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A novel eggplant AP2/ERF transcription factor *StPti5* confers resistance to Verticillium wilt across different species

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Abstract The APETALA 2/ethylene response factors (AP2/ERFs) are considered essential in plant disease resistance responses. In this study, a novel eggplant AP2/ERF transcription factor gene, *StPti5*, was identified and functionally described from wild eggplant (*Solanum torvum* Sw.). The results of bioinformatics analysis show that *StPti5* protein contains a DNA-binding AP2/ERF domain and shares high degree of amino acid similarity with the other known AP2/ERF domain. Subcellular localization assay shows that *StPti5* protein was localized in the nucleus. Expression of *StPti5* was induced by infection with *Verticillium dahliae*, ethylene, and methyl jasmonate. Analysis of expression patterns suggests that the function of *StPti5* in resistance to Verticillium wilt was most closely linked to ethylene signaling. Overexpression of *StPti5* in *Arabidopsis thaliana* could improve disease resistance to *V. dahliae* and activate genes linked to the ethylene signaling pathway. Studies on the *A. thaliana* defence response revealed that the formation of reactive oxygen species (ROS) increased considerably following overexpression of *StPti5*. In summary, our analysis suggest that *StPti5* gene is a functional gene that could improve eggplant's resistance to *V. dahliae*.

Keywords: *Solanum torvum;* ERF transcription factors; *Verticillium dahliae;* disease resistance; ethylene signaling

Eggplant (*Solanum melongena* L.) is one of the most significant vegetable crops worldwide, and China has the largest eggplant production area. In 2014, the cultivation area of eggplant in China was 800 000 ha, with a total production of 29.5 million t, accounting for 60% of world production (Yang et al. 2019). However, several diseases seriously affect eggplant yield and fruit quality, one of which is eggplant Verticillium wilt caused by the fungal pathogen *Verticillium dahliae* and brings about economic losses worldwide. Verticillium wilt is a vascular disease that can result in yellow discolouration or defoliation and, eventually death of plants (Fradin & Thomma 2006; Wally & Punja 2010).

Traditional control methods such as chemical and biological control cannot effectively control the disease for eggplant Verticillium wilt. The lack of resistant cultivars to this pathogen in eggplant limits the progress of genetic breeding for disease resistance. *Solanum torvum* Sw., also known as tur-

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key berry, is a wild eggplant relative frequently used as a rootstock for protected cultivation (Yamaguchi et al. 2010). *S. torvum* is resistant to Verticillium wilt (Bletsos et al. 2003), which is essential to discovering disease resistance genes in improving eggplant Verticillium wilt disease resistance.

Plant transcription factors (TFs) play critical roles in plant growth, development, and response to various biotic and abiotic stresses (Rashid et al. 2012; Shu et al. 2016). The AP2/ERF superfamily is one of the largest groups of TFs in plants (Nakano et al. 2006), which contains one or two AP2 domains with 60–70 conserved amino acid residues. These amino acid residues are all made up of a three-stranded anti-parallel beta-sheet and an alpha helix. Previously, through the analysis of suppression subtractive hybridization (SSH) libraries, we have isolated genes in a *S. torvum*, that were responsive to *V. dahliae* infection. Among the sequences identified, we found a cDNA fragment representing the transcriptional activator PTI5 gene of pathogenesis-related genes (designated *StPti5*) significantly increased in response to *V. dahliae* infection. The protein encoded by PTI5 is a typical plant transcription factor in tomatoes, which belongs to Ethylene responsive element binding factor (ERF) (He et al. 2001; Wu et al. 2015). ERFs, a major subfamily of the AP2/ERF transcription factor superfamily, specifically bind to the promoter of genes with a GCC frame and then induce the expression of downstream genes (Ohme-Takagi & Shinshi 1995). The AP2/ERF family has important roles in plant response to abiotic and biotic stresses (Gilmour et al. 2000; Sharabi-Schwager et al. 2009; Zhao et al. 2012; Mishra et al. 2015; Chen et al. 2022). Ethylene signal activates the expression of many pathogenesis-related (PR) proteins and other genes that facilitate defence responses (Eyal et al. 1993). Overexpression of ERF genes in *A. thaliana*, wheat, cotton, tomato can induce the expression of pathogenesis-related genes (PRs), enhance reactive oxygen species (ROS) scavenging capacity and increase the resistance of plants to fungi, bacteria and viruses (He et al. 2001; Pré et al. 2008; Meng et al. 2013; Guo et al. 2016; Hawku et al. 2021; Zhu et al. 2021). The AP2/ERF, transcription factor superfamily also participates in several hormone signaling pathways, such as the ethylene, jasmonic acid, and salicylic acid pathways (Fujimoto et al. 2000; Mantiri et al. 2008).

Although many investigations have shown that AP2/ERF transcription factors played important roles in plant pathogen defence, only a few genes have been found in eggplant (Shen et al. 2022). To explore the potential role of *StPti5* gene in plant resistance to Verticillium wilt, the gene (named *StPti5*) was cloned and characterized from *S. torvum*. The putative *StPti5* protein contains a DNA-binding AP2/ERF domain and shares a high degree of amino acid similarity with the other known AP2/ERF domain. Subcellular localization assay shows that *StPti5* protein was localized in the nucleus. The expression of the *StPti5* gene was induced when subjected to infection by *V. dahliae* and treatment with disease-related hormones. We then heterologously expressed *StPti5* in *Arabidopsis* and found that the transgenic *Arabidopsis* plants exhibited increased resistance to *V. dahliae*. We found a significant increase in reactive oxygen species (ROS) levels, and genes related to the ethylene signaling pathway and pathogenesis-related genes were induced during *V. dahliae* infection. This study will contribute to understanding the functions of *StPti5* and the regulation of defence responses against *V. dahliae* infection.

MATERIAL AND METHODS

Plant materials and inoculation method. The highly resistant eggplant species (*S. torvum* SW.) and *A. thaliana* seeds were sprouted on a wet cloth, and the seedling plants were transplanted into substrate cultures in a greenhouse. The eggplant and *A. thaliana* were cultured at 25 ℃ and 22 ℃, respectively with a light/dark cycle of 16/8 h.

A *V. dahliae* strain was identified from the diseased plants in the field and identified with pathogenicity. The pathogenic fungus was placed on potato dextrose agar (PDA) plates and incubated at 25 °C for 14 days to collect spores. The infection tests of wild eggplant and *A. thaliana* with *V. dahliae* were conducted using a root-dip method. (Yang et al. 2013). The steps were as follows: the seedlings were uprooted, and the roots were submerged in a conidial suspension of *V. dahliae* (5 mL per seedling) for 2 minutes. The roots of non-inoculated plants were treated with sterile water for 2 minutes. Subsequent to this, the seedlings were placed back into potting soil. No additional procedures were carried out to wound the roots throughout the process.

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Isolation of full-length cDNA. An SSH library of the resistant wild eggplant species *S. torvum* was constructed. A differentially expressed transcript was isolated, representing the ethylene response factor *Pti5* gene. To clone *StPti5*, root samples were collected at 6 h after inoculating 4-week-old *S. torvum* seedlings with 5 mL of 5×10^6 conidia mL conidial suspension. The EasyPure® Plant RNA Kit (TransGen Biotech, China) was used to extract total RNA, and then cDNA was synthesized with the One-Step cDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, China). The primers for middle segments were designed [Electronic Supplementary Material (ESM) Table S1] based on the EST sequences, and the full-length sequence was obtained using the RACE method, following the manufacturer's instructions (Clontech).

Bioinformatic and phylogenetic analysis. The BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST/) was used for sequence identities and homology analysis. The domain sequence of *StPti5* was analyzed using a conserved-domain search tool (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The analysis was conducted on the physical and chemical properties of the predicted protein by using the Protparam tool (https://web.expasy.org/protparam/), and the secondary structure was predicted by Predictprotein (https://predictprotein.org/). Gene localization was predicted using the WoLF PSORT II tool (https://www.genscript.com/wolf-psort.html?src=leftbar). Phylogenetic analysis of the *StPti5* was carried out using MEGA software (version 5.0).

Gene expression analysis. To analyze the expression of *StPti5* in eggplant, 4-week-old *S. torvum* seedlings were inoculated with 5 mL of conidial suspension (5×10^6 conidia/mL) of *V. dahliae* using a root-dip method (Yang et al. 2013). Samples were collected from inoculated roots at six different time points (0, 2, 6, 12, 24, 48, and 72 hours post-inoculation) from three seedlings for each time point to analyze the expression of *StPti5*. To analyze the expression of *StPti5* in hormone-treated eggplant, *S. torvum* leaves were sprayed with 10 Mm salicylic acid (SA), 5 Mm ethephon (ETH) and 0.1 Mm methyl jasmonate (MeJA). Then, leaves of hormone-treated plants were collected at different time intervals, including 2, 6, 12, 24, 48 and 72 h after spraying. The plants sprayed with sterile water were used as the control. For the expression analysis of ethylene signaling pathway-related genes and pathogen defence genes in transgenic *A. thaliana*, the roots of four-week-old *StPti5*-overexpression transgenic line (OE1) and wild-type (Col-0) *A. thaliana* were immersed in a suspension of *V. dahliae* spores (1×10^7 conidia/mL) for 5 minutes, while a sterile water treatment was used as a control. The treated seedlings were then replanted into nutrient bowls.

Three root samples were collected per treatment 24 hours after inoculation. qRT-PCR test steps were performed using a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The eggplant *ubiquitin* gene was used as an internal control. The gene expression data represent the mean value obtained from three independent replicates. The relative expression of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen 2001).

Subcellular localization analysis of *StPti5*. The full-length coding region of *StPti5* was inserted into the pRTL2 vector to create a C-terminal fusion with the GFP gene. This construct was placed under the control of the CaMV35S promoter and designated as p35S:*StPti5* to investigate the subcellular localization of *StPti5*. The plasmid construct was confirmed by sequencing. The plasmids harboring GFP alone (empty vector, p35S:*GFP*) were used as controls. Both p35S:*GFP* and p35S:*StPti5* vectors were transiently expressed in tobacco epidermal cells through *Agrobacterium* infiltration. The subcellular localization of the p35S:*GFP* and p35S:*StPti5* fusion proteins was observed using laser scanning confocal microscopy (LSMT-PMT) with an excitation wavelength of 488 nm and an emission wavelength of 510 nm.

Generation and analysis of transgenic *A. thaliana*. The full-length coding sequence of *StPti5* was amplified using primers containing specific (Sac I and BstB I) enzyme sites and was inserted into the vector pFAST-G02 to construct the vector CaMV35S:*StPti5* for overexpression. After the overexpressing plasmid (pFAST-G02:*StPti5*) was constructed, it was transformed into *A. tumefaciens* (strain GV3101). The transformed *A. tumefaciens* was subsequently used to introduce the overexpressing plasmid into 4-week-old *A. thaliana* ecotype Col-0 plants through the floral dip method (Clough & Bent 1998). The transgenic *A. thaliana* plants were selected by growing them on an MS medium containing 50 mg/L Basta. The T3 homozygous transgenic plants were then identified using RT-PCR with cDNA samples, and wild-type

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A. thaliana cDNA was used as a control. For PCR amplification, the following conditions were used: an initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds. The control used for this PCR amplification was *ubiquitin*. For the Verticillium wilt resistance of transgenic plants, 3-week-old seedlings of wild type and transgenic *A. thaliana* were subjected to inoculation with 5 mL of conidial suspension (1×10^7 conidia/mL) of *V. dahliae* using the root-dip method, respectively. Plants treated with sterile water were used as the control. For quantifying the fungal biomass, the aboveground parts of three inoculated *A. thaliana* plants per gene target (one per replicate) were reaped 21 days post-inoculation, as described by Santhanam et al. (2013). Following DNA extraction, the biomass was quantified using qPCR with primers explicitly designed for *V. dahliae* elongation factor 1-α (EF-1α). *A. thaliana* ubiquitin extension protein 1 (*UBQ1*, NM_115119.4) was used as an internal reference standard (ESM Table S1).

Detecting ROS accumulation using DAB staining. After infiltration with 50 µL *V. dahliae* conidia suspension (1×10^7 conidia/mL), transgenic *A. thaliana* and wild-type (Col-0) leaves from 3-week-old plants were used to detect the generation of ROS using 3'3-diaminobenzidine (DAB) solution (Li et al. 2018). The sterile water treatment served as the control. The detection process involved putting the leaves into the DAB solution and infiltrating them under a gentle vacuum. The reaction was incubated at 25 ℃ in the dark and terminated at 10–12h post-inoculation. To remove chlorophyll, the DAB solution was removed with distilled water and replaced with ethanol (75%). The decolourized leaves were then placed in 30% glycerol.

RESULTS

Cloning and sequence analysis of the *StPti5* gene from *S. torvum*. An SSH cDNA library of roots from *S. torvum* was generated after inoculation with *V. dahliae*. A cDNA fragment showed increased expression of an ethylene response factor (designated as *StPti5*) in wild eggplant during infection. By using the RACE method, the full-length *StPti5* gene was obtained. The gene was found to contain a 537-bp open reading frame (ORF), which is predicted to encode 178 amino acids. (MK248476.1, Figure 1A). The predicted protein weight is 19.43 kD, and the protein theoretical PI is 7.85. It is predicted that *StPti5* does not contain signal peptide and is located in other parts of the cell except mitochondria and chloroplasts. The deduced *StPti5* protein sequence contained a 58 amino acid (59–116) AP2/ERF DNA binding domain that shared high amino acid homology with the other AP2/ERF conserved

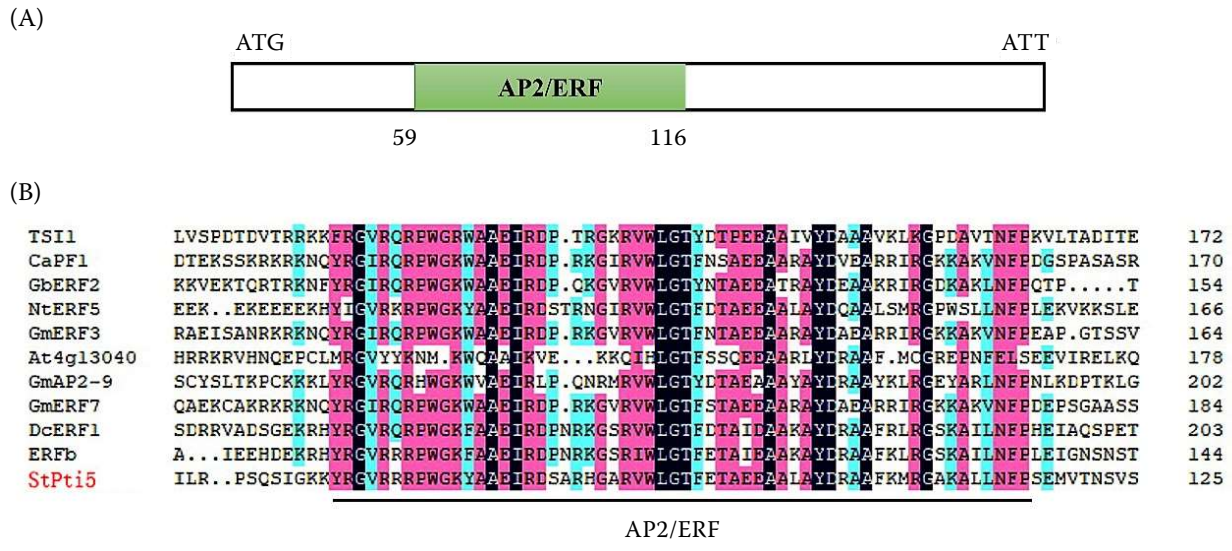

Figure 1. *StPti5* encodes an AP2/ERF protein

(A) Domains predicted for *StPti5*. The numbers represent the amino acid positions of the domain

(B) Multiple sequence alignment of *StPti5*'s AP2/ERF domain with other plant AP2/ERF proteins; the conserved amino acids are highlighted in blue and pink backgrounds, respectively, and the AP2/ERF domain is indicated

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domain (Figure 1B). The Neighbour-joining phylogenetic tree revealed that *StPti5* showed a closer evolutionary relationship (59.23%) to NtERF5 in *Nicotiana tabacum* (Figure 2).

The expression pattern of *StPti5* induced by *V. dahliae*, ET, MeJA, and SA. To confirm *StPti5* up-regulated expression in response to pathogen inoculation, we used qRT-PCR to compare *StPti5* expression in resistant eggplant *S. torvum* after *V. dahliae* inoculation to that in distilled water treatment controls. The relative expression of *StPti5* showed two up-regulation peaks at 6 and 24 h post-inoculation, respectively. Among them, the expression at 24 h post-inoculation was the highest, which was 10.34 folds over the control (Figure 3A). The expression pattern of *StPti5* was also up-regulated by spraying ETH and MeJA in leaves. After treatment with ETH, the expression pattern of *StPti5* was similar to treatment with *V. dahliae* (Figure 3B). On the contrary, treatment with MeJA resulted in a rapid increase in expression, which peaked 12 h after inoculation and then decreased to a level similar to that of mock-inoculated plants (Figure 3C). Treatment with SA had a less significant effect on *StPti5* expression than treatments with ETH and MeJA (Figure 3D).

***StPti5* is localized to the nucleus.** Subcellular localization analysis of the *StPti5* peptide sequence using WoLF PSORT II indicated that *StPti5* has no distinct signal peptide or any transmembrane structure, and its subcellular localization was most likely in the nucleus. (kNN value: nuclear, 6; nuclear plasma, 5.5; plasma membrane, 3; cytoskeleton, 2; cytoplasm, 1; mitochondrion, 1). To test the subcellular location of *StPti5*, the localization of a *StPti5*-GFP fusion protein was assessed by transient expression in tobacco. As predicted, the target protein p35S:*StPti5* was obviously localized in cell nuclei; however, the control p35S:GFP protein was found throughout the foliar cells in tobacco (Figure 4), implying that the protein of *StPti5* was localized in the nucleus.

Heterologous overexpression of *StPti5* enhanced Verticillium wilt resistance in *A. thaliana*. To determine the function of *StPti5* in the defence against *V. dahliae*, we transferred the *StPti5* gene into the *A. thaliana* genome. The CaMV35S promoter (P35S:*StPti5*) was used to drive

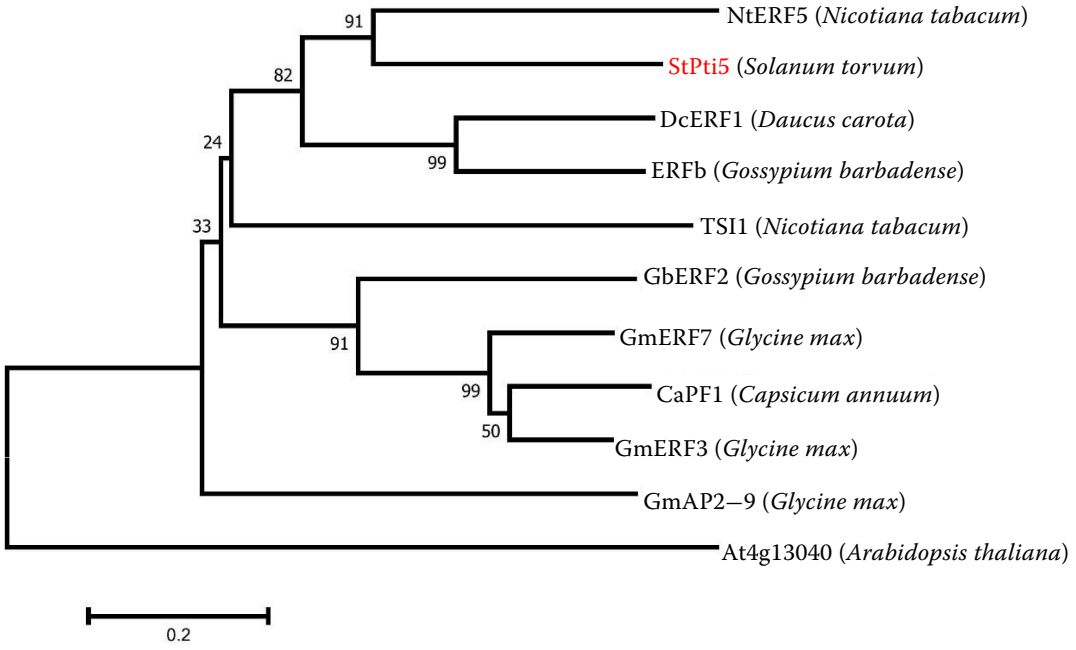

Figure 2. *StPti5* phylogenetic tree with orthologous proteins from other plants

MEGA (version 7.0) was used to create the phylogenetic analysis using the bootstrap method based on full amino acid sequences; at each node, the bootstrap values for 1 000 bootstrap trails are displayed; the analysis included the following protein sequences: NtERF5 (AAU81956.1), *StPti5* (QDA34242.1), DcERF1 (BAF75651.1), ERFb (ALK82289.1), TSI1 (AAC14323.1), GbERF2 (AAT77191.1), GmERF7 (NP_001341213.1), CaPF1 (NP_001312013.1), GmERF3 (NP_001238300.2), GmAP2-9 (ACJ37443.1), At4g13040 (Q56XP9.2)

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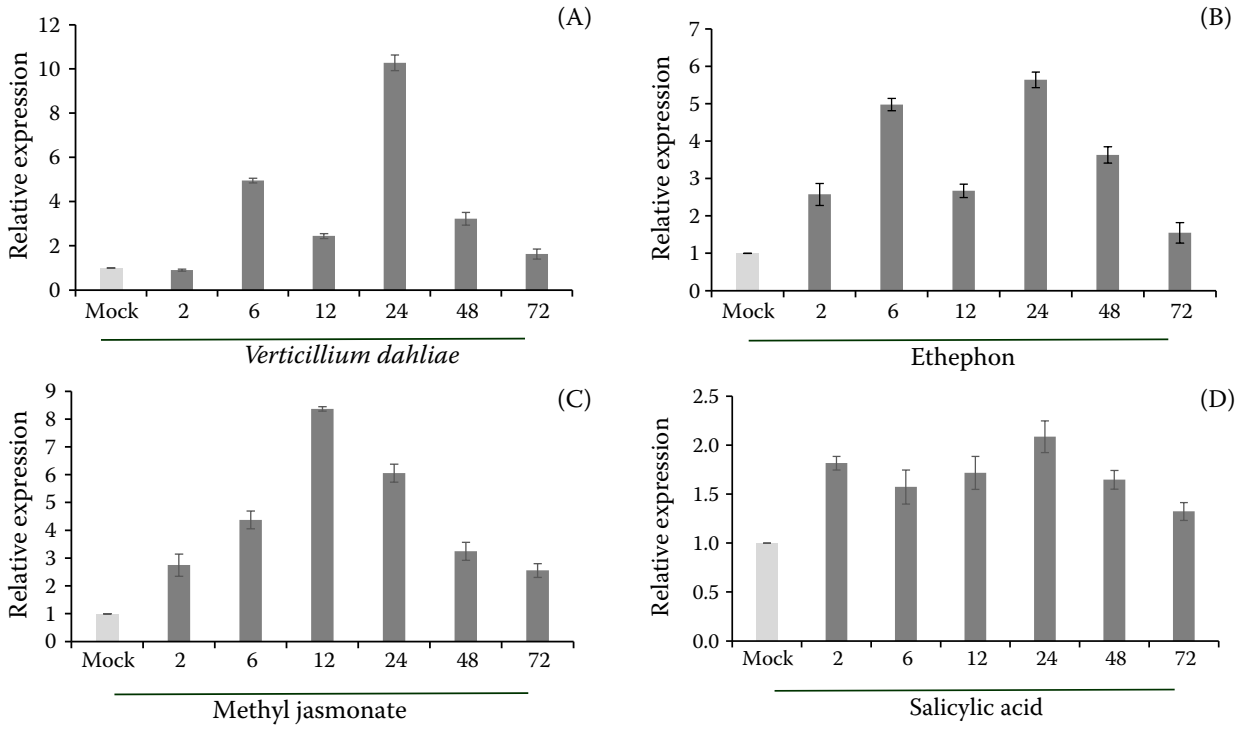

Figure 3. Gene expression patterns of *StPti5* in wild eggplant

(A–D) Expression analysis of *StPti5* in eggplant at different time points after inoculation with *Verticillium dahliae* or treatment with ethephon (ETH), methyl jasmonate (MeJA) and salicylic acid (SA). The expression levels of *StPti5* were determined by qRT-PCR, using the eggplant ubiquitin gene as a control, and compared with the expression of wild eggplant plants treated with sterile water (Mock). All samples were collected at 2, 6, 12, 24, 48 and 72 h. The relative expression levels of the StPti5 gene were represented by the mean of three independent biological replicates for each of the three plants. The error bars represent standard deviations, ** indicating $P < 0.01$

the overexpression plasmid (pFAST-G02:*StPti5*), which was then transferred into *A. thaliana* ecotype Col-0. Three transgenic lines (OE1-OE3) were chosen for further analysis. The *StPti5* transcript was detected in the three transgenic lines

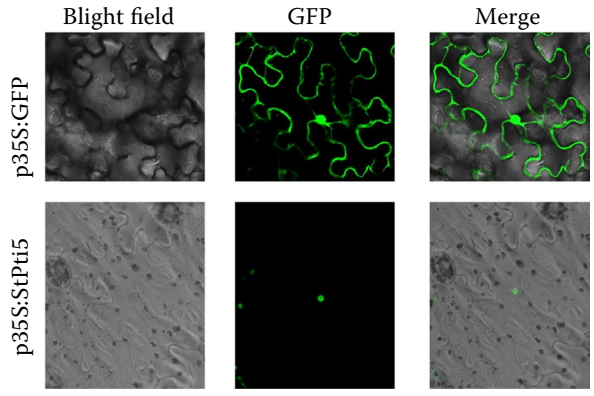

Figure 4. Subcellular localization of *StPti5*

A p35S:*StPti5* containing the full-length coding sequence of *StPti5* was inserted into the pRTL2 vector and introduced into tobacco by *Agrobacterium infiltration*; the empty vector p35S:GFP served as a control

but not in the wild-type Col-0 by reverse transcription-PCR (RT-PCR), confirming that the transferred genes were successfully expressed (Figure 5A). For Verticillium wilt resistance tests, the result showed that all three overexpression of lines increased the resistance of *A. thaliana* to *V. dahliae* compared with the WT, as evidenced by reduced leaf chlorosis and withering. (Figure 5B). Furthermore, *StPti5* overexpression lines had significantly less fungal biomass than wild-type plants (Figure 5C). The statistics suggested that the wild eggplant gene *StPti5* conferred resistance to *V. dahliae* in *A. thaliana*.

***StPti5*-mediated resistance against *V. dahliae* by an ethylene signaling pathway.** Previous hormone response expression testing results show that the *StPti5* gene in eggplant had similar expression patterns after treatment with *V. dahliae* and *ETH,* suggesting that *StPti5*, which plays a disease-resistance role in *A. thaliana,* may also be associated with ethylene signaling. To assess the correlation, the RT-qPCR method was utilized to measure the

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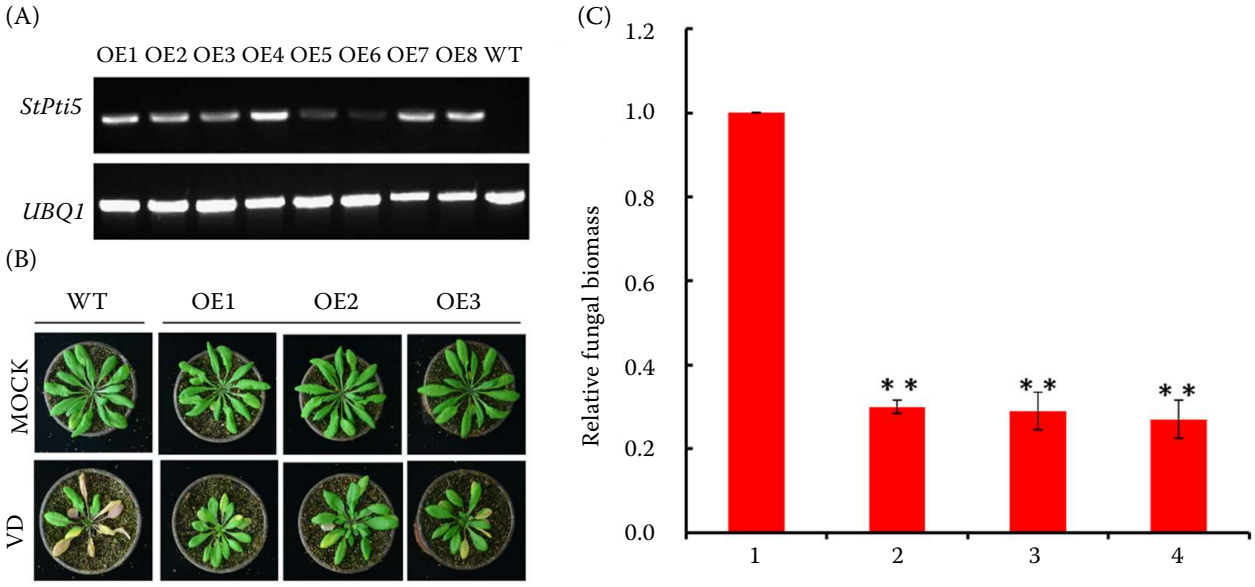

Figure 5. Identify the resistance of *StPti5* overexpression lines to *Verticillium dahliae*

(A) Amplification of *StPti5* cDNA by RT-PCR in transgenic *Arabidopsis thaliana* lines; the *UBQ1* gene is depicted as an internal reference

(B) *StPti5* transgenic *A. thaliana* inoculated with *V. dahliae* phenotype analysis; three transgenic lines and wild-type (Col-0) were inoculated with *V. dahliae* or sterile water (Mock), disease symptoms were observed 14 days after inoculation

(C) *V. dahliae* biomass was quantified in *StPti5* transgenic *A. thaliana* plants and compared to wild-type plants (Col-0), genomic DNA extracted from three plants were used to determine relative fungal biomass by quantitative real-time PCR; the data represent the mean ± SD of the three biological replicates, and statistical significance was determined using an unpaired Student's t-test (** indicates $P < 0.01$)

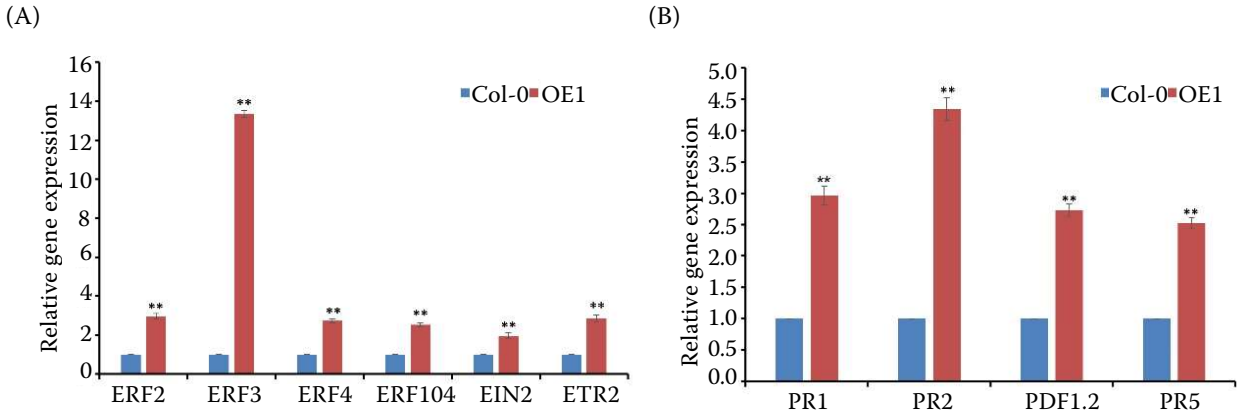

Figure 6. The expression levels of ethylene signaling-associated genes were analyzed in a *StPti5*-overexpression transgenic line using RT-qPCR

(A) Relative expression of ETH signaling pathways; (B) defense marker genes were analyzed in the *StPti5*-overexpression transgenic line by RT-qPCR

For relative expression analysis, root samples of wild type Col-0, transgenic *Arabidopsis thaliana* line OE1 were collected for RNA isolation and cDNA synthesis 24 h after inoculation. Using the comparative threshold $2^{-\Delta\Delta Ct}$ method and *A. thaliana UBQ1* as a reference, the relative expression of ethylene signaling-associated genes was determined through RT-qPCR; the values presented are the averages of three independent biological replicates, and the error bars indicate standard errors Statistical significance was determined using an unpaired Student's t-test (** indicates $P < 0.01$)

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relative expression levels of seven genes, namely *ERF2*, *ERF3*, *ERF4*, *ERF13*, *ERF104*, *EIN2*, and *ETR2*, involved in the ethylene signaling pathway. The measurement was conducted on both Col-0 and *StPti5* transgenic line OE1, 24 hours after being infected with *V. dahliae*. According to Figure 6A, the expression levels of these genes, except for *ERF13*, were significantly elevated in the *StPti5* transgenic lines compared to the wild-type plants after inoculation with *V. dahliae*. Moreover, the marker genes *PR1*, *PR4*, *PDF1.2*, and *PR5* that ETH induces were considerably up-regulated upon overexpression of *StPti5* in the wild-type Col-0 (Figure 6B). These findings indicate that ethylene signaling plays a critical role in the defence responses mediated by *StPti5* against *V. dahliae*.

The defence response of ROS activation was enhanced by the overexpression of *StPti5*. To investigate the defence responses against *V. dahliae* mediated by *StPti5*, the accumulation of ROS was measured in the leaves of *A. thaliana* wild ecotype Col-0 and *StPti5* transgenic line OE1 at 12 hours post-infiltration with conidia suspension of *V. dahliae*. Compared to infiltration with sterile water, the results showed that wild-type Col-0 leaves exhibited higher levels of ROS accumulation around the infiltration sites, which were visible as dark brown deposits in the leaves (Figure 7). *StPti5* transgenic *A. thaliana* OE1 displayed significantly more ROS accumulation than the wild-type Col-0 plants after being infiltrated with *V. dahliae*. (Figure 7). These findings suggest that *StPti5* improves the resistance to *V. dahliae* through *StPti5*-mediated ROS activation.

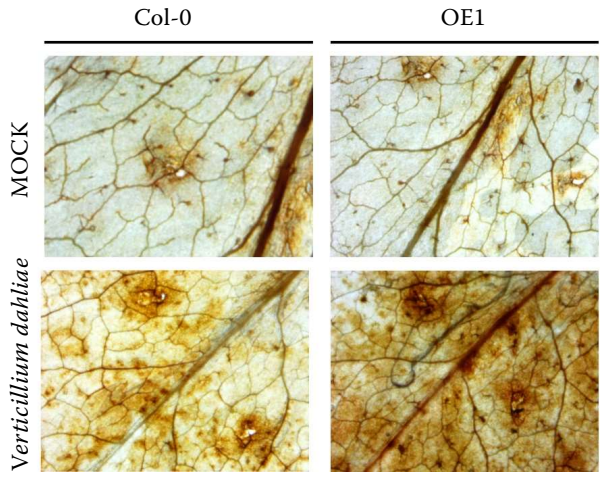

Figure 7. Eggplant *StPti5* regulate ROS accumulation in *Arabidopsis thaliana*

ROS — inducing activities were evaluated in *StPti5* transgenic *A. thaliana* (OE1) and wild-type (Col-0) plants after inoculation with *Verticillium dahliae*, with sterile water treatments serving as controls (Mock)

DISCUSSION

Verticillium wilt, a devastating soil-borne vascular disease caused primarily by *V. dahliae*, poses a significant threat to eggplant production and significantly reduces yield and quality (Bletsos et al. 2003; Fradin & Thomma 2006). The AP2/ERF transcription factors, one of the largest transcription factor families with one-two well-conserved AP2 domain in a plant, has been shown to play a significant role in both biotic and abiotic stress responses (Pré et al. 2008; Mizoi et al. 2012; Licausi et al. 2013; Shoji et al. 2013; Yang et al. 2019). Studies on eggplant Verticillium wilt resistance have identified a few genes with resistance function against Verticillium wilt (Ali et al. 2022; Yang et al. 2013). However, AP2/ERF resistance genes in eggplant were rarely reported. This study isolated and characterized a novel AP2/ERF transcription factor named *StPti5* from the wild eggplant *Solanum torvum*. Through bioinformatics analysis, we discovered that *StPti5* contained a nuclear localization signal (K^{58}YRGVRRRPW) in a basic amino acid domain, indicating its ability to target the nucleus. Subsequently, we conducted a subcellular localization test using tobacco epidermal cells and the p35S:GFP vector and the results showed that *StPti5* was localized in the nucleus. Meanwhile, *StPti5* contained a conserved AP2/ERF DNA binding domain (58 amino acids) that shared high amino acid homology with other conserved domains of the AP2/ERF family (Figure 1B). The most related protein is NtERF5 from *Nicotiana tabacum* (Fischer & Dröge-Laser 2004). Therefore, *StPti5* may function as a transcriptional activator in plants. It has been demonstrated that ET, MeJA, and SA play a significant role as signaling molecules in the defence response of plants (Reymond & Farmer 1998; Liu et al. 2017). Plant defence responses are typically regulated by specific hormones, with SA involved in defending against biological pathogens and JA activating resistance against necrotrophic pathogens (Glazebrook et al. 2003). The ERFs act as a cross factor of ET/SA and ET/JA signaling pathways in response to biotic and abiotic stress-

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es. (Yao et al. 2017). The expression patterns of most ERF genes are different by hormone-induced (Mazarei et al. 2007; Gao 2008; Pré et al. 2008). The ERFs genes in tomato, both ET, JA and SA activate Pti4, while Pti5 and Pti6 are activated only by JA and ET (Gu et al. 2000). A similar situation exists in *A. thaliana*, ERF1 and ERF14 are induced by JA and ET. (Pré et al. 2008), while ERF5 and ERF6 could be induced by ET, JA and SA (Son et al. 2012; Moffat et al. 2012). In this study, the gene expression of *StPti5* in *S. torvum* could be strongly up-regulated hormone treatment, including by ETH and MeJA (Figures 3B and 3C). These results suggested *StPti5* responds to disease stress via various signaling pathways. Notably, the expression pattern following ETH treatment was comparable to the induction caused by *V. dahliae in vivo* (Figures 3A and 3B), and *StPti5* was significantly up-regulated at 24 h after treatment with ETH, the primary association of *StPti5* with Verticillium wilt resistance function was with ET signaling.

Several ERF genes, including those from *A. thaliana* (Oñate-Sanchez et al. 2007; Pré et al. 2008; Moffat et al. 2012), rice (Cao et al. 2006), wheat (Dong et al. 2010), cotton (Zuo et al. 2007; Guo et al. 2016), tomato (He et al. 2001), and tobacco (Guo et al. 2004), have been shown to play important roles in disease resistance. For example, overexpression of *OPBP1* in tobacco increases resistance against *Phytophthora parasiticavar* nicotianae and *Pseudomonas syringae* pv tabaci pathogens (Guo et al. 2004). However, there have been no reports of ERF genes involved in Verticillium wilt resistance in eggplant. This study aimed to confirm the role of *StPti5* in the resistance to *V. dahliae* by generating *A. thaliana* lines that overexpress *StPti5* and validating their resistance to Verticillium wilt. The results showed that the overexpression lines of *A. thaliana* exhibited increased resistance to *V. dahliae* compared with the WT, and there was a significant reduction in fungal biomass in the *StPti5* overexpression lines (Figures 5B and 5C). The statistics suggested that *StPti5* is a positive regulator transcription factor involved in Verticillium wilt resistance in eggplant by participating in complex various signal pathways.

Furthermore, some disease-resistant genes were up-regulated in transgenic *Arabidopsis* lines after infection with *V. dahliae*. The ERF proteins can mediate the defence responses of ethylene signaling activation and pathogenesis-related genes. For example, overexpression of the soybean ERF transcription factor gene, *GmERF5*, enhanced resistance to *Phytophthora sojae*, significant up-regulation of the expression of the ethylene synthesis genes and PR genes (Dong et al. 2015). In this study, the ethylene pathway-related genes dramatically up-regulated expression (Figure 6A). For example, the expression of the *ERF3* gene in overexpression lines increased by more than ten times. In addition, the pathogenesis-related proteins *PR1*, *PR2*, *PDF1.2* and *PR5* were also significantly expressed after infection (Figure 6B), implying that the ET signaling pathway plays an important role in *StPti5*-mediated resistance against *V. dahliae*. Existing research has indicated that transcription factors can both positively and negatively regulate the accumulation of ROS (Wang et al. 2020; Wang et al. 2022). In this study, *StPti5* transgenic *A. thaliana* displayed significantly more ROS accumulation than the wild-type plants after infiltrating with *V. dahliae*. This result suggests that *StPti5* enhances plant resistance to *V. dahliae* by positively regulating ROS accumulation. Although multiple genes and signaling pathways are induced in disease resistance responses, the key genes and pathways that plants inhibit pathogen growth are not clear. The process of plant disease resistance may result from multiple signaling pathways or different signaling pathways for different types of pathogens.

CONCLUSION

In summary, *StPti5* is a novel AP2/ERF transcription factor encoding a protein that is localized in the nucleus. It involves hormones (ETH and MeJA) and Verticillium wilt-induced reaction. Overexpression of *StPti5* gene in *A. thaliana* enhanced resistance to *V. dahliae*, activating genes related to the ethylene signaling pathway and increased ROS production. Our study demonstrated that the *StPti5* gene plays a critical role in conferring resistance to Verticillium wilt in eggplant.

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