

RESEARCH

Open Access



Suberin feruloyl transferase FHT confers the antiviral activity by targeting the coat protein of cucumber green mottle mosaic virus

Niu Ai¹, Tingyuan Fu², Lina Xie¹, Han Fu¹, Lianwei Yu¹, Ling Shen¹, Qijia Ai¹, Heng Sun¹, Xiaoyu Han¹, Honglian Li¹, Xue Yang^{1*} and Yan Shi^{1,3*}

Abstract

Fatty acid ω -hydroxyacid/fatty alcohol hydroxycinnamoyl transferase (FHT) is a key enzyme involved in suberin biosynthesis. The phytohormone abscisic acid (ABA) positively regulates suberin formation by promoting FHT expression, however the role of FHT regulated by ABA in viral infection remains unclear. Immunoprecipitation coupled with mass spectrometry (IP-MS) screening indicated that NbFHT interacting with the coat protein (CP) of cucumber green mottle mosaic virus (CGMMV). This interaction was further confirmed by bimolecular fluorescence complementation (BiFC) assay and co-immunoprecipitation (Co-IP) assays. Quantitative real-time polymerase chain reaction (qPCR) analysis revealed that the *NbFHT* gene expression was induced during CGMMV infection. Virus-induced gene silencing (VIGS) and overexpression analysis further demonstrated that NbFHT in *Nicotiana benthamiana* enhances host resistance to CGMMV. Notably, NbFHT interferes with the CGMMV CP self-interaction and promotes CP degradation. Moreover, exogenous ABA treatment significantly stabilized NbFHT and accelerated CP degradation, contributing to the increased resistance to CGMMV. Collectively, this study makes a unique contribution by shedding light on the previously unexplored relationship between the suberin synthesis-related enzyme FHT and ABA during viral infection.

Keywords Abscisic acid (ABA), Antiviral immunity, Cucumber green mottle mosaic virus (CGMMV), Fatty acid ω -hydroxyacid/fatty alcohol hydroxycinnamoyl transferase (FHT), Suberin

Background

Suberin is a complex biopolymer composed of fatty acids and glycerol, which is associated with lignin-like polymers that mainly consist of ferulates (Beisson et al. 2012; Graça et al. 2015). Suberin is deposited in specific tissues situated between the plasma membrane and the primary cell wall, such as the exodermis of roots, endodermis, periderm, and seed coat. Crucially, it undergoes de novo synthesis in response to diverse environmental stresses, including wounding, salt stress, drought, and pathogen infection (Ranathunge et al. 2011; Vishwanath et al. 2015; Holbein et al. 2019; Salas-González et al. 2021). For example, suberin deposition isolates the stele from the remainder of the root, acting as a barrier

*Correspondence:

Xue Yang
yangxuepphappy@126.com
Yan Shi
shiyan@henau.edu.cn

¹ College of Plant Protection, Henan Agricultural University, Zhengzhou 450002, Henan, China

² College of Plant Protection, China Agricultural University, Beijing 100083, China

³ State Key Laboratory of High-Efficiency Production of Wheat-Maize Double Cropping, Zhengzhou 450002, Henan, China



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

that restricts nematode entry and pathogen invasion into the xylem, thereby limiting their systemic spread throughout the plant (Holbein et al. 2019; Kashyap et al. 2021, 2022). The suberin induction is tightly regulated by phytohormonal signaling. Notably, the phytohormone abscisic acid (ABA), a pivotal hormone involved in plant growth, development, and response to environmental stresses (Finkelstein 2013; Brookbank et al. 2021), has been demonstrated to contribute to suberin biosynthesis in multiple plant species, including *Arabidopsis thaliana* (Xu et al. 2022; Binenbaum et al. 2023), rice (Lu et al. 2022), potato (Boher et al. 2013), and kiwifruit (Han et al. 2022). A key enzyme in suberin biosynthesis is fatty acid ω -hydroxyacid/fatty alcohol hydroxycinnamoyl transferase (FHT), a member of the HxxxD-type acyl-transferase BAHD family. FHT facilitates the transfer of ferulic acid from feruloyl-CoA to ω -hydroxy fatty acids/alcohols, thereby generating suberin monomers, an essential step in suberin synthesis (Gou et al. 2009; Molina et al. 2009; Serra et al. 2010). Previous studies showed that ABA regulates the accumulation of suberin feruloyl transferase FHT under wounding stress (Boher et al. 2013; Wei et al. 2020). Recently, root-associated microbiota have also been reported to influence ABA-dependent suberin deposition in the endodermis (Salas-González et al. 2021). Despite significant progress in understanding suberin biosynthesis and its induction by ABA under stress conditions, a critical gap exists in our understanding of the specific molecular mechanisms linking ABA signaling to enhanced suberin-based defenses, particularly against viral pathogens.

Here, we used cucumber green mottle mosaic virus (CGMMV, *Tobamovirus viridimaculae*) as a model to study the molecular mechanisms by which ABA induces suberin-associated defenses under biotic stress. CGMMV consists of a single-stranded, positive-sense genomic RNA of approximately 6.4 kb in length (Ugaki et al. 1991). In a previous study, we demonstrated that interaction between CGMMV coat protein (CP) and movement protein (MP) suppresses host antiviral defence, thereby facilitating systemic virus infection (Shi et al. 2023). In this study, we identified NbFHT as a protein that interacts with the CP of CGMMV via immunoprecipitation-mass spectrometry (IP-MS) screening. NbFHT inhibited CP self-interaction and promoted its degradation, conferring antiviral defense to the host. Furthermore, exogenous ABA treatment impeded CGMMV accumulation while significantly promoting NbFHT accumulation. This research advances our understanding by revealing the previously unexplored relationship between the suberin biosynthesis-related enzyme FHT and ABA in the context of viral infection.

Results

NbFHT is highly expressed during CGMMV infection

To identify host proteins interacting with CGMMV CP, CP into the flag-tagged vector (CP-flag) was expressed in *Nicotiana benthamiana* leaves. Protein complexes were isolated by flag-affinity purification and subsequently analyzed by mass spectrometry, which identified NbFHT as a novel CP-interacting protein (Additional file 2: Table S1). To elucidate the evolutionary relationships of FHTs in plants, amino acid sequences of the FHT family members from the species in the families of *Cucurbitaceae*, *Solanaceae*, *Brassicaceae*, and *Poaceae* were aligned, and a phylogenetic tree was constructed. The results indicated that NbFHT is distantly related to FHT proteins in other plant species (Fig. 1a). We then amplified the full-length open reading frame of *NbFHT* from *N. benthamiana* cDNA and cloned it into the pGEM-T vector for sequencing and subsequent analysis. The NbFHT fused with the N-terminus of red fluorescent protein (RFP) was transiently expressed in *N. benthamiana* leaves. NbFHT fluorescence was observed in the cytoplasm, cell wall, and the nucleus (Fig. 1b, upper panel). After mannitol-induced plasmolysis of the leaf epidermal cells, NbFHT localization was confined to the cytoplasm and excluded from the cell walls (Fig. 1b, bottom panel). Previous studies have shown that FHT transcription and protein accumulation are restricted to tissues undergoing suberization, including the root, the tuber periderm, and wounding tissues (Serra et al. 2010; Boher et al. 2013; Wei et al. 2020). Consistent with this, our RT-qPCR analysis revealed that *NbFHT* transcript levels were relatively high in mechanically wounded leaves (Fig. 1c). Notably, *NbFHT* expression was strongly induced in locally inoculated and systemically infected leaves during CGMMV infection (Fig. 1d). Furthermore, a comparative analysis revealed that *NbFHT* expression in infected leaves was approximately 5.5-fold higher than that in roots of the same plants (Fig. 1e).

NbFHT interacts with CGMMV CP

Previous studies had shown that CGMMV CP localizes to the cytoplasm and nucleus (Shi et al. 2023). To further confirm the interaction between the CGMMV CP and NbFHT, NbFHT-RFP and CP fused to green fluorescent protein (CP-GFP) were co-expressed in *N. benthamiana* leaf cells. At 48 h post infiltration (hpi), NbFHT-RFP was observed to co-localize with CP-GFP in both the cytoplasm and nucleus (Fig. 2a). We confirmed the interaction between NbFHT and CP using bimolecular fluorescence complementation (BiFC) assay. NbFHT and CP were individually fused to the N and C terminals of yellow fluorescent protein (YFP) and transformed into *Agrobacterium tumefaciens*. YFP fluorescent signal was

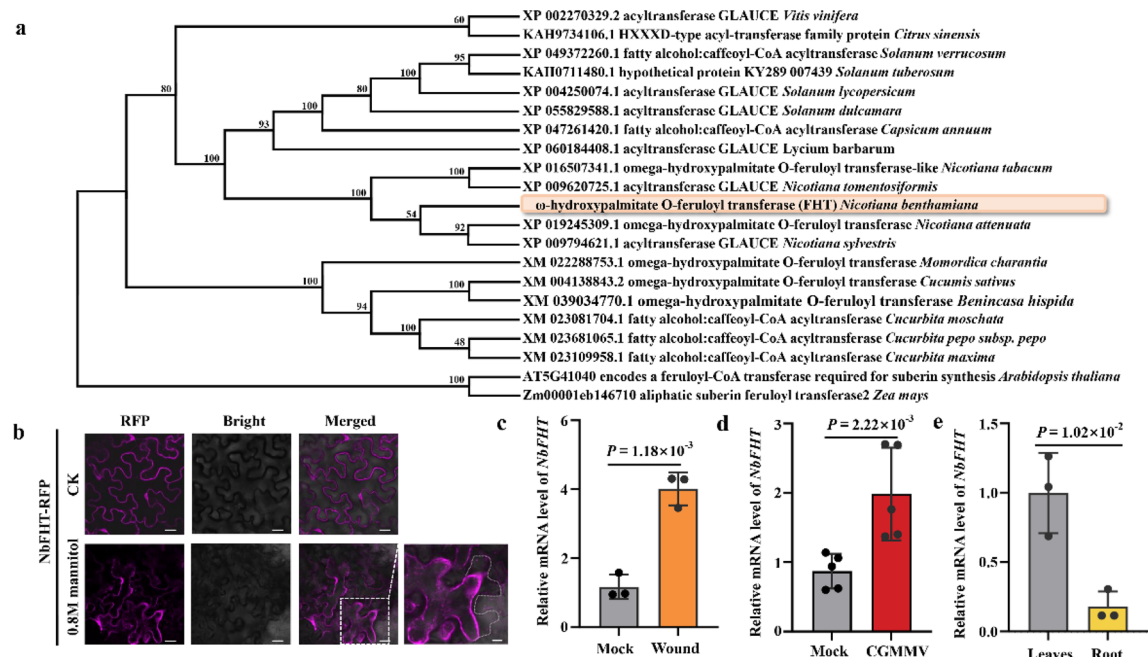


Fig. 1 Phylogenetic analysis, subcellular localization, and expression profiles of *NbFHT*. **a** Phylogenetic tree of FHT constructed based on amino acid sequences. **b** *N. benthamiana* leaves transiently expressing NbFHT-RFP were treated with 0.8 M mannitol, and NbFHT localization was observed in the cytoplasm and nucleus. Scale bar = 50 μ m. **c** *N. benthamiana* leaves were mechanically wounded with quartz sand, and the rubbed leaves were collected 7 days later for RT-qPCR analysis of *NbFHT* transcript levels. **d** RT-qPCR analysis of *NbFHT* mRNA levels in both inoculated and systemic leaves of *N. benthamiana* at 7 days post inoculation (dpi) with CGMMV. **e** RT-qPCR analysis of *NbFHT* mRNA levels in the roots and leaves of *N. benthamiana* at 7 dpi with CGMMV. Error bars represent standard error of the mean (SEM). The significant difference was analyzed by an unpaired Student's *t*-test (two-tailed). All experiments were repeated independently three times with similar results

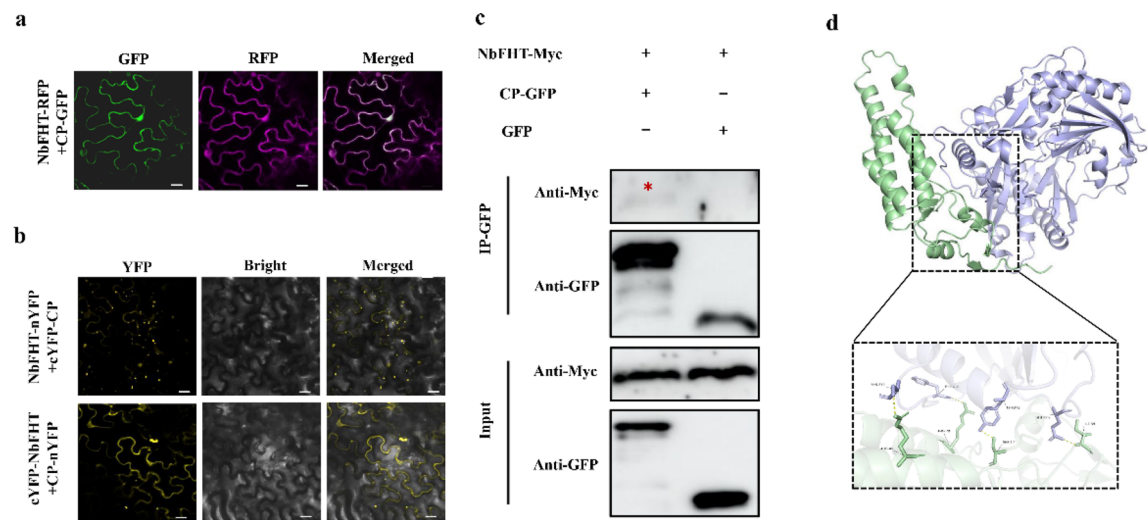


Fig. 2 NbFHT interacts with CGMMV CP. **a** Confocal micrographs of *N. benthamiana* leaf epidermal cells after 48 hpi co-expressing NbFHT-RFP and CP-GFP. **b** Interaction between NbFHT-nYFP and cYFP-CP/nYFP and cYFP-NbFHT was observed through BiFC in *Agrobacterium*-infiltrated *N. benthamiana* leaves. Scale bar = 50 μ m. **c** Interaction between NbFHT-Myc and CP-GFP was verified by Co-IP. Protein extracts were incubated with GFP magnetic beads, and samples before (Input) and after (IP) immunoprecipitation were analyzed by immunoblotting using anti-GFP or anti-Myc antibodies. NbFHT-Myc is marked with an asterisk. **d** Predicted protein structures generated using DMFold with NbFHT shown in purple and CP shown in green (pTM-scores: 0.7, ipTM-scores: 0.17). All experiments were repeated independently three times with similar results

observed when the combination of cYFP-NbFHT and CP-nYFP or NbFHT-nYFP and cYFP-CP in *N. benthamiana* leaf cells, indicating an *in vivo* interaction between NbFHT and CP (Fig. 2b). In contrast, no fluorescence signal was detected in negative control combinations (Additional file 1: Figure S1a). These results confirmed that CP interacted with NbFHT *in vivo*, producing YFP fluorescent aggregation in the cytoplasm and nucleus. To further explore the interaction between NbFHT and CP, we performed co-immunoprecipitation (Co-IP) assays. NbFHT-Myc and CP-GFP were transiently expressed in *N. benthamiana*, with NbFHT-Myc and GFP alone serving as a negative control. Then GFP-Trap_M magnetic beads were used to purify GFP-tagged proteins and all their interacting partners for verifying the *in vivo* interaction between NbFHT and CGMMV CP. NbFHT-Myc was co-precipitated only in the presence of CP-GFP, and not when co-expressed with GFP alone (Fig. 2c). However, no interaction between CP and FHT was detected using a yeast two-hybrid assay (Additional file 1: Figure S1b). In addition, we also used the DMfold program (Zheng et al. 2024) to predict the interfaces between NbFHT and CP. Structural modeling identified multiple potential interaction residues, including NbFHT residues Ser210, Phe216, Glu225, and Tyr375, which were predicted to interact with CP residues Agr46, Agr78, Ile59,

and Ser17, respectively (pTM-scores: 0.7, ipTM-scores: 0.17) (Fig. 2d). These results demonstrated that NbFHT interacted with CP.

NbFHT confers antiviral defense to host plants

To investigate the role of NbFHT in response to CGMMV infection, we utilized tobacco rattle virus-induced gene silencing (TRV VIGS) to knock down *NbFHT* expression. *NbFHT*-silenced plants exhibited no phenotypic changes compared with control plants (Fig. 3a, upper panel), with a silencing efficiency detected at 56% (Fig. 3b). At 10 days post silencing, both TRV:*NbFHT* and TRV:00 treated plants were inoculated with CGMMV. By 6 dpi, all plants showed leaf curling and mosaic symptoms, and the upper systemic leaves of TRV:*NbFHT* plants displayed more severe leaf curling and mosaic symptoms than that of the TRV:00 control (Fig. 3a, bottom panels). Western blotting and RT-qPCR results indicated significantly higher levels of CP mRNA and protein in the systemic leaves of TRV:*NbFHT* plants than in TRV:00 plants (Fig. 3c, d). These findings suggest that silencing *NbFHT* enhances the systemic infection of CGMMV in *N. benthamiana*. To further confirm the antiviral function of NbFHT, NbFHT-flag was transiently co-expressed with CGMMV multiplication, with free RFP-flag with CGMMV serving as controls in separate regions of the same leaves. At

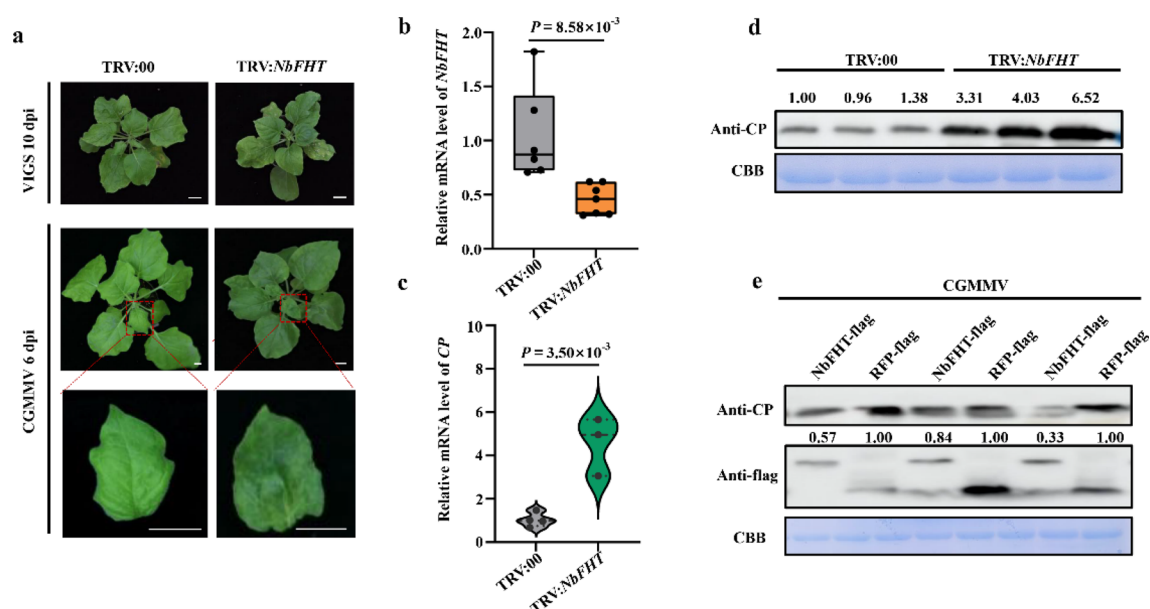


Fig. 3 NbFHT positively regulates plant resistance to CGMMV. **a** Biological phenotypes of TRV:*NbFHT* and TRV:00 inoculated *N. benthamiana* plants before and after CGMMV infection. Scale bar = 1 cm. **b** Silencing efficiency of *NbFHT* detected by RT-qPCR. **c** Accumulation of CP transcripts detected by RT-qPCR. **d** Accumulation of CP in systemic leaves analyzed by Western blotting using an anti-CGMMV CP antibody. **e** Co-inoculation of NbFHT-flag and CGMMV in the same leaf, with RFP-flag and CGMMV as controls. Accumulation of CP and NbFHT-flag at 2 dpi was detected by Western blotting using anti-CGMMV CP and anti-flag antibodies, respectively. Each treatment had three biological replicates. Each lane represents an individual plant. Two-sample unequal variance directional *t*-test was used to test the significance of differences. Relative protein levels were calculated using ImageJ. All experiments were repeated independently three times with similar results

2 dpi, CGMMV accumulation was assessed by Western blotting, which revealed markedly reduced CP levels in plants expressing NbFHT-flag compared with control treatments (Fig. 3e). Together, these results suggest that NbFHT plays crucial roles in host antiviral defense against CGMMV.

NbFHT impedes the self-interaction of CP and promotes its degradation

Self-interaction of CGMMV CP is crucial for viral structure, replication, and transmission (Liu et al. 2020; Yin et al. 2025). To investigate whether NbFHT influences the self-interaction of CP, we co-infiltrated NbFHT with CP-nYFP and cYFP-CP into *N. benthamiana* leaves. The YFP fluorescence intensity was markedly reduced when CP was coexpressed with NbFHT-flag compared with co-expression with RFP-flag (Fig. 4a, b), indicating that NbFHT inhibits CP self-interaction. This inhibitory effect was further confirmed by Co-IP assays (Fig. 4c). We next examined whether NbFHT influences CP protein stability. We co-expressed NbFHT-Myc and CP-GFP, and analyzed protein accumulation at 3 dpi. Western

blotting confirmed that CP accumulation was reduced to approximately 67% of the control level in the presence of NbFHT (Fig. 4d). Additionally, co-expression of NbFHT-Myc and CP-GFP revealed that CP also promotes the degradation of NbFHT, reducing its abundance to approximately 53% of the control level (Fig. 4e). To identify the pathway responsible for CP degradation mediated by NbFHT, we used a 26S proteasome inhibitor MG132, or an autophagy inhibitor E64d to treat the leaves co-expressing CP and NbFHT. Western blotting showed that CP accumulation in the presence of NbFHT was slightly increased by treatment with either MG132 or E64d (Fig. 4f, g). It was previously shown that autophagy could impact the 26S proteasome's degradative route and remove excess or damaged proteasomes when the proteasome was inhibited by chemical or genetic deficiency (Marshall et al. 2015). To assess the combined effect of these pathways, we treated samples with both E64d and MG132 and then examined the CP accumulation in the presence of NbFHT-Myc (Fig. 4f, g). CP protein level was significantly increased compared with the mock treated controls, whereas NbFHT accumulation was reduced

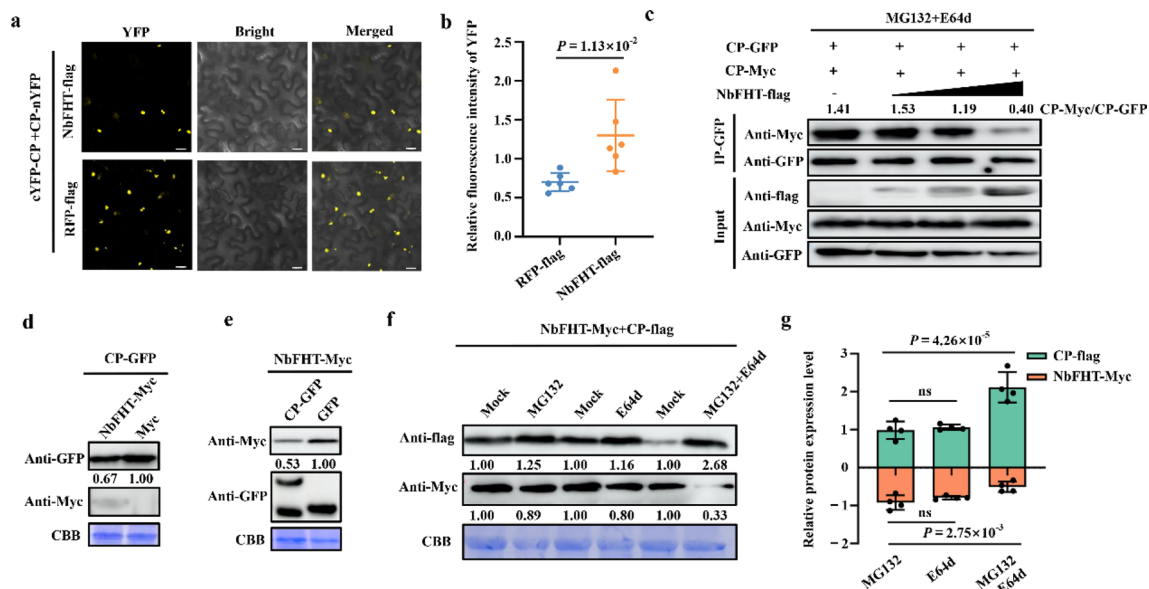


Fig. 4 NbFHT impedes CP self-interaction and promotes degradation of CGMMV CP. **a** NbFHT-flag was co-inoculated with cYFP-CP and CP-nYFP in the same leaf, with RFP-flag co-inoculated with cYFP-CP and CP-nYFP serving as controls. Leaves were observed under confocal microscopy 48 hpi. Scale bar = 50 μm. **b** YFP fluorescence intensity was quantified using ImageJ. **c** Co-IP assay of CP-GFP with CP-Myc in the presence of increasing concentrations of NbFHT-flag. Total protein extracts from leaves were immunoprecipitated using GFP beads, followed by immunoblotting with anti-Myc and anti-GFP antibodies. Input lanes show the expression levels of each protein prior to Co-IP. Quantification of the relative interaction between CP-GFP and CP-Myc. The band intensity of co-precipitated protein CP-Myc was normalized to that of CP-GFP for each sample (CP-Myc/CP-GFP). This ratio was then normalized to the value obtained from the controls without NbFHT-flag (set as 1.00). **d** NbFHT-Myc was co-expressed with CP-GFP in the same leaf, with CP-GFP and Myc as controls. **e** NbFHT-Myc was co-inoculated with CP-GFP in the same leaf, with NbFHT-Myc and free GFP as controls. **f, g** Following co-expression of NbFHT-Myc and CP-flag at 48 hpi, treatments with MG132, E64d, or both inhibitors, with DMSO as the control. Protein accumulation was analyzed by Western blotting at 60 hpi. Each treatment had three biological replicates. Each lane represents an individual plant. Two-sample unequal variance directional *t*-test was used to test the significance of differences. Relative protein levels were quantified using ImageJ software. All experiments were repeated independently three times with similar results

to approximately 33% of control in the treatment of MG132 and E64d. The treatment of MG132 and E64d did not significantly affect CP accumulation when co-expressed with GUS-Myc (Additional file 1: Figure S1c). Together, these results indicate that NbFHT inhibits the CP self-interaction and promotes the degradation of both CGMMV CP and NbFHT.

ABA treatment induces NbFHT accumulation and inhibits CGMMV infection

Prior research has reported that the phytohormone ABA facilitates the accumulation of FHT under wounding stress (Boher et al. 2013; Wei et al. 2020). To determine whether ABA induced the expression of NbFHT, we expressed NbFHT-flag with ABA, with ddH₂O treatment serving as a control. Western blotting results revealed that NbFHT protein level increased by 2.16-fold compared with that of the control (Fig. 5a), whereas NbFHT transcript levels remained unchanged (Additional file 1: Figure S1d). We also found that ABA treatment decreased the accumulation of CP protein (Fig. 5b). Subsequently, we inoculated CGMMV onto *NbFHT*-silenced *N. benthamiana* plants, followed by ABA treatment. The results showed that ABA treatment alleviated

the viral infection symptoms of *NbFHT*-silenced plants and reduced the viral accumulation level in the plants (Fig. 5c–f). To further study the role of the ABA in anti-viral defense, we treated the plants with 100 μ M ABA or ddH₂O for 3 days prior to CGMMV inoculation. At 9 dpi, curling and chlorosis were observed in systemic leaves. CGMMV symptoms were milder in plants treated with ABA (Fig. 5g). RT-qPCR and Western blot analyses revealed lower CP transcript and protein levels in ABA-treated plants compared with controls (Fig. 5h, i). Thus, these results indicate that exogenous ABA enhanced *N. benthamiana* resistance to CGMMV. While NbFHT accumulation is induced by ABA and contributes to viral inhibition, the observation that ABA still attenuated infection in *NbFHT*-silenced plants suggests that its anti-viral activity is not solely dependent on NbFHT and may involve additional pathways.

Discussion

Suberin is a complex biopolymer found in land plants that serves as a natural barrier, regulating water and nutrient uptake while also providing defense against invading pathogens (Nichol et al. 2025). Suberization is a process that is both developmentally regulated and

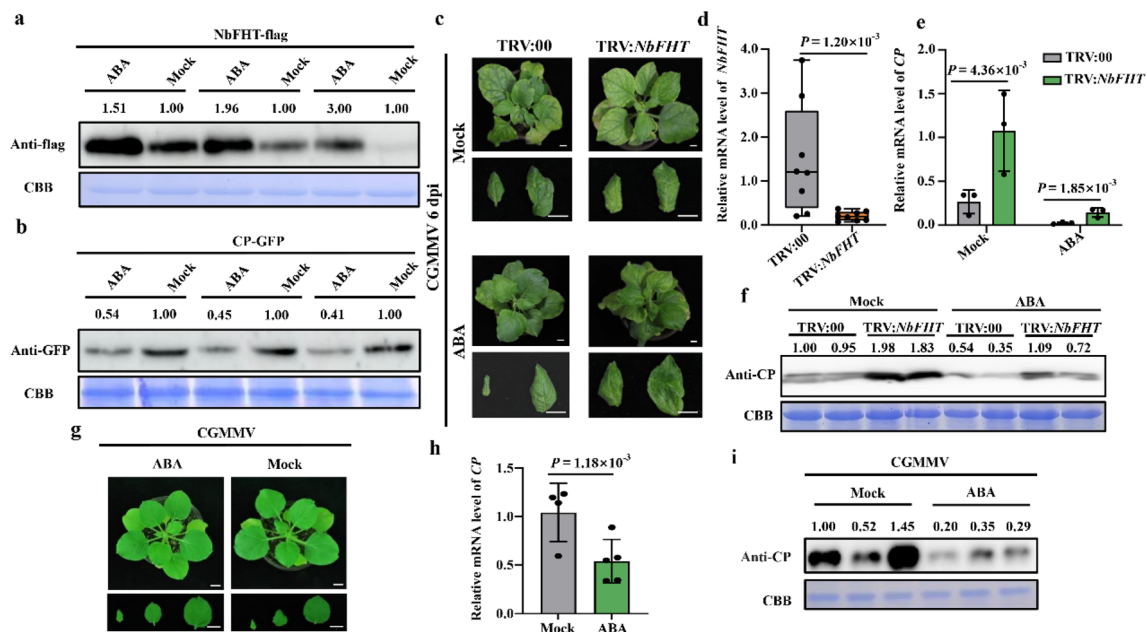


Fig. 5 ABA treatment induces NbFHT accumulation and inhibits CGMMV infection. **a, b** After expressing NbFHT-flag or CP-GFP for 48 hpi, leaves were treated with ABA, with ddH₂O serving as the control. Protein accumulation was analyzed by Western blotting at 60 hpi. **c** Biological phenotypes of TRV:NbFHT and TRV:00 plants sprayed with exogenous ABA after CGMMV infection. Scale bar = 1 cm. **d** Silencing efficiency of *NbFHT* detected by RT-qPCR. **e** CP transcript levels in *NbFHT*-silenced plants following ABA treatment, measured by RT-qPCR. **f** CGMMV CP protein levels in *NbFHT*-silenced plants following ABA treatment, measured by CGMMV CP antibody. **g** Symptoms in *N. benthamiana* inoculated with CGMMV at 9dpi and treated with exogenous ABA applied 3 days before CGMMV inoculation. **h** RT-qPCR analysis of CGMMV CP transcript levels after ABA treatment in CGMMV-infected plants. **i** Western blotting of CGMMV CP protein levels after ABA treatment in CGMMV infected plants using CGMMV CP antibody. Two-sample unequal variance directional t-test was used to test the significance of differences. Relative protein levels were calculated using ImageJ. All experiments were repeated independently three times with similar results

inducible, such as wound-induced suberization (Fabri et al. 2000; Mertz and Brutnell 2014). The phytohormone ABA level increases rapidly following wounding and leads to suberization in potato tubers and tomato, suggesting a general requirement of ABA to stimulate suberization (Leide et al. 2012; Tao et al. 2016). Despite studies have been conducted to suberin deposition to confer resistance to necrotrophic pathogens and nematodes (Holbein et al. 2019; Kashyap et al. 2021, 2022), the precise regulatory mechanisms remain largely unknown. Moreover, as pathogens regulate ABA biosynthesis and signal transduction, the relationship between suberin biosynthesis and ABA under biotic stress is poorly understood. Here, we demonstrate that NbFHT, a key enzyme in the synthesis of suberin, recognizes and interacts with CGMMV CP during viral infection. NbFHT inhibits CP self-interaction and facilitates its degradation, thereby conferring antiviral defense. Exogenous ABA impeded CGMMV accumulation while significantly promoting NbFHT accumulation. Our results make a unique contribution by shedding light on the previously unexplored relationship between suberin and ABA during viral infection, enriching our understanding of the sophisticated regulatory mechanisms for the FHT-mediated antiviral immunity.

Typical suberized tissues include root endodermis and epidermis, bark, tuber skin, and seed coats. Our results demonstrate that NbFHT is induced in leaves upon viral infection. This finding contrasts with the expression profile of FHT in potato, which localizes to periderm and root tissues but is absent in stems, leaves, and tuber parenchyma (Boher et al. 2013). We speculate that viral invasion triggers heterologous expression of FHT in leaves, consistent with evidence of wound-induced suberin formation in mature *Arabidopsis* leaves (Lee et al. 2025). Notably, during CGMMV infection, NbFHT directly targets the CP, inhibits CP self-interaction, and facilitates its degradation, a function independent of its feruloyl transferase activity involved in suberin biosynthesis (Molina et al. 2009). This antiviral defense function of NbFHT may lead to the expression of NbFHT in leaves. Collectively, NbFHT may act as a dual-function enzyme, contributing to both suberin biosynthesis and antiviral defense under viral stress.

We further found that NbFHT and CP mutually promote each other's degradation. Interestingly, treatment with MG132 and E64d inhibits CP degradation, while promotes NbFHT degradation. Previous studies have shown that autophagy can impinge on the 26S proteasome's degradative pathway and eliminate excess or damaged proteasomes when the proteasome is inhibited by chemical or genetic deficiency, which indicates that UPS and autophagy could complement one another to manage

the recycling of nutrients and mitigate proteotoxic stress (Marshall et al. 2015; Yang et al. 2020). However, the degradative pathways by which NbFHT represses CP require further investigation. We speculate that enhanced CP expression promotes NbFHT degradation, overriding the effects of proteasome and autophagy inhibitor. Additionally, CP-mediated degradation of FHT probably impedes the suberin synthesis, thereby promoting viral infection.

Increasing evidence suggests that the phytohormone ABA participates in plant responses to viral infection, such as rice stripe virus (RSV), rice black streaked dwarf virus (RBSDV), etc. (Xie et al. 2018; Cui et al. 2021). Our results show that exogenous ABA treatment represses CP expression and enhances the resistance of *N. benthamiana* to CGMMV. ABA treatment also stabilizes NbFHT, which is consistent with previous reports demonstrating that ABA modulates the accumulation of the suberin feruloyl transferase FHT under wounding stress (Boher et al. 2013; Wei et al. 2020). Based on our findings, we propose that ABA enhances antiviral defense, at least in part, through the induction of NbFHT. We speculate that ABA likely induces NbFHT accumulation, which in turn degrades CPs and impedes the self-interaction of the CPs. Notably, our work is the first to uncover the function of the ABA-regulated NbFHT in antiviral defense. In contrast to prior studies focused on fungal and bacterial pathogens (Serra et al. 2010), we demonstrate that NbFHT confers antiviral activity in the host by targeting CP in an ABA-dependent manner, providing novel evidence for the involvement of suberin biosynthetic proteins in antiviral immunity.

Methods

Plant growth and virus inoculation

The *N. benthamiana* plants were grown in a growth chamber at 25°C under a 16-h light/8-h dark photoperiod and 60% relative humidity. CGMMV was obtained from a previously published source and maintained in our laboratory (Shi et al. 2023). For agroinfiltration, *Agrobacterium tumefaciens* GV3101 carrying individual infectious viral clones was suspended in infiltration buffer (10 mM MgCl₂, 10 mM MES, and 200 µM acetosyringone, pH 5.6) to OD₆₀₀=1. The suspension was incubated at room temperature for 4 h and infiltrated into leaves of *N. benthamiana* leaves using 1-mL needleless syringes.

Construction of the homologous phylogenetic tree of NbFHT

Amino acid sequences of FHTs from various plant species were retrieved from the database at the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/>). Multiple sequence alignments were performed using the ClustalW program in BioEdit with

default parameters. Phylogenetic analysis was conducted using the neighbor-joining (NJ) method. A phylogenetic tree was constructed using MEGA 7.0.

Plasmid construction

The full-length coding sequence (CDS) of *NbFHT* was amplified from *N. benthamiana*. For BiFC analysis, the full-length CDS of *NbFHT* and *CP* were cloned into both cYFP and nYFP vectors. For Co-IP assays, the full-length CDS of *NbFHT* was cloned into a Myc-tagged vector, and the full-length CDS of *CP* was cloned into a GFP vector. For subcellular localization and transient expression, *NbFHT* was cloned into RFP, flag and Myc vectors, while *CP* was cloned into Myc and flag vectors. For VIGS assays, a 300-bp fragment of *NbFHT* was cloned into the tobacco rattle virus (TRV) vector to generate TRV:*NbFHT*. All vectors were verified by DNA sequencing.

IP-MS assay

CP-flag and the empty vector RFP-flag were expressed in *N. benthamiana* leaves. After 48 hpi, leaves were harvested for total protein extraction and preliminarily analyzed by SDS-PAGE. Subsequently, flag magnetic beads were used for protein enrichment, and enriched proteins were denatured and submitted to Shanghai Bioprofile Biotechnology Co., Ltd. for mass spectrometry analysis.

RNA extraction and RT-qPCR

Total RNA was isolated from leaves using TRIzol reagent (TaKaRa) according to the manufacturer's instructions and treated with RNase-free DNase I. First-strand cDNA was synthesized using 1 µg of total RNA per 20 µL reaction using random primers and M-MLV reverse transcriptase. The tenfold diluted cDNA products and SYBR Green Master Mix (Vazyme) were used for qPCR on an Eppendorf Real-Time PCR system. The *NbUBC* gene (GenBank accession number AB026056.1) was used as an internal control. The relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001).

Western blotting analysis

Nicotiana benthamiana leaves were collected and extracted from 0.1 g in 400 µL lysis buffer (100 mM Tris-HCl pH 8.8, 60% SDS, 2% β-mercaptoethanol). The total proteins were separated by 12% SDS-PAGE and transferred to a nitrocellulose membrane. Subsequently, detection was performed with primary monoclonal antibodies, including anti-GFP, anti-flag, anti-Myc (Abmart) at a dilution of 1:5000, and anti-CGMMV CP (prepared in our laboratory), followed by HRP-conjugated secondary antibodies (Sigma-Aldrich). Hybridization signals

were detected using an enhanced chemiluminescence system (eECL Western Blot Kit; YEASEN, Shanghai, China). The expression levels were quantified using ImageJ.

Confocal laser scanning microscopy

For BiFC and colocalization assays, plasmids were transformed into *A. tumefaciens* strain GV3101 and co-infiltrated into 4-week-old *N. benthamiana* leaves at an OD₆₀₀ of 0.5. Samples were observed using a Zeiss LSM 710 laser scanning microscope at 48 hpi. YFP was excited at 514 nm and emission was captured at 565–585 nm. Three independent experiments were performed.

VIGS

The TRV vectors pYL196 (TRV1) and pTRV (TRV2) were kindly provided by Dr. Yule Liu from Tsinghua University (Beijing, China) (Liu et al. 2002). A 300-bp fragment of *NbFHT* was inserted into the pTRV2 vector to mediate its silencing, using empty pTRV2 vector as the control (TRV:00). The primers used for these constructs are listed in Additional file 2: Table S2. Silenced plants and control plants at 10 dpi were used for further analysis.

Co-IP assay

Protein extracts from *N. benthamiana* leaves were prepared at 48 hpi. For the Co-IP assay, 15 µL of GFP-Trap agarose beads (Proteintech, Nanjing, China) were incubated with 2 mL of crude protein at 4°C for 10 h. Beads were washed five times, and Myc and GFP antibodies were diluted at 1:10,000. Western blotting was performed and visualized using a chemiluminescence detection system (YEASEN, Shanghai, China).

Quantification and statistical analysis

Data are presented as mean ± standard deviation (SD) using GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA). The statistical significance of RT-qPCR results was evaluated using Student's *t*-test, the *P*-value < 0.05 was considered to indicate statistical significance. ImageJ software was used to quantify the intensity of bands in Western blotting. All experiments were repeated independently three times with similar results.

Abbreviations

ABA	Abscissic acid
BiFC	Bimolecular fluorescence complementation
CGMMV	Cucumber green mottle mosaic virus
CP	Coat protein
dpi	Days post inoculation
FHT	ω-Hydroxyacid/fatty alcohol hydroxycinnamoyl transferase
GFP	Green fluorescent protein
hpi	Hours post inoculation
IP-MS	Immunoprecipitation-Mass Spectrometry
MP	Movement protein
RBSDV	Rice black streaked dwarf virus

RFP	Red fluorescent protein
RSV	Rice stripe virus
RT-qPCR	Quantitative real-time polymerase chain reaction
VIGS	Virus-induced gene silencing
YFP	Yellow fluorescent protein

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42483-026-00436-7>.

Additional file 1: Figure S1. Supplementary information on the interaction and function of NbFHT with CP.

Additional file 2: Table S1. IP-MS analysis of *N. benthamiana* proteins interacting with CGMMV CP. **Table S2.** Primers used in the study.

Acknowledgements

Not applicable.

Author contributions

XY and YS planned and designed the research. NA, TF, and LX performed experiments, conducted fieldwork, analyzed data etc. HF, LS, LY, QA, HS, and XH analyzed data. XY and NA wrote the manuscript. XY, YS, and HL reviewed the manuscript.

Funding

This work was funded by the Zhongyuan Science and Technology Innovation Youth Talents Program (30603407) and the Program for Innovative Research Team (in Science and Technology) in University of Henan Province (25IRTSTHN031).

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 20 August 2025 Accepted: 30 March 2026

Published online: 18 June 2026

References

- Beisson F, Li-Beisson Y, Pollard M. Solving the puzzles of cutin and suberin polymer biosynthesis. *Curr Opin Plant Biol.* 2012;15:329–37. <https://doi.org/10.1016/j.pbi.2012.03.003>.
- Binenbaum J, Wulff N, Camut L, Kiradjiev K, Anfang M, Tal I, et al. Gibberellin and abscisic acid transporters facilitate endodermal suberin formation in *Arabidopsis*. *Nat Plants.* 2023;9:785–802. <https://doi.org/10.1038/s41477-023-01391-3>.
- Boher P, Serra O, Soler M, Molinas M, Figueras M. The potato suberin feruloyl transferase FHT which accumulates in the phellogen is induced by wounding and regulated by abscisic and salicylic acids. *J Exp Bot.* 2013;64:3225–36. <https://doi.org/10.1093/jxb/ert163>.
- Brookbank BP, Patel J, Gazzarrini S, Nambara E. Role of basal ABA in plant growth and development. *Genes.* 2021. <https://doi.org/10.3390/genes12121936>.
- Cui W, Wang S, Han K, Zheng E, Ji M, Chen B, et al. Ferredoxin 1 is downregulated by the accumulation of abscisic acid in an ABI5-dependent manner to facilitate rice stripe virus infection in *Nicotiana benthamiana* and rice. *Plant J.* 2021;107:1183–97. <https://doi.org/10.1111/tpj.15377>.
- Fabbri AA, Fanelli C, Reverberi M, Ricelli A, Camera E, Urbanelli S, et al. Early physiological and cytological events induced by wounding in potato tuber. *J Exp Bot.* 2000;51:1267–75. <https://doi.org/10.1093/jexbot/51.348.1267>.
- Finkelstein R. Abscisic acid synthesis and response. *Arabidopsis Book.* 2013;11:e0166. <https://doi.org/10.1199/tab.0166>.
- Gou JY, Yu XH, Liu CJ. A hydroxycinnamoyltransferase responsible for synthesizing suberin aromatics in *Arabidopsis*. *Proc Natl Acad Sci U S A.* 2009;106:18855–60. <https://doi.org/10.1073/pnas.0905555106>.
- Graça J, Cabral V, Santos S, Lamosa P, Serra O, Molinas M, et al. Partial depolymerization of genetically modified potato tuber periderm reveals intermolecular linkages in suberin polyester. *Phytochemistry.* 2015;117:209–19. <https://doi.org/10.1016/j.phytochem.2015.06.010>.
- Han X, Wei X, Lu W, Wu Q, Mao L, Luo Z. Transcriptional regulation of KCS gene by bZIP29 and MYB70 transcription factors during ABA-stimulated wound suberization of kiwifruit (*Actinidia deliciosa*). *BMC Plant Biol.* 2022;22:23. <https://doi.org/10.1186/s12870-021-03407-6>.
- Holbein J, Franke RB, Marhavý P, Fujita S, Górecka M, Sobczak M, et al. Root endodermal barrier system contributes to defence against plant-parasitic cyst and root-knot nematodes. *Plant J.* 2019;100:221–36. <https://doi.org/10.1111/tpj.14459>.
- Kashyap A, Planas-Marquès M, Capellades M, Valls M, Coll NS. Blocking intruders: inducible physico-chemical barriers against plant vascular wilt pathogens. *J Exp Bot.* 2021;72:184–98. <https://doi.org/10.1093/jxb/eraa444>.
- Kashyap A, Jiménez-Jiménez AL, Zhang W, Capellades M, Srinivasan S, Laro-mainé A, et al. Induced ligno-suberin vascular coating and tyramine-derived hydroxycinnamic acid amides restrict *Ralstonia solanacearum* colonization in resistant tomato. *New Phytol.* 2022;234:1411–29. <https://doi.org/10.1111/nph.17982>.
- Lee JM, Jeon WT, Han M, Choi MS, Kwon M, Kim K, et al. Wounding induces multilayered barrier formation in mature leaves via phytohormone signalling and ATML1-mediated epidermal specification. *Nat Plants.* 2025;11:1298–315. <https://doi.org/10.1038/s41477-025-02028-3>.
- Leide J, Hildebrandt U, Hartung W, Riederer M, Vogt G. Abscisic acid mediates the formation of a suberized stem scar tissue in tomato fruits. *New Phytol.* 2012;194:402–15. <https://doi.org/10.1111/j.1469-8137.2011.04047.x>.
- Liu Y, Schiff M, Dinesh-Kumar SP. Virus-induced gene silencing in tomato. *Plant J.* 2002;31:777–86. <https://doi.org/10.1046/j.1365-313X.2002.01394.x>.
- Liu M, Liu LL, Wu HJ, Kang BS, Gu QS. Mapping subgenomic promoter of coat protein gene of Cucumber green mottle mosaic virus. *J Integr Agric.* 2020;19:153–63. [https://doi.org/10.1016/S2095-3119\(19\)62647-X](https://doi.org/10.1016/S2095-3119(19)62647-X).
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) method. *Methods.* 2001;25:402–8. <https://doi.org/10.1006/meth.2001.1262>.
- Lu HP, Gao Q, Han JP, Guo XH, Wang Q, Altosaar I, et al. An ABA-serotonin module regulates root suberization and salinity tolerance. *New Phytol.* 2022;236:958–73. <https://doi.org/10.1111/nph.18397>.
- Marshall RS, Li F, Gemperline DC, Book AJ, Vierstra RD. Autophagic degradation of the 26S proteasome is mediated by the dual ATG8/ubiquitin receptor RPN10 in *Arabidopsis*. *Mol Cell.* 2015;58:1053–66. <https://doi.org/10.1016/j.molcel.2015.04.023>.
- Mertz RA, Brutnell TP. Bundle sheath suberization in grass leaves: multiple barriers to characterization. *J Exp Bot.* 2014;65:3371–80. <https://doi.org/10.1093/jxb/eru108>.
- Molina I, Li-Beisson Y, Beisson F, Ohlrogge JB, Pollard M. Identification of an *Arabidopsis* feruloyl-coenzyme A transferase required for suberin synthesis. *Plant Physiol.* 2009;151:1317–28. <https://doi.org/10.1104/pp.109.144907>.
- Nichol JB, Yeung LS, Bernards MA, Samuel MA. Establishing a suberin tool kit for determining suberization within classical and “orphan” tissues. *Trends Plant Sci.* 2025. <https://doi.org/10.1016/j.tplants.2025.03.009>.
- Ranathunge K, Schreiber L, Franke R. Suberin research in the genomics era—new interest for an old polymer. *Plant Sci.* 2011;180:399–413. <https://doi.org/10.1016/j.plantsci.2010.11.003>.
- Salas-González I, Rey G, Flis P, Custódio V, Gopaulchan D, Bakhoum N, et al. Coordination between microbiota and root endodermis supports plant

- mineral nutrient homeostasis. *Science*. 2021. <https://doi.org/10.1126/science.abd0695>.
- Serra O, Hohn C, Franke R, Prat S, Molinas M, Figueras M. A feruloyl transferase involved in the biosynthesis of suberin and suberin-associated wax is required for maturation and sealing properties of potato periderm. *Plant J*. 2010;62:277–90. <https://doi.org/10.1111/j.1365-313X.2010.04144.x>.
- Shi YJ, Yang X, Yang LL, Li QL, Liu XM, Han XY, et al. Interaction between cucumber green mottle mosaic virus MP and CP promotes virus systemic infection. *Mol Plant Pathol*. 2023;24:208–20. <https://doi.org/10.1111/mpp.13287>.
- Tao X, Mao L, Li J, Chen J, Lu W, Huang S. Absciscic acid mediates wound-healing in harvested tomato fruit. *Postharvest Biol Technol*. 2016;118:128–33. <https://doi.org/10.1016/j.postharvbio.2016.04.002>.
- Ugaki M, Tomiyama M, Kakutani T, Hidaka S, Kiguchi T, Nagata R, et al. The complete nucleotide sequence of cucumber green mottle mosaic virus (SH strain) genomic RNA. *J Gen Virol*. 1991;72(7):1487–95. <https://doi.org/10.1099/0022-1317-72-7-1487>.
- Vishwanath SJ, Delude C, Domergue F, Rowland O. Suberin: biosynthesis, regulation, and polymer assembly of a protective extracellular barrier. *Plant Cell Rep*. 2015;34:573–86. <https://doi.org/10.1007/s00299-014-1727-z>.
- Wei X, Lu W, Mao L, Han X, Wei X, Zhao X, et al. ABF2 and MYB transcription factors regulate feruloyl transferase FHT involved in ABA-mediated wound suberization of kiwifruit. *J Exp Bot*. 2020;71:305–17. <https://doi.org/10.1093/jxb/erz430>.
- Xie K, Li L, Zhang H, Wang R, Tan X, He Y, et al. Absciscic acid negatively modulates plant defence against rice black-streaked dwarf virus infection by suppressing the jasmonate pathway and regulating reactive oxygen species levels in rice. *Plant Cell Environ*. 2018;41:2504–14. <https://doi.org/10.1111/pce.13372>.
- Xu H, Liu P, Wang C, Wu S, Dong C, Lin Q, et al. Transcriptional networks regulating suberin and lignin in endodermis link development and ABA response. *Plant Physiol*. 2022;190:1165–81. <https://doi.org/10.1093/plphys/kiac298>.
- Yang X, Tian Y, Zhao X, Jiang L, Chen Y, Hu S, et al. NbALY916 is involved in potato virus X P25-triggered cell death in *Nicotiana benthamiana*. *Mol Plant Pathol*. 2020;21:1495–501. <https://doi.org/10.1111/mpp.12986>.
- Yin L, Li X, Zhao C, Song R, Song B. CGMMV capsid protein hijacks host proviral factors to generate condensates and remodel photosynthesis: strategic antiviral protocol. *Sci Bull*. 2025;9:S2095–9273(25)00495–5. <https://doi.org/10.1016/j.scib.2025.05.012>.
- Zheng W, Wuyun Q, Li Y, Zhang C, Freddolino L, Zhang Y. Improving deep learning protein monomer and complex structure prediction using DeepMSA2 with huge metagenomics data. *Nat Methods*. 2024;21:279–89. <https://doi.org/10.1038/s41592-023-02130-4>.