functional conservation of thiol peroxidase *TPX1* across *Solanaceae* species, suggesting its potential broad applicability in breeding programs aimed at improving disease resistance in *Solanaceous* crops.

In immune-stimulated cells, *TPX* is sulfenylated or directly oxidized at Cys51, which subsequently transduces the endogenous H_2O_2 signal to downstream target proteins through intermolecular disulfide bonds, thus contributing to the regulation of plant immunity (Bela et al. 2015; Bi et al. 2022). To functionally characterize this redox-sensitive residue, the key cysteine (C) residue was substituted with a serine (S) to generate the *CaTPX1*^{C51S} point mutant. Subsequently, *GUS-GFP* or *CaTPX1*^{C51S}-*GFP* was transiently expressed in *N. benthamiana* and pepper leaves, followed by pathogen inoculation assays. The results revealed that *CaTPX1*^{C51S}-*GFP* lost its disease resistance to *P. capsici*. These findings establish Cys51 as essential for *CaTPX1*-mediated immunity against *P. capsici*, suggesting that mutation of the Cys51 residue disrupts the transduction of oxidative signals.

The thiol peroxidase *TPX1* has been identified as a positive regulator of plant immunity, acting as an atypical resistance-related gene involved in disease resistance. Expression of *CaTPX1* is induced by *P. capsici*. Mutation of the cysteine (C) residue leads to a loss of resistance, suggesting its critical role. Further investigation revealed that *CaTPX1* enhances plant immunity by interacting with its downstream component, *CaCDC48*, promoting the accumulation of *CaCDC48*. Mutation of the Cys51 residue weakened this interaction. Thiol peroxidases are pivotal in $H_2O_2^-$ and redox-dependent signaling, and *TPX* may function as an H_2O_2 sensor in response to environmental stress (Miao et al. 2006; Calvo et al. 2013; Bi et al. 2022; Vogelsang and Dietz 2022). Therefore, *TPX1* in pepper is likely involved in regulating plant disease resistance through its role as an H_2O_2 sensor. The Cys51 mutant, however, fails to transduce the endogenous H_2O_2 signal to the downstream target protein. The molecular mechanisms behind *CaTPX1*-mediated stabilization of *CaCDC48* and the potential role of *CaCDC48* downstream of *CaTPX1* in the defense signaling pathway require further investigation.

Using IP-MS analysis, *CaCDC48* was identified as a target protein of *CaTPX1*. *CDC48* encodes a hexameric AAA-type ATPase, a key component in the cell division cycle (Ines et al. 2025). Detached leaf assays

demonstrated that *CaCDC48* positively regulates plant immune responses and induces the expression of SA-related defense genes, *CaPR1* and *CaPR10*. These findings suggest that *CaCDC48* may enhance disease resistance by modulating *PR* gene expression, which is regulated through the SA signaling pathway (Li et al. 2025b; Molinari et al. 2014). Additionally, *CaCDC48* expression was found to increase following *P. capsici* induction, implying that *P. capsici* infection triggers *CaCDC48* gene expression, which, in turn, induces *PR* gene expression and contributes to disease resistance. Additionally, *CDC48* regulates plant immunity by maintaining ubiquitin homeostasis (Ao et al. 2021; Ines et al. 2025; Nicolas-Frances et al. 2024; Niehl et al. 2012; Copeland et al. 2016), influencing the accumulation of ubiquitinated proteins and processing of ubiquitinated substrates (Ao et al. 2021; Rosnoblet et al. 2017). Therefore, further research is required to elucidate the precise mechanism by which *CaCDC48* mediates the positive regulation of plant immunity through *CaTPX1*.

Conclusions

In conclusion, VIGS screening of the *N. benthamiana* cDNA library led to the identification of the thiol peroxidase *TPX1* as a positive regulator of plant immunity against *P. capsici*. IP-MS analysis revealed that *CaTPX1* interacts with *CaCDC48*, a protein that positively regulates pepper defense against *P. capsici*. Biochemical analyses confirmed that *CaTPX1* binds to *CaCDC48*, promoting its accumulation. Further investigation showed that the oxidation signal mutant, *CaTPX1*^{C51S}, lost the ability to regulate immunity, and mutation of the Cys51 residue weakened the interaction and stabilization between *CaTPX1* and *CaCDC48*. These findings suggest that *CaTPX1* functions as an H_2O_2 sensor, transducing oxidative signals to *CaCDC48*, thereby modulating pepper resistance to *P. capsici* infection.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44281-025-00087-2>.

Additional file 1. Figure S1. Plant morphology of tobacco rattle virus-*Capsicum annuum* Thiol Peroxidase 1 (TRV-*CaTPX1*) and TRV-*Capsicum annuum* Cell Division Cycle 48 (*CaCDC48*) inoculated pepper plants. a, c The developmental phenotype of 3-week-old pepper plants was examined following inoculation with TRV constructs, including TRV-*GUS* (negative control), TRV-*CaTPX1* or TRV-*CaCDC48* (gene silencing targets), and TRV-*Capsicum annuum* Phytoene desaturase (*CaPDS*, used as a control), with representative images for each treatment group. b, d RT-qPCR data showing relative expression of *CaTPX1* and *CaCDC48* genes in pepper plants inoculated with the indicated TRV clones ($n \geq 8$). *CaActin* was used as the reference gene for normalization. One-sided *t*-tests were used to assess significance; **, $P \leq 0.01$. e Expression levels of *CaTPX1* at different time points after inoculation with *P. capsici*. f Expression levels of *CaCDC48* at different times after inoculation with *P. capsici*.

Additional file 2. Figure S2. Plant morphology of tobacco rattle virus-*Solanum lycopersicum* Thiol Peroxidase 1 (TRV-SITPX1) and *Capsicum annuum* TPX1 (CaTPX1) overexpression transgenic tomato plants. a Developmental phenotype of 3-week-old tomato plants inoculated with TRV-GUS, TRV-SITPX1, and TRV-*Solanum lycopersicum* Phytoene desaturase (SIPDS, used as a control). Representative images are shown for each TRV construct. b RT-qPCR data showing relative expression of SITPX1 genes in tomato plants inoculated with the indicated TRV clones ($n \geq 8$). Slactin was used as the reference gene for normalization. One-sided t-tests were used to assess significance; **, $P \leq 0.01$. c DNA identification of CaTPX1-GFP transgenic tomato plants at T₀ generation. d Morphology of wild-type tomato Ailsa Craig (WT) and CaTPX1-GFP overexpression transgenic tomato plants (T₀ generation). e DNA identification of CaTPX1-GFP in T₁ generation transgenic tomato plants. f Morphology of wild-type (WT) tomato AC and CaTPX1-GFP overexpression transgenic tomato plants (T₁ generation). g Leaf phenotype of WT and CaTPX1-GFP transgenic plants in the whole-plant infection assay (T₁ generation)

Additional file 3. Figure S3. Thiol Peroxidase 1 (TPX1) Sequence alignment of orthologous proteins from *Arabidopsis* and several *Solanaceae* plants. Accession numbers of the protein sequences used for alignment are as follows: AtTPX1 (Thiol Peroxidase 1 of *Arabidopsis thaliana*), AT1G65980; CaTPX1 (TPX1 of *Capsicum annuum*), CA01g23340; SITPX1 (TPX1 of *Solanum lycopersicum*), Solyc07g020860; StTPX1 (TPX1 of *Solanum tuberosum*), PGSC0003DMP400009065; NbTPX1 (TPX1 of *Nicotiana benthamiana*), Niben101Scf03711g01005. Protein sequences of selected accessions were aligned using DNAMAN software. The black star indicates the conserved cysteine

Additional file 4. Figure S4. Expression levels of *Capsicum annuum* Pathogenesis-Related 1 (CaPR1, a) and CaPR10 (b) genes after overexpression of *Capsicum annuum* Cell Division Cycle 48 (CaCDC48)-GFP and GUS-GFP (control) for 2 days. CaActin was used as the reference gene for normalization

Additional file 5. Table S1. Primers used in the study. The red font indicates the restriction enzyme cutting sites. CaTPX1, *Capsicum annuum* Thiol Peroxidase 1; SITPX1, *Solanum lycopersicum* TPX1; CaCDC48, *Capsicum annuum* Cell Division Cycle 48

Additional file 6. Table S2. Sequence information of genes with immune functions screened from the *Nicotiana benthamiana* cDNA-VIGS library

Additional file 7. Table S3. Information on *Capsicum annuum* Thiol Peroxidase 1 (CaTPX1)-interacting candidate proteins

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Authors' contributions

YD designed the research. YYG, RXQ, WJT, and QY performed the experiments and analysed the data. RXQ wrote the manuscript and YD revised the manuscript. All authors reviewed the manuscript.

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Data availability

All data are available in the main text or in the supplementary materials.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests. Prof. Yu Du is an editorial board member of *Horticulture Advances* and was not involved in the journal's review or decisions related to this manuscript.

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