Arabidopsis AtERF014 acts as a dual regulator that differentially modulates immunity against *Pseudomonas syringae pv. tomato* and *Botrytis cinerea*

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ERF transcription factors play critical roles in plant immune responses. Here, we report the function of AtERF014, a nucleus-localized transcriptional activator, in Arabidopsis immunity. Expression of AtERF014 was induced by *Pseudomonas syringae pv. tomato* (Pst) and *Botrytis cinerea* (Bc). AtERF014-overexpressing (OE) plants displayed increased Pst resistance but decreased Bc resistance, whereas AtERF014-RNAi plants exhibited decreased Pst resistance but increased Bc resistance. After Pst infection, expression of salicylic acid (SA)-responsive genes AtPR1 and AtPR5 in AtERF014-OE plants and of a jasmonic acid/ethylene-responsive gene AtPDF1.2 in AtERF014-RNAi plants was intensified but expression of AtPDF1.2 in AtERF014-OE plants and of AtPR1 and AtPR5 in AtERF014-RNAi plants was weakened. After Bc infection, expression of AtPR1 and AtPR5 in AtERF014-OE plants was attenuated but expression of AtPR1, AtPR5 and AtPDF1.2 in AtERF014-RNAi plants was strengthened. Pathogen- and flg22-induced ROS burst, expression of PTI genes and SA-induced defense were partially suppressed in AtERF014-RNAi plants, whereas pathogen-induced ROS and flg22-induced immune response were strengthened in AtERF014-OE plants. Altered expression of AtERR014 affected expression of pectin biosynthetic genes and pectin content in AtERF014-RNAi plants was decreased. These data demonstrate that AtERF014 acts as a dual regulator that differentially modulates immunity against Pst and Bc in Arabidopsis.

Plants are frequently exposed to attack by potential microbes during their lifespan and thus they have developed to possess arrays of complicated molecular mechanisms to cope with the invading pathogens. The plant innate immunity system comprises of two layers of immune responses, called pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI)1,2. PTI and ETI are activated upon recognition of PAMPs such as flagellin, EF-Tu and chitin3–6 and pathogen-derived specific effectors7,8 by pattern-recognition receptors or specified R proteins in plants, respectively. In addition, plants have also developed to possess several forms of inducible immunity, e.g. systemic acquired resistance and induced systemic resistance, which becomes activated upon pathogen infection or treatment of elicitors9,10.

Upon perception of pathogen-derived signals, plants often activate a network of defense hormone-mediated signaling pathways11, which ultimately lead to transcriptional reprogramming that coordinately regulates expression of a large set of genes. For example, one-third of the Arabidopsis genome changes in expression during the first 48 hr after infection by *Botrytis cinerea*12, whereas approximately 4000 genes are differentially expressed in immune response induced by *Pseudomonas syringae pv. tomato* DC3000hrpA13. Such large-scale transcriptional reprogramming of gene expression in a specific immune response obviously requires a concerted function of different types of transcription factors (TFs) in both temporal and spatial manners. Recent genetic studies have demonstrated that a number of TFs from the families of WRKY, AP2/ERF (Apetala2/ethylene responsive factor) and NAC play crucial roles in immune responses against pathogens14–17.
The AP2/ERF superfamily is a large plant-specific TF family and is characterized by the presence of one or two AP2/ERF domains that consist of 58 or 59 conserved amino acid residues. The AP2/ERF superfamily can be divided into three subfamilies, namely AP2, RAV and ERF, and 122 out of 147 members in Arabidopsis are ERF TFs. The ERFs can act as transcription activators or repressors; for example, Arabidopsis AtERF1, AtERF2 and AtERF5 are activators while AtERF3, AtERF4 and AtERF7 are repressors. Generally, ERF TFs regulate the expression of genes, whose promoters harbor at least one core GCC box, and most of these GCC box-containing genes are defense-related.

ERF TFs are heavily linked with the regulation of immune response in plants and are integrators of different defense hormone pathways. In Arabidopsis, a total of 53 AP2/ERF TFs are differentially expressed following B. cinerea infection. Functional studies using knockout/knockdown mutants have revealed that 10 out of 17 members in the group IX of the ERF family, including AtERF92 (AtERF1), AtERF93 (AtERF15), AtERF94 (ORA59), AtERF96, AtERF97 (AtERF14), AtERF100 (AtERF1), AtERF101 (AtERF2), AtERF102 (AtERF5), AtERF103 (AtERF6) and AtERF104, play important roles in regulating immune response against pathogens including B. cinerea. In addition, AtERF078 (AtERF4) and AtERF080 (AtERF9), belonging to group VIII, act as negative regulators of resistance to Fusarium oxysporum and B. cinerea, respectively, whereas AtERF075 (AtRAP2.2), a member of group VII, functions as an important regulator in resistance to B. cinerea. In addition, overexpression of AtERF11 (AtDEAR1), a member of group II, resulted in lesion-like cell death, elevated SA level, constitutive expression of defense genes and increased resistance to P. syringae pv. tomato DC3000. Most of the reported ERF TFs, including AtERF1, AtERF5, AtERF6, AtERF14, ORA59 and AtERF96, function in Arabidopsis immune response through modulating jasmonic acid (JA)/ethylene (ET)-mediated signaling pathway, resulting in expression of defense genes including AtPDF1.2. It was also found that AtERF5 and AtERF15 are involved in PTI and positively regulate salicylic acid (SA)-mediated signaling pathway that is involved in resistance to P. syringae pv. tomato DC3000. Collectively, these data strongly demonstrate the importance of ERF TFs in regulation of Arabidopsis immune responses through modification of different defense signaling pathways.

AtERF014, a member of group II in the ERF family, was recently reported to be involved in pectin biosynthesis. However, the biological function of AtERF014 remains elusive. The present study focused on the function of AtERF014 in disease resistance to P. syringae pv. tomato DC3000 and B. cinerea. Functional analyses using AtERF014-OE and AtERF014-RNAi lines suggest that AtERF014 acts as a positive regulator of resistance against Pst DC3000 but functions as a negative regulator of resistance to B. cinerea. Furthermore, AtERF014 is involved in flg22-induced PTI response and SA-induced defense response. Our data demonstrate that AtERF014 is a dual regulator that differentially modulates immunity against P. syringae pv. tomato DC3000 and B. cinerea in Arabidopsis.

**Results**

**AtERF014 is responsive to pathogen infection and defense signaling hormones.** To explore the involvement in disease resistance, we examined whether expression of AtERF014 could be induced by pathogen infection and defense signaling hormones such as SA and JA. As shown in Fig. 1A, the transcript level of AtERF014 increased as early as 12 h post-inoculation (hpi), peaked with ~6 folds at 24 hpi, maintained at relatively higher level at 48 hpi and then decreased to basal level at 72 hpi after infection by Pst DC3000. Similar kinetics of change in the transcript level of AtERF014 was observed in B. cinerea-infected plants, showing a peak of 7 folds at 24 hpi (Fig. 1B). In addition, the expression of AtERF014 was also induced by SA and MeJA but showed different patterns. In SA-treated plants, the transcript level of AtERF014 increased rapidly at 3 h post-treatment (hpt), peaked within 6 folds at 12 hpt and maintained at relatively higher level until 24 hpt (Fig. 1C). By contrast, the transcript level of AtERF014 in methyl jasmonate (MeJA)-treated plants increased gradually and peaked with 6 folds at 12 hpt and maintained at relatively higher level until 24 hpt (Fig. 1C). These data suggest that AtERF014 is responsive to pathogen infection and defense signaling hormones.

**AtERF014 is a transcriptional activator that is localized in nucleus.** The biochemical characters of AtERF014 protein were examined by analyzing the transactivation activity in yeast and subcellular localization in planta. In transactivation activity assay, yeasts carrying pBD-AtERF014, pBD-GAL4 (a positive control) or pBD empty vector (a negative control) grew on SD/Trp medium but only yeasts carrying pBD-AtERF014 or pBD-GAL4 exhibited β-galactosidase activity, as revealed by the production of blue pigment after addition of X-α-gal (Fig. 1D). In subcellular localization assay, the GFP alone was seen ubiquitously in cells without specific compartmental localization, whereas the GFP-AtERF014 fusion was solely localized in nucleus, co-localized with the known nucleus marker RFP–H2B protein (Fig. 1E). These results indicate that AtERF014 may be a transcriptional activator that is localized in nucleus.

**Altered expression of AtERF014 does not affect the growth and development in AtERF014-OE and AtERF014-RNAi plants.** To better understand the biological function of AtERF014, we generated transgenic lines with overexpression or RNA interfering (RNAi)-mediated suppression of AtERF014. Two homozygous single-copy AtERF014-OE and AtERF014-RNAi lines (T3 generation) were chosen for further studies. The transcript levels of AtERF014 in AtERF014-OE lines were 6.56 and 5.84 times higher than that in WT while the transcript levels in AtERF014-RNAi lines were 18% and 29% of that in WT (Fig. 2A). The AtERF014-OE and AtERF014-RNAi plants grew and developed normally, indistinguishable from WT, at vegetable and reproductive stages (Fig. 2B). Thus, it is likely that altered expression of AtERF014 does not affect the growth and development in AtERF014-OE and AtERF014-RNAi plants, implying a limited role for AtERF014 in growth and development.
Altered expression of AtERF014 affects the basal immunity in AtERF014-OE and AtERF014-RNAi plants against Pst DC3000. Having shown that the expression of AtERF014 was induced by Pst DC3000, we first examined whether altered expression of AtERF014 affected the resistance of AtERF014-OE and AtERF014-RNAi plants to this bacterial pathogen. At 4 days post-inoculation (dpi), typical Pst DC3000-provoked disease with chlorotic lesion was seen in WT plants (Fig. 3A). At the same time, AtERF014-RNAi plants displayed much severe disease with extensive chlorotic lesion while AtERF014-OE plants showed less severe disease (Fig. 3A). Accordingly, bacterial populations in AtERF014-RNAi plants were 7 and 21 folds higher while the populations in AtERF014-OE plants were 13 and 32 times lower, as compared to those in WT at 2 and 4 dpi, respectively (Fig. 3B). These data indicate that overexpression of AtERF014 leads to an increased resistance while suppression of AtERF014 results in a decreased resistance against Pst DC3000.

Altered expression of AtERF014 affects the basal immunity in AtERF014-OE and AtERF014-RNAi plants against B. cinerea. We next assessed whether altered expression of AtERF014 affected the resistance of AtERF014-OE and AtERF014-RNAi plants to B. cinerea, a necrotrophic fungus causing grey mold disease. After infection, typical B. cinerea-provoked disease symptom was seen in WT plants (Fig. 4A). At the same time, AtERF014-OE plants displayed much severe disease with extensive chlorotic lesion while AtERF014-RNAi plants showed less severe disease (Fig. 3A). Accordingly, bacterial populations in AtERF014-RNAi plants were 7 and 21 folds higher while the populations in AtERF014-OE plants were 13 and 32 times lower, as compared to those in WT at 2 and 4 dpi, respectively (Fig. 3B). These data indicate that overexpression of AtERF014 leads to an increased resistance while suppression of AtERF014 results in a decreased resistance against Pst DC3000.

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content in AtERF014-OE plants decreased gradually with disease development and exhibited 30–35% lower while chlorophyll content in AtERF014-RNAi plants decreased slowly and showed 6–9% higher, than that in WT, at 10 dpi (Fig. 4C). Furthermore, more AtERF014-OE plants died (38–41% of death) while more AtERF014-RNAi plants survived (7–11% of death), as compared to that in WT (16% of death) (Fig. 4D). These results indicate overexpression of AtERF014 weakens while suppression of AtERF014 strengthens the resistance against B. cinerea.

Altered expression of AtERF014 affects Pst DC3000- and B. cinerea-induced defense response in AtERF014-OE and AtERF014-RNAi plants. To examine whether altered expression of AtERF014 affected the defense response, we analyzed and compared the patterns for accumulation of reactive oxygen species (ROS) and expression of defense genes among WT, AtERF014-OE and AtERF014-RNAi plants after infection by Pst DC3000 or B. cinerea. In in situ detection of ROS assays, accumulation of superoxide anion and H$_2$O$_2$ in leaves was detected by nitroblue tetrazolium (NBT) and 3, 3-diaminobenzidine (DAB) staining, respectively. Without pathogen challenge, no difference in accumulation of superoxide anion and H$_2$O$_2$ was seen among WT, AtERF014-OE and AtERF014-RNAi plants (Fig. 5A,B). At 24 hr after infection by Pst DC3000 or B. cinerea, accumulation of superoxide anion and H$_2$O$_2$ in inoculated leaves increased obviously except the case of H$_2$O$_2$ in B. cinerea-infected leaves (Fig. 5A,B). However, more staining for superoxide anion and H$_2$O$_2$ in AtERF014-OE leaves while less in AtERF014-RNAi leaves was observed, as compared to that in WT (Fig. 5A,B). On the other hand, the expression levels of some selected defense genes including AtPR1, AtPR3, AtPR5 and AtPDF1.2 in AtERF014-OE and AtERF014-RNAi plants were comparable to those in WT without infection with Pst DC3000 or B. cinerea (Fig. 5C,D). As compared with those in WT, higher expression levels of AtPR1 and AtPR5 and a lowered expression level of AtPDF1.2 in AtERF014-OE plants were observed, whereas lowered expression levels of AtPR1 and AtPR5 and a higher expression level of AtPDF1.2 in AtERF014-RNAi plants were detected, at 24 hr after infection by Pst DC3000 (Fig. 5C,D). By contrast, lowered expression levels of AtPR1 and AtPR5 and a higher expression level of AtPDF1.2 in AtERF014-OE plants were seen, while higher expression levels of AtPR1 and AtPR5 and a lowered expression level of AtPDF1.2 in AtERF014-RNAi plants were observed, as compared to those in WT at 24 hr after infection with B. cinerea (Fig. 5C,D). There was no difference in pathogen-induced expression of AtPR3 among WT, AtERF014-OE and AtERF014-RNAi plants (Fig. 5C,D). Taken together, these
data suggest that altered expression of *AtERF014* affects the pathogen-induced defense responses including ROS accumulation and expression of defense genes in *AtERF014-OE* and *AtERF014-RNAi* plants.

**Altered expression of *AtERF014* affects flg22-triggered immune response in *AtERF014-OE* and *AtERF014-RNAi* plants.** To examine whether *AtERF014* has a function in Arabidopsis PTI response, we compared the occurrence of flg22-induced ROS burst and flg22-induced expression of PTI marker genes among WT, *AtERF014-OE* and *AtERF014-RNAi* plants. In ROS burst assay, no significant ROS burst was observed in WT, *AtERF014-OE* and *AtERF014-RNAi* leaves without treatment of flg22 (Fig. 6A). Compared with a significant flg22-induced ROS burst in WT leaves at 6 min, much enhanced flg22-induced ROS bursts were seen in *AtERF014-OE* leaves while only a much reduced ROS burst was detected in *AtERF014-RNAi* leaves after addition of flg22 (Fig. 6A). Similarly, the expression levels of *FLG22-INDUCED RECEPTOR-LIKE KINASE 1 (AtFRK1)* and *AtWRKY53*, two well-known PTI-responsive genes,48,49, in *AtERF014-OE* and *AtERF014-RNAi* leaves were comparable to those in WT without flg22 treatment or at 0 hr after addition of flg22 (Fig. 6B,C), suggesting that altered expression of *AtERF014* did not affect the expression of these two PTI genes. At 60 min after treatment, flg22 significantly upregulated the expression of *AtFRK1* and *AtWRKY53* in WT, *AtERF014-OE* and *AtERF014-RNAi* leaves with distinct patterns (Fig. 6B,C). As compared with the levels in flg22-treated WT leaves, the flg22-upregulated expression of *AtFRK1* and *AtWRKY53* in *AtERF014-OE* leaves was further increased, resulting in 1.4–1.6 times and 4–5 times of increases, whereas the expression of these two genes in *AtERF014-RNAi* leaves was markedly suppressed, leading to 25–30% and 50–60% of reduction (Fig. 6A,B). These data indicate that overexpression of *AtERF014* strengthens while suppression of *AtERF014* attenuates the flg22-induced PTI response, implying a function for *AtERF014* in PTI response in Arabidopsis.

**Suppression of *AtERF014* attenuates the SA-induced defense response in *AtERF014-RNAi* plants.** To explore whether *AtERF014* is required for SA-induced defense response, we evaluated the SA-induced resistance between *AtERF014-RNAi* and WT plants by comparing disease phenotype and bacterial population in water- and SA-treated plants after inoculation with *Pst* DC3000. SA-treated WT plants showed less disease than water-treated plants at 4 dpi (Fig. 7A). Disease in SA-treated *AtERF014-RNAi* plants was less than that in water-treated plants but was much severe than that in SA-treated WT plants (Fig. 7A). Accordingly, in planta growth of *Pst* DC3000 in inoculated leaves was consistent with the disease severity observed. At 0 dpi, no significant difference in bacterial growth was seen between *AtERF014-RNAi* and WT plants treated with or without 1 mM SA. At 3 dpi, the bacterial titers in inoculated leaves of water-treated WT, *AtERF014-RNAi-1* and *AtERF014-RNAi-2* plants increased to $1.10 \times 10^7$ CFU/cm², $1.12 \times 10^8$ CFU/cm² and $2.04 \times 10^8$ CFU/cm²,
respectively, whereas the titers in inoculated leaves of SA-treated WT, AtERF014-RNAi-1 and AtERF014-RNAi-2 plants were 8.51 $\times$ 10^5 CFU/cm^2, 2.29 $\times$ 10^7 CFU/cm^2 and 2.88 $\times$ 10^7 CFU/cm^2, respectively, showing 12.1, 5.6 and 6.7 times lower than those in corresponding water-treated plants (Fig. 7B). The expression levels of AtPR1 and AtPR5 in AtERF014-RNAi plants were comparable to those in WT without SA treatment; their expression was significantly induced by SA in both AtERF014-RNAi and WT plants (Fig. 7C). However, the SA-induced expression of AtPR1 and AtPR5 in AtERF014-RNAi plants was weakened as compared to those in WT (Fig. 7C). These results indicate that suppression of AtERF014 weakens the SA-induced defense response in AtERF014-RNAi plants.

Altered expression of AtERF014 affects the pectin content and expression of pectin biosynthetic genes in AtERF014-OE and AtERF014-RNAi plants. It was previously reported that overexpression of AtERF012 (DREB26) resulted in deformed plants, indicating an involvement in developmental processes^56. AtERF06 (AtRAP2.1) was found to function as a transcriptional repressor that plays a role in abiotic stress response^57. AtERF011 (AtDEAR1) acts as an upstream regulator that mediates plant defense and freezing

**Discussion**

Fifteen ERF TFs are classified into group II^21 and some of them have recently been functionally characterized. Overexpression of AtERF012 (DREB26) resulted in deformed plants, indicating an involvement in developmental processes^56. AtERF06 (AtRAP2.1) was found to function as a transcriptional repressor that plays a role in abiotic stress response^57. AtERF011 (AtDEAR1) acts as an upstream regulator that mediates plant defense and freezing
stress responses in Arabidopsis while AtERF018 seems to be involved in JA signaling pathway in response to wounding. In the present study, we explored in detail the biological function of AtERF014 in immunity against different pathogens using AtERF014-OE and AtERF014-RNAi lines and found that AtERF014 acts as a dual regulator that differentially modulates immunity against *Pst* DC3000 and *B. cinerea*. These findings, together with a

**Figure 5.** Altered pathogen-induced accumulation of ROS accumulation and expression of defense genes in AtERF014-OE and AtERF014-RNAi plants after infection with *P. syringae pv. tomato* DC3000 or *B. cinerea*. Four-week-old plants were inoculated with *Pst* DC3000, *B. cinerea* spore suspension or similar volume of solutions as mock controls. Leaf samples were collected at 24 hr after inoculation. (A,B) **In situ** detection of superoxide anion (A) and H$_2$O$_2$ (B) accumulation in leaves of inoculated plants, detected by NBT or DAB staining, respectively. (C,D) Changes in expression of defense genes in AtERF014-OE and AtERF014-RNAi plants after infection with *Pst* DC3000 (C) or *B. cinerea* (D). Data presented are the means ± standard deviation from three independent experiments and * above the columns indicate significant differences at $p < 0.05$ level between AtERF014-OE/AtERF014-RNAi and WT plants.
previous observation that AtERF014 is involved in pectin deposition in cultured Arabidopsis cells, demonstrate a clear biological function of AtERF014 in biotic stress response.

Group II of the ERF subfamily consists of three subgroups, IIa, IIb, and IIc. ERF TFs in this group all contain a common AP2/ERF domain at N-terminal and a conserved motif CMII-1 at C-terminals but also have several additional conserved motifs. The six IIa members contain a CMII-2 motif that is similar to the EAR (ethylene response factor-associated amphiphilic repression) motif, which functions as a repression domain. For instance, AtERF06 (AtRAP2.1) and AtERF011 (AtDEAR1) in subgroup IIa were experimentally demonstrated to be active transcriptional repressors that repress the transcription of some stress-responsive genes whose promoters harbor DRE (dehydration-responsive elements)/CRT (dehydration-responsive element) cis-elements. By contrast, the members in subgroup IIb, except AtERF015, have a CMII-3 motif at the C-terminal instead of the EAR-like CMII-2 motif. ERF TFs lacking an EAR motif are expected to act as transcriptional activators. We found that AtERF014 displayed transactivation activity in yeasts, implying that AtERF014 may be a transcriptional activator. Another member of subgroup IIb, AtERF012 (DREB26), was also found to have transactivation activity in yeast. Thus, it seems likely that the group II ERFs have distinct biochemical activity and can act as transcriptional activators or repressors. Further transactivation assays in vivo systems like Arabidopsis protoplasts or tobacco leaves are required to confirm the transactivation activity of AtERF014 and its closely related members in group II.

We showed in the present study that AtERF014 plays differential functions in immunity against *Pst* DC3000 and *B. cinerea*, demonstrating that AtERF014 is a dual regulator of immunity against these two pathogens. It is generally accepted that immune response against (hemi)biotrophic pathogens such as *Pst* DC3000 is modulated...
through the SA signaling while immune response against necrotrophic pathogens like *B. cinerea* is regulated by the JA/ET signaling. Antagonistic interaction between the SA and JA/ET signaling pathways often occurs and allows plants to mount appropriate defense responses against different invading pathogens. The expression of *AtERF014* was induced by *Pst* DC3000 and *B. cinerea* as well as by SA and MeJA (Figs. 1A–C). However, overexpression of *AtERF014* led to increased resistance to *Pst* DC3000 but decreased resistance to *B. cinerea*, whereas suppression of *AtERF014* resulted in decreased resistance to *Pst* DC3000 but increased resistance to *B. cinerea* (Figs 3 and 4), indicating that *AtERF014* is a positive regulator of immunity to *Pst* DC3000 but a negative regulator of immunity to *B. cinerea*. This is similar to the functions of *AtERF1*, *AtERF5* and *AtERF6*, which have opposite functions in immunity against *Pst* DC3000 and necrotrophic fungi including *B. cinerea* and *Alternaria brassicicola*. The expression of SA-responsive and JA-responsive defense genes exhibited opposite patterns in *AtERF014*-OE and *AtERF014*-RNAi plants after infection of *Pst* DC3000 (Fig. 5C), indicating that *AtERF014* is involved in modulating an antagonistic interaction between the SA and JA/ET signaling pathways, resulting in the expression of corresponding defense genes. It was previously shown that some of the B3 group members including ERF1, *AtERF5*, *AtERF6*, *AtERF14* and ORA59 act as regulators of the JA/ET signaling pathway. In contrast, only a few of ERFs, e.g. *AtERF15* and *AtERF5*, were shown to be involved in the SA signaling pathway. The involvement of *AtERF014* in the SA signaling pathway can be further validated by the facts that the SA-induced *Pst* DC3000 resistance and SA-upregulated expression of defense genes were partially suppressed in *AtERF014*-RNAi plants (Fig. 7). The pathogen-induced ROS in *AtERF014*-OE plants (Fig. 5A,B) may function differentially in immunity against *Pst* DC3000 or *B. cinerea*. In the *AtERF014*-OE plants, the pathogen-induced ROS may largely contribute to signaling in immunity against *Pst* DC3000, whereas this pathogen-induced ROS may benefit the infection by *B. cinerea*, correlating with the altered resistance against these pathogens (Figs 3 and 4). Notably, *B. cinerea* did not induce significant accumulation of H$_2$O$_2$ but caused...
remarkable accumulation of superoxide anion in infected Arabidopsis plants (Fig. 5A, B). It was suggested that the kinetics of ROS accumulation is the better indicator of defense response than the absolute levels of ROS in many interactions. Quantitative analysis of the kinetics of ROS accumulation in AtERF014-OE and AtERF014-RNAi plants after pathogen infection should clarify the involvement of H$_2$O$_2$ in AtERF014-mediated defense response against _B. cinerea_. In addition, the flg22-induced ROS burst and the expression of PTI marker genes _AtFRK1_ and _AtWRKY53_ were fostered in AtERF014-OE plants but was weakened in AtERF014-RNAi plants (Fig. 6), demonstrating that AtERF014 plays a role in innate immunity. AtERF5 and AtERF15 were also found to be involved in flg22- or chitin-induced PTI.

Plant cell walls consist of three major components cellulose, hemicelluloses and pectins, and pectins make up approximately 50% of Arabidopsis leaf cell walls. It was previously shown that overexpression of _AtERF014_ coordinately activated the pectin biosynthetic pathway genes and increases the pectin content in cultured Arabidopsis cells. We found in the present study that the pectin content in AtERF014-RNAi plants was significantly decreased due to the downregulated expression of pectin biosynthetic gene such as _AtASX2_, _AtGAE1_, _AtMUM4_, _AtQUA1_ and _AtUGP1_. Although overexpression of _AtERF014_ upregulated the expression of these pectin biosynthetic genes, the pectin content in AtERF014-OE plants was comparable to that in WT (Fig. 8). This is contrary to the observation in cultured Arabidopsis cells, where overexpression of _AtERF014_ led to increased pectin content.

Plant cell walls act as preformed structural barriers against pathogen entry and modifications of cell walls can induce plant defense responses, which are able to reduce pathogen penetration and growth. In the present study, we found that suppression of _AtERF014_ resulted in reduced pectin content in AtERF014-RNAi plants as compared to WT (Fig. 8A); however, the contribution of the reduced pectin content to the resistance against _Pst_ DC3000 and _B. cinerea_ may differ (Figs 3 and 4). It seems reasonable that the reduced pectin content in AtERF014-RNAi plants may be responsible for the decreased resistance against _Pst_ DC3000, as decrease in pectin content should weaken the cell wall strength and integrity and thereby make it more susceptible to _Pst_ DC3000 infection. This is in agreement with a recent observation that mutation in _GAE6_, coding for a glucuronate 4-epimerase involved in the biosynthesis of pectin, impaired the immunity to _P. syringae pv. maculicola_ ES4326. However, the Arabidopsis _powdery mildew-resistant_ (pmr) _5_ and _pmr6_ mutant plants, whose cell walls contain enriched pectin, are fully susceptible to _Pst_ DC3000, as the WT plants, indicating that increased pectin content in _pmr5_ and _pmr6_ plants is not responsible for the resistance to this bacterial pathogen. On the other hand, most of the necrotrophic fungal pathogens like _B. cinerea_ secrete a variety of cell wall-degrading enzymes including polygalacturonases that can degrade pectin in cell walls during pathogenesis. _B. cinerea_ was also shown to metabolize pectin and probably utilize pectin as a carbon source during _in planta_ growth. In this regard, reduced pectin content in AtERF014-RNAi plants (Fig. 8A) may contribute to increased resistance to _B. cinerea_ (Fig. 4A), as the AtERF014-RNAi plants supported less _in planta_ growth of _B. cinerea_ in comparison to that in WT (Fig. 4B). This finding is different from the observation that the _gae1 gae6_ plants, which are reduced in pectin.

![Figure 8](image-url)
content, impaired the resistance to specific *B. cinerea* isolates and allowed enhanced *in planta* growth of these specific isolates\(^6^9\). The AtERF014OE plants had similar pectin content to WT (Fig. 8A) but showed enhanced susceptibility to *B. cinerea* and *in planta* fungal growth (Fig. 3A,B), indicating that utilization of pectin as a carbon source may not contribute to immunity against *B. cinerea*. It is likely that the pectin content itself may not be a contributing factor that determines the immunity to pathogens; instead, the degree and pattern of pectin modification such as methylesterification are critical to immunity against *P. syringae*\(^74\)–\(^76\) or *B. cinerea*\(^2\)\(^7\)\(^8\). It was previously shown that pectin fragments known as oligogalacturonides can trigger plant immune responses\(^69\),\(^79\),\(^80\). How AtERF014 regulates the biosynthesis of pectin and thereby the immunity against different pathogens need to be further investigated.

The involvement of ERFs in plant growth and development has been well-documented\(^81\). No morphological and developmental changes (Fig. 2) and altered expression of defense genes (Fig. 5C,D) were introduced into yeast strain AH109 and the yeast transformants were plated and grown on SD/Trp– medium. For construction of overexpression vector, the resulting plasmid pMD19-AtERF014 was used as templates for all experiments described below. For construction of RNAi vector, a fragment was amplified with a pair of primers AtERF014-RNAi-F (5′-GCG GAATTC ATG GTG AAA ACA CTT-3′) and AtERF014-RNAi-R (5′-ATA CCCGGG TCA GCA GAA GTT CCA TAA-3′), a Sall site underlined) and inserted into pCAMBIA 99-1 under the control of CaMV 35S promoter. For construction of RNAi vector, a fragment was amplified with a pair of primers AtERF014-RNAi-F (5′-GCG GAATTC ATG GTG AAA ACA CTT-3′) and AtERF014-RNAi-R (5′-ATA CCCGGG TCA GCA GAA GTT CCA TAA-3′), a Sall site underlined) and inserted into pCAMBIA 99-1 under the control of CaMV 35S promoter. For construction of RNAi vector, a fragment was amplified with a pair of primers AtERF014-RNAi-F (5′-GCG GAATTC ATG GTG AAA ACA CTT-3′) and AtERF014-RNAi-R (5′-ATA CCCGGG TCA GCA GAA GTT CCA TAA-3′), a Sall site underlined) and inserted into pCAMBIA 99-1 under the control of CaMV 35S promoter. For construction of RNAi vector, a fragment was amplified with a pair of primers AtERF014-RNAi-F (5′-GCG GAATTC ATG GTG AAA ACA CTT-3′) and AtERF014-RNAi-R (5′-ATA CCCGGG TCA GCA GAA GTT CCA TAA-3′), a Sall site underlined) and inserted into pCAMBIA 99-1 under the control of CaMV 35S promoter. For construction of RNAi vector, a fragment was amplified with a pair of primers AtERF014-RNAi-F (5′-GCG GAATTC ATG GTG AAA ACA CTT-3′) and AtERF014-RNAi-R (5′-ATA CCCGGG TCA GCA GAA GTT CCA TAA-3′), a Sall site underlined) and inserted into pCAMBIA 99-1 under the control of CaMV 35S promoter. For construction of RNAi vector, a fragment was amplified with a pair of primers AtERF014-RNAi-F (5′-GCG GAATTC ATG GTG AAA ACA CTT-3′) and AtERF014-RNAi-R (5′-ATA CCCGGG TCA GCA GAA GTT CCA TAA-3′), a Sall site underlined) and inserted into pCAMBIA 99-1 under the control of CaMV 35S promoter. For construction of RNAi vector, a fragment was amplified with a pair of primers AtERF014-RNAi-F (5′-GCG GAATTC ATG GTG AAA ACA CTT-3′) and AtERF014-RNAi-R (5′-ATA CCCGGG TCA GCA GAA GTT CCA TAA-3′), a Sall site underlined) and inserted into pCAMBIA 99-1 under the control of CaMV 35S promoter. For construction of RNAi vector, a fragment was amplified with a pair of primers AtERF014-RNAi-F (5′-GCG GAATTC ATG GTG AAA ACA CTT-3′) and AtERF014-RNAi-R (5′-ATA CCCGGG TCA GCA GAA GTT CCA TAA-3′), a Sall site underlined) and inserted into pCAMBIA 99-1 under the control of CaMV 35S promoter. For construction of RNAi vector, a fragment was amplified with a pair of primers AtERF014-RNAi-F (5′-GCG GAATTC ATG GTG AAA ACA CTT-3′) and AtERF014-RNAi-R (5′-ATA CCCGGG TCA GCA GAA GTT CCA TAA-3′), a Sall site underlined) and inserted into pCAMBIA 99-1 under the control of CaMV 35S promoter. For construction of RNAi vector, a fragment was amplified with a pair of primers AtERF014-RNAi-F (5′-GCG GAATTC ATG GTG AAA ACA CTT-3′) and AtERF014-RNAi-R (5′-ATA CCCGGG TCA GCA GAA GTT CCA TAA-3′), a Sall site underlined) and inserted into pCAMBIA 99-1 under the control of CaMV 35S promoter.
collected 24 hr later. Fluorescence signals were excited at 488 nm and detected under a confocal laser scanning microscope (LSM 510 Meta, Zeiss, Oberkochen, Germany) using a 500–530 nm emission filter.

**Disease assays and measurement of in planta pathogen growth.** *Pst* DC3000 was grown in King’s B (KB) broth and collected by centrifugation, followed by re-suspending in 10 mM MgCl₂ solution to OD₆₀₀ = 0.002. The bacterial inoculation was performed by hand infiltration using 1-ml syringes without needle into rosette leaves and the inoculated plants were kept in sealed containers to facilitate disease development under high humidity. For quantification of *in planta* bacterial growth, leaf discs from inoculated leaves were collected at different time points and homogenized in 10 mM MgCl₂. After a series of gradient dilutions, the homogenate was subjected to statistical analysis using the Student’s t-test at p = 0.05 level.

**Measurement of ROS burst and in situ detection of ROS accumulation.** Measurement of ROS burst was carried out using a luminol-based luminescence method⁴⁶. Briefly, 4-mm leaf discs were floated in 200 μL H₂O overnight and then transferred into 100 μL 100 nM flg22 solution containing 34 μg/ml lumino (Sigma, St. Louis, MO, USA) and 20 μg horseradish peroxidase (Sigma, St. Louis, MO, USA) or in solution without luminol as controls. Luminescence was recorded at a 2 min interval over 20 min using a Synergy HT plate reader (Biotek Instruments Inc. Winooski, VT, USA). *In situ* detection of superoxide anion and H₂O₂ was carried out by NBT and DAB staining, respectively⁴⁵. Accumulation of ROS in stained leaves was visualized by a digital camera.

**Measurement of pectin content.** Pectin content was determined using a Pectin Measurement kit (Keming Biotechnology Co., Suzhou, China) according to the manufacturer’s instruction. Briefly, 100 mg of leaf tissues was immersed into 1 mL sterile water and then ground to homogeneate. After centrifugation at 8000 × g at 4 °C for 10 min, the supernatant was used for measurement of pectin.

**qRT-PCR analysis of gene expression.** Total RNA was extracted by Trizol reagent (Invitrogen, Shanghai, China) according to the manufacturer’s instruction. First-strand cDNA was synthesized from 1 μg total RNA using PrimerScript RT kit (TaKaRa, Dalian, China) following the supplier’s recommendation. Quantitative RT-PCR was prepared using SYBR Premix Ex Taq Kit (TaKaRa, Dalian, China) according to the manufacturer’s instruction. Briefly, 100 mg of leaf tissues was immersed into 1 mL sterile water and then ground to homogenate. After centrifugation at 8000 × g at 4 °C for 10 min, the supernatant was used for measurement of pectin.

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Author Contributions
F.S. conceived the study. H.Z. and F.S. designed the experiments. H.Z., Y.H., L.H. and D.L. performed the experiments. H.Z. and F.S. analyzed the data. F.S. drafted the manuscript, and all authors read and approved the final manuscript.

Additional Information
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