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Research Article

MdERF114 enhances the resistance of apple roots to *Fusarium solani* by regulating the transcription of *MdPRX63*

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Abstract

As the main fungal etiologic agent of apple (*Malus domestica*) replant disease (ARD), *Fusarium solani* seriously damages apple roots. Ethylene response factors (ERFs) play an important role in plant resistance to biotic stress. Here, we show that *MdERF114* is expressed during *F. solani* infections and positively regulates the resistance of apple roots to *F. solani*. Yeast one-hybrid, dual-luciferase, electrophoretic mobility shift assays and determinations of lignin content indicated that MdERF114 directly binds the GCC-box of the *MdPEROXIDASE63* (*MdPRX63*) promoter and activates its expression, resulting in lignin deposition in apple roots and increased resistance to *F. solani*. We identified a WRKY family transcription factor, *MdWRKY75*, that binds to the W-box of the *MdERF114* promoter. Overexpression of *MdWRKY75* enhanced resistance of apple roots to *F. solani*. MdMYB8 interacted with MdERF114 to enhance resistance to *F. solani* by promoting the binding of MdERF114 to the *MdPRX63* promoter. In summary, our findings reveal that the MdWRKY75-MdERF114-MdMYB8-MdPRX63 module is required for apple resistance to *F. solani* and the application of this mechanism by *Agrobacterium rhizogenes*-mediated root transformation provides a promising strategy to prevent ARD.

Introduction

Apple (*Malus domestica*) replant disease (ARD) is a soil-borne disease that severely inhibits the growth of young apple trees and reduces yield during apple replanting (Grunewaldt-Stöcker et al. 2019). The etiology of ARD is complex yet the accumulation of harmful soil fungi is hypothesized as its main cause (Mazzola 1998; Manici et al. 2003; van Schoor et al. 2009). Indeed, *Fusarium* species are the causal agents of ARD in the Bohai Gulf, South Africa, and Italy (van Schoor et al. 2009; Kelderer et al. 2012; Wang et al. 2018). A current study showed that *Fusarium solani* destroyed the reactive oxygen species scavenging system, causing both oxidative damage and growth inhibition of apple rootstocks (Xiang et al. 2021b). Moreover, *F. solani* blocked water transport, resulting in water deficit stress and disruption of the apple photosystem (Yan et al. 2018). Despite them posing a threat to both the environment and human health, fumigation and fungicide use are the current main methods of controlling ARD. The most effective strategy for prevention and control of ARD is to breed ARD-resistant rootstocks using molecular biological techniques (Zhu et al. 2014). However, the mechanisms by which apples defend themselves against *F. solani* remain unclear. This thus necessitates investigations into the mechanism of interactions between apples and *F. solani*.

To mitigate the adverse effects of harmful fungi, plants evolved a series of effective mechanisms to defend against fungal infections. Relatedly, transcription factors (TFs) contribute to biotic stress defenses by activating the expression

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of target genes via binding to specific DNA sequences (Reboledo et al. 2022). The ethylene response factors (ERFs) TF is involved in plant defense responses to biotic stress (Feng et al. 2020). ERFs belong to APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) superfamily and thus contain a conserved AP2 domain (Nakano et al. 2006). ERFs specifically bind to GCC-boxes to activate downstream defense genes involved in the plant response to biological stress. For example, in Arabidopsis (*Arabidopsis thaliana*), ERF11 activated *BTB AND TAZ DOMAIN PROTEIN 4* (*BT4*) transcription to regulate immunity to *Pseudomonas syringae* (Zheng et al. 2019). AtERF114 enhanced the resistance of Arabidopsis to PevD1 by increasing lignin content via binding to the *PAL1* promoter (Li et al. 2022). *ZmERF105* positively regulated maize (*Zea mays* L.) resistance to *Exserohilum turcicum* by regulating the expression of several pathogenesis-related (PR) genes (Zang et al. 2020). Relatedly, the apple rootstock G.935, which was resistant to ARD, had numerous highly expressed ERFs (Zhu et al. 2016). Apple roots responding to *F. solani* had many highly expressed ERFs (Xiang et al. 2021a). However, the function of ERFs in defending the apple against ARD is unclear, necessitating the determination of the specific transcriptional regulation mechanisms of ERFs in ARD.

Other families of TFs contribute to plant resistance to biotic stress, such as WRKY and MYB TFs (Jiang et al. 2017; Ma et al. 2022). The *Gossypium hirsutum* WRKY genes *GhWRKY39-1* and *GhWRKY40* mediated the resistance of *Nicotiana benthamiana* to *Ralstonia solanacearum* (Shi et al. 2014). In Arabidopsis, *WRKY8* regulated their susceptibility to *P. syringae* and *Botrytis cinerea*, to confer resistance to TMC-cg by coordinating abscisic acid and ethylene signaling pathways (Chen et al. 2013). *NtWRKY12* regulated the transcription of *PR-1a*, which is a salicylic acid (SA)-inducible defense gene (van Verk et al. 2008). MYB TFs also contributed to plant defenses against biotic stress. Heterologous expression of *MdMYB30* in Arabidopsis enhanced resistance to various bacterial pathogens (Zhang et al. 2019). *AtMYB96* enhanced Arabidopsis resistance to *P. syringae* by regulating SA-signaling pathway defense genes (Seo and Park 2010). Several WRKY and MYB TFs were involved in genetic responses to *F. solani* infections (Xiang et al. 2021a).

Lignin is an important phenolic polymer in plants with resistance to biotic and abiotic stresses (Mottiar et al. 2023). The biosynthesis of lignin occurs in plants via phenylpropanoid pathway. Three monolignol precursors (*p*-coumaryl, coniferyl, and sinapyl alcohols) are formed from phenylalanine through a series of catalytic reactions and then oxidized and polymerized by cross-linking reaction triggered to form lignin (Mottiar et al. 2023). Peroxidases (PRXs) play an important role in the oxidative polymerization of three monolignol precursors to form lignin (Barceló et al. 2004; Marjamaa et al. 2009). In Arabidopsis, the suppression of *AtPRX72* lead to the decrease of lignin content (Fernandez-Perez et al. 2015). Overexpression of *OsPRX38* promotes the deposition of lignin in Arabidopsis which confer the tolerance to arsenic (Kidwai et al. 2019). The seeds from *prx2 prx25* double mutant plants shows changes in lignin content which leads to shortened seed longevity (Renard et al. 2020). However, there are few studies on the function and regulatory relationship of PRXs in interaction between roots and pathogenic fungi.

In this study, the ERF TF MdERF114 positively regulated apple resistance to *F. solani*. MdERF114 bound directly to the *MdPRX63* promoter to increase the deposition of lignin, which resulted in resistance to *F. solani*. MdWRKY75 enhanced apple resistance to *F. solani* by binding to the *MdERF114* promoter. Moreover, MdMYB8 participated in the apple defense against *F. solani* by interacting with MdERF114 to enhance its binding to the *MdPRX63* promoter. Our study reveals a molecular mechanism by which MdWRKY75-MdERF114-MdMYB8-MdPRX63 mediates apple resistance to *F. solani*. These insights will aid the breeding of resistant rootstocks. It provides an important means to obtain ARD-resistance rootstock by *Agrobacterium rhizogenes*-mediated root transformation producing overexpression transgenic plant.

Results

The expression pattern and subcellular localization of MdERF114

The RNA-seq results (Xiang et al. 2021a) showed that *F. solani* up-regulated *MdERF114* (MD06G1130400) (ERF TF). Further analysis showed that *MdERF114* contains an 807 bp open reading frame and encodes a protein with 269 amino acids. RT-qPCR showed that *F. solani* induced *MdERF114* expression, peaking at 72 h (Supplemental Fig. S1A). MeJA also induced *MdERF114* expression (Supplemental Fig. S1B). However, SA treatment did not significantly change *MdERF114* expression (Supplemental Fig. S1C). *MdERF114* was highly expressed in roots and stems (Supplemental Fig. S1D). Analysis of the *MdERF114* promoter sequence revealed that it contains several cis-acting elements related to stress and hormonal responses (Supplemental Fig. S1E). These results indicate that *MdERF114* may play an essential role in root response to stress.

The ORF sequence of *MdERF114* was cloned and inserted into pBIN-GFP vector, and transformed into *Agrobacterium tumefaciens* strain GV3101, then observed using a confocal laser scanning microscope (Leica SP5, Leica Microsystems, Buffalo Grove, IL). Results revealed that *MdERF114* was localized in the nucleus (Supplemental Fig. S2).

MdERF114 positively regulates apple resistance to *F. solani*

The *MdERF114*-overexpressing and *MdERF114*-RNAi roots were obtained via *A. rhizogenes*-mediated transformation (Supplemental Fig. S3), then inoculated with *F. solani* to explore the function of *MdERF114* in apple response to *F. solani*. There was no obvious difference in growth between *MdERF114*-overexpressing lines and WT-pBIN line or *MdERF114*-RNAi lines and WT-pK7 line before *F. solani* inoculation. However, *F. solani* inoculation inhibited the growth of all the plants (Fig. 1A). *Fusarium solani* inoculation

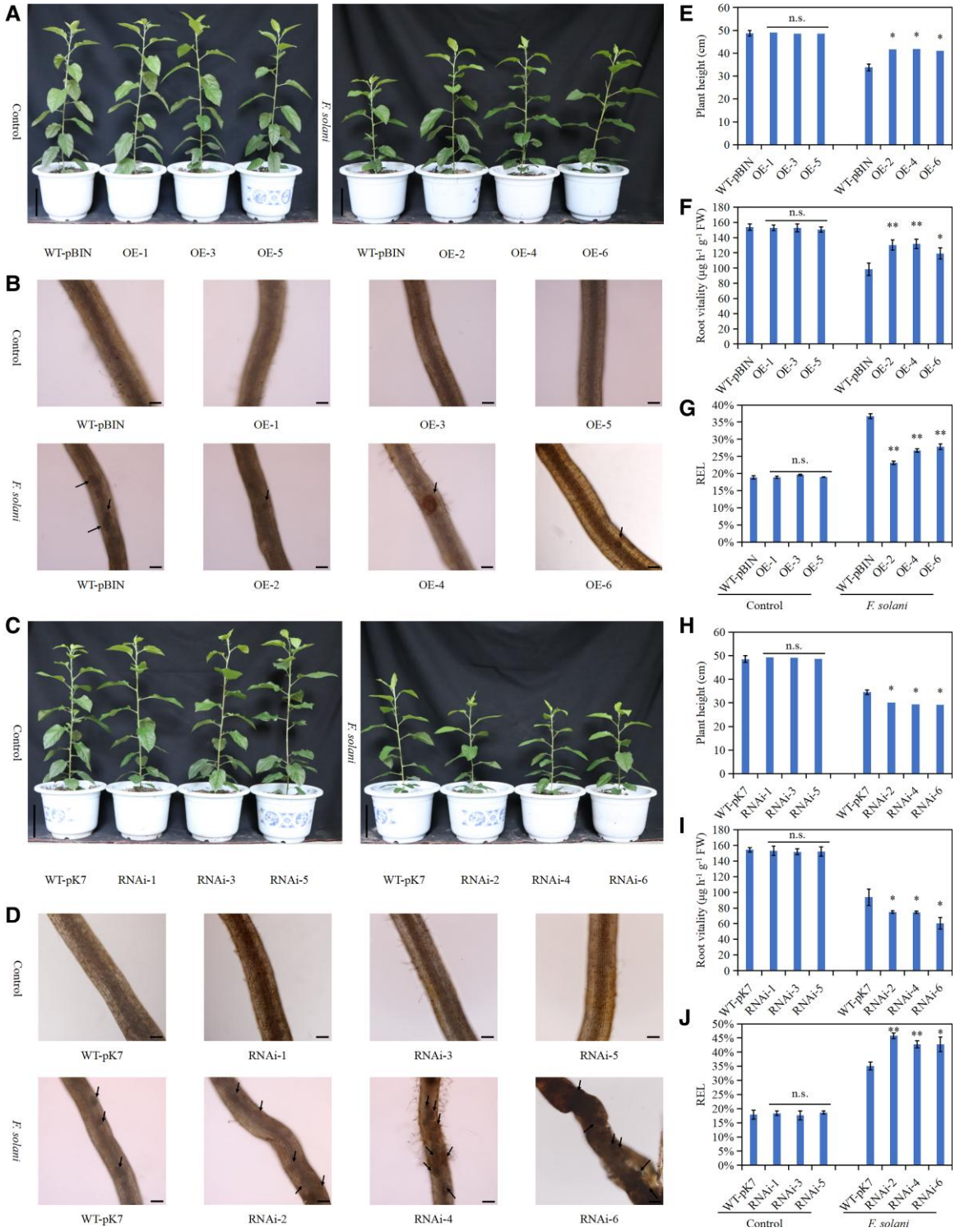

Figure 1 *MdERF114* positively regulates the resistance of apple to *Fusarium solani*. A) Phenotypic analysis of plants overexpressing *MdERF114* in the roots under *F. solani* treatment. Scale bar = 10 cm. B) Observation of *MdERF114*-overexpressing roots under *F. solani* treatment. The black arrow indicates the wound. Scale bar = 100 μm. C) Phenotypic analysis of plants with *MdERF114*-RNAi roots under *F. solani* treatment. Scale bar = 10 cm. D) Observation of *MdERF114*-RNAi roots under *F. solani* treatment. The black arrow indicates the wound. Scale bar = 100 μm. E) Height of plants overexpressing *MdERF114* in the roots under *F. solani* treatment. F) Root vitality of *MdERF114*-overexpressing roots under *F. solani* treatment. G) REL of *MdERF114*-overexpressing roots under *F. solani* treatment. H) Height of plants with *MdERF114*-RNAi roots under *F. solani* treatment. I) Vitality of *MdERF114*-RNAi roots under *F. solani* treatment. J) Root REL of *MdERF114*-RNAi roots under *F. solani* treatment. WT-pBIN, GL3 transformed with an pBIN-GFP empty vector; OE 1–6, different *MdERF114*-overexpressing root lines. WT-pK7, GL3 transformed with an pK7GWIWG2 empty vector; RNAi 1–6, different *MdERF114*-RNAi root lines. Data are shown as means ± standard deviation (SD) with three biological replicates. Different letters indicate significant differences between treatments based on Student's *t*-test (*$P \leq 0.05$; **$P \leq 0.01$).

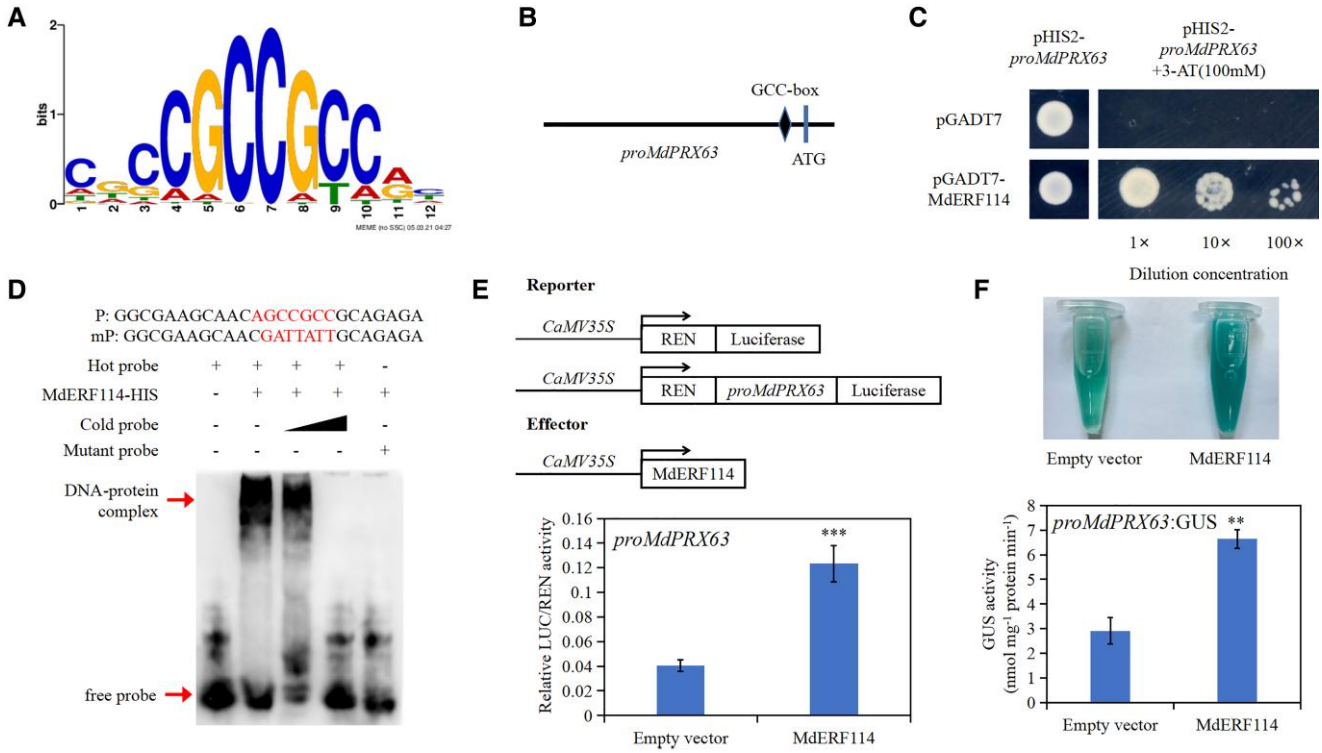

Figure 2 MdERF114 directly binds to the *MdPRX63* promoter. A) Potential binding elements of MdERF114 identified by DNA affinity purification sequencing (DAP- seq). B) *MdPRX63* promoter sequence analysis revealed the presence of GCC-box binding element. C) Yeast one-hybrid (Y1H) assay showed that MdERF114 binds to the *MdPRX63* promoter. The yeast strain co-transformed with pGADT7 and pHIS2-*proMdPRX63* was used as the control. The screening concentration of 3-AT was 100 mM. D) EMSA indicates that MdERF114 binds to the GCC-box in *MdPRX63* promoter. The "+" represents the presence of relevant probes or proteins and "-" represents the absence of relevant probes or proteins. The 5′-AGCCGCC-3′ is the motif bound by MdERF114 in "P" and the 5′-AGCCGCC-3′ motif was replaced by 5′-GATTATT-3′ in "mP". E) Relative LUC/REN activity in *Nicotiana benthamiana* co-expressing *MdERF114* and *proMdPRX63*. F) GUS staining assay showing that MdERF114 can activate *proMdPRX63* expression. Data are shown as means ± standard deviation (SD) with three biological replicates in E and F. Different letters indicate significant differences between treatments based on Student's *t*-test (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$).

significantly inhibited the growth of plants with WT-pBIN roots than those with *MdERF114*-overexpressing roots. The plant heights of OE-2, OE-4, and OE-6 were 23.41%, 24.06%, and 21.43% higher than those of WT-pBIN, respectively (Fig. 1E). Moreover, *F. solani* inoculation significantly inhibited the growth of plants with *MdERF114*-RNAi roots than WT-pK7 plants. The plant heights of RNAi-2, RNAi-4, and RNAi-6 were 87.57%, 84.98%, and 84.56% of those of WT-pK7 plants, respectively (Fig. 1H). A microscope showed that no damage occurred in the roots before *F. solani* treatment. However, the *MdERF114*-overexpressing roots were slightly injured after *F. solani* treatment, with a few brown spots and minor wounds (Fig. 1B). Besides, dense brown lesions were observed on *MdERF114*-RNAi roots, some of which were necrotic (Fig. 1D). The root vitality and relative electrolytic leakage (REL) were also measured to evaluate the root damage. The *MdERF114*-overexpressing roots had higher root vitality and lowered REL after *F. solani* treatment than WT-pBIN (Fig. 1, F and G). However, *MdERF114*-RNAi roots had lower root vitality and higher REL than WT-pK7 (Fig. 1, I and J). These results indicate that *MdERF114* positively regulates apple resistance to *F. solani*.

MdERF114 directly binds to the MdPRX63 promoter

The downstream target genes of MdERF114 were predicted via DNA affinity purification sequencing (DAP-seq) to explore the regulatory network of MdERF114 under *F. solani* treatment. A total of 2,483 potential downstream target genes and the highest scoring cis-acting element, GCC-box (GCCGCC), were identified (Fig. 2A). A previous study found that *AtERF114* is involved in lignin synthesis in Arabidopsis (Li et al. 2022). Herein, DAP-seq found several genes related to lignin synthesis (MD17G1040100, MD04G1101700, MD16G1085600, MD13G1086200, MD09G1226600, MD03G1059000, MD09G1039100, MD16G1062800). However, the identification of the promoter of these genes revealed that only the peroxidase *MdPRX63* (MD17G1040100) had a GCC-box (Fig. 2B), indicating that *MdPRX63* might function as a downstream target gene of MdERF114. Yeast one-hybrid assay (Y1H), electrophoretic mobility shift assay (EMSA), dual-luciferase assay, and β-glucuronidase (GUS) staining assay were then performed to further verify this hypothesis. Y1H assay showed that the Y187 yeast strain co-transformed with pGADT7-*MdERF114* and pHIS2-*proMdPRX63* could grow on the medium supplemented with 100 mM 3-amino-1, 2,

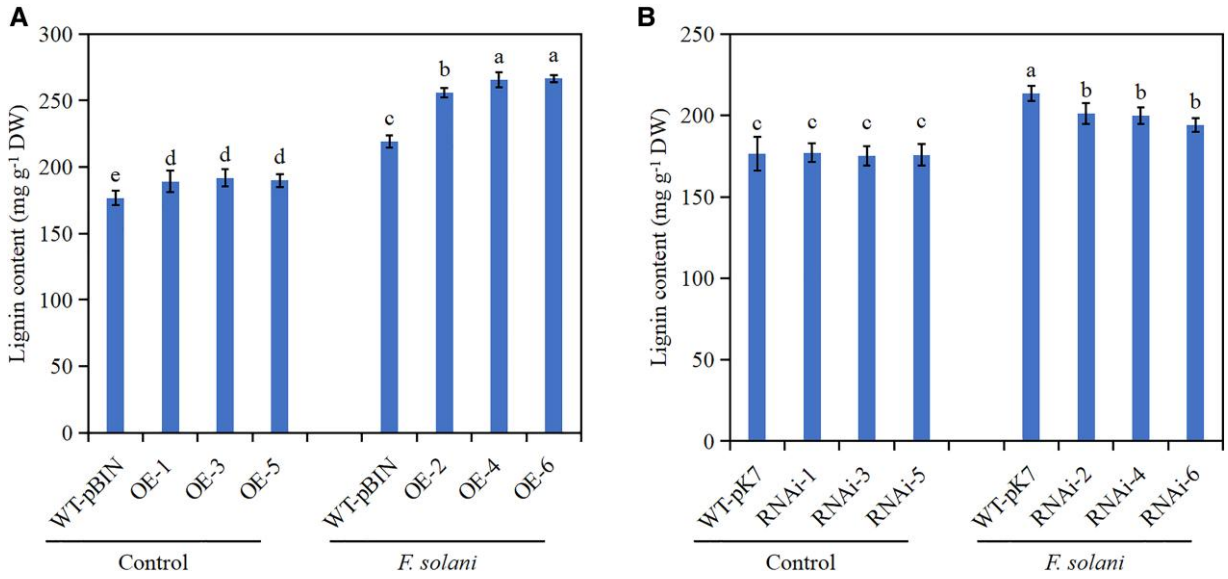

Figure 3 Determination of lignin content in *MdERF114* transgenic roots under *Fusarium solani* treatment. A) Determination of lignin content in *MdERF114*-overexpressing roots. B) Determination of lignin content in *MdERF114*-RNAi roots. WT-pBIN, GL3 transformed with an pBIN-GFP empty vector; OE 1–6, different *MdERF114*-overexpressing root lines. WT-pK7, GL3 transformed with an pK7GWIWG2 empty vector; RNAi 1–6, different *MdERF114*-RNAi root lines. Data are shown as means ± standard deviation (SD) with three biological replicates. Different letters indicate significant differences between treatments based on Tukey's test ($P < 0.05$).

4-triazole (3-AT), while the yeast strain transformed with pHIS2-*proMdPRX63* could not grow on this medium (Fig. 2C). EMSA results also showed that MdERF114 could bind to the promoter of *MdPRX63* while the mutant probe could not be bind by MdERF114 (Fig. 2D). In addition, luciferase assay was conducted by constructing the ORF sequence of *MdERF114* in pGreenII 62-SK vector and the *MdPRX63* promoter in pGreenII 0800-LUC vector. The *N. benthamiana* co-injected with the *MdERF114* and *MdPRX63* promoters had higher firefly luciferase (LUC) activity than control (Fig. 2E). Similarly, GUS staining assay showed that apple callus co-expressing *MdERF114* and *MdPRX63* promoters had higher GUS activity than callus expressing *MdPRX63* promoter (Fig. 2F). Taken together, these results suggest that MdERF114 directly binds to the promoter of *MdPRX63*.

Detection of lignin content in MdERF114 transgenic roots under *F. solani* treatment

Previous studies have shown that PRX genes are associated with lignin synthesis. In this study, we determined the lignin content of *MdERF114* transgenic roots under *F. solani* treatment, and the results showed that the lignin content of *MdERF114*-overexpressing roots (OE-2, OE-4, and OE-6) was significantly higher under *F. solani* treatment than that of WT-pBIN roots by 1.16, 1.21 and 1.22 times, respectively (Fig. 3A). However, the lignin content of *MdERF114*-RNAi roots under *F. solani* treatment was lower than that of WT-pK7 roots (Fig. 3B). These results indicate that *MdERF114* affects root lignin deposition under *F. solani* treatment by regulating *MdPRX63* transcription.

MdPRX63 overexpression enhances the resistance of apple roots to *F. solani*

The *MdPRX63*-overexpressing roots (Supplemental Fig. S4) were inoculated with *F. solani* to explore the function of *MdPRX63* in apple root response to *F. solani*. The results showed that plants with *MdPRX63*-overexpressing roots had better growth status and higher plant height under *F. solani* treatment than WT-pBIN plants (Fig. 4, A and C). Moreover, *MdPRX63*-overexpressing roots had higher root vitality and lower REL after *F. solani* treatment (Fig. 4, D and E). In addition, microscopic observation showed that the brown wound in *MdPRX63*-overexpressing roots was much less than that of WT-pBIN roots after *F. solani* inoculation (Fig. 4B). These results indicate that *MdPRX63* overexpression enhances root resistance to *F. solani*.

MdWRKY75 binds to the MdERF114 promoter

The *MdERF114* promoter was constructed in pHIS2 vector as bait for screening upstream proteins of *MdERF114* using Y1H system to further understand the signaling pathway of *MdERF114* involved in apple root defense against *F. solani*. Results showed that a WRKY family TF, *MdWRKY75* (MD09G1008800), could bind the *MdERF114* promoter. From the analysis of the *MdERF114* promoter, it was found that there is a W-box (TTGACC) element on the *MdERF114* promoter (Fig. 5A). Y1H assay was performed to further verify this result. The yeast strain co-transformed with pGADT7-*MdWRKY75* and pHIS2-*proMdERF114* could grow on the medium supplemented with 50 mM 3-AT, while that co-transformed with pGADT7 and pHIS2-*proMdERF114* could not grow on this medium (Fig. 5B). Meanwhile, the MdWRKY75-GST fusion protein

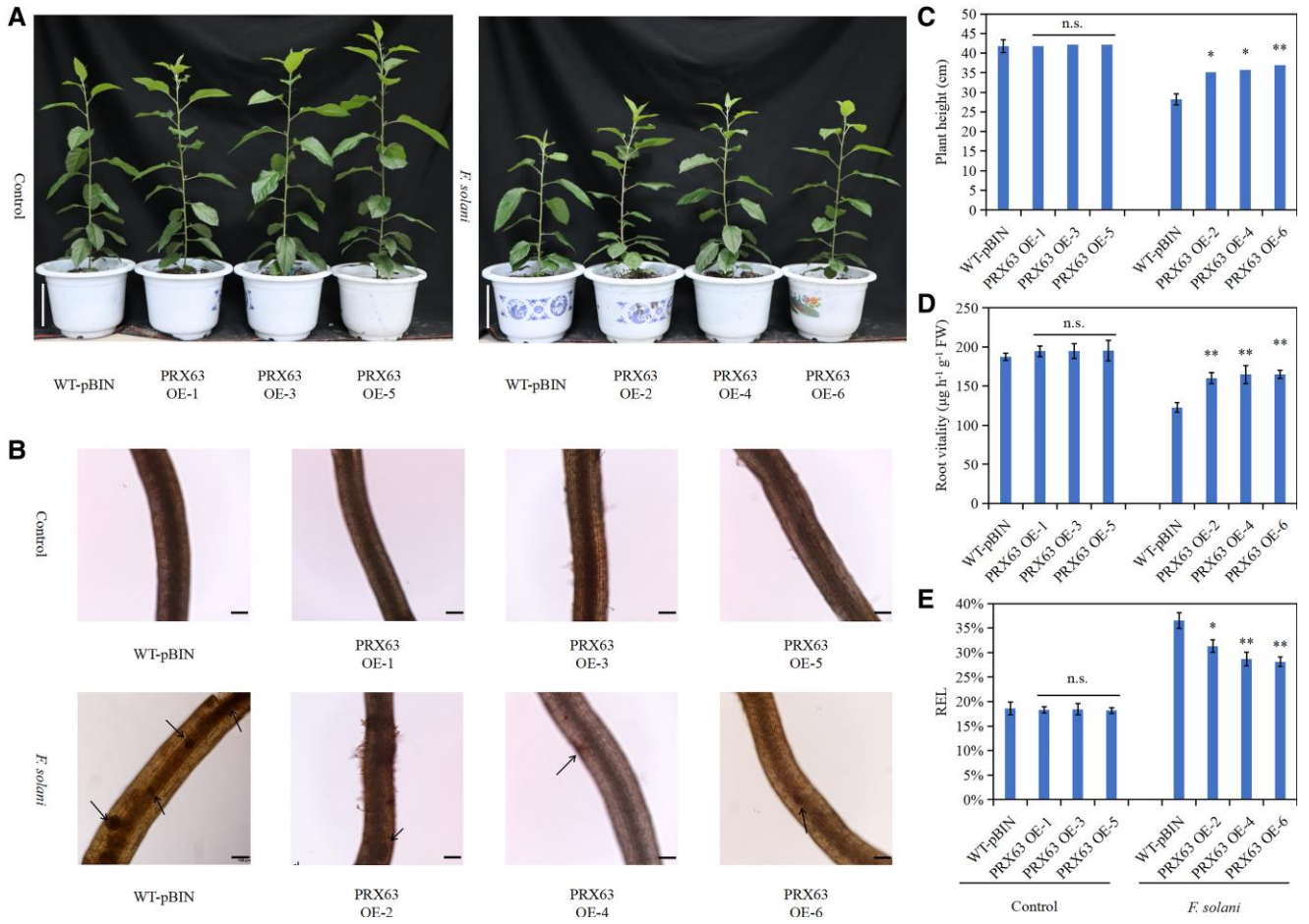

Figure 4 Overexpression of *MdPRX63* enhances apple resistance to *Fusarium solani*. A) Phenotype of plants overexpressing *MdPRX63* in the roots under *F. solani* treatment. Scale bar = 10 cm. B) Observation of *MdPRX63*-overexpressing roots under *F. solani* treatment. The black arrow indicates the wound. Scale bar = 100 μm. C) Height of plants overexpressing *MdPRX63* in the roots under *F. solani* treatment. D) Vitality of *MdPRX63*-overexpressing roots under *F. solani* treatment. E) REL of *MdPRX63*-overexpressing roots under *F. solani* treatment. WT-pBIN, GL3 transformed with an empty vector containing the GFP tag; PRX63 OE 1–6, different *MdPRX63*-overexpressing root lines. Data are shown as the means ± standard deviation (SD) with three biological replicates. Different letters indicate significant differences between treatments based on Student's *t*-test (*$P \leq 0.05$; **$P \leq 0.01$).

was purified, then the probe containing W-box (TTGACC) was synthesized for EMSA. The results showed that MdWRKY75-GST fusion protein could bind to the *MdERF114* promoter, while the mutant probe could not be bound by MdWRKY75-GST (Fig. 5D). Dual-luciferase assay was performed to further verify when MdWRKY75 can directly regulate *MdERF114* in vivo. The results showed that the LUC activity of *N. benthamiana* leaves co-expressing MdWRKY75 and *MdERF114* promoter was significantly increased compared with the negative control (Fig. 5C). These results suggest that MdWRKY75 binds to the *MdERF114* promoter.

MdWRKY75 overexpression enhances the resistance of apple roots to *F. solani*

The expression of *MdWRKY75* in roots under *F. solani* was assessed to investigate whether *MdWRKY75* is involved in the resistance of apple roots to *F. solani*. RT-qPCR results showed that *F. solani* induced *MdWRKY75* expression, peaking at 48 h (Supplemental Fig. S5A). Further results showed that the growth of plants with *MdWRKY75*-overexpressing roots was better than that of WT-pBIN. Although *F. solani* inhibited the growth of all plants, the height of plants with *MdWRKY75*-overexpressing roots was significantly higher than that of WT-pBIN plants (Fig. 6, A and C). Furthermore, WT-pBIN roots had dense wounds and even necrosis, while *MdWRKY75*-overexpressing roots only had fewer brown wounds (Fig. 6B). Meanwhile, *MdWRKY75*-overexpressing roots (WRKY75 OE-2, WRKY75 OE-4, and WRKY75 OE-6) had higher root vitality than WT-pBIN roots by 1.22, 1.25, and 1.33 times, respectively (Fig. 6D). However, *MdWRKY75*-overexpressing roots had a significantly lower REL than WT-pBIN roots (Fig. 6E). These results indicate that *MdWRKY75* overexpression increases the resistance of apple roots to *F. solani*.

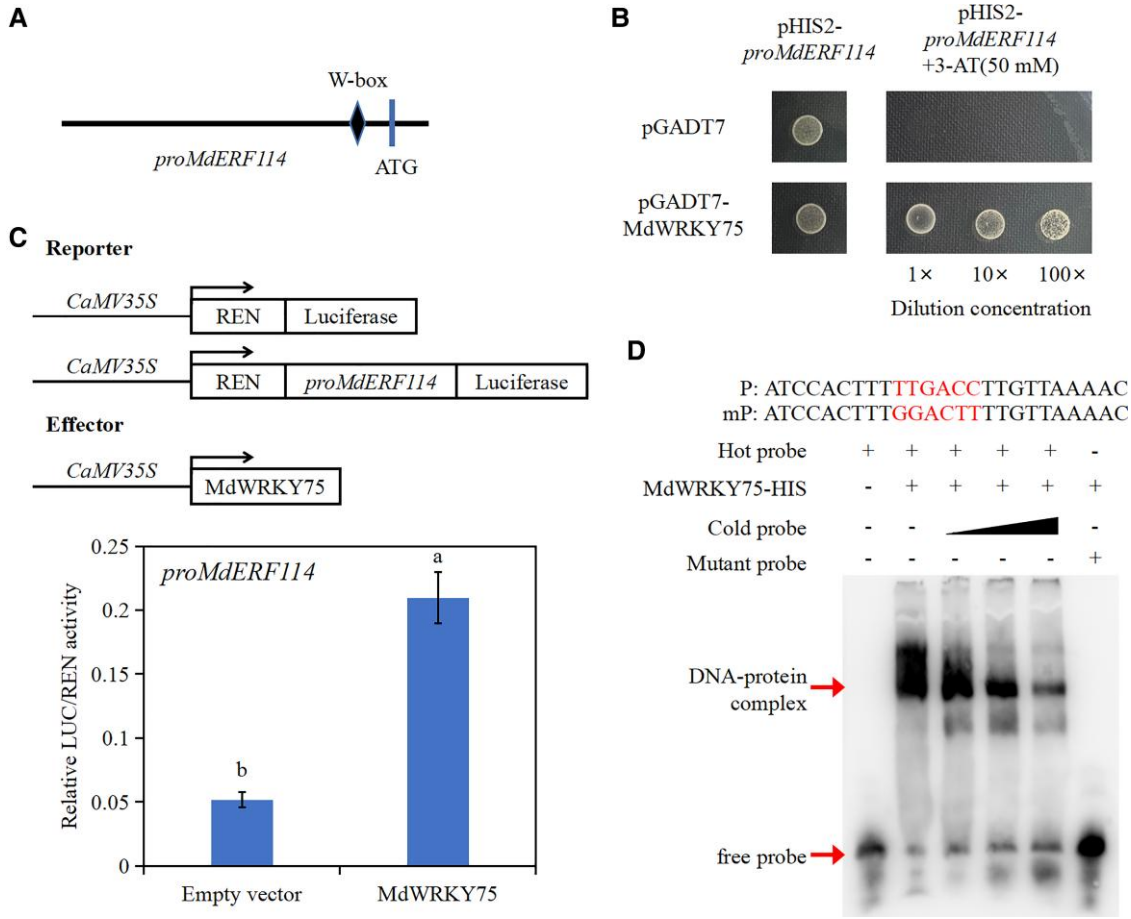

Figure 5 MdWRKY75 directly binds to the *MdERF114* promoter. A) *MdERF114* promoter sequence analysis reveals the presence of W-box binding element. B) Yeast one-hybrid (Y1H) assay shows that MdWRKY75 binds to the *MdERF114* promoter. The yeast strain co-transformed with pGADT7 and pHIS2-*proMdERF114* was used as the control. The screening concentration of 3-AT was 50 mM. C) Relative LUC/REN activity of *Nicotiana benthamiana* co-expressing *MdWRKY75* and *proMdERF114*. Data are shown as means ± standard deviation (SD) with three biological replicates in C. Different letters indicate significant differences between treatments based on Tukey's test ($P < 0.05$). D) EMSA indicates that MdWRKY75 binds to the W-box in promoter of *MdERF114*. The "+" represents the presence of relevant probes or proteins and "-" represents the absence of relevant probes or proteins. The 5′-TTGACC-3′ is the motif bound by MdWRKY75 in "P" and the 5′-TTGACC-3′ motif was replaced by 5′-GGACTT-3′ in "mP".

MdMYB8 interacts with MdERF114

The MdERF114 interacting proteins were screened using a yeast two-hybrid (Y2H) system to further analyze the regulatory network of *MdERF114* involved in apple defense against *F. solani*. The results showed that an MYB family TF *MdMYB8* (MD06G1217200) could interact with MdERF114. The Y2H assay was performed to elucidate whether MdMYB8 interacts with MdERF114. The results showed that the yeast strain co-expressing MdERF114-pGBD and MdMYB8-pGAD could grow normally in SD/-L/-H/-T/-A, while the control could not grow in SD/-L/-H/-T/-A (Fig. 7A). Meanwhile, luciferase complementarity assay was performed to verify the interaction between MdMYB8 and MdERF114. Fluorescence signal was detected in sites co-injected with MdERF114-Nluc and MdMYB8-Cluc (Fig. 7B). Bimolecular fluorescence complementation (BiFC) assay was further used to verify the interaction between MdMYB8 and MdERF114. The MdERF114-YFP^C and MdMYB8-YFP^N vectors were constructed and transferred into *A. tumefaciens*, then injected into *N. benthamiana*. Confocal microscope showed that MdMYB8 interacted with MdERF114 in the nucleus (Fig. 7C). In addition, pull-down assay was conducted using the fusion proteins of GST, MdERF114-HIS, and MdMYB8-GST to verify the interaction between MdMYB8 and MdERF114. The MdERF114-HIS could be captured by MdMYB8-GST, but could not be captured by GST (Fig. 7D). Taken together, these results indicate that MdMYB8 can interact with MdERF114, indicating that MdMYB8 may affect the binding of MdERF114 to the *MdPRX63* promoter.

MdMYB8 promotes the binding of MdERF114 to the MdPRX63 promoter

Luciferase assay was performed to verify whether MdMYB8 can affect the binding of MdERF114 to the *MdPRX63* promoter. The ORF of *MdMYB8* and *MdERF114* were inserted into pGreenII 62-SK vector, while the *MdPRX63* promoter

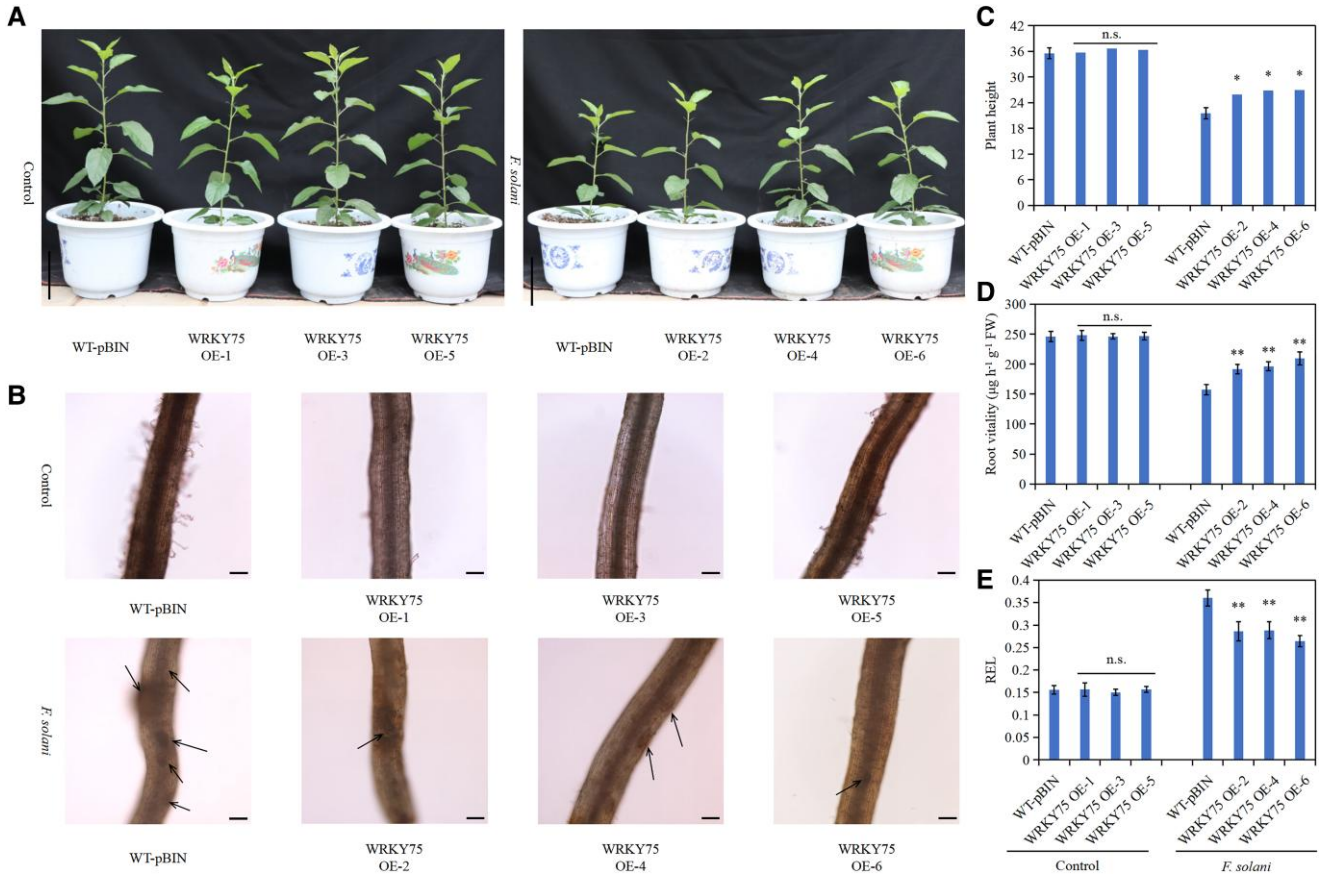

Figure 6 Overexpression of *MdWRKY75* enhances apple resistance to *Fusarium solani*. A) Phenotype of plants overexpressing *MdWRKY75* in the roots under *F. solani* treatment. Scale bar = 10 cm. B) Observation of *MdWRKY75*-overexpressing roots under *F. solani* treatment. The black arrow indicates the wound. Scale bar = 100 μm. C) Height of plants overexpressing *MdWRKY75* in the roots under *F. solani* treatment. D) Vitality of *MdWRKY75*-overexpressing roots under *F. solani* treatment. E) REL of *MdWRKY75*-overexpressing roots under *F. solani* treatment. WT-pBIN, GL3 transformed with an empty vector containing the GFP tag; WRKY75 OE 1–6, different *MdWRKY75*-overexpressing root lines. Data are shown as means ± standard deviation (SD) with three biological replicates. Different letters indicate significant differences between treatments based on Student's *t*-test (*$P \leq 0.05$; **$P \leq 0.01$).

was inserted into pGreenII 0800-LUC vector. The luciferase activity of *N. benthamiana* leaves co-expressing MdMYB8, MdERF114, and *MdPRX63* promoter was significantly higher than that of *N. benthamiana* leaves co-expressing MdERF114 and *MdPRX63* promoter (Fig. 8, A and B). GUS staining assay was performed to further confirm this result. Three vectors (*35S::MdMYB8*, *35S::MdERF114*, and *proMdPRX63*-GUS) were constructed, then transformed into apple callus. The callus co-expressing *MdMYB8*, *MdERF114*, and *proMdPRX63* had higher GUS activity than the callus co-expressing *MdERF114* and *proMdPRX63* (Fig. 8, C and D). These results indicate that the interaction between MdMYB8 and MdERF114 promotes the binding of MdERF114 to the *MdPRX63* promoter.

MdMYB8 overexpression enhances the resistance of apple roots to *F. solani*

The above results indicated that *MdMYB8* may be involved in the mechanism of apple root resistance to *F. solani*. The expression pattern of *MdMYB8* in apple roots was determined under *F. solani* treatment to assess this hypothesis. RT-qPCR results showed that *F. solani* induced *MdMYB8* expression (Supplemental Fig. S6A). The *MdMYB8*-overexpressing roots were then inoculated with *F. solani* to explore the function of *MdMYB8* (Supplemental Fig. S6, B and C). The growth status of plants with *MdMYB8*-overexpressing roots was better than that of WT-pBIN plants under *F. solani* treatment (Fig. 9A). Furthermore, plant height and root vitality of plants with *MdMYB8*-overexpressing roots were significantly higher than those of WT-pBIN, while REL was lower in plants with *MdMYB8*-overexpressing roots than in WT-pBIN plants (Fig. 9, C–E). In addition, *F. solani* caused less damage to *MdMYB8*-overexpressing roots than WT-pBIN roots (Fig. 9B). These results indicate that *MdMYB8* overexpression increases the resistance of apple roots to *F. solani*.

Discussion

MdERF114 enhances the resistance of apples to *F. solani* by regulating lignin biosynthesis

Fusarium solani, the main pathogenic fungus associated with ARD, causes substantial damage to apple roots (Yan et al. 2018;

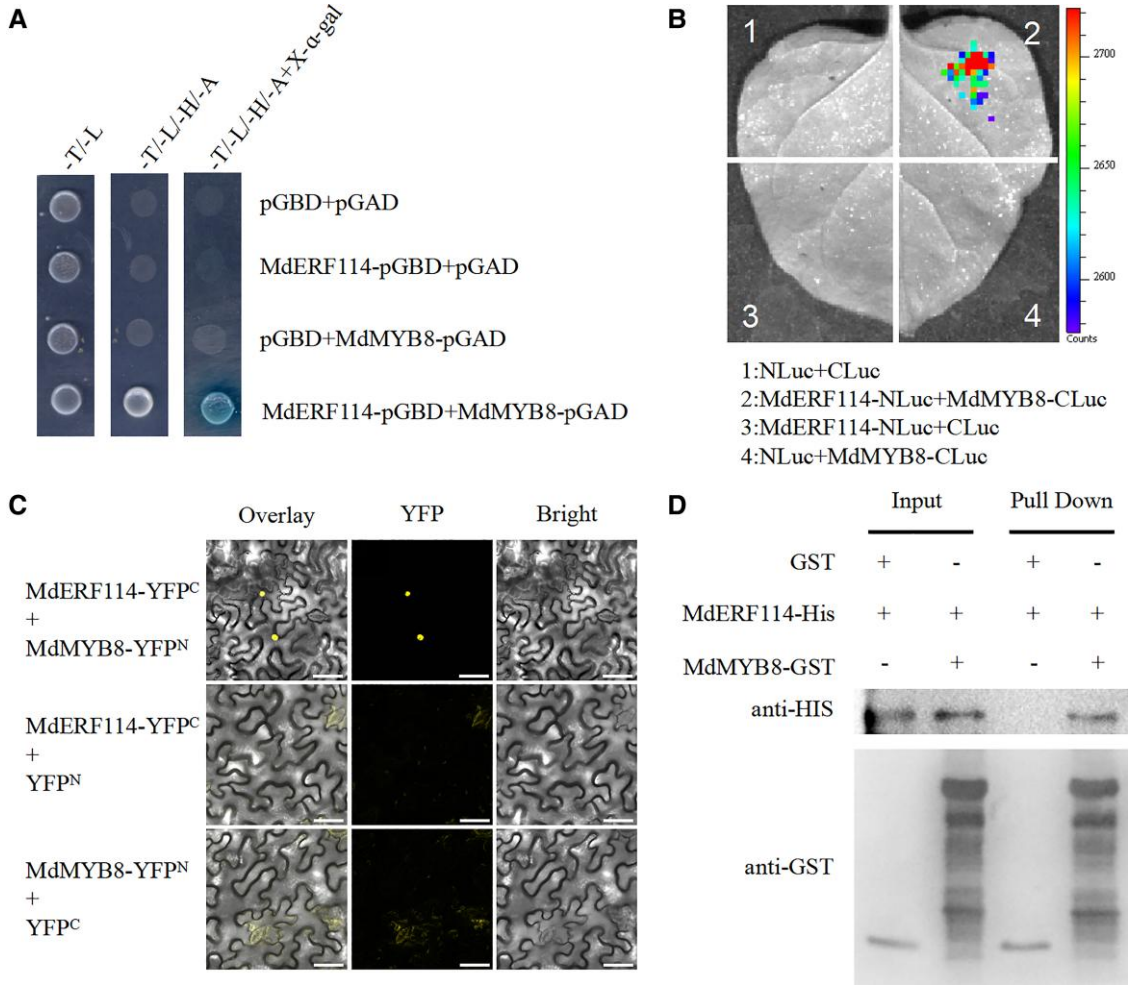

Figure 7 MdMYB8 interacts with MdERF114. A) Yeast two-hybrid (Y2H) assay showing that MdMYB8 interacts with MdERF114 in yeast. B) Luciferase complementarity assay showing that fluorescence signal was only observed in sites co-injected with MdERF114-Nluc and MdMYB8-Cluc. C) BiFC assay showing that MdMYB8 interacts with MdERF114 in the nucleus. Scale bar = 50 μm. D) Pull-down assay showed that MdERF114-HIS could be captured by MdMYB8-GST and not Glutathione S-transferase (GST). The "+" represents the presence of relevant proteins and "-" represents the absence of relevant proteins.

Xiang et al. 2021b). ERF TFs are plant-specific TFs involved in plant growth, development, and response to biotic and abiotic stresses (Licausi et al. 2013; Feng et al. 2020). A previous study reported numerous ERF TFs after conducting RNA-seq of apple rootstock M9T337 in response to *F. solani* (Xiang et al. 2021a). A combination of the above RNA-seq data and expression pattern analysis revealed that *MdERF114* was upregulated by *F. solani*, suggesting that *MdERF114* might play a role in the interaction of apple roots with *F. solani*. Herein, *MdERF114* transgenic roots, obtained by *A. rhizogenes*-mediated transformation, were inoculated with *F. solani* to explore the function of *MdERF114*. Results demonstrated that overexpression of *MdERF114* enhanced the resistance of apple roots to *F. solani*, whereas *MdERF114*-RNAi roots exhibited higher susceptibility. These results suggest that *MdERF114* mediates the defense response of apple roots against to *F. solani*.

Lignin, the main component of plant cell wall, plays a critical role in determining the cell wall's mechanical strength and oxidation resistance (Xie et al. 2018; Cesarino 2019; Vanholme et al. 2019). When pathogenic microorganisms infect plants, lignin is rapidly deposited in the cell wall, thereby limiting the diffusion of mycelia and toxins into the plant cell and preventing pathogens from absorbing nutrients from the plant cell. This process provides a physical barrier for plants to resist the invasion of pathogenic microorganisms (Ma et al. 2018; Dong and Lin 2021). Here, a *PRX* gene, *MdPRX63*, was identified as the target gene of MdERF114, and MdERF114 was further verified to bind to the *MdPRX63* promoter by Y1H, luciferase, and EMSA assay. A previous study showed that *PRX* genes are involved in the last step of the lignin biosynthesis pathway, which mediates the final synthesis of Guajacyl lignin, Syringyl lignin, and Hydroxy-phenyl lignin (Quan et al. 2019). The result of

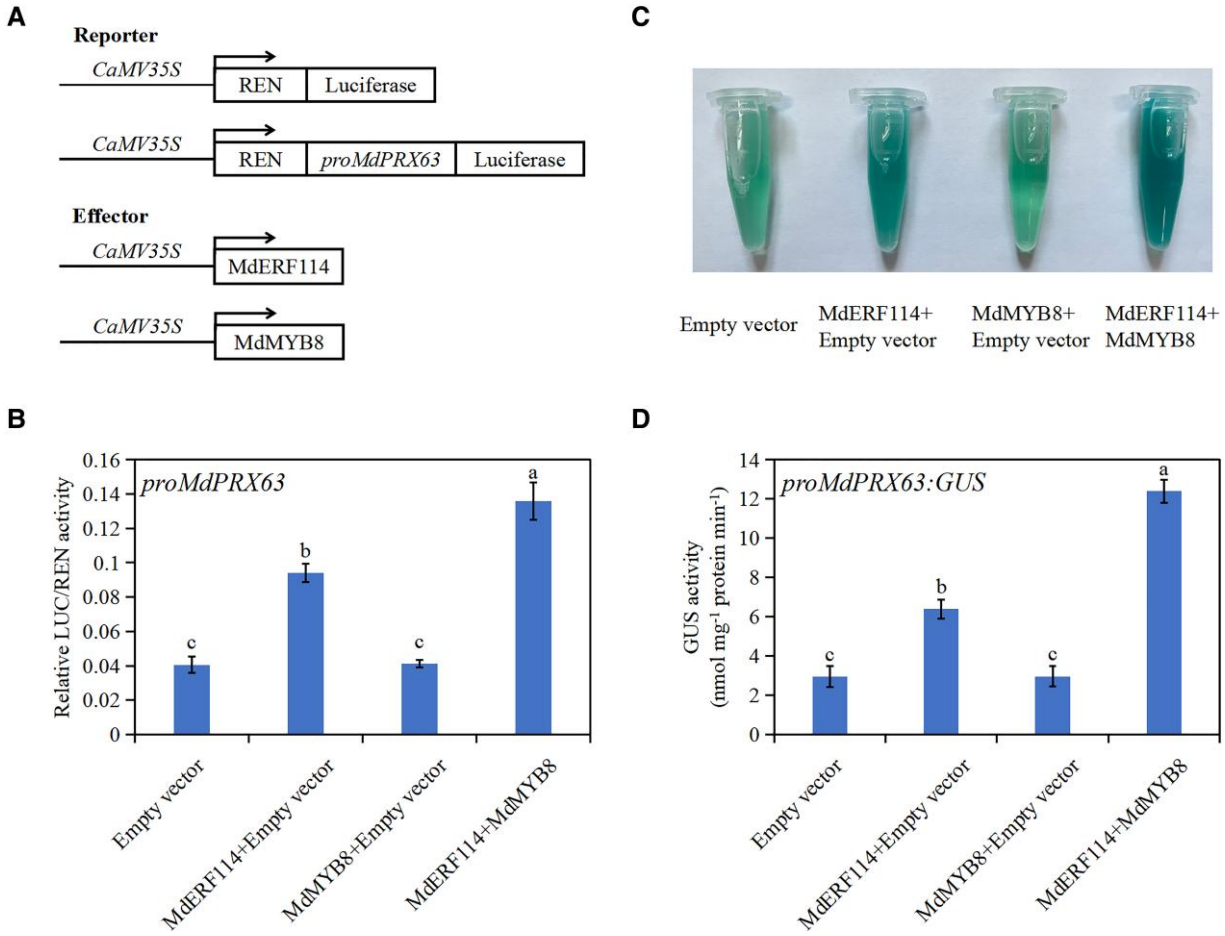

Figure 8 MdMYB8 promotes MdERF114 binding to the *MdPRX63* promotor. A, B) Relative LUC/REN activity of *Nicotiana benthamiana* co-expressing *MdMYB8*, *MdERF114* and *proMdPRX63*. C, D) Relative GUS activity analysis. The *35s::MdMYB8*, *35s::MdERF114* and *proMdPRX63*-GUS were co-transformed into apple calli and stained. The apple calli co-expressing *35s::MdERF114* and *proMdPRX63*-GUS, *35s::MdMYB8* and *proMdPRX63*-GUS, *proMdPRX63*-GUS were used as control. Data are shown as means ± standard deviation (SD) with three biological replicates in B and D. Different letters indicate significant differences between treatments based on Tukey's test ($P < 0.05$).

the lignin content determination in *MdERF114* transgenic roots showed that the transcriptional regulation of MdERF114 on *MdPRX63* affected the lignin biosynthesis under *F. solani* treatment. Similar to the function of *ERF114* in Arabidopsis (Li et al. 2022), *ERF114* affected lignin content by regulating the expression of *PAL1*. The deposition of lignin had a substantial effect on the disease resistance of apple roots (Zhu et al. 2021). In addition, we also found that overexpression of *MdPRX63* enhanced the resistance of apple roots to *F. solani*. Thus, the resistance of apple roots to *F. solani* mediated by *MdERF114* may be achieved by regulating the transcription of *MdPRX63* to influence lignin biosynthesis.

MdWRKY75 increased apple resistance to *F. solani* by regulating MdERF114

WRKY TF, one of the largest family of TFs in land plants, modulate plant response to biotic and abiotic stress through the hormone signal pathway (Jiang et al. 2017). Accumulating evidence has revealed that WRKY TFs are involved in plant defense pathways to pathogens, growth and development, senescence, biosynthesis, and regulation of hormone signaling (Rushton et al. 2010; Bakshi and Oelmuller 2014). In addition, they play an important role in plant disease resistance. A previous study showed that nine WRKY TFs in *Brochypodium distachyon* were induced upon infection with *Fusarium* (Wen et al. 2014), and *MdWRKY74* in apple is involved in the resistance to *F. solani* (Xiang et al. 2022). Interestingly, the Y1H system performed in this study found that *MdWRKY75*, a WRKY TF, binds the *MdERF114* promoter, and the result was verified by Y1H, luciferase, and EMSA assays. A previous report has revealed that *MdWRKY75e*, a homolog of *MdWRKY75*, could confer elevated apple resistance to *Alternaria alternata* through regulating lignin biosynthesis (Hou et al. 2021). Interestingly, our data also showed that *MdWRKY75* was induced by *F. solani*, and *MdWRKY75* overexpression apple roots led to enhanced apple resistance to *F. solani*. We

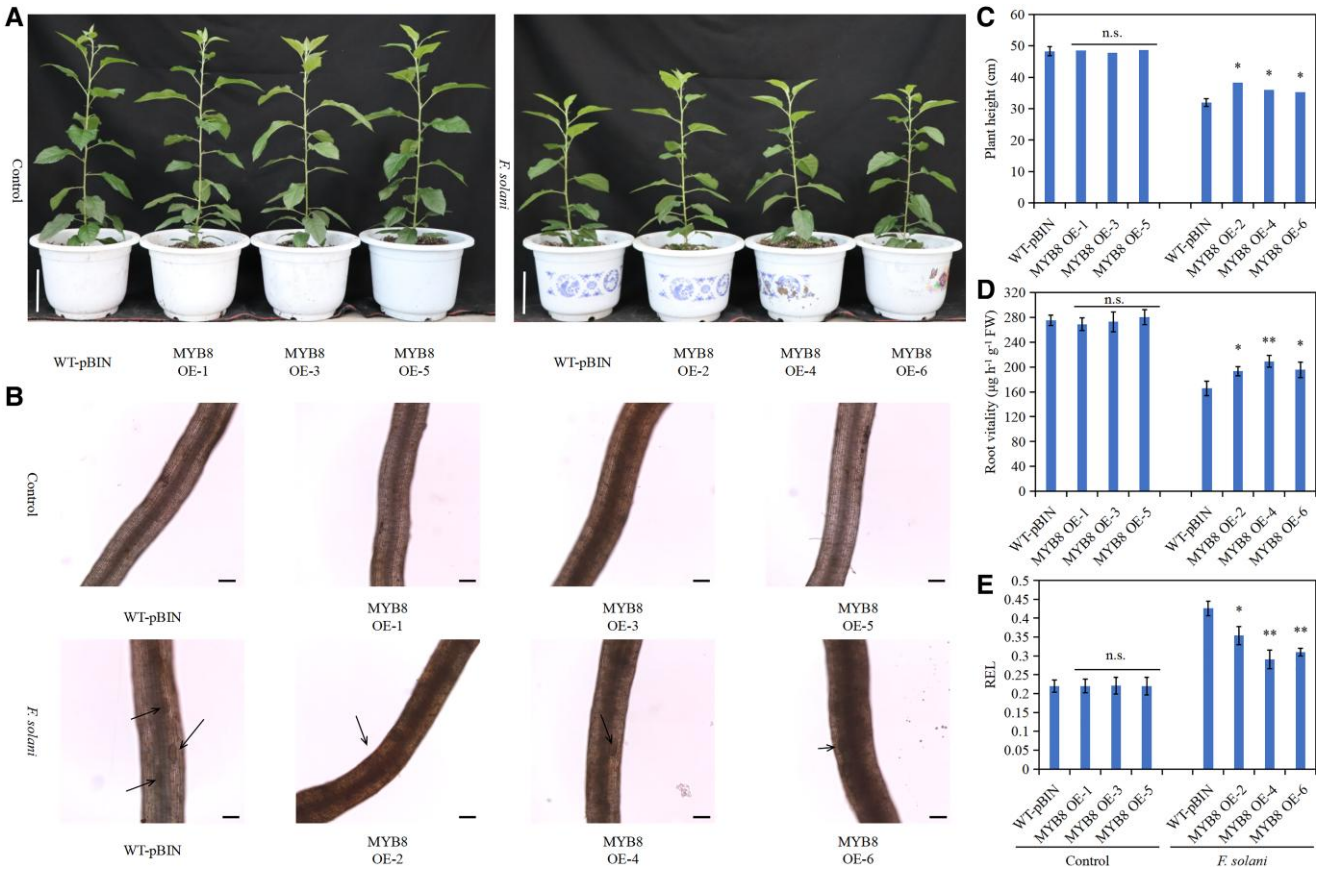

Figure 9 Overexpression of *MdMYB8* enhances apple resistance to *Fusarium solani*. A) Phenotype of plants overexpressing *MdMYB8* in the roots under *F. solani* treatment. Scale bar = 10 cm. B) Observation of *MdMYB8*-overexpressing roots under *F. solani* treatment. The black arrow indicates the wound. Scale bar = 100 μm. C) Height of plants overexpressing *MdMYB8* in the roots under *F. solani* treatment. D) Vitality of *MdMYB8*-overexpressing roots under *F. solani* treatment. E) REL of *MdMYB8*-overexpressing roots under *F. solani* treatment. WT-pBIN, GL3 transformed with an empty vector containing the GFP tag; MYB8 OE1–6, different *MdMYB8*-overexpressing root lines. Data are as means ± standard deviation (SD) with three biological replicates. Different letters indicate significant differences between treatments based on Student's *t*-test (*$P \leq 0.05$; **$P \leq 0.01$).

speculate that *MdWRKY75* may confer enhanced resistance to *F. solani* by regulating the transcription of *MdERF114*, considering that MdWRKY75 can bind to the *MdERF114* promoter. However, future work is needed to determine the underlying mechanism by which *MdWRKY75* manipulates pathway for apple resistance to *F. solani*.

MdMYB8 enhances resistance to *F. solani* by interacting with MdERF114

It is well known that ERF TFs can interact with other proteins to play a role in the plant disease resistance process (Meng et al. 2013). For example, the interaction between *VaERF16* and *VaMYB306* improves the resistance of grapevine to *Botrytis cinerea* (Zhu et al. 2022). MdERF100 interacts with MdbHLH92 to enhance apple resistance to powdery mildew (Zhang et al. 2021). In this study, a Y2H was employed to screen the interaction between MdMYB8 and MdERF114, and the results were verified by Y2H, BiFC, luciferase complementation, and pull-down assays. Previous studies have shown that *MYB8* is involved in the synthesis of secondary metabolites in *Nicotiana attenuate* and hop (*Humulus lupulus* L.) (Onkokesung et al. 2012; Schäfer et al. 2017; Kocábek et al. 2018). Our results also demonstrated that the interaction between MdMYB8 and MdERF114 enhanced the binding of MdERF114 to the *MdPRX63* promoter. Moreover, under *F. solani* treatment, *MdMYB8* was induced to up-regulate and enhance the resistance of apple to *F. solani*. Therefore, we speculate that *MdMYB8* participates in the defense response of apple roots against *F. solani* by interacting with MdERF114 to promote the binding of MdERF114 to the *MdPRX63* promoter.

In conclusion, this study identified a defense mechanism against *F. solani* infection in apple roots mediated by *MdERF114* (Fig. 10). Specifically, *MdERF114* promotes lignin deposition in apple roots by binding to the promoter of *MdPRX63* and regulating its transcription, thereby enhancing the resistance to *F. solani*. We also found that the expression of *MdWRKY75* was upregulated by *F. solani* infection. Subsequently, *MdWRKY75* regulates the expression of

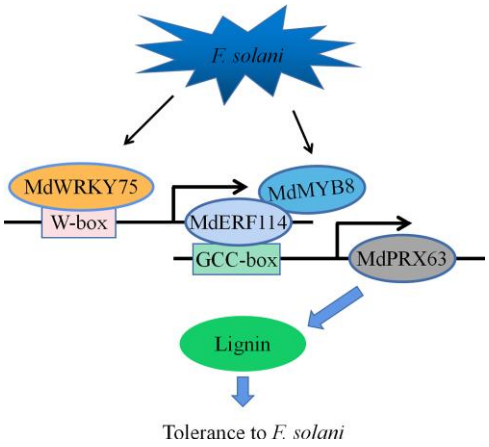

Figure 10 A working model illustrating the role of MdWRKY75-MdERF114-MdMYB8-MdPRX63 in apple response to *Fusarium solani*. *Fusarium solani* induces the expression of *MdWRKY75*, which directly binds to the W-box motif in the promoter of *MdERF114*. MdERF114 directly binds to the GCC-box motif of the *MdPRX63* promoter and activates its expression, resulting in lignin deposition. The expression of *MdMYB8* is also induced by *F. solani*. The interaction between MdMYB8 and MdERF114 enhances the binding of MdERF114 to the *MdPRX63* promoter. The deposition of lignin increases the resistance to *F. solani* in apple roots.

MdERF114 by binding to the W-box element of the *MdERF114* promoter, ultimately conferring resistance to *F. solani*. In addition, results revealed that *MdMYB8* is involved in the *MdERF114*-mediated defense network against *F. solani* by interacting with MdERF114 to promote binding of MdERF114 to the *MdPRX63* promoter. The application of the *A. rhizogenes*-mediated root transformation makes it possible to obtain transgenic rootstocks with resistance genes quickly and efficiently. Transgenic roots with defense mechanisms were obtained by *A. rhizogenes*-mediated root transformation and applied to grafting, providing a promising strategy to prevent ARD. Overall, this study provides a reference on the *MdERF114*-mediated root defense response to pathogens and lays the theoretical foundation for breeding resistant rootstocks and prevention ARD.

Materials and methods

Plant materials and growth conditions

Tissue culture seedlings of apple cultivar GL-3 (*Malus* × *domestica* cv. Gala) were used for apple root transformation. The seedlings were cultured on MS basal medium containing 30 gL^{-1} sucrose, 0.3 mgL^{-1} 6-BA, and 0.2 mgL^{-1} IAA under a 14 h photoperiod with 60 µmol m^{-2} s^{-1} light intensity for 1 month. The *35S::MdERF114-GFP*, *MdERF114-RNAi* *35S::MdPRX63-GFP*, *35S::MdWRKY75-GFP*, and *35S::MdMYB8-GFP* fusion vectors were constructed. Then, *A. rhizogenes* K599 (Weidi Biotechnology, Shanghai, China) was transformed with pBIN-GFP, pK7GWIWG2, and the fusion vectors to obtain transgenic roots as described by Meng et al. (2019). The primers are listed in Supplemental Table S1.

Positive transgenic root transformants were used to inoculate *F. solani* as per the method of Liu et al. (2022a). Briefly, potato glucose liquid medium was inoculated with *F. solani* strain (MG836251.1) and incubated at 24°C in the dark for 48 h. The spore suspension was obtained by filtering the medium through eight layers of sterile gauze. Its concentration was adjusted to 10^5 cells/mL^{-1} using sterile water. Treatments and control plants were watered with 200 mL of spore suspension and water only, respectively.

Physiological analysis

Root vitality was measured using a root vitality analysis kit from Suzhou Grace Biotechnology Co. Ltd (Suzhou, China). Three biological replicates were performed for each treatment.

According to the method of Liu et al. (2022a), fresh roots were used to determine the REL of roots. Three biological replicates were performed for each treatment.

The lignin content of roots was determined using a lignin content analysis kit (Suzhou Comin Biotechnology Co., Ltd., Suzhou, China). Three biological replicates were performed for each treatment.

Y1H assay

Inserts containing the ORFs of *MdERF114* and *MdWRKY75* were cloned into the pGADT7 vector. Likewise, inserts containing the promoters of *MdPRX63* and *MdERF114* were cloned into the pHIS2 vector. The 3-AT was used to suppress the expression of pHIS2-*proMdPRX63* and pHIS2-*proMdERF114*. Then, according to the method of Liu et al. (2022b), the yeast strain Y187 was transformed with fusion vectors for Y1H assays. The primers are listed in Supplemental Table S1.

Dual-luciferase assays

Each of the ORF sequences for *MdERF114*, *MdMYB8*, and *MdWRKY75* were individually cloned into pGreenII 62-SK vectors. Each of the promoters for *MdERF114* and *MdPRX63* was individually cloned into pGreenII 0800-LUC vectors. *Agrobacterium tumefaciens* strain GV3101 (Weidi Biotechnology, Shanghai, China) was transformed with fusion vectors. Then, LUC and renilla luciferase (REN) activities were assessed using a dual-luciferase reporter gene assay kit (Yeasen, Shanghai, China). Three biological replicates were performed for each treatment. The primers are listed in Supplemental Table S1.

EMSA assay

The ORFs of *MdERF114* and *MdWRKY75* were cloned into the pET-32a (+) vector and upon expression, MdERF114-HIS and MdWRKY75-HIS fusion proteins were obtained. Biotin-labeled probes for *MdPRX63* and *MdERF114* were synthesized by Sangon Biotech (Shanghai, China). The EMSA assay was done with a LightShift Chemiluminescent EMSA kit

(Thermo Scientific, Waltham, MA, USA). The primers are listed in Supplemental Table S1.

GUS analysis

The promoter of *MdPRX63* was cloned into the pC0390-GUS vector, and apple callus was co-transformed with *35S::MdERF114* and *proMdPRX63*-GUS (*35S::MdERF114*, *35S::MdMYB8*, and *proMdPRX63*-GUS) using a vacuum method. Histochemical staining was performed to measure the GUS activity in the transformants (An et al. 2018). Three biological replicates were performed for each treatment. The primers are listed in Supplemental Table S1.

Y2H assay

The ORFs of *MdERF114* and *MdMYB8* were cloned into the pGBKT7 and pGADT7 vectors, respectively. Y2H Gold yeast (Weidi Biotechnology, Shanghai, China) was transformed with both plasmids. Positive transformants were selected for by cultivation on media lacking Trp and Leu, and also media lacking Ade, Trp, Leu, and His. The primers are listed in Supplemental Table S1.

Pull-down assay

The ORFs of *MdERF114* and *MdMYB8* were cloned into the pET-32a (+) and pGEX-4T-1 vectors, respectively. Pull-down assays were carried out using a Pierce GST Protein Interaction Pull-Down Kit (Thermo Scientific, Waltham, MA, USA). The primers are listed in Supplemental Table S1.

BiFC assay

The ORFs of *MdERF114* and *MdMYB8* were cloned into the pSPYCE-35S and pSPYNE-35S vectors, respectively. *Agrobacterium tumefaciens* strain GV3101 (Weidi Biotechnology, Shanghai, China) was transformed with the two vectors, which were also co-injected into *N. benthamiana*. Yellow fluorescent protein (YFP) fluorescence was observed under a confocal laser scanning microscope (Leica SP5, Leica Microsystems, Buffalo Grove, IL, USA). The excitation wavelength was 514 nm and the fluorescence was detected with a 522–560-nm band-pass filter. The primers are listed in Supplemental Table S1.

Luciferase complementation assay

MdERF114-NLuc and *MdMYB8*-CLuc vectors were constructed. *Agrobacterium tumefaciens* strain GV3101 (Weidi Biotechnology, Shanghai, China) was then transformed with these two vectors, which were also co-injected into *N. benthamiana*. The fluorescence intensity was measured with an in vivo imaging system (Xenogen, Boston, USA) (Jiang et al. 2021). The primers are listed in Supplemental Table S1.

Statistical analysis

Statistical analysis was conducted using SPSS Statistics version 18.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA and Student's *t*-test were used to determine the statistical significance of differences between various treatments.

Accession numbers

Sequence data from this article can be found in the NCBI data libraries under accession numbers MdERF114 (XM_017328133.2), MdPRX63 (XM_008343268.3), MdWRKY75 (XM_008380876.3), and MdMYB8 (DQ267899.1).

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Author contributions

C.Li., F.M., and Y.L. conceived and designed the study. Y.L., Q.L., X.L., Z.Z., S.A. and C.Liu. performed the analyses. Y.L. drafted the manuscript. F.M. and C.Li. supervised the process of this research. F.M. and C.Li. provided financial support for the study. All authors critically revised and provided final approval of this manuscript.

Supplemental data

The following materials are available in the online version of this article.

Supplemental Fig. S1. Analysis of *MdERF114* expression patterns under different conditions and *cis*-acting elements of *MdERF114* promoter.

Supplemental Fig. S2. Subcellular localization of MdERF114.

Supplemental Fig. S3. Determination of *MdERF114* transgenic roots.

Supplemental Fig. S4. Determination of *MdPRX63* transgenic roots.

Supplemental Fig. S5. Analysis of MdWRKY75 expression pattern under *Fusarium solani* treatment and determination of *MdWRKY75* transgenic roots.

Supplemental Fig. S6. Analysis of *MdMYB8* expression pattern under *Fusarium solani* treatment and determination of *MdMYB8* transgenic roots.

Supplemental Table S1. The primers used in this study.

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Conflict of interest statement. None declared.

References

An JP, Li R, Qu FJ, You CX, Wang XF, Hao YJ. R2R3-MYB transcription factor *MdMYB23* is involved in the cold tolerance and proanthocyanidin accumulation in apple. Plant J. 2018:**96**(3): 562–577 https://doi.org/10.1111/tpj.14050

Bakshi M, Oelmuller R. WRKY Transcription factors: jack of many trades in plants. Plant Signal Behav. 2014:**9**(2):e27700 https://doi.org/10.4161/psb.27700

Barceló AR, Gómez Ros LV, Gabaldón C, López-Serrano M, Pomar F, Carrión JS, Pedreño MA. Basic peroxidases: the gateway for lignin evolution? Phytochem Rev. 2004:**3**(1–2):61–78 https://doi.org/10.1023/B:PHYT.0000047803.49815.1a

Cesarino I. Structural features and regulation of lignin deposited upon biotic and abiotic stresses. Curr Opin Biotechnol. 2019:**56**:209–214 https://doi.org/10.1016/j.copbio.2018.12.012

Chen L, Zhang L, Li D, Wang F, Yu D. *WRKY8* transcription factor functions in the TMV-cg defense response by mediating both abscisic acid and ethylene signaling in *Arabidopsis*. Proc Natl Acad Sci U S A. 2013:**110**(21): E1963–E1971 https://doi.org/10.1073/pnas.1221347110

Dong NQ, Lin HX. Contribution of phenylpropanoid metabolism to plant development and plant-environment interactions. J Integr Plant Biol. 2021:**63**(1):180–209 https://doi.org/10.1111/jipb.13054

Feng K, Hou XL, Xing GM, Liu JX, Duan AQ, Xu ZS, Li MY, Zhuang J, Xiong AS. Advances in AP2/ERF super-family transcription factors in plant. Crit Rev Biotechnol. 2020:**40**(6):750–776 https://doi.org/10.1080/07388551.2020.1768509

Fernandez-Perez F, Pomar F, Pedreno MA, Novo-Uzal E. Suppression of *Arabidopsis* peroxidase 72 alters cell wall and phenylpropanoid metabolism. Plant Sci. 2015:**239**:192–199 https://doi.org/10.1016/j.plantsci.2015.08.001

Grunewaldt-Stöcker G, Mahnkopp F, Popp C, Maiss E, Winkelmann T. Diagnosis of apple replant disease (ARD): microscopic evidence of early symptoms in fine roots of different apple rootstock genotypes. Sci Hortic. 2019:**243**:583–594 https://doi.org/10.1016/j.scienta.2018.09.014

Hou Y, Yu X, Chen W, Zhuang W, Wang S, Sun C, Cao L, Zhou T, Qu S. *MdWRKY75e* enhances resistance to *Alternaria alternata* in *Malus domestica*. Hortic Res. 2021:**8**(1):225 https://doi.org/10.1038/s41438-021-00701-0

Jiang J, Ma S, Ye N, Jiang M, Cao J, Zhang J. WRKY transcription factors in plant responses to stresses. J Integr Plant Biol. 2017:**59**(2):86–101 https://doi.org/10.1111/jipb.12513

Jiang H, Ma QJ, Zhong MS, Gao HN, Li YY, Hao YJ. The apple palmitoyltransferase *MdPAT16* influences sugar content and salt tolerance via an *MdCBL1-MdCIPK13-MdSUT2.2* pathway. Plant J. 2021:**106**(3):689–705 https://doi.org/10.1111/tpj.15191

Kelderer M, Manici LM, Caputo F, Thalheimer M. Planting in the 'inter-row' to overcome replant disease in apple orchards: a study on the effectiveness of the practice based on microbial indicators. Plant Soil. 2012:**357**(1–2):381–393 https://doi.org/10.1007/s11104-012-1172-0

Kidwai M, Dhar YV, Gautam N, Tiwari M, Ahmad IZ, Asif MH, Chakrabarty D. Oryza sativa class III peroxidase (*OsPRX38*) overexpression in *Arabidopsis thaliana* reduces arsenic accumulation due to apoplastic lignification. J Hazard Mater. 2019:**362**:383–393 https://doi.org/10.1016/j.jhazmat.2018.09.029

Kocábek T, Mishra AK, Matoušek J, Patzak J, Lomnická A, Khare M, Krofta K. The R2R3 transcription factor *HlMYB8* and its role in flavonoid biosynthesis in hop (*Humulus lupulus* L.). Plant Sci. 2018:**269**:32–46 https://doi.org/10.1016/j.plantsci.2018.01.004

Li Z, Zhang Y, Ren J, Jia F, Zeng H, Li G, Yang X. Ethylene-responsive factor *ERF114* mediates fungal pathogen effector PevD1-induced disease resistance in *Arabidopsis thaliana*. Mol Plant Pathol. 2022:**23**(6):819–831 https://doi.org/10.1111/mpp.13208

Licausi F, Ohme-Takagi M, Perata P. APETALA2/ethylene responsive factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs. New Phytol. 2013:**199**(3):639–649 https://doi.org/10.1111/nph.12291

Liu YJ, An JP, Gao N, Wang X, Chen XX, Wang XF, Zhang S, You CX. *MdTCP46* interacts with *MdABI5* to negatively regulate ABA signalling and drought response in apple. Plant Cell Environ. 2022b:**45**-(11):3233–3248 https://doi.org/10.1111/pce.14429

Liu Y, Liu Q, Li X, Tang Z, Zhang Z, Gao H, Ma F, Li C. Exogenous dopamine and *MdTyDC* overexpression enhance apple resistance to *Fusarium solani*. Phytopathology. 2022a:**112**(12):2503–2513. https://doi.org/10.1094/PHYTO-04-22-0142-R

Ma R, Liu B, Geng X, Ding X, Yan N, Sun X, Wang W, Sun X, Zheng C. Biological function and stress response mechanism of MYB transcription factor family genes. J Plant Growth Regul. 2022:**42**(1):83–95. https://doi.org/10.1007/s00344-021-10557-2

Ma QH, Zhu HH, Qiao MY. Contribution of both lignin content and sinapyl monomer to disease resistance in tobacco. Plant Pathol. 2018:**67**(3):642–650 https://doi.org/10.1111/ppa.12767

Manici LM, Ciavatta C, Kelderer M, Erschbaumer G. Replant problems in South Tyrol: role of fungal pathogens and microbial population in conventional and organic apple orchards. Plant Soil. 2003:**256**(2):315–324 https://doi.org/10.1023/A:1026103001592

Marjamaa K, Kukkola EM, Fagerstedt KV. The role of xylem class III peroxidases in lignification. J Exp Bot. 2009:**60**(2):367–376 https://doi.org/10.1093/jxb/ern278

Mazzola M. Elucidation of the microbial complex having a causal role in the development of apple replant disease in Washington. Phytopathology. 1998:**88**(9):930–938 https://doi.org/10.1094/PHYTO.1998.88.9.930

Meng X, Xu J, He Y, Yang KY, Mordorski B, Liu Y, Zhang S. Phosphorylation of an ERF transcription factor by *Arabidopsis MPK3/MPK6* regulates plant defense gene induction and fungal resistance. Plant Cell. 2013:**25**(3): 1126–1142 https://doi.org/10.1105/tpc.112.109074

Meng D, Yang Q, Dong B, Song Z, Niu L, Wang L, Cao H, Li H, Fu Y. Development of an efficient root transgenic system for pigeon pea and its application to other important economically plants. Plant Biotechnol J. 2019:**17**(9): 1804–1813 https://doi.org/10.1111/pbi.13101

Mottiar Y, Smith RA, Karlen SD, Ralph J, Mansfield SD. Evolution of p-coumaroylated lignin in eudicots provides new tools for cell wall engineering. New Phytol. 2023:**237**(1):251–264 https://doi.org/10.1111/nph.18518

Nakano T, Suzuki K, Fujimura T, Shinshi H. Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice. Plant Physiol. 2006:**140**(2):411–432 https://doi.org/10.1104/pp.105.073783

Onkokesung N, Gaquerel E, Kotkar H, Kaur H, Baldwin IT, Galis I. *MYB8* controls inducible phenolamide levels by activating three novel hydroxycinnamoyl-coenzyme A: polyamine transferases in *Nicotiana attenuata*. Plant Physiol. 2012:**158**(1):389–407 https://doi.org/10.1104/pp.111.187229

Quan M, Du Q, Xiao L, Lu W, Wang L, Xie J, Song Y, Xu B, Zhang D. Genetic architecture underlying the lignin biosynthesis pathway involves noncoding RNAs and transcription factors for growth and wood properties in *Populus*. Plant Biotechnol J. 2019:**17**(1):302–315 https://doi.org/10.1111/pbi.12978

Reboledo G, Agorio A, De Leon IP. Moss transcription factors regulating development and defense responses to stress. J Exp Bot. 2022:**73**-(13):4546–4561 https://doi.org/10.1093/jxb/erac055

Renard J, Martinez-Almonacid I, Sonntag A, Molina I, Moya-Cuevas J, Bissoli G, Munoz-Bertomeu J, Faus I, Ninoles R, Shigeto J, et al. *PRX2* and *PRX25*, peroxidases regulated by *COG1*, are involved in seed longevity in *Arabidopsis*. Plant Cell Environ. 2020:**43**(2):315–326 https://doi.org/10.1111/pce.13656

Rushton PJ, Somssich IE, Ringler P, Shen QJ. WRKY transcription factors. Trends Plant Sci. 2010:**15**(5):247–258 https://doi.org/10.1016/j.tplants.2010.02.006

Schäfer M, Brütting C, Xu S, Ling Z, Steppuhn A, Baldwin IT, Schuman MC. *NaMYB8* regulates distinct, optimally distributed herbivore defense traits. J Integr Plant Biol. 2017:**59**(12):844–850 https://doi.org/10.1111/jipb.12593

Seo PJ, Park CM. *MYB96*-mediated abscisic acid signals induce pathogen resistance response by promoting salicylic acid biosynthesis in *Arabidopsis*. New Phytol. 2010:**186**(2):471–483 https://doi.org/10.1111/j.1469-8137.2010.03183.x

Shi W, Hao L, Li J, Liu D, Guo X, Li H. The *Gossypium hirsutum* WRKY gene *GhWRKY39-1* promotes pathogen infection defense responses and mediates salt stress tolerance in transgenic *Nicotiana benthamiana*. Plant Cell Rep. 2014:**33**(3):483–498 https://doi.org/10.1007/s00299-013-1548-5

Vanholme R, De Meester B, Ralph J, Boerjan W. Lignin biosynthesis and its integration into metabolism. Curr Opin Biotechnol. 2019:**56**:230–239 https://doi.org/10.1016/j.copbio.2019.02.018

van Schoor L, Denman S, Cook NC. Characterisation of apple replant disease under South African conditions and potential biological management strategies. Sci Hortic. 2009:**119**(2):153–162 https://doi.org/10.1016/j.scienta.2008.07.032

van Verk MC, Pappaioannou D, Neeleman L, Bol JF, Linthorst HJM. A novel WRKY transcription factor is required for induction of *PR-1a* gene expression by salicylic acid and bacterial elicitors. Plant Physiol. 2008:**146**(4):1983–1995 https://doi.org/10.1104/pp.107.112789

Wang G, Yin C, Pan F, Wang X, Xiang L, Wang Y, Wang J, Tian C, Chen J, Mao Z. Analysis of the fungal community in apple replanted soil around Bohai Gulf. Hortic Plant J. 2018:**4**(5):175–181 https://doi.org/10.1016/j.hpj.2018.05.003

Wen F, Zhu H, Li P, Jiang M, Mao W, Ong C, Chu Z. Genome-wide evolutionary characterization and expression analyses of WRKY family genes in *Brachypodium distachyon*. DNA Res. 2014:**21**(3):327–339 https://doi.org/10.1093/dnares/dst060

Xiang L, Wang M, Huang J, Jiang W, Yan Z, Chen X, Yin C, Mao Z. *MdWRKY74* is involved in resistance response to apple replant disease. Plant Growth Regul. 2022:**96**(1):145–156 https://doi.org/10.1007/s10725-021-00766-w

Xiang L, Wang M, Pan F, Wang G, Jiang W, Wang Y, Chen X, Yin C, Mao Z. Transcriptome analysis *Malus domestica* 'M9T337' root molecular responses to *Fusarium solani* infection. Physiol Mol Plant Pathol. 2021a:**113** 101567 https://doi.org/10.1016/j.pmpp.2020.101567

Xiang L, Zhao L, Wang M, Huang J, Chen X, Yin C, Mao Z. Physiological responses of apple rootstock M.9 to infection by *Fusarium solani*. HortScience. 2021b:**56**(9):1104–1111 https://doi.org/10.21273/HORTSCI15945-21

Xie M, Zhang J, Tschaplinski TJ, Tuskan GA, Chen JG, Muchero W. Regulation of lignin biosynthesis and its role in growth-defense tradeoffs. Front Plant Sci. 2018:**9**:1427 https://doi.org/10.3389/fpls.2018.01427

Yan K, Han G, Ren C, Zhao S, Wu X, Bian T. *Fusarium solani* infection depressed photosystem performance by inducing foliage wilting in apple seedlings. Front Plant Sci. 2018:**9**:479 https://doi.org/10.3389/fpls.2018.00479

Zang Z, Lv Y, Liu S, Yang W, Ci J, Ren X, Wang Z, Wu H, Ma W, Jiang L, et al. A novel ERF transcription factor, *ZmERF105*, positively regulates maize resistance to *Exserohilum turcicum*. Front Plant Sci. 2020:**11**:850 https://doi.org/10.3389/fpls.2020.00850

Zhang Y, Zhang L, Ma H, Zhang Y, Zhang X, Ji M, van Nocker S, Ahmad B, Zhao Z, Wang X, et al. Overexpression of the apple (*Malus* × *domestica*) *MdERF100* in *Arabidopsis* increases resistance to *Powdery mildew*. Int J Mol Sci. 2021:**22**(11):5713 https://doi.org/10.3390/ijms22115713

Zhang YL, Zhang CL, Wang GL, Wang YX, Qi CH, Zhao Q, You CX, Li YY, Hao YJ. The R2R3 MYB transcription factor *MdMYB30* modulates plant resistance against pathogens by regulating cuticular wax biosynthesis. BMC Plant Biol. 2019:**19**(1):362 https://doi.org/10.1186/s12870-019-1918-4

Zheng X, Xing J, Zhang K, Pang X, Zhao Y, Wang G, Zang J, Huang R, Dong J. Ethylene response factor *ERF11* activates *BT4* transcription to regulate immunity to *Pseudomonas syringae*. Plant Physiol. 2019:
180(2):1132–1151 https://doi.org/10.1104/pp.18.01209

Zhu Y, Fazio G, Mazzola M. Elucidating the molecular responses of apple rootstock resistant to ARD pathogens: challenges and opportunities for development of genomics-assisted breeding tools. Hortic Res. 2014:**1**(1):14043 https://doi.org/10.1038/hortres.2014.43

Zhu Y, Li G, Singh J, Khan A, Fazio G, Saltzgiver M, Xia R. Laccase directed lignification is one of the major processes associated with the defense response against *Pythium ultimum* infection in apple roots. Front Plant Sci. 2021:**12**:629776 https://doi.org/10.3389/fpls.2021.629776

Zhu Y, Shin S, Mazzola M. Genotype responses of two apple rootstocks to infection by *Pythium ultimum* causing apple replant disease. Can J Plant Pathol. 2016:**38**(4):483–491 https://doi.org/10.1080/07060661.2016.1260640

Zhu Y, Zhang X, Zhang Q, Chai S, Yin W, Gao M, Li Z, Wang X. The transcription factors *VaERF16* and *VaMYB306* interact to enhance resistance of grapevine to *Botrytis cinerea* infection. Mol Plant Pathol. 2022:**23**(10):1415–1432 https://doi.org/10.1111/mpp.13223