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A plant natriuretic peptide GhEG45 plays a significant role in regulating resistance to *Verticillium dahliae*

GAO Linying¹, HU Menghui¹, YAN Xin¹, LIU Yang¹ and HOU Yuxia^{1*}

Abstract

Background Plant natriuretic peptides (PNPs) are a class of peptide hormones that regulate plant responses to salt stress, water balance, and pathogen attacks. However, the precise underlying role of PNPs in plant defense mechanisms remains poorly understood.

Results In this study, we investigated the role of the cotton gene *GhEG45* in plant response to *Verticillium dahliae* infection. *GhEG45* overexpression in cotton (*Gossypium hirsutum*) enhanced resistance to *V. dahliae* by increasing the expression levels of salicylic acid (SA)-related defense genes and upregulating antioxidant activities. *GhEG45* expression was significantly induced by both *V. dahliae* infection and exogenous treatments with SA, hydrogen peroxide (H₂O₂), and other stress signals, which indicates its potential involvement in modulating plant defense mechanisms via SA signaling, oxidative stress pathways, and cell wall-based defenses. Transcriptomic analysis showed that *GhEG45* regulates SA signaling and reactive oxygen species (ROS) metabolism. *GhEG45* silencing via virus-induced gene silencing (VIGS) increased susceptibility to *V. dahliae*, impaired SA signaling, and disrupted ROS regulation.

Conclusions This study provides evidences that *GhEG45* plays a pivotal role in defense against *V. dahliae* infection in cotton, primarily by regulating SA signaling and ROS metabolism. Although GhEG45 shares some functional characteristics with PNPs, further structural and biochemical studies are needed to comprehensively categorize GhEG45 as a natriuretic peptide. Our findings suggest that *GhEG45* enhances cotton resistance to *V. dahliae* by potentiating defense responses.

Keywords *Gossypium hirsutum*, *Verticillium dahliae*, Plant natriuretic peptide, *GhEG45*, Salicylic acid, Reactive oxygen species

Introduction

Upland cotton (*Gossypium hirsutum*) is the primary source of natural textile fibers, which sustains global economies by contributing to more than 90% of the global cotton production. However, the most cultivated *G. hirsutum* varieties are highly susceptible to *Verticillium dahliae* infection. *V. dahliae* is a soil-borne fungal

pathogen that severely compromises cotton yield (Li et al. 2022). It survives for years as microsclerotia in soil and infects the roots through germinated hyphae that colonize plant vascular tissues to cause systemic plant infection. Infected cotton exhibits leaf yellowing, wilting, and premature death, but conventional control methods have proven ineffective against the soil-dwelling microsclerotia. Therefore, implementing advanced breeding strategies to develop genetic resistance is imperative for sustainable wilt management. This necessitates a thorough exploration of the mechanism underlying the

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cotton-pathogen interaction and identification of durable resistance genes.

Plant natriuretic peptides (PNPs) are homologous to vertebrate atrial natriuretic peptides (ANPs) and constitute a distinct class of mobile signaling molecules that regulate osmotic homeostasis, stress adaptation, and immune modulation (Gehring et al. 2013; Lee et al. 2020). Exogenous PNP application induces stomatal opening, activates membrane H^+ -ATPase (Maryani et al. 2000), increases cyclic guanosine monophosphate (cGMP) levels in maize root tissues, guards cell protoplasts (Pharmawati et al. 1998), and enhances osmoticum-driven volume changes in isolated mesophyll protoplasts (Maryani et al. 2000). *Arabidopsis thaliana* PNP (AtPNP-A) (At2g18660) is a well-characterized PNP that is involved in stomatal aperture regulation, ion flux modulation, and osmotic adjustments (Gehring et al. 2013). AtPNP-A induces rapid cGMP signaling with reactive oxygen species (ROS) acting as secondary messengers (Turek et al. 2016). In fact, ROS mediates both abiotic stress tolerance and pathogenic defense mechanisms. AtPNP-A interacts with catalase 2 (CAT2) to modulate intracellular hydrogen peroxide (H_2O_2) homeostasis (Turek et al. 2021), and based on transcriptomic analyses, it has been classified as a novel pathogenesis-related (PR) protein that is induced under biotic stress (Meier et al. 2008).

Additionally, PNPs have been implicated in plant defense against pathogens. For example, CjBAP12 from *Citrus jambhiri* contributes to citrus blight resistance (Ceccardi et al. 1998), whereas the overexpression of *ScPNP-A* from *Stellera chamaejasme* enhances resistance to *Pseudomonas syringae* and induces PR gene expression in *Arabidopsis* (Liu et al. 2020). Additionally, Plants overexpressing *AtPNP-A* exhibit higher resistance to saline and oxidative stresses and improved pathogenic defense compared with those of the PNP-deficient lines (Ficarra et al. 2018). Furthermore, *AtPNP-A* is responsive to salicylic acid (SA) and modulates SA-induced immune responses by interacting with the receptor-like protein PNP-R2 (Lee et al. 2020). Recent studies have shown that PNP exhibits enzymatic activity. For example, VaEG45 is a novel PNP from *Vigna angularis* that inhibits fungal uredospore germination, suppresses mycelial growth, and exhibits β -1,3-glucanase activity, which enhances resistance against fungal pathogens (Sun et al. 2023). These findings highlight the diverse mechanisms that underlie PNP-regulated plant immunity and stress responses. However, critical knowledge gaps exist regarding the enzymatic functions and biological activities of PNPs during host-pathogen interactions. Therefore, this study has been designed to elucidate the functional and mechanistic aspects of PNP-mediated responses in

cotton, thereby advancing our knowledge of their roles in stress tolerance.

Plant defense mechanisms integrate sophisticated signaling networks with SA signaling and ROS homeostasis to constitute the central components of biotic stress responses (Salinas et al. 2025). In cotton plants, SA pathway activation and ROS regulation are critical in determining the resistance to *V. dahliae*-induced Verticillium wilt (Zhang et al. 2025). Transcriptomic analyses have shown the pronounced upregulation of the key SA biosynthesis pathway genes *PAL* and *4CL* in resistant cotton varieties, which indicates the essential role in defense responses (Ma et al. 2024). Therefore, SA-mediated systemic acquired resistance (SAR) activates defense-related PR1/PR2 expression upon pathogenic invasion (Ali et al. 2018), whereas ROS function as dual-effect signaling molecules that trigger defense activation during controlled accumulation and cause oxidative damage at excessive levels; hence, the maintenance of ROS homeostasis is imperative (Ray et al. 2012), and the strategic manipulation of these pathways may enhance resistance against *V. dahliae*. In addition to SA signaling and ROS homeostasis, plant stress responses are regulated by intricate transcriptional, hormonal, and antioxidant defense networks. Sugarcane *ScSEC14-1* activates phosphatidylinositol/calcium/mitogen-activated protein kinase (MAPK) signaling to increase chlorophyll content and antioxidant capacity (Su et al. 2024), whereas grapevine *VvTGA8* transcriptionally activates SA-responsive *PR* genes against *Coniella vitis* (Li et al. 2023). Poplar *PdbGSTU10* enhances the resistance to *Alternaria alternata* through ROS scavenging and SA/PR gene induction (Huang et al. 2024), and cotton *GauERF105* coordinates lignin deposition and ethylene-mediated defense against *V. dahliae* (Wang et al. 2023a, b). Collectively, these findings illustrate the multidimensional regulation of plant immunity through transcriptional, hormonal, and redox networks. As PNPs exhibit high potential to modulate SA biosynthesis and ROS homeostasis, elucidating their interactions with these pathways may help identify novel mechanisms underlying pathogenic resistance and stress tolerance.

Verticillium wilt remains one of the most devastating diseases that affects cotton yield and quality. Although previous studies have highlighted the involvement of SA signaling and ROS homeostasis in plant defense, the upstream regulatory components that coordinate these responses remain unclear. GhEG45 was initially identified as a member of the PNPs that regulate water-salt balance and defense signaling. However, its role in biotic stress responses has not been elucidated. In this study, we aimed to investigate the functional role of *GhEG45* in cotton immunity against *V. dahliae* by integrating gene

expression analysis, genetic modification, and transcriptomic approaches. Our work provides new insights into the molecular mechanisms underlying *GhEG45*-mediated resistance and offers potential genetic targets for improving disease resistance in cotton.

Materials and methods

Plant materials and growth conditions

The resistant upland cotton cultivar ‘Zhongzhimian 2’ was used in this study. The seeds were delinted with 98% (v/v) sulfuric acid for 5 min under constant agitation, rinsed thoroughly with sterile water five times, and germinated in an incubator in the dark on moistened filter paper (Wen et al. 2022). After 48 h, the germinated seeds were transferred to pots containing nutrient-rich soil and grown at $(25 \pm 1)^\circ\text{C}$ under a 16-h light/8-h dark cycle with natural light intensity ($150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Then, the plant tissues (roots, stems, and leaves) were harvested at the two-leaf-one-heart stage, frozen immediately in liquid nitrogen, and stored at -80°C for downstream analyses. This procedure was performed in three biological replicates.

Additionally, the wild-type (WT) *A. thaliana* Columbia-0 (Col-0) and T_3 generation homozygous transgenic lines overexpressing *GhEG45* were used in this study. The seeds were sterilized by immersion for 5 min in 75% ethanol containing 0.1% (v/v) Tween-20, followed by a 15 min treatment with 2% (v/v) NaClO solution and rinsing 5–7 times with sterile water. The sterilized seeds were germinated on half-strength Murashige and Skoog (1/2 MS) medium (Li et al. 2016; Subbaiyan et al. 2017). For subcellular localization studies, *Arabidopsis* seedlings were cultivated in growth chambers set to $(22 \pm 1)^\circ\text{C}$ under a 16-h light/8-h dark cycle.

Gene cloning and bioinformatic analyses

Total RNA was extracted from cotton tissues infected with *V. dahliae* using a Biomed Plant RNA Rapid Extraction Kit (Code: RA106-02, Tiangen Biotech, Beijing, China) by following the manufacturer’s protocol. RNA integrity was verified by performing 1.2% agarose gel electrophoresis and analysis with NanoDrop 2000 (Thermo Fisher Scientific Inc., USA) ($A_{260}/A_{280} = 1.8\text{--}2.0$, $A_{260}/A_{230} > 2.0$). First-strand cDNA was synthesized from 1 μg of RNA using a reverse transcription kit (Code: KR118-01, Biomed, Beijing, China). Polymerase chain reaction (PCR) amplification was performed using gene-specific primers designed based on the coding sequence of *GhEG45* (Table S1). The reaction mixture (25 μL) contained 12.5 μL of $2\times$ Taq Master Mix, 1 μL of forward and reverse primers (10 μM each), 2 μL of template cDNA, and 8.5 μL of nuclease-free water. The thermal cycling conditions were as follows: initial denaturation at 95°C

for 3 min, followed by 34 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 5 min. The amplified products were verified using agarose gel electrophoresis.

The phylogenetic relationship was determined using a neighbor-joining tree constructed using MEGA X software with 1 000 bootstrap replicates. Conserved domains and signal peptides were predicted using SMART (<http://smart.embl-heidelberg.de>). Multiple sequence alignments were performed using ClustalX2, visualized with ESPript, and compared with the protein structural data from the Protein Data Bank (PDB, <https://www.rcsb.org/>).

Vector construction and genetic transformation

The *GhEG45* coding sequence was amplified and cloned into the pCambia1300 expression vector under the control of the 35S promoter. The resulting plasmid (35S::GhEG45-GFP) was introduced into *Agrobacterium tumefaciens* strain GV3101. Genetic transformation of *Arabidopsis* was performed using the floral dip method (Clough et al. 1998), and transgenic plants were selected on 1/2 MS medium supplemented with 50 $\text{mg}\cdot\text{L}^{-1}$ hygromycin. Lines with a 3:1 segregation ratio were propagated to the T_3 generation for further analysis.

Subcellular localization

Preliminary prediction of the subcellular localization of GhEG45 was performed using the ProtCompB version 9.0 program (<http://www.softberry.com/>). Transgenic *Arabidopsis* (T_3 generation) expressing GhEG45-GFP fusion proteins under the control of the pCambia1300 vector were germinated on MS solid medium and cultured under standard conditions for 7 d to observe GFP fluorescence. The roots of the seedlings were excised and treated with 0.8% mannitol to induce plasmodesmata separation. The samples were imaged using a Nikon A1 SIM confocal laser scanning microscope (Nikon, Tokyo, Japan) at 488 nm excitation and 530 nm emission detection (GFP signal) wavelengths. The images were processed using the NIS-Elements software to confirm GhEG45 localization.

Expression analysis using reverse transcription-quantitative PCR (RT-qPCR)

RT-qPCR was performed to analyze the *GhEG45* expression pattern under various abiotic and biotic stress conditions in cotton (*G. hirsutum* cv. Zhongzhimian 2) seedlings at the two-leaf stage (two fully expanded true leaves and one emerging leaf). The seedlings were subjected to the following treatments: spraying with 1 $\text{mmol}\cdot\text{L}^{-1}$ SA, 1 $\text{mmol}\cdot\text{L}^{-1}$ abscisic acid (ABA), and 1 $\text{mmol}\cdot\text{L}^{-1}$ H_2O_2 until the leaf surfaces were uniformly wet; mechanical wounding by gently puncturing the leaves using sterile needles; and inoculation with *V.*

dahliae spores at a concentration of 1×10^7 spores·mL⁻¹ using a root-dip method for 5 min. The leaf tissues were harvested at 0, 1, 3, 6, 12, and 24 h after SA, ABA, H₂O₂, and wounding treatments and at 0, 12, 24, 72, 120, and 168 h post *V. dahliae* inoculation. All samples were immediately frozen in liquid nitrogen and stored at -80 °C until RNA extraction was performed.

Total RNA extraction, cDNA synthesis, and RT-qPCR analyses were performed as described earlier, and relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method (Wei et al. 2024) with *GhUBQ7* as the reference gene. The primer sequences are shown in Table S1.

Antioxidant enzyme activity analysis

Leaf samples (0.1 g) from gene-silenced cotton plants and controls were homogenized in 1 mL ice-cold enzyme extraction buffer (50 mmol·L⁻¹ PBS pH 7.8, containing 1 mmol·L⁻¹ EDTA and 1% PVP) and centrifuged at 12 000 r·min⁻¹ for 10 min at 4 °C (Chen et al. 2019). The supernatant was used to assay the enzyme activities. Catalase (CAT) activity was quantified by monitoring H₂O₂ decomposition at 240 nm using the ultraviolet (UV) absorption method. Additionally, superoxide dismutase (SOD) activity was measured via nitroblue tetrazolium (NBT) photoreduction inhibition at 560 nm, whereas peroxidase (POD) activity was determined using the guaiacol oxidation method at 470 nm. All assays were performed with three biological replicates, each containing pooled leaves from five individual plants per genotype.

H₂O₂ determination

The transgenic *Arabidopsis* and gene-silenced cotton plants (cultivar Zhongzhimian 2) were inoculated with *V. dahliae* conidial suspension (5 µL per leaf, 1×10^7 spores·mL⁻¹). The leaves were collected at 72 h post-inoculation (hpi). For H₂O₂ extraction, the infected leaves were ground in liquid nitrogen, homogenized in 25 mmol·L⁻¹ HCl (400 µL), and centrifuged at 12 000 r·min⁻¹ for 10 min. A 100 -µL aliquot of the supernatant was mixed with 900 µL of FOX reagent (ammonium iron (II) sulfate (250 µmol·L⁻¹), sorbitol (100 µmol·L⁻¹), xylene orange (100 µmol·L⁻¹), H₂SO₄ (25 mmol·L⁻¹), and 1% ethanol (v/v)). The mixture was incubated at 30 °C for 30 min, and absorbance was measured at 560 nm using a spectrophotometer (model TU-1900, Beijing Purkinje General Instrument Co., Ltd., Beijing, China). Three independent biological replicates (n ≥ 20 plants per group) were performed to ensure statistical reliability. The assay was adapted from established protocols (Cheeseman 2006; Pei et al. 2019).

Virus-induced gene silencing (VIGS) of *GhEG45*

The function of *GhEG45* was studied using a tobacco rattle virus (TRV)-based VIGS approach. A partial coding sequence of *GhEG45* (targeting the gene-specific region) from the cotton seedling cDNA was amplified, cloned into the TRV2 vector, and validated through sequencing. The recombinant plasmids TRV2:*GhEG45* (target), TRV2:00 (negative control), and TRV2:*GhCLA1* (positive control) were introduced into *A. tumefaciens* strain GV3101. *Agrobacterium* cultures containing TRV1 and TRV2 constructs were mixed in a 1:1 ratio (OD₆₀₀ = 1.0–1.2) and infiltrated into the cotyledons of 10-day-old cotton seedlings. After incubation for 24 h in the dark, the plants were maintained under a 16-h light/8-h dark cycle at 25 °C/20 °C (day/night). The *GhCLA1*-silenced plants showed a characteristic albino phenotype at 14 days post-inoculation (dpi), which confirmed VIGS efficacy. Silencing efficiency was validated at 21 days post-infiltration by performing RT-qPCR using the RNA extracted from the treated plants. Successfully silenced *GhEG45* plants and controls were used for subsequent assays, including *V. dahliae* infection evaluation.

Transcriptomic profiling of *GhEG45*-OE

Total RNA was extracted from *Arabidopsis* WT and *GhEG45*-overexpressing (OE4) plants at 0 and 48 hpi with *V. dahliae*. Three biological replicates were analyzed for each genotype-time combination; thus, 12 samples were analyzed in total. The samples were frozen in liquid nitrogen and stored at -80 °C. The cDNA libraries were sequenced on the Illumina sequencing platform by Metware Biotechnology Co. Ltd. (Wuhan, China). RNA sequencing libraries were prepared and sequenced on the Illumina HiSeq platform. Raw reads were trimmed using Cutadapt (v3.1) to remove adapters and low-quality sequences. Quality control was performed using FastQC (v0.12.0). The clean reads were aligned to the *A. thaliana* genome using Bowtie2. Gene expression levels were quantified as fragments per kilobase million (FPKM) using RNA-Seq by Expectation-Maximization (RSEM).

Transcript count matrices underwent normalization via the DESeq2 variance stabilizing transformation (vst) in R. Next, the top 500 most variable genes, which were meticulously selected using the prcomp function (R stats package v4.2.1), were subjected to a principal component analysis (PCA). Furthermore, intricate sample clustering patterns were projected and vividly visualized in 2D space using ggplot2 (v3.4.0).

Differentially expressed genes (DEGs) were identified using edgeR under the following conditions: false discovery rate (FDR) < 0.05 and log₂ fold change ≥ 2. Enrichment analyses for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were

performed using TopGO and ClusterProfiler, respectively. DEG comparisons and overlaps were visualized using OmicStudio and OmicShare tools.

Weighted gene co-expression network analysis (WGCNA) was performed using the WGCNA (1.71) package in R software. A soft-thresholding power (β) of 20 was selected to achieve scale-free topology ($R^2 > 0.85$). The modules were defined using dynamic tree cutting ($\text{minModuleSize} = 50$), and correlations between module eigengenes and physiological traits were calculated using Pearson correlation ($P < 0.01$). Hub genes were identified within key modules by ranking the intramodular connectivity (k_{Within}) values.

Disease evaluation and fungal biomass assessment

The aggressive *V. dahliae* strain V991 was initially cultured on potato dextrose agar (PDA) at 25 °C for 4–6 d, followed by incubation in Czapek's liquid medium at 200 r·min⁻¹ for 7 d. The spore suspension was adjusted to a final concentration of 1×10^7 spores·mL⁻¹. For VIGS assays, cotton seedlings were inoculated 21 days post-treatment, whereas 21-day-old *Arabidopsis* plants were inoculated using the root-dipping method and transplanted into fresh soil.

Disease severity was evaluated on a scale of 0–4, and the disease index (DI) was calculated using the formula: $\text{DI} = (\sum \text{disease grade} \times \text{plant number}) / (\text{total plants} \times 4) \times 100$. At 21 dpi, the fungal biomass was quantified through RT-qPCR using *V. dahliae*-specific primers (ITS1-F/STVe1-R) and plant reference genes (*AtEF1- α* or *GhUBQ7*) (Table S1). Fungal colonization was further confirmed through stem recovery assays, where surface-sterilized stem segments were cultured on PDA. All experiments included three biological replicates.

Ion chromatographic analysis of leaf Na⁺ and K⁺ concentrations

Leaf samples (> 1.0 g) were oven-dried, finely ground into ash, and accurately weighed. Cations were extracted by dissolving the required quantity of ash in ultrapure water. Na⁺ and K⁺ concentrations in the leaf samples were determined using an ion chromatograph (ICS-1500, Thermo Fisher Scientific Inc., USA) equipped with a conductivity detector by following a previously described protocol (Iseki et al. 2016; Yoshida et al. 2015). Ion separation was performed using a Dionex IonPac[™] CS12A RFIC[™] analytical column (4 × 250 mm; P/N 046122, Thermo Fisher Scientific, Waltham, MA, USA). Methanesulfonic acid (12 mmol·L⁻¹) was used as the mobile phase, which was degassed and pumped at a flow rate of 1.0 mL·min⁻¹. The injection volume was set to 25 μ L, and the column temperature was maintained at 35 °C. An anion electrolytic suppressor (AERS[™] 500, 4 mm, Thermo Fisher Scientific)

was used with a suppression current of 25 mA. Detection was performed using a conductivity detector with a total runtime of 12 min per sample. Standard Na⁺ and K⁺ solutions (5 g·kg⁻¹) were used for calibration and quantification. The retention times for Na⁺ and K⁺ were approximately 5.9 min and 8.6 min, respectively. A chromatographic data system (Shimadzu C-R6A Integrator, Shimadzu Corporation, Japan) was used for data processing and quantification of ion concentrations.

Statistical analysis

The data were organized in Microsoft Excel and analyzed using GraphPad Prism v.9.2 (GraphPad Software Inc., San Diego, CA, USA). Statistical differences were determined using one-way analysis of variance (ANOVA) with Tukey's or Duncan's post-hoc tests, or using two-way ANOVA with least significant difference (LSD) multiple comparisons. Student's *t*-test was used for pairwise comparisons ($P < 0.05$, $P < 0.01$). The statistical significance threshold was set at $P < 0.05$ for all analyses. Heatmaps and Venn diagrams were created using TBtools.

Results

Characterization and stress-responsive functional analysis of GhEG45

GhEG45 (Ghcr24_A08G099400.1) is located on chromosome A08 of *G. hirsutum*, originating from the At subgenome. BLAST searches against the CottonMD database identified several homologs, including the Dt subgenome counterpart Ghcr24_D08G103500 (Fig. S1). *GhEG45* encodes a protein with a length of 135 amino acids. The predicted protein structure comprises an N-terminal signal peptide (residues 1–28) and a conserved double- ψ β -barrel (DPBB) structural fold. This protein was classified as a member of the DPBB-1 superfamily (Fig. 1A). Multiple sequence alignment showed that GhEG45 shares high homology with EG45-like proteins from various plant species, especially AtPNP-A. This suggests a potential evolutionary conservation and functional relevance in stress adaptation. The phylogenetic tree indicated that GhEG45 clusters within a clade of dicot EG45-like proteins and is closely related to AtPNP-A and other homologs (Fig. 1B). This supports the hypothesis that the function of GhEG45 may be similar to those of PNP proteins, potentially in cell-wall dynamics, hormone signaling, or biotic stress response pathways.

Tissue-specific expression analysis using RT-qPCR showed that *GhEG45* is predominantly expressed in the leaves at transcript levels that are 3.2- and 2.8-fold higher than those observed in the roots and stems, respectively (Fig. 1C). This indicates a role in leaf-associated physiological processes. Abiotic stress treatments induced significant *GhEG45* expression. SA treatment rapidly

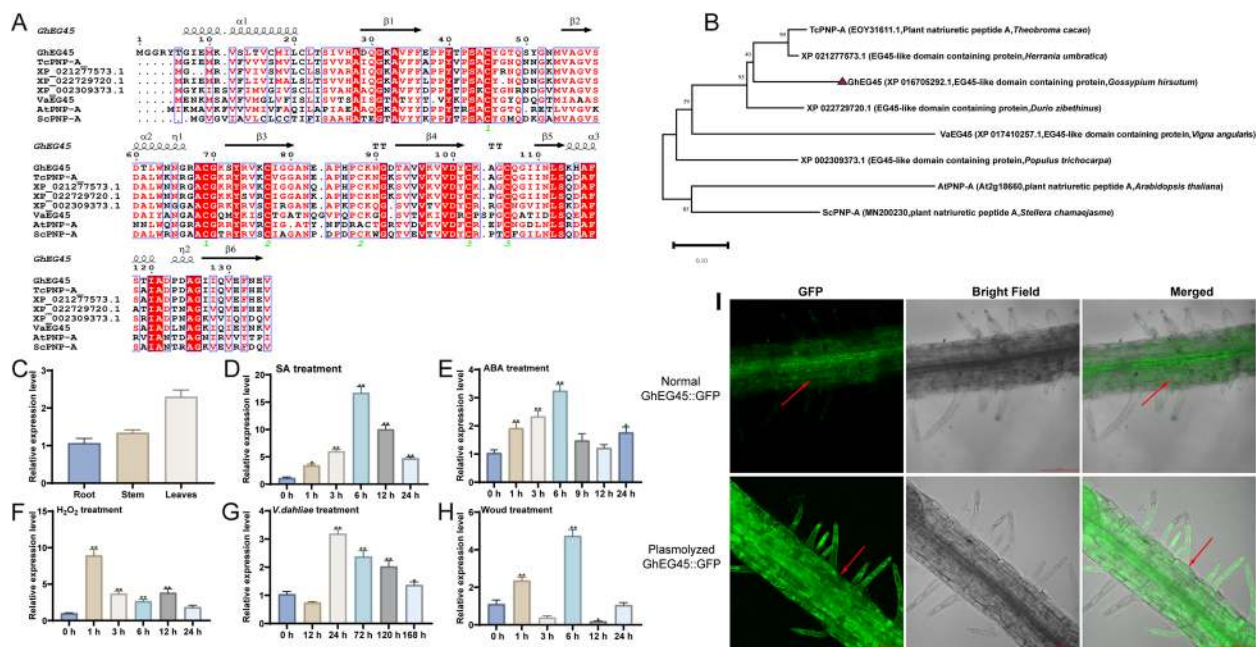


Fig. 1 Molecular characterization, phylogenetic analysis, expression profiling, and subcellular localization of GhEG45. **A** Multiple sequence alignment of GhEG45 with homologous proteins from other plant species. Conserved amino acids were highlighted, and secondary structure elements (α -helices and β -sheets) were annotated based on prediction results. **B** Phylogenetic tree of GhEG45 and related proteins constructed using the neighbor-joining method in MEGA X. **C** Tissue-specific expression analysis of *GhEG45* in cotton roots, stems, and leaves under normal growth conditions. **D–H** Time-course expression of *GhEG45* under salicylic acid (SA), abscisic acid (ABA), hydrogen peroxide (H_2O_2) treatments, *V. dahliae* inoculation, and mechanical wounding. Expression levels were determined using RT-qPCR and normalized to the internal reference gene *GhUBQ7*. Data are expressed as means $\pm$ standard deviation (SD) ($n = 3$). Student's *t*-test: $*P < 0.05$ and $**P < 0.01$ indicate significant differences compared with 0 h, respectively. **I** Subcellular localization of GhEG45 fused with GFP in *Arabidopsis* epidermal cells. The GFP fluorescence remains associated with the cell wall or apoplast in both normal and plasmolyzed conditions. Red arrows indicate GFP fluorescence localization. Scale bars = 100 μm

increased *GhEG45* transcript levels, which peaked at 16.7-fold after 6 h (Fig. 1D). ABA treatment led to a transient increase in expression, which peaked at 3.25-fold within 3–6 h (Fig. 1E). H_2O_2 elicited a rapid and substantial 8.9-fold increase in the expression level at 1 h post-treatment (Fig. 1F), suggesting the potential involvement of *GhEG45* in ROS signaling pathways. Under biotic stress conditions, inoculation with *V. dahliae* induced a gradual upregulation of *GhEG45*, which peaked to a 3.4-fold increase within 24 h post-infection (Fig. 1G). Mechanical wounding triggered a biphasic expression pattern with an initial modest 2.4-fold increase at 1 h and a robust secondary peak indicating a 7.3-fold increased expression level at 6 h post-treatment (Fig. 1H). These results imply possible roles in early pathogen recognition and subsequent repair signaling pathways.

Subcellular localization analysis predicted GhEG45 protein to be extracellularly localized (ProtComp score = 8.9). Confocal microscopy analysis of GhEG45::GFP fusion proteins transiently expressed in *Arabidopsis* epidermal cells confirmed extracellular (apoplast or cell wall) localization. Plasmolysis treatment with 0.8% mannitol did

not alter GFP localization, which was further confirmed (Fig. 1I). These findings support a functional role for *GhEG45* in cell wall-mediated immunity and stress adaptation mechanisms.

GhEG45 silencing in cotton significantly compromised plant resistance to *V. dahliae*

The VIGS approach of suppressing *GhEG45* expression (Fig. 2A, B) to investigate the functional role of *GhEG45* in cotton defense showed that *GhEG45*-silenced plants (TRV:*GhEG45*) exhibited significantly more severe symptoms of Verticillium wilt, such as leaf chlorosis, wilting, and necrosis, compared with those observed in the control plants (TRV:00) (Fig. 2C). The DI and fungal biomass were substantially increased in the silenced plants (Fig. 2D, E), and vascular browning was more pronounced than that in the controls (Fig. 2F). Furthermore, pathogen recovery assays showed enhanced fungal growth from the infected stem segments in TRV:*GhEG45* plants, which indicates the increased colonization capacity of *V. dahliae* (Fig. 2G). Trypan blue staining showed extensive necrotic lesions in silenced plants, which is

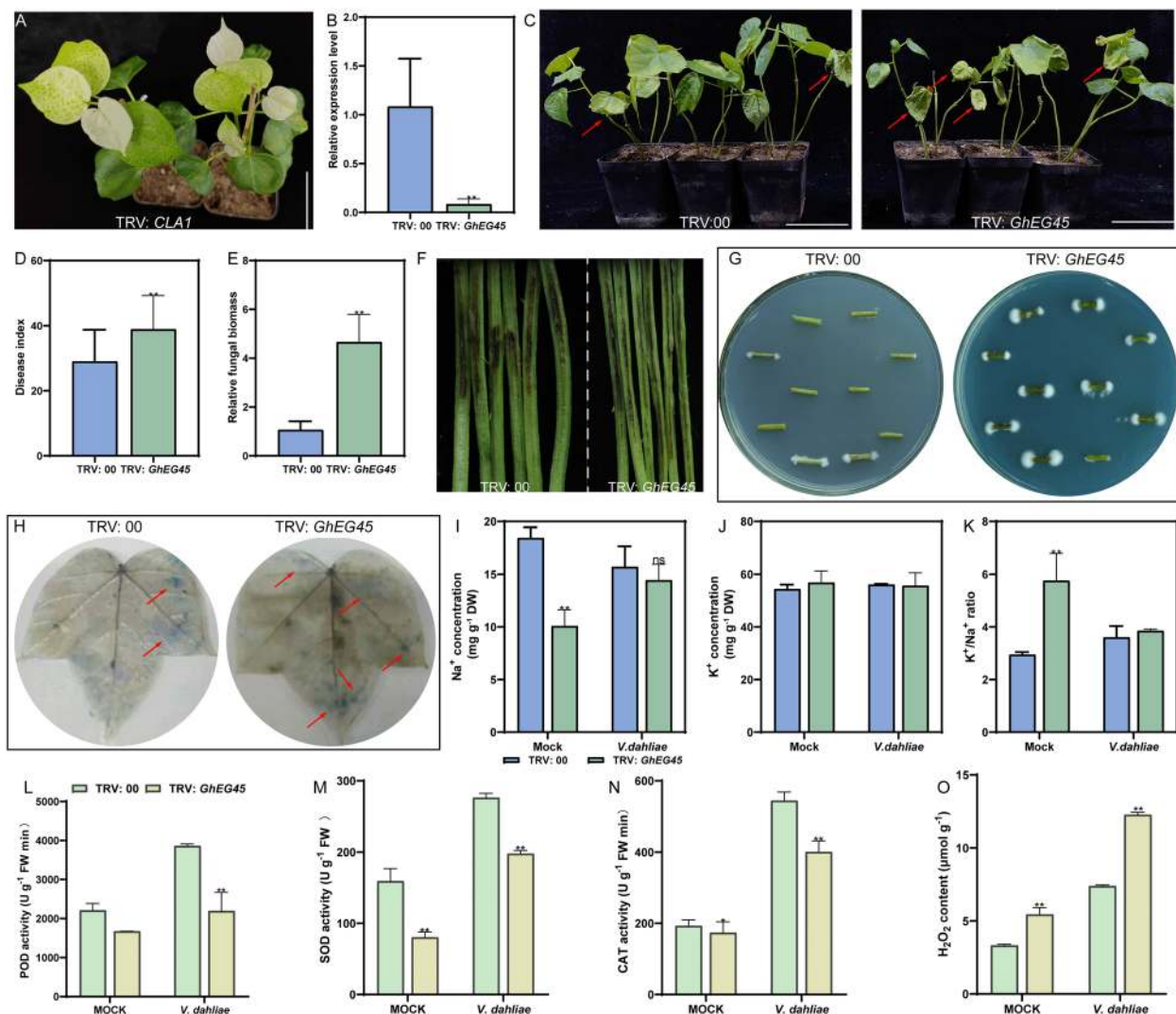


Fig. 2 Sensitivity of *GhEG45*-silenced cotton plants to *V. dahliae*. **A** Bleaching phenotype in TRV: *CLA1* confirms silencing efficiency. **B** RT-qPCR analysis showing suppressed *GhEG45* expression. **C** Disease symptoms in TRV:00 and TRV: *GhEG45* plants at 14 dpi. **D** Statistical analysis of disease index. **E** Relative fungal biomass quantified using RT-qPCR. **F** Vascular browning in TRV:00 and TRV: *GhEG45* plants. **G** Pathogen recovery assay in TRV:00 and *GhEG45*-silenced stems. **H** Trypan blue staining in TRV:00 and *GhEG45*-silenced leaves. **I–K** Changes in Na⁺ and K⁺ contents and K⁺/Na⁺ ratio before and after *V. dahliae* infection. **L** Peroxidase activity. **M** Superoxide dismutase activity. **N** Catalase activity. **O** H₂O₂ content. Error bars represent the SD of three biological replicates. Statistical significance ($n = 3$; ns, not significant, $*P < 0.05$ and $**P < 0.01$) was determined using two-way ANOVA and denotes differences between TRV:00 and TRV:*GhEG45* within the same treatment (Mock or *V. dahliae*) ($n = 3$)

indicative of aggravated cell death and fungal invasion (Fig. 2H). These findings collectively show that *GhEG45* is critical for restricting fungal proliferation and maintaining cellular integrity during *V. dahliae* infection.

To assess whether *GhEG45* is involved in ion homeostasis, the Na⁺ and K⁺ concentrations and K⁺/Na⁺ ratio were analyzed under mock and *V. dahliae*-infected conditions. Prior to inoculation, the Na⁺ levels were slightly lower in TRV:*GhEG45* plants than those in the control group (Fig. 2I), whereas the K⁺ levels and K⁺/Na⁺ ratios did not differ significantly (Fig. 2J, K). However, after

infection, the K⁺/Na⁺ ratio decreased significantly in *GhEG45*-silenced plants, which indicates impaired Na⁺ regulation under pathogenic stress. This result suggests that *GhEG45* is involved in the maintenance of ion balance during disease challenge, which may be linked to membrane integrity and stress tolerance.

Subsequently, the antioxidant response was evaluated to understand the molecular mechanism underlying *GhEG45*-mediated resistance. After pathogen infection, the control plants (TRV:00) exhibited robust induction of POD, SOD, and CAT activities, whereas

the TRV:*GhEG45* plants showed significantly attenuated enzyme activation (Fig. 2L–N). This diminished antioxidant capacity correlated with the excessive accumulation of H_2O_2 in *GhEG45*-silenced plants (Fig. 2O). These data indicate that *GhEG45* plays a central role in modulating redox homeostasis by regulating ROS during resistance responses. In conclusion, compromised cotton plant defense against *V. dahliae* by disrupting ROS balance, weakening antioxidant enzyme activities, impairing K^+/Na^+ homeostasis, and enabling increased fungal colonization. These results highlight the crucial regulatory role of *GhEG45* in orchestrating oxidative stress responses and ionic equilibrium to enhance cotton plant resistance to *V. dahliae*.

GhEG45 overexpression enhances resistance to *V. dahliae*

Transgenic *Arabidopsis* lines overexpressing *GhEG45* were generated, and the transcript levels were assessed.

Among the 12 independent lines analyzed, lines 1, 4, and 12 exhibited significantly higher *GhEG45* expression than the others (Fig. S2). These three lines were confirmed to be genetically stable in the T_3 generation and were selected for subsequent disease resistance assays to evaluate their responses to *V. dahliae* infection. To elucidate the functional role of *GhEG45* in xylem development and defense responses, ultrastructural and physiological analyses were performed in transgenic *Arabidopsis* overexpressing *GhEG45* (*GhEG45*-OE). Transmission electron microscopy (TEM) images showed that both fiber cells and vessel elements of *GhEG45*-OE *Arabidopsis* exhibited notably thicker secondary cell walls compared with those of the WT controls (Fig. 3A). This indicates a potential enhancement of vascular mechanical strength. Moreover, these structural changes were consistent with the results of safranin-fast green staining of the stem cross-sections, which showed increased lignin deposition

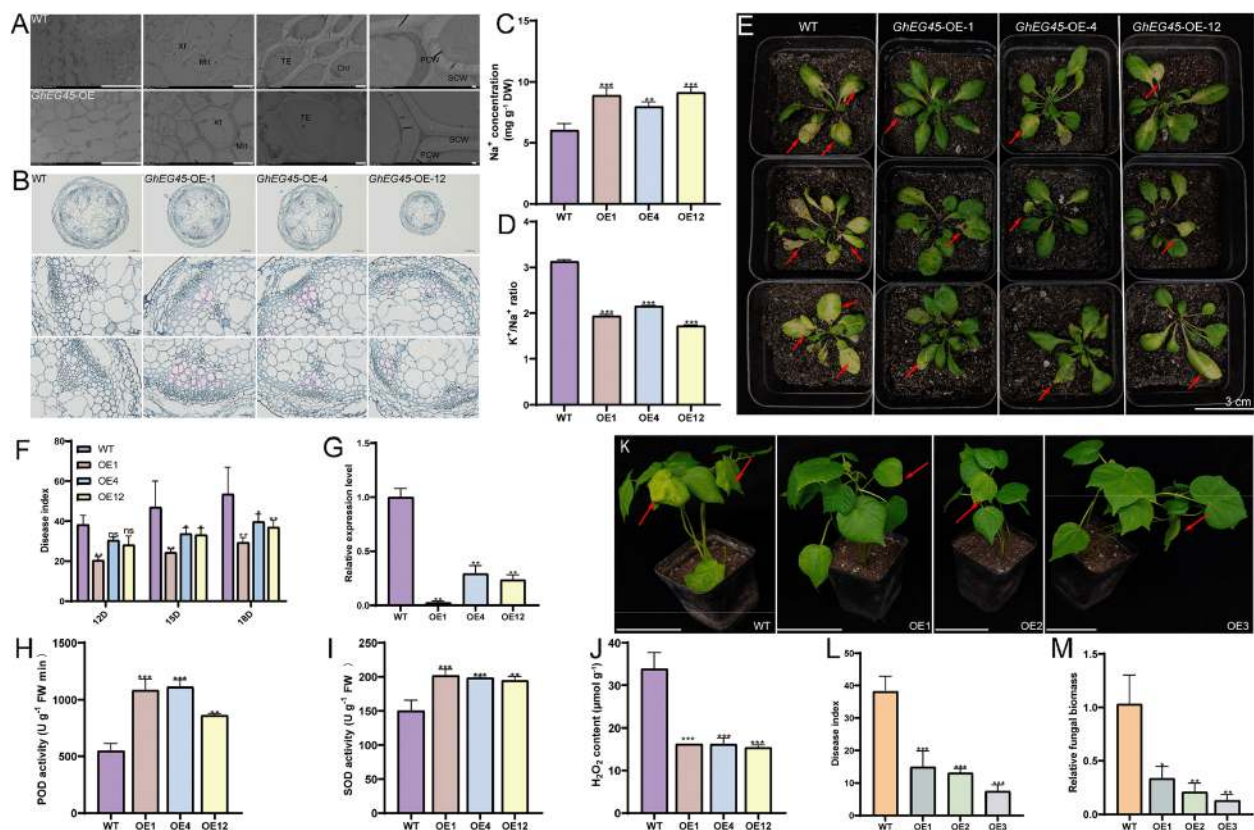


Fig. 3 *GhEG45* overexpression enhances resistance to *V. dahliae*. **A** Transmission electron microscopy (TEM) images of xylem tissues from *GhEG45*-overexpressing *Arabidopsis* plants showing xylem fiber and tracheary element cell wall structure. **B** Safranin-fast green staining of stem sections in cotton *GhEG45*-OE lines (OE-1, OE-4, OE-12). **C**, **D** Ion content analysis for Na^+ (**C**), and K^+/Na^+ ratio (**D**) in *Arabidopsis* lines. **E** Phenotypes of *GhEG45*-OE and WT *Arabidopsis* plants at 15 days post-inoculation (dpi) with *V. dahliae*. **F** Disease index assessment at 12, 15, and 18 dpi. **G** RT-qPCR analysis of *GhEG45* transcript levels at 18 dpi. **H**, **I** Enzymatic activities of peroxidase (POD) and superoxide dismutase (SOD). **J** Hydrogen peroxide (H_2O_2) content. **K** Phenotypic comparison of cotton transgenic lines (OE1–OE3) and WT under inoculation. **L** Disease index assessment at 15 dpi. **M** Quantification of fungal biomass by RT-qPCR. Statistical significance was determined using one-way ANOVA ($n = 3$; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, not significant)

in *GhEG45*-OE lines (Fig. 3B). The result of ion chromatography showed a significant increase in Na^+ level in the transgenic lines (Fig. 3C), whereas K^+ concentration remained unchanged (Fig. S3). This resulted in a significantly lower K^+/Na^+ ratio in the *GhEG45*-OE plants than in the WT (Fig. 3D). This altered ratio suggests that *GhEG45* may be involved in regulating Na^+ transport or sequestration during pathogen challenge, thereby contributing to plant stress adaptation capability.

After *V. dahliae* inoculation, *GhEG45*-OE *Arabidopsis* plants showed significantly less severe symptoms with only mild chlorosis and limited necrosis compared with that observed in the WT, which developed more extensive leaf damage (Fig. 3E). Quantitative DI analysis confirmed that *GhEG45* overexpression reduced susceptibility at 12, 15, and 18 dpi (Fig. 3F). Similarly, fungal biomass showed notably lower *V. dahliae* colonization in *GhEG45*-OE lines than in the WT (Fig. 3G). Transgenic cotton plants overexpressing *GhEG45* showed similarly enhanced resistance phenotypes, as evidenced by the low disease severity and reduced fungal accumulation (Fig. 3K–M). These findings collectively demonstrate the conserved role of *GhEG45* in enhancing resistance across species.

Under pathogen stress, POD and SOD activities were substantially upregulated in all transgenic *Arabidopsis* lines overexpressing *GhEG45* (Fig. 3H, I), and CAT activity showed a modest but significant increase in line OE12 (Fig. S4), whereas the H_2O_2 level was significantly lower in the *GhEG45*-OE lines than in the WT plants (Fig. 3J). In three independent *GhEG45*-overexpressing cotton lines (OE-1, OE-2, OE-3), POD activity was significantly higher in all transgenic lines compared with that in the WT, although OE-2 exhibited the highest induction (Fig. S5A). Similarly, SOD activity showed a pronounced increase in the OE lines (Fig. S5B). Additionally, CAT activity showed significant enhancement (Fig. S5C). Notably, H_2O_2 accumulation was significantly reduced in all *GhEG45*-overexpressing lines compared with that in the WT, which suggests highly efficient ROS scavenging (Fig. S5D).

Transcriptomic analysis and functional integration of *GhEG45* in *V. dahliae* resistance

To elucidate the molecular mechanisms underlying *GhEG45*-mediated enhanced resistance to *V. dahliae*, we performed transcriptomic sequencing of samples from *GhEG45*-overexpressing (OE) *Arabidopsis* and WT plants before and after pathogen infection. Transcriptomic sequencing generated 46.17–114.79 million reads per sample with Q30 values >96% and GC >44%

(Table S2), which indicated high sequencing quality that is suitable for further analysis. Most samples showed unique alignment results (Table S3), and the three biological replicates showed high correlation. PCA showed significant differences in gene expression between the *GhEG45* transgenic plants and WT before and after *V. dahliae* treatment (Fig. 4A). Venn diagram analysis indicated that 814 DEGs were commonly affected across all groups, whereas 450 DEGs were shared between the two *GhEG45*-related comparisons (Fig. 4B). This suggests both shared and genotype-specific responses to *GhEG45* under pathogen stress.

To assess the biological significance of these DEGs, we performed GO and KEGG enrichment analyses. GO enrichment analysis further revealed that DEGs in *GhEG45*-transgenic plants were enriched in response to oxygen levels, plastid thylakoid membrane, and photosynthetic membrane categories, which are closely associated with stress responses and photosynthetic regulation (Fig. 4C). KEGG analysis revealed significant enrichment in key defense-related pathways such as plant–pathogen interactions, MAPK signaling, phenylpropanoid biosynthesis, plant hormone signal transduction, ABC transporters, and ROS-related pathways (Fig. 4D). Notably, the *GhEG45*-Vd group showed upregulation of 415 DEGs in the plant–pathogen interaction pathway and 207 DEGs in MAPK signaling. Further analysis of the *GhEG45*-Vd vs. *GhEG45*-CK comparison showed the differential expression of key genes in the phenylpropanoid biosynthesis pathway, including *CCoAOMT*, *C4H*, and *UGT* family genes and ABC transporter genes, which highlights their potential involvement in *GhEG45*-mediated defense responses (Fig. S6). Together, these results indicate that *GhEG45* contributes to plant defense by regulating key metabolic pathways, hormone signaling, and oxidative stress responses.

We confirmed the reliability of the transcriptomic data by performing an RT-qPCR validation of 15 key genes involved in defense-related pathways (MAPK cascade, transcription factors, ROS detoxification, and hormone signaling). The expression trends of these genes were consistent with the RNA-seq FPKM values, which confirm the robustness and reproducibility of our transcriptomic dataset (Fig. 5). The results show that these genes showed significantly upregulated expression levels in *GhEG45*-OE plants and that the expression patterns were highly consistent with the FPKM values derived from RNA-seq analysis. These results confirm the high reliability of the transcriptomic data (Fig. 5).

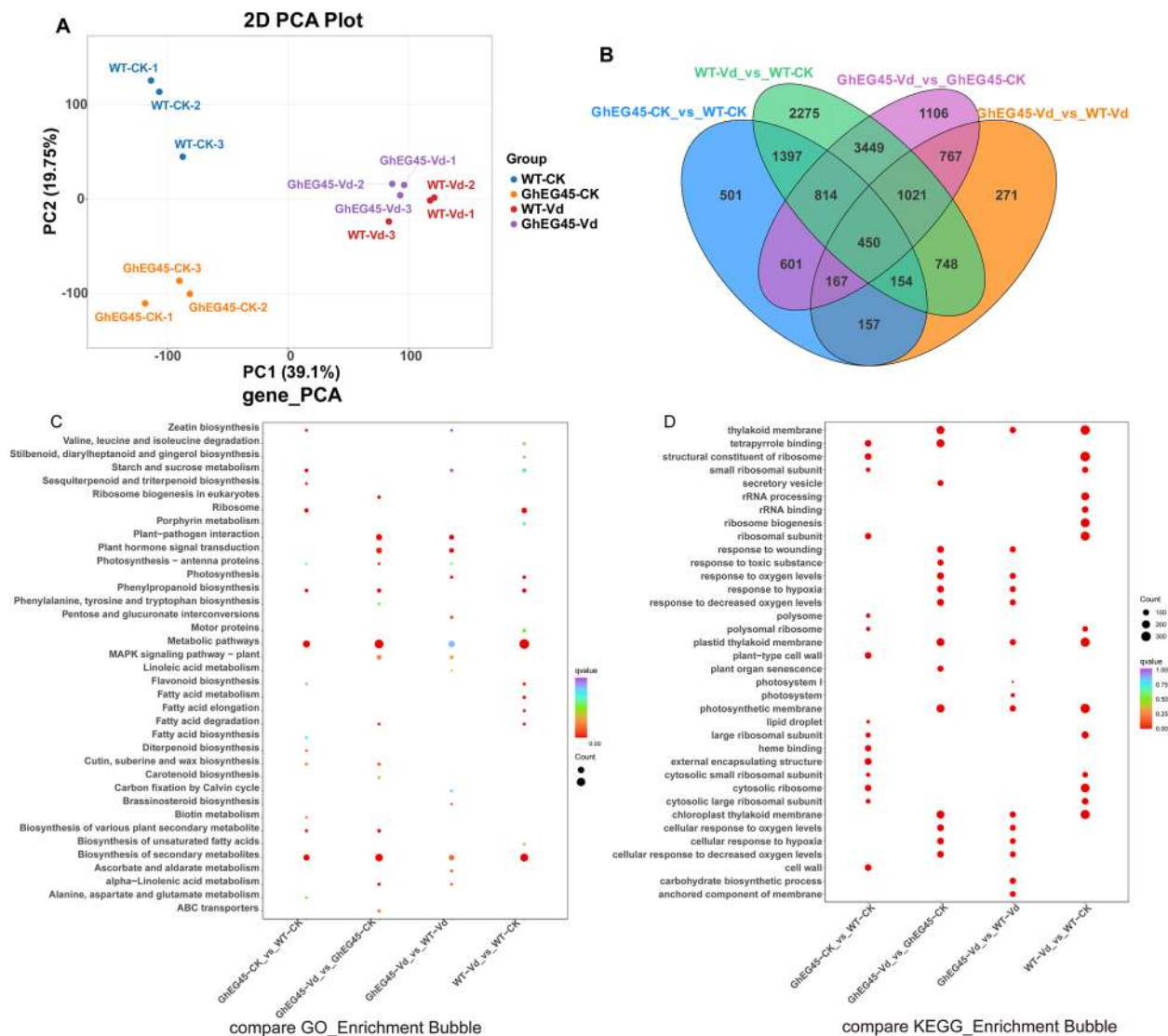


Fig. 4 Transcriptomic analysis of *GhEG45*-OE and WT plants under *V. dahliae* stress. **A** Principal component analysis (PCA) among different samples. **B** Venn diagram illustrating overlapping and unique DEGs across comparisons. **C** GO enrichment bubble plot indicating top biological processes affected in *GhEG45*-OE plants. **D** KEGG pathway enrichment identifying top pathways associated with pathogen resistance

Furthermore, WGCNA identified 12 gene modules from the DEGs. The green module showed the strongest positive correlation with *GhEG45* expression under infection conditions (Fig. 6A–C). Within this module, 23 hub genes (e.g. *JAZ5*, *LOX3*, *PUB17*, *NPR3*) related to plant hormone signaling and immune responses were identified (Fig. 6D). The close co-expression of *GhEG45* with these hub genes further supports its pivotal role in coordinating hormone-mediated immune signaling and ROS regulation during *V. dahliae* infection. In summary, these results indicate that *GhEG45* plays a central role in modulating plant defense by activating key immune-related pathways and co-regulating critical

genes associated with ROS homeostasis, hormone-mediated pathways, and defense signaling cascades.

GhEG45 positively regulates SA signaling to enhance defense gene expression

The role of *GhEG45* in SA-mediated immunity was elucidated by analyzing the expression of key SA pathway marker genes in both *GhEG45*-silenced cotton and *GhEG45*-OE *Arabidopsis* under pathogenic stress. In *GhEG45*-silenced plants, *GhPR1*, *GhPR5*, and *GhEDS1* were significantly downregulated compared with those in the TRV:00 controls under both mock and *V. dahliae*-inoculated conditions (Fig. 7A–C). This

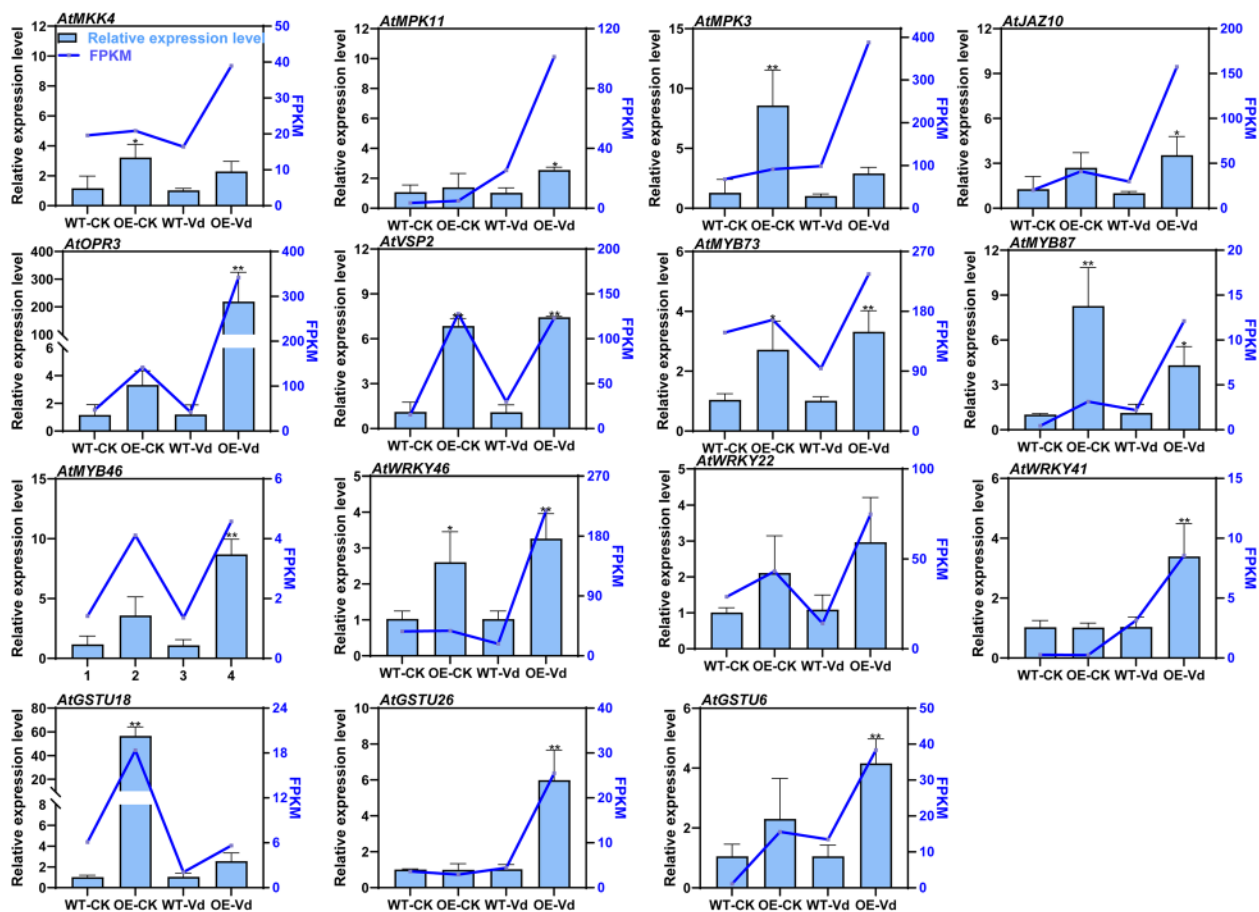


Fig. 5 RT-qPCR validation of 15 differentially expressed genes in *GhEG45*-overexpressing *Arabidopsis*. Relative expression levels (bar graphs, left axis) and RNA-seq derived FPKM values (blue lines, right axis) of 15 key genes across treatments. These genes are vital for regulating signaling, transcriptional regulators, and antioxidant enzymes. Statistical significance was determined using one-way ANOVA ($n = 3$; * $P < 0.05$, ** $P < 0.01$)

indicates that *GhEG45* suppression compromises basal and induced SA signaling responses. We observed consistent results in *GhEG45*-OE *Arabidopsis*, where the expression of canonical SA-responsive genes such as *AtPR1*, *AtPR5*, *AtNPR1*, *AtPAD4*, *AtEDS16*, and *AtTGA7* showed pronounced upregulation with fold-changes ranging from 2.1 to 10.7 in all OE lines compared with those in the WT plants following pathogenic infection (Fig. 7D). These transcriptional changes suggest that *GhEG45* positively regulates the SA signaling cascade. Additionally, a similar expression trend was observed in *GhEG45*-OE cotton, where SA pathway components such as *GhPR1*, *GhPR5*, *GhPAD4*, and *GhTGA7* showed consistent upregulation following *V. dahliae* infection (Fig. S7). This indicates that *GhEG45* enhances resistance by promoting SA-dependent transcriptional reprogramming. Taken together, *GhEG45* acts as a positive regulator of SA-mediated defense by enhancing both constitutive and

inducible expression of immunity genes to counter *V. dahliae*.

***GhEG45* promotes antioxidant defense and maintains ROS homeostasis**

We found that *GhEG45* contributes to ROS regulation by modulating the expression of antioxidant-related genes during *V. dahliae* infection. *GhEG45*-silenced cotton (TRV:*GhEG45*) showed significantly lower expression of *GhPOD1*, *GhSOD1*, and *GhGST1* than that in control plants (TRV:00) both under mock and *V. dahliae*-inoculated conditions (Fig. 8A–C). This transcriptional suppression suggests a compromised ROS detoxification capacity in silenced lines, which likely caused excessive ROS accumulation and increased susceptibility to pathogenic invasion. In contrast, *GhEG45*-overexpressing *Arabidopsis* lines showed that the transcript levels of ROS-related genes such as *AtPOD4*, *AtRbohD*, *AtGST1*, and *AtCAT2* were significantly elevated in the OE lines compared with in the WT controls (Fig. 8D), which

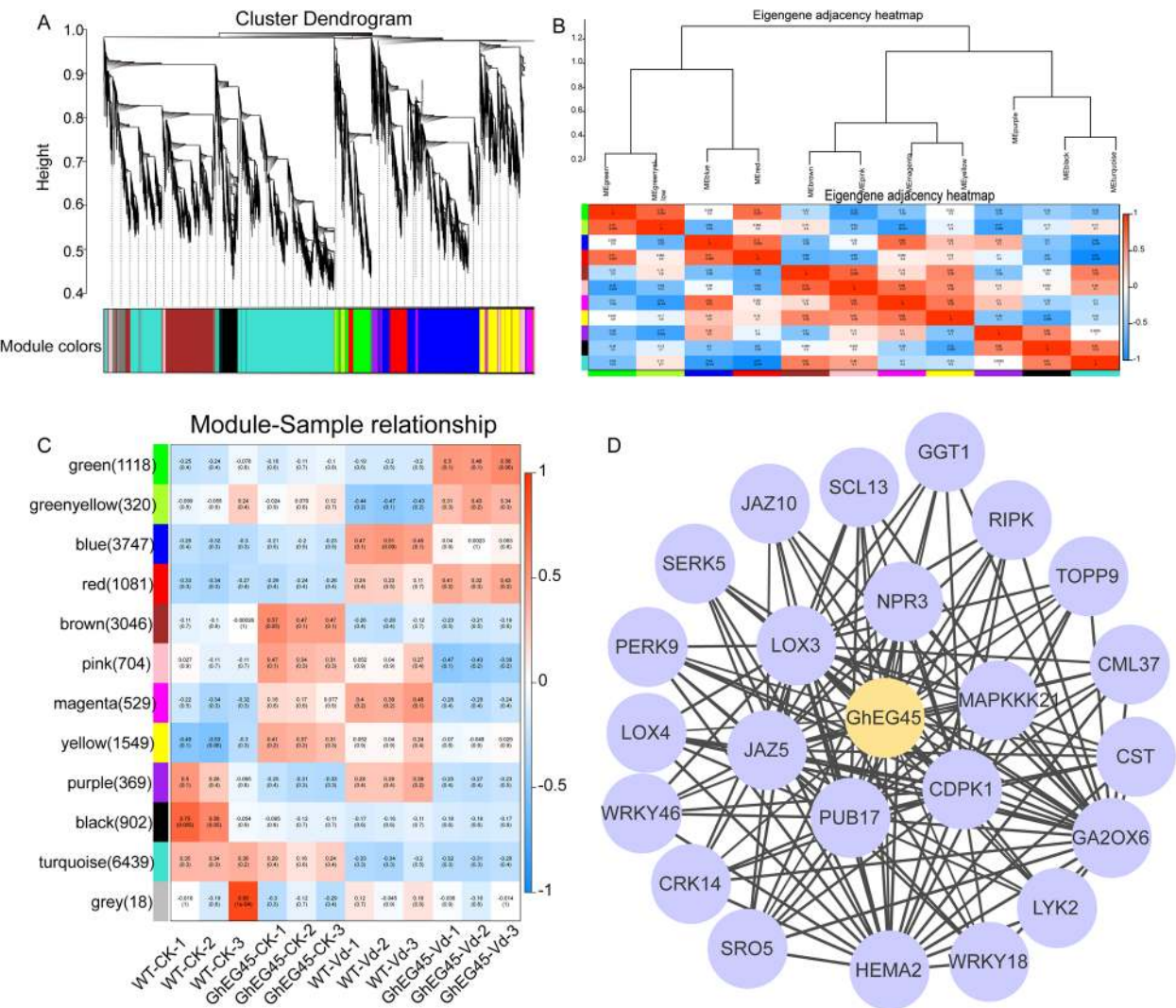


Fig. 6 WGCNA-based co-expression module analysis. **A** Dendrogram showing hierarchical clustering of DEGs into modules. **B** Eigengene adjacency heatmap visualizing relationships among gene modules. **C** Module-sample correlation heatmap indicating module relationships with sample traits. Green module strongly correlates with *GhEG45* expression post-infection. **D** Co-expression network highlighting 23 hub genes from the green module, centered around *GhEG45*, showing close interactions with hormone signaling and immune response genes

indicates enhanced antioxidative capacity. Furthermore, these patterns were observed in transgenic cotton lines overexpressing *GhEG45* with pronounced upregulation of *GhPOD4*, *GhSOD1*, *GhGST1*, and *GhCAT2* expression after pathogenic challenge (Fig. 8E). The upregulation of both ROS-generating and ROS-scavenging genes in *GhEG45*-OE plants suggests a dual role for *GhEG45* in orchestrating redox signaling and detoxification for effective oxidative stress management. This result suggests that *GhEG45* may modulate intracellular ROS homeostasis to enhance resistance against *V. dahliae*. These genes are essential for ROS production and scavenging (Mittler et al. 2022). These findings support the conclusion that *GhEG45* positively regulates ROS homeostasis

by activating the transcription of key antioxidant defense genes, thereby enhancing plant resilience against *V. dahliae* infection.

Discussion

The *GhEG45* gene is a member of the plant *PNP* family and has been recognized as a critical regulator in cotton plant defense against *V. dahliae*. This study provides comprehensive evidence supporting its role in enhancing plant immunity through multifaceted mechanisms, including ROS signaling, cell-wall modification, and ion homeostasis regulation. These findings concur with those of previous studies on *PNP* family members such as *AtPNP-A* in *Arabidopsis*, which regulates stomatal

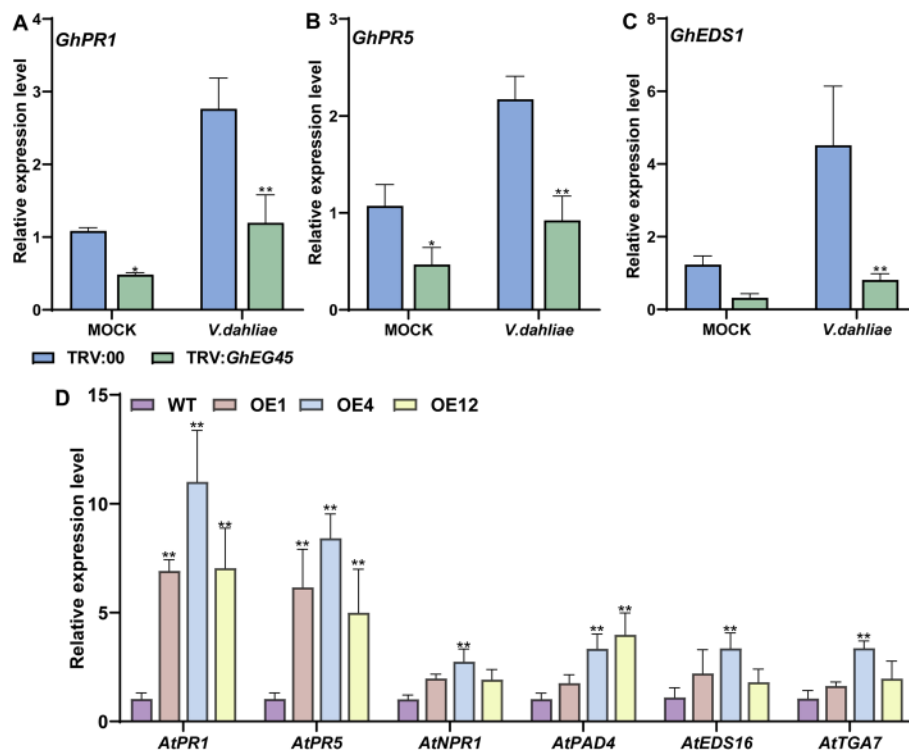


Fig. 7 *GhEG45* regulates resistance to *V. dahliae* through the SA signaling pathway. **A–C** Relative expression of *GhPR1*, *GhPR5*, and *GhEDS1* in TRV:00 and *GhEG45*-silenced cotton plants. **D** Relative expression of SA-related genes *AtPR1*, *AtPR5*, *AtNPR1*, *AtPAD4*, *AtEDS16*, and *AtTGA7* in WT and *GhEG45*-overexpressing *Arabidopsis*. Error bars represent the SD of three biological replicates, and statistical significance was evaluated using one-way ANOVA ($n = 3$; * $P < 0.05$ and ** $P < 0.01$)

aperture and transpiration, thereby aiding in pathogenic defense (Billington et al. 1997; Gehring 1999). Furthermore, members of the PNP family modulate plant responses to abiotic stress, which indicates their broad role in environmental stress adaptation (Wang et al. 2011). The involvement of *GhEG45* in cotton immunity further highlights the conserved regulatory functions of this gene family across plant species.

This study showed that *GhEG45* was significantly induced after *V. dahliae* infection. This finding supports the role of *GhEG45* as a positive regulator of plant immunity (Fig. 2). *GhEG45*-overexpressing *Arabidopsis* and cotton lines exhibited enhanced disease resistance characterized by reduced fungal colonization, less severe leaf necrosis, and increased lignification of vascular bundles compared with those of the controls. In contrast, *GhEG45*-silenced plants displayed compromised resistance, showing extensive necrotic lesions and increased pathogen biomass. *GhEG45*-silenced cotton leaves exhibited widespread necrotic lesions, which are indicative of extensive cell death and fungal proliferation (Fig. 2H). The observed structural enhancement of the cell wall in *GhEG45*-overexpressing plants (Fig. 3A) concurs with the findings of recent studies that have emphasized the

importance of cell-wall fortification in plant immunity (Wang et al. 2023a, b). *GhEG45* silencing was reflected in the altered K^+/Na^+ ratios (Fig. 2I–K). This indicates its role in maintaining cellular ionic balance, which is critical for stress adaptation. This function aligns with findings on other PNP family members, although the outcomes differ depending on the host–pathogen context, as observed in the case of *XacPNP* in *Xanthomonas* (Ficarra et al. 2018; Lee et al. 2020).

PCA and heatmaps of DEGs showed distinct transcriptional divergence between *GhEG45*-overexpressing and WT plants, which suggests a substantial reprogramming of the defense-associated gene expression (Fig. 4A, B). GO and KEGG enrichment analyses identified significant upregulation in pathways related to ROS metabolism, cell-wall biosynthesis, phenylpropanoid metabolism, hormone signaling, and pathogen interactions (Fig. 4C, D). Enhanced expression of genes encoding cell-wall modifying enzymes, including those for CCoAOMT and C4H, was observed in *GhEG45*-overexpressing lines (Fig. S5). These enzymes are key players in lignin biosynthesis and the phenylpropanoid pathway, respectively, and aid in the mechanical strengthening of plant cell walls during pathogenic attack (Gomez-Cano et al. 2020; Liu et al. 2016).

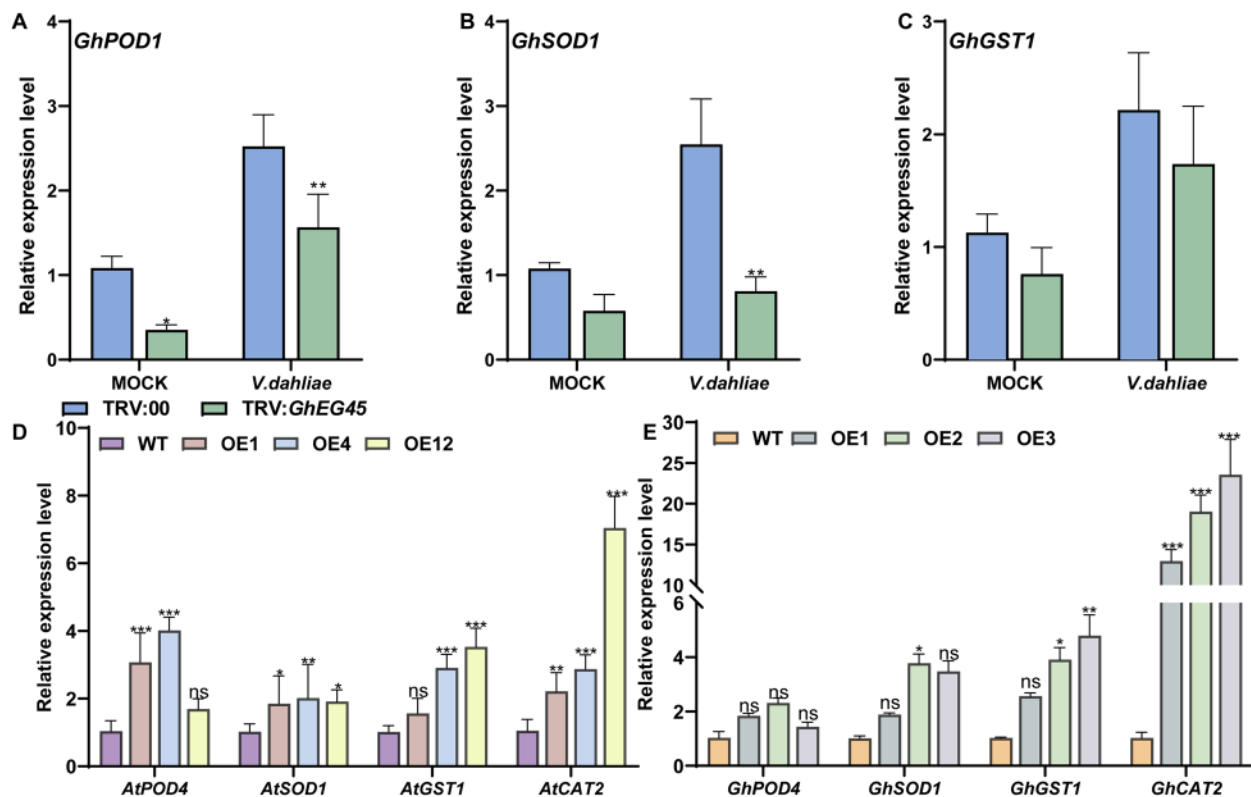


Fig. 8 *GhEG45* regulates ROS-related pathways to enhance antioxidant capacity. **A–C** Relative expression of *GhPOD1*, *GhSOD1*, and *GhGST1* in TRV:00 and *GhEG45*-silenced cotton. **D** Relative expression of antioxidant genes in WT and *GhEG45*-overexpressing *Arabidopsis*. **E** Relative expression of antioxidant genes in *GhEG45*-overexpressing cotton. Error bars represent the SD of three biological replicates, and statistical significance was evaluated using one-way ANOVA ($n = 3$; ns, not significant, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$)

Additionally, glycosyltransferase family genes (*UGTs*) and ABC transporter family members such as *ABCG40* and *ABCC5* were significantly induced (Fig. S5), which suggests enhanced transport and accumulation of defense-related secondary metabolites and hormones. This transcriptional activation supports the hypothesis that *GhEG45* mediates immune responses both by enhancing antioxidant and hormonal defenses and by reinforcing physical barriers. Additionally, WGCNA analyses identified modules and hub genes highly correlated with *GhEG45* expression and resistance phenotypes, reaffirming its integrative role in plant immune signaling networks (Fig. 6).

The SA signaling pathway is central to plant immune responses. This study distinctly shows that *GhEG45* positively regulates the SA signaling cascade. In transgenic *GhEG45*-overexpressing *Arabidopsis* and cotton plants, key SA signaling components—*NPR1*, *EDS1*, *PAD4*, and *TGA7*—were significantly upregulated following pathogenic challenge (Fig. 7). Notably, *NPR1* is a central transcriptional regulator of *PR* genes (Chen et al. 2019). *GhEG45* silencing reduced the expression of these genes,

highlighting *GhEG45*'s essential role in SA-dependent transcriptional defense activation.

Besides SA signaling, *GhEG45* also positively modulates ROS homeostasis. ROS are dual-functional molecules with antimicrobial activity and signaling roles in defense pathways (Ray et al. 2012). *GhEG45* overexpression induced antioxidant enzyme activities (POD, SOD, and CAT), reducing oxidative damage during infection. The transcriptional activation of antioxidant genes (*POD1*, *GST1*, and *CAT2*) further supports its role in redox regulation (Fig. 8). *GhEG45* silencing severely impaired the antioxidant system, which disrupted ROS metabolism and increased the susceptibility to oxidative stress. Notably, RT-qPCR and biochemical assays confirmed the significantly reduced H_2O_2 levels in *GhEG45*-overexpressing plants compared with those in the wild-type after pathogenic challenge, which confirmed effective ROS scavenging and corrected earlier inaccuracies in ROS interpretation.

Comprehensive transcriptomic analyses provided deeper insights into the regulatory landscape modulated by *GhEG45*. Overall, these transcriptomic data present a systemic-level overview of the functions of

GhEG45 and show that it simultaneously regulates ROS balance, hormone signaling, and cell-wall biosynthesis to establish robust and multilayered defense strategies. These findings clarify the molecular framework through which *GhEG45* confers disease resistance and present potential targets for future functional validation and breeding applications.

Conclusion

In summary, this study shows that *GhEG45* plays a crucial role in the plant immune response of the cotton plant to *V. dahliae* infection by potentially enhancing disease resistance through the regulation of ROS accumulation and the SA signaling pathway. As a member of the PNP family, *GhEG45* shows substantial potential as a molecular target for breeding disease-resistant cotton cultivars. Although the precise mechanism underlying the actions of *GhEG45* remains to be fully elucidated, its potential as a disease-resistant gene has been clearly established in this study. Further studies should investigate the detailed regulatory mechanisms involving *GhEG45* to expand its utility in broader crop protection strategies.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42397-025-00240-2>.

Supplementary Material 1.

Supplementary Material 2

Acknowledgements

We are grateful to/thank Wuhan Metware Biotechnology Co., Ltd. for assisting in sequencing and/or bioinformatics analysis. We appreciate the VIGS constructs provided by Dr. Liu YL from Tsinghua University and Dr. Li FJ from College of Agronomy and Biotechnology, China Agricultural University.

Authors' contributions

Hou YX developed experimental design. Gao LY drafted the manuscript. Hou YX revised the article. Gao LY conducted bioinformatics analysis and carried out most of the experiments. Hu MH and Yan X provided the breeding of wild-type and transgenic plants. Gao LY and Liu Y assisted with data analysis and material preparation. Hou YX supervised the project. All authors reviewed the manuscript and agreed on its content.

Funding

The National Natural Science Foundation of China (grant number: 32272631) supports this work.

Data availability

Data will be made available by the corresponding author upon reasonable request.

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. Author Hou YX is a Guest editor to the Special issue 'Celebrate the 120th Anniversary of China Agricultural University'. Author Hou YX was not involved in the journal's review of, or decision related to this manuscript.

Received: 4 July 2025 Accepted: 27 August 2025

Published online: 11 October 2025

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