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Heterologous expression of Chinese wild grapevine *VqERFs* in *Arabidopsis thaliana* enhance resistance to *Pseudomonas syringae* pv. *tomato* DC3000 and to *Botrytis cinerea*

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ABSTRACT

When a plant is attacked by a pathogen, an immune response is activated to help protect it from harm. ERF transcription factors have been reported to regulate immune responses in plants. Here, three ERF transcription factors from Chinese wild *Vitis quinquangularis*, *VqERF112*, *VqERF114* and *VqERF072*, are shown to respond to pathogen inoculation by powdery mildew, *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 and *Botrytis cinerea* and to hormone treatments including with ET, SA, MeJA or ABA. Tissue specific expression analysis shows the highest expression levels of *VqERF112* and *VqERF114* were in mature berries and of *VqERF072* was in tendrils. A GUS activity assay indicates that the promoters of *VqERF112*, *VqERF114* and *VqERF072* can be induced by powdery mildew inoculation and by hormone treatment, including with ET, SA and MeJA. Overexpression of *VqERF112*, *VqERF114* and *VqERF072* in transgenic *Arabidopsis* enhanced the resistance to *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) and *B. cinerea*, and it increased the expression of the SA signaling-related genes *AtNPR1* and *AtPR1* and of the JA/ET signaling-related genes *AtPDF1.2*, *AtLOX3*, *AtPR3* and *AtPR4*. Compared to Col-0 plants, the H_2O_2 accumulation in transgenic *Arabidopsis* increased after *Pst* DC3000 inoculation but decreased after *B. cinerea* inoculation. These results demonstrate that *VqERF112*, *VqERF114* and *VqERF072* positively regulate resistance to *Pst* DC3000 and *B. cinerea*.

1. Introduction

A plant is able to sense changes in its immediate environment and to adjust its internal workings so as to minimize the negative impacts of those changes. As well as the regular diurnal and annual cycles of environmental factors such as light, temperature and water availability, plants are also able to respond to the more extreme excursions of these factors so as to minimize the associated stress. In addition to these abiotic stress factors, plants are also exposed to a range biotic stress factors. These are associated with their interactions with animals, with other plants and particularly with microorganisms - including with pathogens.

Plant pathogens can be divided into two major types according to their modes of nutrition [1,2]. The first are the biotrophic pathogens which gain their nutrition from the living tissues of a host. For grapevine, such pathogens include *Golovinomyces cichoracearum* and *Uncinula necator* (Schw.) Burr. [1,3]. The second type are the necrotrophic pathogens which gain their nutrition from dead (or dying) host tissues. Such pathogens include *Botrytis cinerea* [1,2]. Meanwhile, the hemi-biotroph pathogens present as both biotrophs and necrotrophs, their nutritional balance depending on the stage of their life cycle and on environmental conditions. Such hemi-biotroph pathogens include *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 [1].

Plants have evolved a two-tier immune system to protect themselves

Abbreviations: ERF, Ethylene Responsive Factor; *Pst* DC3000, *Pseudomonas syringae* pv. *tomato* DC3000; ET, ethylene; SA, salicylic acid; MeJA, methyl jasmonate; ABA, abscisic acid; GA, gibberellin; MAMPs/PAMPs, microbe/pathogen-associated molecular patterns; PRRs, pattern-recognition receptors; PTI, pattern-triggered immunity; NLR, nucleotide-binding leucine-rich repeat; ETI, effector-triggered immunity; PCD, programmed cell death; ROS, reactive oxygen species; SAR, systemic acquired resistance; H_2O_2, hydrogen peroxide; JA, jasmonic acid; *NPR1*, *NON-EXPRESSOR OF PR GENES 1*; *PR1*, *PATHOGENESIS-RELATED PROTEIN 1*; *ICS*, *ISOCHORISMATE SYNTHASE*; *PR5*, *THAUMATIN-LIKE PROTEIN*; *PDF1.2*, *PLANT DEFENSIN 1.2*; *LOX3*, *LIPOXYGENASE 3*; *PR3*, *BASIC CHITINASE*; *PR4*, *HEVEIN-LIKE*; qRT-PCR, quantitative realtime polymerase chain reaction; OE, Overexpressing; DAB, 3,3′-diaminobenzidine; cDNA, complementary DNA; pro, promoter

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from pathogen attack [4]. The first tier is triggered by microbe/pathogen-associated molecular patterns (MAMPs/PAMPs) and is governed by cell surface pattern-recognition receptors (PRRs) which activate a basal resistance response called pattern-triggered immunity (PTI) [5,6]. The second tier is triggered by pathogen effectors and is mediated by nucleotide-binding leucine-rich repeat (NLR) receptors encoded by *R* genes which activate effector-triggered immunity (ETI) [7,8]. During PTI and ETI responses, plants trigger a variety of immune reactions. These include programmed cell death (PCD) [9], the accumulation of reactive oxygen species (ROS) [10,11] and the production of hormones [12]. When plants are attacked by pathogens, systemic acquired resistance (SAR) is triggered, to enhance the resistance of systemic tissues to pathogens [11]. ROS, such as hydrogen peroxide (H_2O_2), may be linked to PCD in the hypersensitive response and may also interact with salicylic acid (SA) signaling in SAR [13]. There are three major plant hormones - SA, jasmonic acid (JA) and ethylene (ET). These represent cellular signal molecules which participate in the regulation of plant defense to pathogens. The SA signaling pathway is connected with biotrophic pathogens, while the JA/ET signaling pathway is effective against necrotrophic pathogens [12]. Meanwhile, some hormone-specific defense genes are induced during the immune responses. *ISOCHORISMATE SYNTHASE* (*ICS*) is involved in SA synthesis and *NON-EXPRESSOR OF PR GENES 1* (*NPR1*) controls SA signaling downstream [12]. The pathogen-inducible genes *PATHOGENESIS-RELATED PROTEIN 1* (*PR1*) and *THAUMATIN-LIKE PROTEIN* (*PR5*) can be activated by SA signaling [14]. *LIPOXYGENASE 3* (*LOX3*) is involved in JA synthesis and *PLANT DEFENSIN 1.2* (*PDF1.2*), *BASIC CHITINASE* (*PR3*) and *HEVEIN-LIKE* (*PR4*) are induced by JA/ET in *Arabidopsis* [14,15].

China is one of three major centers of origin for *Vitis* spp. The Chinese wild grapevines offer many valuable genes that play key roles in pathogen immunity. The Chinese wild grapevine, *Vitis quinquangularis* accession 'Danfeng-2' is an important germplasm resource with high resistance to powdery mildew [16,17].

The AP2/ERF superfamily is an important transcription factor family in plants. It is defined by the conserved AP2/ERF domain which is involved in DNA binding [18,19]. Based on the number of AP2 domains, the AP2/ERF superfamily has been divided into subfamilies - the AP2 subfamily, the DREB subfamily, the ERF subfamily and the RAV subfamily [20]. The proteins of the ERF subfamily contain one conserved AP2 domain [21] and specifically bind to the GCC-box, a DNA sequence conserved in the promoters of ethylene-inducible pathogenesis-related protein genes in plants [19]. ERF subfamily proteins have been reported to mediate hormone defense networks and are essential for the plant response to pathogen infections and abiotic stresses [22,23].

In our previous study, expression profiles of ERF subfamily genes from 'Danfeng-2' in response to *U. necator* were analyzed by qRT-PCR. Three ERF transcription factors, designated *VqERF112*, *VqERF114* and *VqERF072*, were found in response to *U. necator*. In this study, we explored the functions of *VqERF112*, *VqERF114* and *VqERF072* from Chinese wild *V. quinquangularis* accession 'Danfeng-2' in immune response to *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) and *B. cinerea*. All three ERF transcription factors responded to inoculation with powdery mildew, *Pst* DC3000 and *B. cinerea*. *VqERF112*, *VqERF114* and *VqERF072* also responded to different hormone treatments, such as to ET, SA, methyl jasmonate (MeJA) and abscisic acid (ABA). A promoter GUS activity assay demonstrated that promoters of *VqERF112*, *VqERF114* and *VqERF072* could be induced by powdery mildew inoculation and by hormone treatment. Overexpressing (OE) transgenic *Arabidopsis* lines were used to investigate the functions of *VqERF112*, *VqERF114* and *VqERF072* in the immune responses to *Pst* DC3000 and *B. cinerea*. Compared with Col-0 plants, more intense cell death and higher levels of H_2O_2 accumulation were observed in *VqERF112*-OE, *VqERF114*-OE and *VqERF072*-OE plants after *Pst* DC3000 inoculation. However, larger lesions and higher levels of H_2O_2 were observed in Col-0 plants than in OE transgenic *Arabidopsis* plants after *B. cinerea* inoculation. Meanwhile, after pathogen inoculation, the expressions of SA- and JA/ET-responsive defense genes were more strongly altered in transgenic plants than in Col-0 plants. Our results demonstrate that heterologous expressions of *VqERF112*, *VqERF114* and *VqERF072* in *Arabidopsis* enhanced resistance against *Pst* DC3000 and *B. cinerea* via the SA and JA/ET signaling pathways.

2. Materials and methods

2.1. Plant materials

The Chinese wild *V. quinquangularis* accession 'Danfeng-2' and *V. vinifera* L. cv. Thompson seedless were grown in the grape germplasm collection at Northwest A&F University, Yangling, Shaanxi, China. The young leaves, mature leaves, inflorescence, young berries (25 days after anthesis), mature berries (80 days after anthesis) and tendrils of 'Danfeng-2' were sampled for tissue-specific expression analysis [24]. *Arabidopsis thaliana* ecotype Col-0 seeds were germinated and transferred to the soil in a chamber at 23 °C under a light/dark cycle of 16/8 h.

2.2. Gene cloning and sequence analysis

Full length coding sequences of *VqERF112*, *VqERF114* and *VqERF072* were isolated using the cDNA of 'Danfeng-2' as template and the special primers listed in Table S1. The phylogenetic tree of three ERF proteins and ERF subfamily proteins from grapevine and *Arabidopsis* was constructed using MEGA 5.0 software.

2.3. Pathogen inoculation and hormone treatments

Pseudomonas syringae pv. *tomato* (*Pst*) DC3000 was grown at 28 °C in liquid LB medium (supplemented with 50 mg/L rifampicin) overnight, then diluted to an OD_{600} of 0.02 with infiltration buffer (10 mM MES pH 5.8, 10 mM $MgCl_2$). The rosette leaves of four-week-old transgenic *Arabidopsis* and ecotype Col-0 were infiltrated with bacterial suspension, then harvested at 0, 24, 48 and 72 h after inoculation for qRT-PCR analysis.

Botrytis cinerea inoculation was carried out according to the method previously described [25] with minor modifications. The *B. cinerea* was cultured on Potato Dextrose Agar medium in the dark at 25 °C for 21 days. Conidia were washed down with distilled water containing 4 % maltose and 1 % peptone, then adjusted to a final concentration of 2.0 $\times$ 10^6 spores/ml. Ten detached rosette leaves from each transgenic line were hanging dropped with 10 μl conidia suspension on the adaxial surface and placed in trays. The trays were covered with a preservative film to keep the relative humidity high (90–100 %) and placed in a chamber for three days. Leaves were collected to measure lesion perimeter and stained with trypan blue and 3,3′-diaminobenzidine (DAB). Meanwhile, leaves of Col-0 plants and transgenic *Arabidopsis* plants were uniformly sprayed with the conidia suspension, then harvested at 0, 24, 48 and 72 h after inoculation for qRT-PCR analysis.

The pathogen *U. necator* was collected from the leaves of susceptible *V. vinifera* cultivars. The leaves of 'Danfeng-2' and Thompson seedless were inoculated with powdery mildew according to the methods previously described [16] and collected at 0, 12, 24, 48, 72, 96 and 120 h after inoculation. The leaves of 'Danfeng-2' and Thompson seedless were uniformly sprayed with *Pst* DC3000 bacterial suspension and *B. cinerea* conidia suspension, then sampled at 0, 24, 48 and 72 h after inoculation. The leaves of 'Danfeng-2' were sprayed with 0.5 g/L ET and 100 μM SA, MeJA, ABA or gibberellin (GA) [26,27]. The leaves were sampled at 0, 0.5, 1, 2, 6 and 10 h after hormone treatments for qRT-PCR analysis. The mock control leaves were sprayed with distilled water.

2.4. qRT-PCR analysis

The stored samples of 'Danfeng-2', Thompson seedless and *Arabidopsis* were ground with liquid nitrogen and total RNA was extracted using a Plant RNA Kit (OMEGA). Total RNA (1 μg) was used to reverse transcribe into complementary DNA (cDNA) with the FastKing RT Kit (With gDNase) (TIANGEN) following the manufacture's protocol. Ten-fold diluted synthetic cDNA was used as template. The PCR reaction mix (20 μl) contained 1 μl cDNA template, 10 μl SYBR (novoprotein), 10 μM of each primer and sterile water. Three step qRT-PCR reaction procedures were run following the manufacture's protocol for NovoStart® SYBR qPCR SuperMix Plus (novoprotein). Grapevine *GAPDH* and *VqActin* genes and *Arabidopsis AtActin* gene were used for normalization and three replicates were analyzed for each sample. The qRT-PCR primers are listed in Table S1. The experimental data were analyzed using the $2^{-\Delta\Delta Ct}$ method.

2.5. Promoters cloning of ERF112, ERF114 and ERF072

The special primers used for promoter cloning were designed according to the reference genome of *V. vinifera* cv. 'Pinot Noir' genome database P40024 and are listed in Table S1. The promoters of *ERF112*, *ERF114* and *ERF072* from 'Danfeng-2' and Thompson seedless were amplified and the Plant-CARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to predict the conserved *cis*-element motifs in each promoter.

2.6. GUS activity assay

The promoters of *ERF112*, *ERF114* and *ERF072* from 'Danfeng-2' and Thompson seedless were inserted into the pC0380-GUS vector and transferred into the GV3101 strain of *Agrobacterium tumefaciens*. Transient expression in leaves of Thompson seedless was carried out following the method previously described [28] with minor modifications. Bacterial cells carrying either the fusion constructs or the control (pC0380-GUS and CaMV35S-GUS) were overnight cultured at 28 °C, pelleted by centrifugation and resuspended with infiltration buffer (10 mM MES pH 5.8, 10 mM $MgCl_2$, 200 μM acetosyringone) to an OD_{600} of 0.4. The leaves of Thompson seedless were transiently infiltrated with resuspended GV3101 cells via vacuum infiltration. The infiltrated leaves were cultured in a chamber for two days, and then inoculated with powdery mildew or sprayed with 0.5 g/L ET, 100 μM SA or MeJA. The mock control was treated with ddH_2O. After cultured for one day, the leaves were collected for GUS staining and protein determination according to the methods previously described [28]. In this assay, CaMV35S-GUS was used as positive control and pC0380-GUS empty vector was used as negative control.

2.7. Generation and characterization of transgenic lines

Full length coding sequences without the stop codons of *VqERF112*, *VqERF114* and *VqERF072* were amplified using the cDNA of 'Danfeng-2' as template and cloned into the pC2300-GFP vector to be fused with GFP reporter. The fusion construct was transferred into *A. tumefaciens* strain GV3101 using electroporation. The bacterial cells were cultured overnight, pelleted by centrifugation, and then resuspended with infiltration buffer (5 % sucrose solution, 0.025 % Silwet L-77) to an OD_{600} of 0.6-0.8. *Agrobacterium* cultures were incubated at 28 °C for 3 h. The floral dip method was used for *Arabidopsis* transformation [29]. T3 homozygous transgenic plants were selected on MS medium containing 50 mg/L kanamycin. Three independent overexpression T3 lines were characterized by qRT-PCR.

2.8. Disease assays

Three days after *Pst* DC3000 inoculation, three leaves from each independent line were selected and two leaf discs (0.5 cm diameter) from each leaf were harvested for bacterial titer determination. Six leaf discs from each independent line were collected into 1.5 ml centrifuge tubes and ground with 1 ml sterile water. The liquid was diluted 10^4-10^6 times and 100 μl of the diluent was cultured on solid LB medium supplemented with 50 mg/L rifampicin at 28 °C for 2–3 d. The bacteria number (cfu/cm^2) was measured for disease assays.

2.9. Detection of cell death and H_2O_2 accumulation

To observe cell death, the rosette leaves were harvested three days after infiltration with *Pst* DC3000 bacterial suspension or inoculation with *B. cinerea* conidia suspension. Leaves were boiled in trypan blue solution (10 ml lactic acid, 10 ml phenol, 10 ml glycerol, 10 ml sterile water, 60 ml ethanol and 0.067 g trypan blue) for 2−5 min, were maintained at room temperature for 1 h and then transferred to 2.5 g/ml chloral hydrate solution for destaining.

To visualize H_2O_2 accumulation, the rosette leaves were harvested three days after infiltration with *Pst* DC3000 bacterial suspension or inoculation with *B. cinerea* conidia suspension. The leaves were stained with DAB (1 mg/ml, pH 3.8) under light condition for 8 h, then transferred to 95 % ethanol for destaining.

2.10. Statistical analysis

Statistical significance analysis used Student's two-tailed *t* test (*, $P < 0.05$; **, $P < 0.01$). Results are indicated as mean values from three biological replicates. Error bars indicate the SD.

3. Results

3.1. Homologous analysis of VqERF112, VqERF114 and VqERF072

It has been reported that there are 73 predicted ERF subfamily proteins in grapevine and 65 in *Arabidopsis* [30]. The phylogenetic tree of VqERF112, VqERF114 and VqERF072 with ERF subfamily proteins from *V. vinifera* and *Arabidopsis* was constructed using the neighbor-joining method. As shown in Fig. S1, VqERF112 is closely related to VvERF112 (Gene ID: VIT_01s0150g00120) in grapevine and AtRAP2.6 L (Gene ID: AT5G13330.1) in *Arabidopsis*. VqERF114 is closely related to VvERF114 (Gene ID: VIT_18s0072g00260) in grapevine and AtABR1 (Gene ID: AT5G64750.1) in *Arabidopsis*. VqERF072 is closely related to VvERF072 (Gene ID: VIT_15s0021g01630) in grapevine and AtERF2 (Gene ID: AT5G47220.1) in *Arabidopsis*.

3.2. Expressions of ERF112, ERF114 and ERF072 respond to pathogens under artificial inoculation

The expression profiles of *ERF112*, *ERF114* and *ERF072* in 'Danfeng-2' and Thompson seedless in response to pathogen inoculation were analyzed by qRT-PCR. Compared to the mock control, the abundance of *VqERF112* transcript increased 1.6-fold by 24 h after powdery mildew inoculation, it peaked at 72 h and then decreased gradually. Compared to the mock control, the abundance of *VvERF112* transcript was lower at 24 h, 72 h and 96 h but peaked 1.4-fold higher at 48 h. Compared to the mock control, the expression of *VqERF114* was lower at 12 h but then increased rapidly at 24 h and peaked at 120 h by 89.2-fold. Compared to the mock control, the expression of *VvERF114* decreased at 12 h, then increased from 24 h to 120 h and peaked at 48 h by 1.6-fold. Compared to the mock control, the transcript level of *VqERF072* was induced significantly at 72 h and 120 h by 2.7-fold and 13.4-fold, respectively. Compared to the mock control, the transcript level of *VvERF072* increased from 24 h to 120 h and peaked at 24 h by 1.6-fold. These results indicated that the expressions of *ERF112*, *ERF114* and *ERF072* respond to powdery mildew inoculation. Moreover, the expression of *VqERF112*, *VqERF114* and *VqERF072* in 'Danfeng-2'

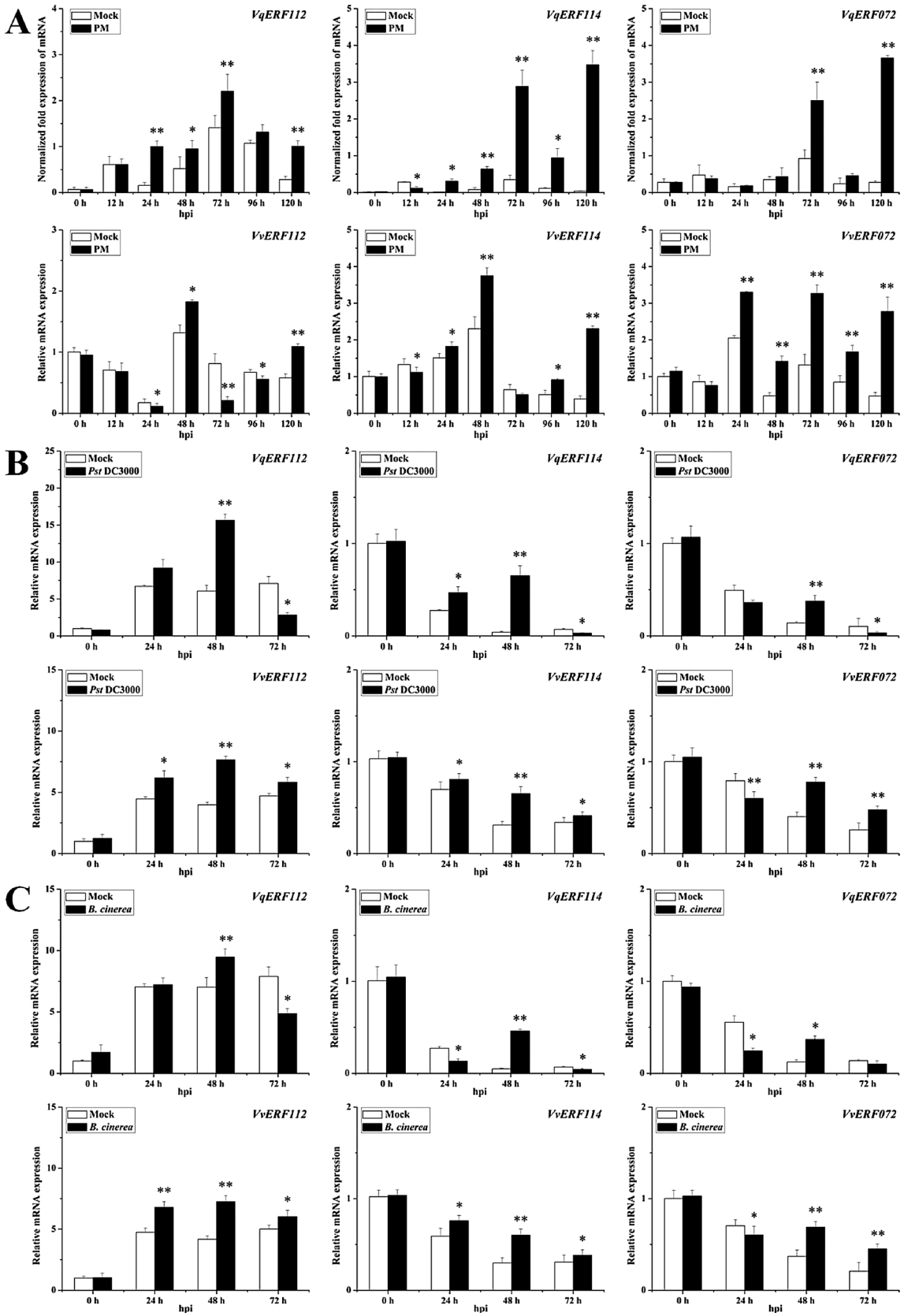

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Fig. 1. Expression profiles of ERF112, ERF114 **and** ERF072 **in response to *U. necator*, *Pst* DC3000 and *Botrytis cinerea* inoculation of Chinese wild *V. quinquangularis* accession 'Danfeng-2' and *V. vinifera* L. cv. Thompson seedless.** (A) Expression analysis of ERF112, ERF114 and ERF072 in response to powdery mildew determined by qRT-PCR. Leaves of 'Danfeng-2' and Thompson seedless were inoculated with *U. necator* and samples were collected at seven time points. PM, powdery mildew inoculation. (B) Expression analysis of ERF112, ERF114 and ERF072 in response to *Pst* DC3000 determined by qRT-PCR. The leaves of 'Danfeng-2' and Thompson seedless were sprayed with *Pst* DC3000 bacterial suspension and samples were collected at four time points. (C) Expression analysis of ERF112, ERF114 and ERF072 in response to *B. cinerea* determined by qRT-PCR. The leaves of 'Danfeng-2' and Thompson seedless were sprayed with *B. cinerea* conidia suspension and samples were collected at four time points. Mock, control leaves sprayed with ddH_2O. hpi, hours post-inoculation. The *VqActin* gene was used as internal control. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$; **, $P < 0.01$).

increased more significantly compared to *VvERF112*, *VvERF114* and *VvERF072* in Thompson seedless after powdery mildew inoculation (Fig. 1A). After *Pst* DC3000 inoculation, the transcript levels of *VqERF112*, *VqERF114* and *VqERF072* in 'Danfeng-2' significantly increased at 48 h by 2.6-fold, 15.9-fold and 2.7-fold compared to mock control, then decreased at 72 h, respectively. Compared to the mock control, the transcript levels of *VvERF112* and *VvERF114* increased, then peaked at 48 h by 1.9-fold and at 24 h by 1.2-fold after *Pst* DC3000 inoculation. Compared to the mock control, the expression of *VvERF072* decreased at 24 h, but then increased and peaked at 48 h by 1.9-fold. These results demonstrated that *ERF112*, *ERF114* and *ERF072* can be induced by *Pst* DC3000 and the increased multiple in 'Danfeng-2' was more than in Thompson seedless (Fig. 1B). After *B. cinerea* inoculation, the expression of *VqERF112* increased and peaked at 48 h by 1.3-fold compared to mock control, then decreased at 72 h. The transcript levels of *VqERF114* and *VqERF072* decreased at 24 h, then significantly increased at 48 h by 9.5-fold and 3.0-fold compared to mock control, respectively. After *B. cinerea* inoculation, the transcript levels of *VvERF112* and *VvERF114* increased, then peaked at 48 h by 1.7-fold and at 24 h by 1.3-fold compared to mock control, respectively. The expression of *VvERF072* decreased at 24 h, but then increased and peaked at 48 h by 1.9-fold compared to mock control. These results indicate that *ERF112*, *ERF114* and *ERF072* respond to *B. cinerea* inoculation and had different expression profiles in 'Danfeng-2' and Thompson seedless (Fig. 1C).

3.3. Tissue specific expression analysis of VqERF112, VqERF114 and VqERF072 from 'Danfeng-2'

The results of qRT-PCR analysis show that *VqERF112*, *VqERF114* and *VqERF072* are constitutively expressed in the young leaves, mature leaves, inflorescence, young berries (25 days after anthesis), mature berries (80 days after anthesis) and tendrils of 'Danfeng-2' (Fig. 2A). The highest expression levels of *VqERF112* and *VqERF114* were in the mature berries. However, the transcript level of *VqERF072* was highest in tendrils.

3.4. VqERF112, VqERF114 and VqERF072 respond to hormone treatments

The expression patterns of *VqERF112*, *VqERF114* and *VqERF072* in response to hormone treatments were analyzed by qRT-PCR (Fig. 2B). After ET treatment, the expression of *VqERF112* increased at 0.5 h and peaked at 6 h. The expression of *VqERF114* and *VqERF072* increased rapidly and peaked at 0.5 h, then gradually decreased. After SA treatment, the expression of *VqERF112* increased at 0.5 h, but then decreased gradually until 2 h, then increased again and peaked at 6 h. The transcript level of *VqERF114* was significantly induced at 0.5, 6 and 10 h. The expression of *VqERF072* decreased at 0.5 h but then increased and peaked at 6 h. After MeJA treatment, the abundance of *VqERF112* transcript increased at 2 h and peaked at 6 h. The expression of *VqERF114* was significantly induced at 1, 2 and 10 h. The transcript level of *VqERF072* increased at 0.5 h and peaked at 1 h but then gradually decreased. After ABA treatment, the expression of *VqERF112* increased at 1 and 10 h. The transcript level of *VqERF114* decreased at 1 h, then increased at 2 h and then returned to the original level. The expression of *VqERF072* was down regulated by the treatment. After GA treatment, the expression level of *VqERF112* and *VqERF072* was down regulated. However, the transcript level of *VqERF114* showed no obvious change compared the mock control. These results indicate that *VqERF112*, *VqERF114* and *VqERF072* respond to hormone treatments, including to ET, SA, MeJA and ABA. *VqERF112* and *VqERF072* also responded to GA treatment, but *VqERF114* did not.

3.5. Activation of VqERF112, VqERF114 and VqERF072 promoters after pathogen inoculation and hormone treatment

To determine the promoter activities of *ERF112*, *ERF114* and *ERF072* in response to pathogen inoculation and hormone treatment, the promoters of *VqERF112*, *VqERF114* and *VqERF072* from 'Danfeng-2' and the promoters of their homologous genes *VvERF112*, *VvERF114* and *VvERF072* from Thompson seedless were used to predict conserved *cis*-element motifs in each promoter (Fig. S2). Promoters of *VqERF112* and *VvERF112* differed in TC-rich repeats (defense and stress responsiveness) and in one TCA-element (salicylic acid responsiveness), while promoters of *VqERF114* and *VvERF114* differed in the ERE element (ethylene-responsive element), HSE element (heat stress responsiveness) and TGACG-motif (involved in MeJA-responsiveness). The promoters of *VqERF072* and *VvERF072* differed in ABRE element (abscisic acid responsiveness), TC-rich repeats and a TCA-element. The promoters of *VqERF112*, *VqERF114*, *VqERF072*, *VvERF112*, *VvERF114* and *VvERF072* were cloned into the pC0380-GUS vector fused to the GUS reporter. Each construct was transiently expressed in leaves of Thompson seedless and tested for GUS activity after powdery mildew inoculation and hormone treatments (ET, SA and MeJA). No GUS activities were observed in the pC0380-GUS empty vector control. The CaMV35S-GUS control showed no significant induction after powdery mildew inoculation or hormone treatment. Compared to the mock control, the GUS activities of *proVqERF112*, *proVqERF114* and *proVqERF072* were significantly enhanced after inoculation with powdery mildew. However, the GUS activities of *proVvERF112*, *proVvERF114* and *proVvERF072* did not differ significantly between the powdery mildew inoculation and the mock control (Fig. 3A). Similar results were obtained with the GUS histochemical staining. Compared to the mock control, the GUS activities of *proVqERF112*, *proVqERF114* and *proVqERF072* were significantly induced by ET, SA and MeJA treatments. However, the GUS activities of *proVvERF112*, *proVvERF114* and *proVvERF072* were not significantly different between the ET treatment and the mock control. The GUS activities of *proVvERF112* and *proVvERF072* were induced only by the SA treatment. The GUS activity of *proVvERF114* was induced by the MeJA treatment (Fig. 3B). These results indicate that the promoters of *VqERF112*, *VqERF114* and *VqERF072* can be induced by powdery mildew inoculation and also by hormone treatment, including by ET, SA and MeJA.

3.6. Characterization of lines overexpressing VqERF112, VqERF114 and VqERF072

To further explore the putative involvement of *VqERF112*, *VqERF114* and *VqERF072* in disease resistance, the CaMV35S promoter-driven overexpressing (OE) lines of *VqERF112*, *VqERF114* and *VqERF072* were generated. The transgenic *VqERF112*-OE, *VqERF114*-OE and *VqERF072*-OE lines were obtained through *Arabidopsis* floral dip

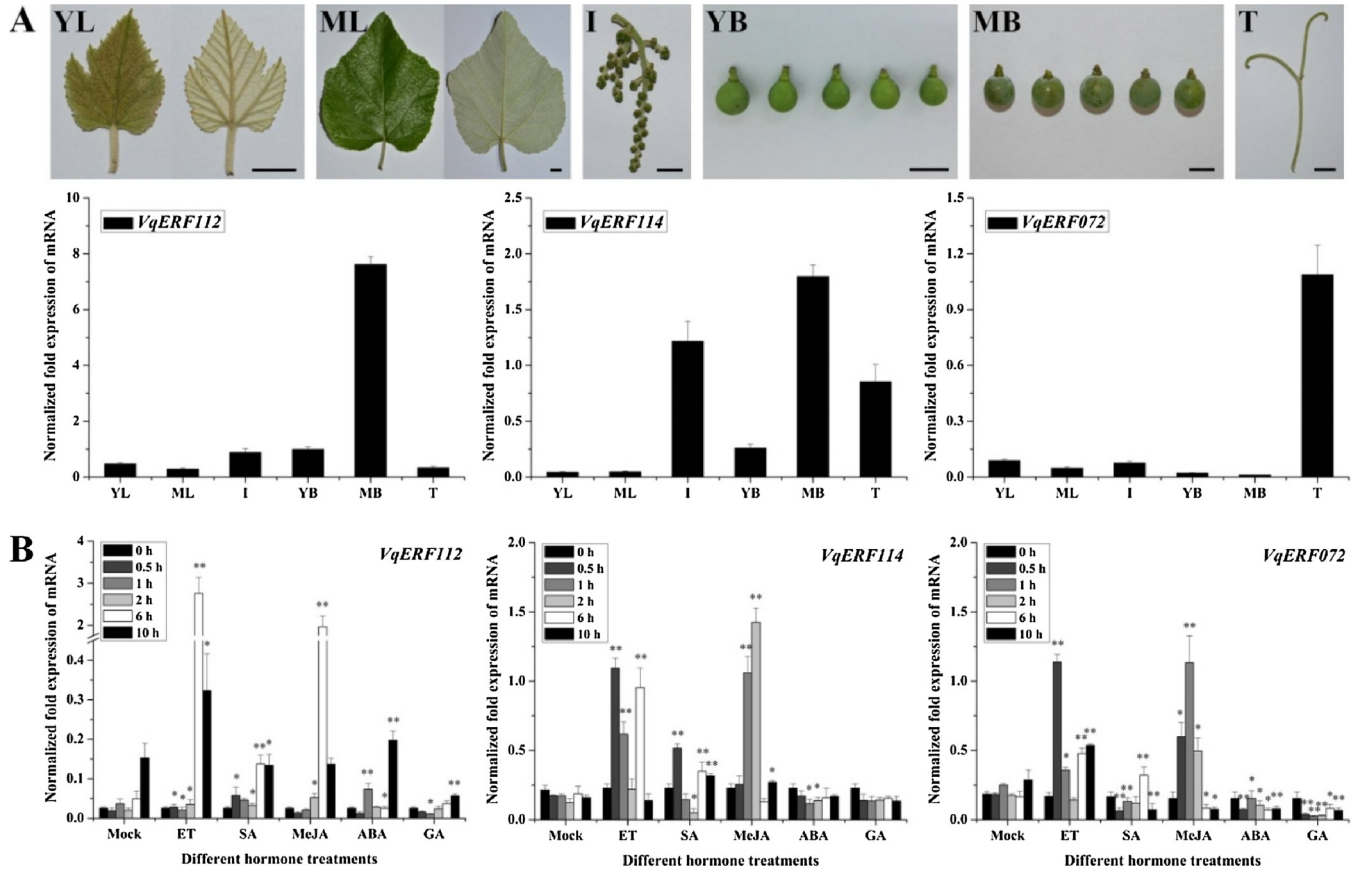

Fig. 2. Expression profiles of *VqERF112*, *VqERF114* and *VqERF072* in different tissues of 'Danfeng-2' and in response to different hormone treatments. (A) Tissue specific expression analysis of *VqERF112*, *VqERF114* and *VqERF072* from 'Danfeng-2'. YL, young leaves; ML, mature leaves; I, inflorescence; YB, young berries (25 days after anthesis); MB, mature berries (80 days after anthesis); T, tendril. Bar = 1 cm. (B) Expression analysis of *VqERF112*, *VqERF114* and *VqERF072* from 'Danfeng-2' in response to different hormone treatments determined by qRT-PCR. The leaves of 'Danfeng-2' were treated with 0.5 g/L ethylene and 100 μM salicylic acid, methyl jasmonate, abscisic acid or gibberellin. The leaves were sampled at 0, 0.5, 1, 2, 6 and 10 h after treatment. Mock, control leaves treated with ddH_2O; ET, treatment with ethylene; SA, treatment with salicylic acid; MeJA, treatment with methyl jasmonate; ABA, treatment with abscisic acid; GA, treatment with gibberellin. *GAPDH* was used as internal control. Results are indicated by mean values from three biological replicates. Error bars indicate SD. Statistical significance was determined by Student's *t* test (*, $P < 0.05$; **, $P < 0.01$).

transformation. Three independent homozygous T3 lines of each *ERF* gene were selected for further functional studies. As shown in Fig. S3A, there were no obvious differences in phenotype morphology among ecotype Col-0 and transgenic lines of *VqERF112*, *VqERF114* and *VqERF072*. The transgenic lines were characterized by qRT-PCR and semi-quantitative PCR. The heterologous expression of *VqERF112*, *VqERF114* and *VqERF072* were detected only in the transgenic lines (Figs. S3B and S3C).

3.7. Overexpression of VqERF112, VqERF114 and VqERF072 in transgenic Arabidopsis improve the resistance to Pst DC3000

Col-0 plants and three independent transgenic *Arabidopsis* lines of *VqERF112*, *VqERF114* and *VqERF072* were inoculated with *Pst* DC3000 to investigate whether these three ERF transcription factors play a role in bacterial resistance. Three days after *Pst* DC3000 inoculation, obvious disease symptoms of chlorosis were observed in leaves of the Col-0 plants, while no such symptoms were apparent in *VqERF112*-OE, *VqERF114*-OE or *VqERF072*-OE plants (Figs. 4 A, 5 A and 6 A). Therefore, the bacteria numbers were measured to assess the bacterial resistance of transgenic lines. At three days post-inoculation, the bacterial quantities in *VqERF112*-OE, *VqERF114*-OE and *VqERF072*-OE plants were significantly lower than in Col-0 plants (Figs. 4B, 5 B and 6 B). These results indicate that overexpression of *VqERF112*, *VqERF114* and *VqERF072* in transgenic *Arabidopsis* improved the resistance to *Pst* DC3000.

3.8. Overexpression of VqERF112, VqERF114 and VqERF072 in transgenic Arabidopsis lines promotes cell death and H_2O_2 accumulation after Pst DC3000 inoculation

To examine whether *VqERF112*-OE, *VqERF114*-OE and *VqERF072*-OE plants show cell death and H_2O_2 accumulation, the rosette leaves of Col-0 and transgenic plants were stained with trypan blue and DAB at three days post-inoculation. The cell death symptoms in leaves of *VqERF112*-OE, *VqERF114*-OE and *VqERF072*-OE plants were more intense compared to in the Col-0 plants (Figs. 4C, 5 C and 6 C). Meanwhile, the *VqERF112*-OE, *VqERF114*-OE and *VqERF072*-OE plants showed higher levels of H_2O_2 accumulation compared to the Col-0 plants (Figs. 4D, 5 D and 6 D). These results indicate that overexpression of *VqERF112*, *VqERF114* and *VqERF072* in transgenic *Arabidopsis* lines promotes cell death and H_2O_2 accumulation.

3.9. VqERF112, VqERF114 and VqERF072 positively regulate the resistance to Pst DC3000 via JA/ET signaling pathway

When plants are attacked by pathogens, hormone signaling pathways will be activated to trigger the defense response. Hence, the expression of SA- and JA/ET-responsive genes were analyzed by qRT-PCR after *Pst* DC3000 inoculation. In *VqERF112*-OE plants, the SA signaling-

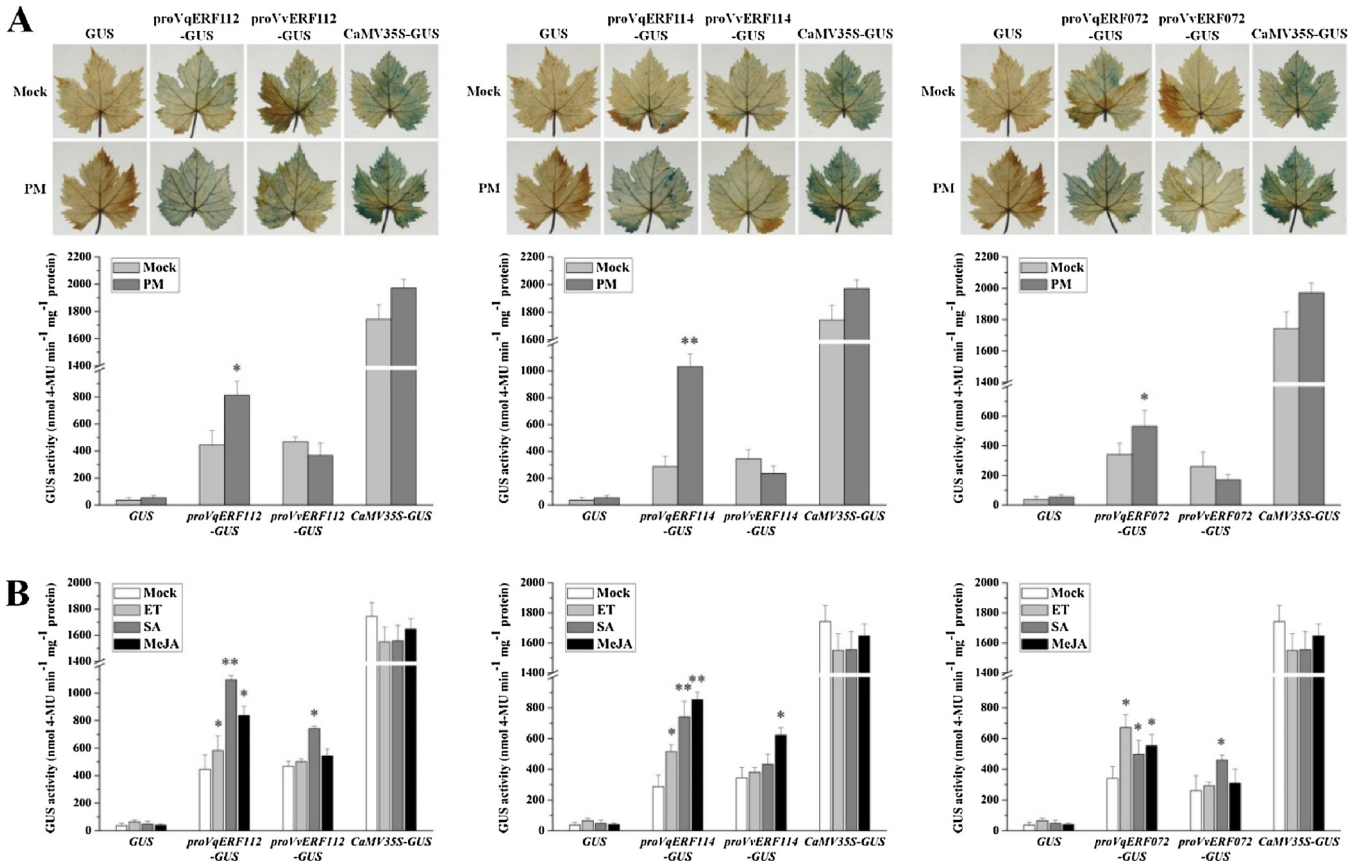

Fig. 3. The promoter activity analysis of *ERF112*, *ERF114* and *ERF072* in response to pathogen inoculation (A) and hormone treatment (B). The promoters of *VqERF112*, *VqERF114* and *VqERF072* from 'Danfeng-2' and the promoters of their homologous genes *VvERF112*, *VvERF114* and *VvERF072* from Thompson seedless were cloned into the pC0380-GUS vector and fused to the GUS reporter. The recombinant constructs were transiently expressed in leaves of Thompson seedless and cultured in a chamber for two days, and then inoculated with powdery mildew or sprayed with 0.5 g/L ET, 100 μM SA or MeJA. After culturing of one day, the infiltrated leaves were collected and tested for GUS activity. In this assay, CaMV35S-GUS was used as positive control and pC0380-GUS empty vector as negative control. Mock control was treated with ddH_2O. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$; **, $P < 0.01$).

related genes, *AtNPR1* and *AtPR1*, were increased at 48 hours post-inoculation (hpi), then decreased at 72 hpi compared to Col-0 plants. Meanwhile, the JA/ET signaling-related gene, *AtLOX3*, was increased at 24 and 48 hpi, then decreased at 72 hpi in *VqERF112*-OE plants compared to Col-0 plants. After *Pst* DC3000 inoculation, the expression of the *Arabidopsis* SA-responsive genes, *AtICS1* and *AtPR5*, decreased significantly in the *VqERF112*-OE transgenic lines compared Col-0. However, the JA/ET-responsive genes *AtPDF1.2*, *AtPR3* and *AtPR4* were all significantly induced after *Pst* DC3000 inoculation in the *VqERF112*-OE plants compared with the Col-0 plants (Fig. 4E). After *Pst* DC3000 inoculation, the expressions of *AtNPR1*, *AtPR1*, *AtPR3* and *AtPR4* increased significantly in the *VqERF114*-OE transgenic lines, compared with in Col-0. However, *AtICS1* and *AtPR5* were down-regulated in the *VqERF114*-OE plants after *Pst* DC3000 inoculation, compared to Col-0 plants. In the *VqERF114*-OE plants, *AtPDF1.2* expression was decreased at 24 hpi, but then increased at 48 and 72 hpi compared to Col-0. The expression of *AtLOX3* was increased at 24 and 48 hpi, but then decreased at 72 hpi in *VqERF114*-OE plants compared to in Col-0 plants (Fig. 5E). After *Pst* DC3000 inoculation, the expression of SA signaling-related genes, *AtICS1* and *AtPR5*, decreased significantly in *VqERF072*-OE transgenic lines compared with in Col-0. However, the expression of JA/ET signaling-related genes, *AtPR3* and *AtPR4*, increased significantly upon-inoculation in *VqERF072*-OE plants compared with Col-0 plants. In *VqERF072*-OE plants, the expression of *AtNPR1*, *AtPR1* and *AtLOX3* increased at 24 and 48 hpi, but then decreased at 72 hpi compared to Col-0 plants. Meanwhile, *AtPDF1.2* expression decreased at 24 hpi, but then increased at 72 hpi in *VqERF072*-OE plants compared with Col-0 plants (Fig. 6E). These results demonstrate that *VqERF112*, *VqERF114* and *VqERF072* are positively involved in the resistance to *Pst* DC3000 via the JA/ET signaling pathway.

3.10. Overexpression of VqERF112, VqERF114 and VqERF072 in transgenic Arabidopsis lines enhance the resistance to B. cinerea

Col-0 plants and three OE transgenic *Arabidopsis* lines of *VqERF112*, *VqERF114* and *VqERF072* were inoculated with *B. cinerea* to investigate whether these three ERF transcription factors participate in resistance to a necrotrophic pathogen. Three days after *B. cinerea* inoculation, more obvious necrotic lesions were seen in Col-0 plants compared with in transgenic *Arabidopsis* plants (Figs. 7A, 8 A and 9 A). The lesion perimeters were measured to assess the resistance of transgenic lines to *B. cinerea*. The lesion perimeters in leaves of *VqERF112*-OE, *VqERF114*-OE and *VqERF072*-OE plants were smaller than in Col-0 plants (Figs. 7B, 8 B and 9 B). These results indicated that overexpression of *VqERF112*, *VqERF114* and *VqERF072* in transgenic *Arabidopsis* lines enhanced the resistance to *B. cinerea*. When plants resist pathogen attack, programmed cell death is accompanied by ROS accumulation and a hypersensitive response. To examine cell death and H_2O_2 accumulation, the rosette leaves of Col-0 plants and transgenic plants were stained with trypan blue and DAB three days after *B. cinerea* inoculation. The cell death symptoms in leaves of Col-0 plants were more intense compared to *VqERF112*-OE, *VqERF114*-OE and *VqERF072*-OE plants (Figs. 7C, 8 C and 9 C). Meanwhile, Col-0 plants showed a higher degree of H_2O_2 accumulation compared to *VqERF112*-OE, *VqERF114*-

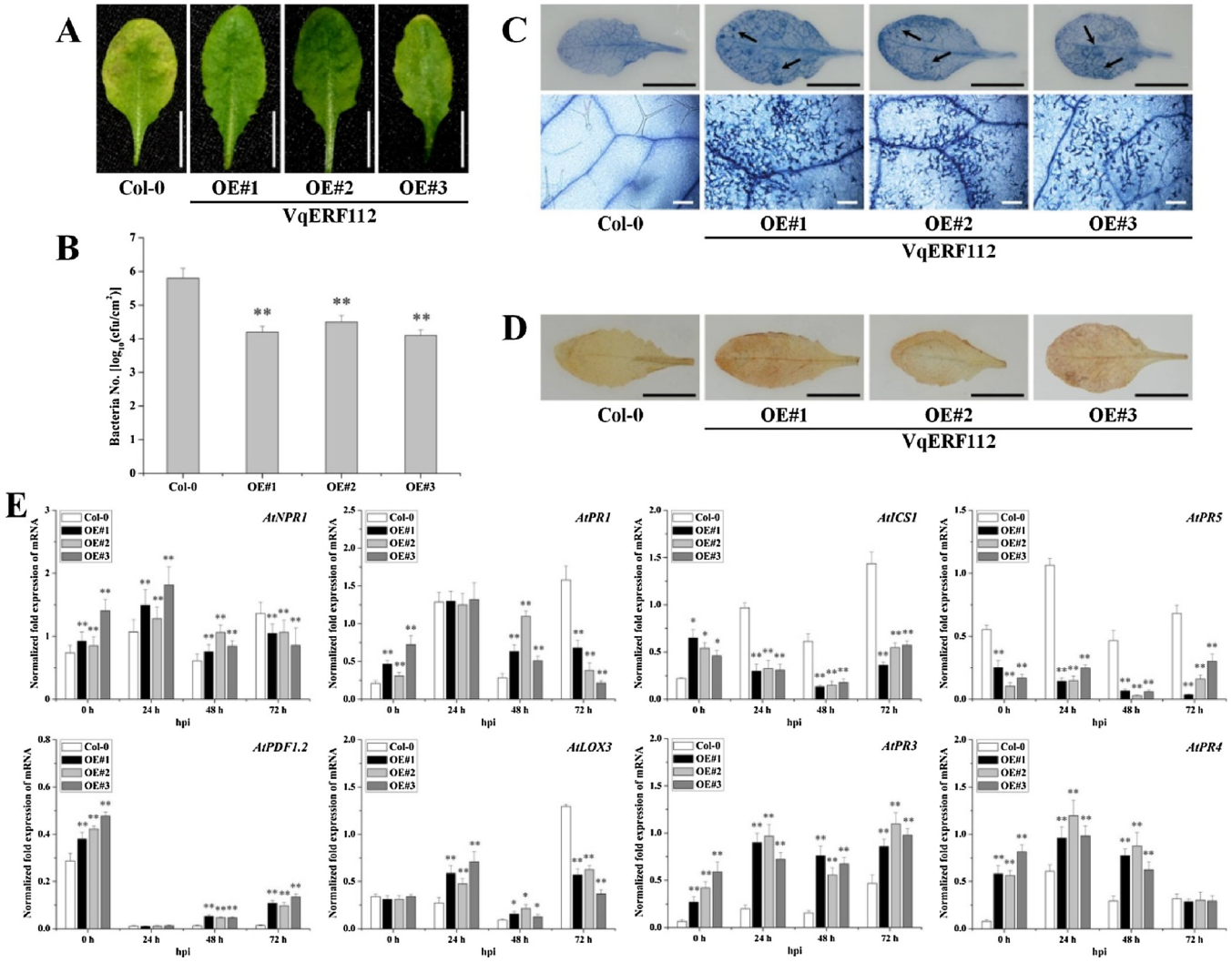

Fig. 4. Overexpression of *VqERF112* in *Arabidopsis thaliana* demonstrates enhanced resistance to *Pst* DC3000 inoculation. (A) Disease symptoms on *VqERF112*-OE transgenic lines and Col-0 leaves three days post-inoculation. Bars =1 cm. (B) Bacterial population assays in inoculated *VqERF112*-OE transgenic lines and Col-0 leaves three days post-inoculation. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (**, $P < 0.01$). (C) Trypan blue straining for cell death. Bars = 1 cm (upper image). Bars = 200 μm (lower image). (D) DAB straining for H_2O_2 accumulation. Bars =1 cm. (E) Expression analysis of SA- and JA/ET-responsive genes determined by qRT-PCR in *VqERF112*-OE transgenic lines and Col-0 plants at 0, 24, 48 and 72 h after *Pst* DC3000 inoculation. The *AtActin* gene was used as internal control. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$; **, $P < 0.01$).

OE and *VqERF072*-OE plants (Figs. 7D, 8 D and 9 D).

3.11. *VqERF112, VqERF114 and VqERF072 positively regulate the resistance to B. cinerea via SA and JA/ET signaling pathways*

The expressions of SA- and JA/ET-responsive genes were analyzed by qRT-PCR after *B. cinerea* inoculation. In the *VqERF112*-OE plants, the expression of the SA-responsive genes (*AtNPR1*, *AtPR1* and *AtICS1*) and the JA/ET-responsive genes (*AtPDF1.2*, *AtLOX3*, *AtPR3* and *AtPR4*) increased significantly after *B. cinerea* inoculation. However, the expressions of the SA-responsive gene, *AtPR5*, decreased in the *VqERF112*-OE plants compared the Col-0 plants at 24 and 72 h after *B. cinerea* inoculation (Fig. 7E). After *B. cinerea* inoculation, the transcript levels of *AtNPR1*, *AtPR1*, *AtICS1*, *AtPDF1.2*, *AtLOX3*, *AtPR3* and *AtPR4* increased in the *VqERF114*-OE plants compared to the Col-0 plants. However, the expression only of *AtPR5* decreased in *VqERF114*-OE plants (Fig. 8E). In the *VqERF072*-OE plants, the expressions of *AtNPR1*, *AtPR1*, *AtICS1*, *AtPDF1.2*, *AtLOX3* and *AtPR3* increased after *B. cinerea* inoculation. The expression of the JA/ET signaling-related gene, *AtPR4*, increased at 24 and 48 h, but then decreased at 72 h after *B. cinerea* inoculation in the *VqERF072*-OE plants compared to the Col-0 plants. However, the transcript level of SA signaling-related gene, *AtPR5*, decreased in *VqERF072*-OE plants after *B. cinerea* inoculation (Fig. 9E). These results indicate that *VqERF112*, *VqERF114* and *VqERF072* positively regulate the resistance to *B. cinerea* via the SA and JA/ET signaling pathways.

4. Discussion

The AP2/ERF superfamily exist widely in the plant kingdom and participate in the response to a range of environmental stimuli, including to pathogen infection [31]. Many ERF transcription factors have been reported to play roles in the regulation of plant disease resistance pathways. The first ERF transcription factor reported to participate in the ET signaling pathway was *Arabidopsis ERF1*. This positively regulates resistance to *B. cinerea* [32]. However, rice *OsERF922* negatively regulates resistance to *Magnaporthe oryzae* [33]. In recent years, a number of ERF transcription factors in grapevine have been identified to respond to biotic and abiotic stresses. In tobacco, overexpression of *VpERF2* and *VpERF3* from Chinese wild grapevine *V. pseudoreticulata* 'Baihe-35-1' showed enhanced resistance to *Ralstonia*

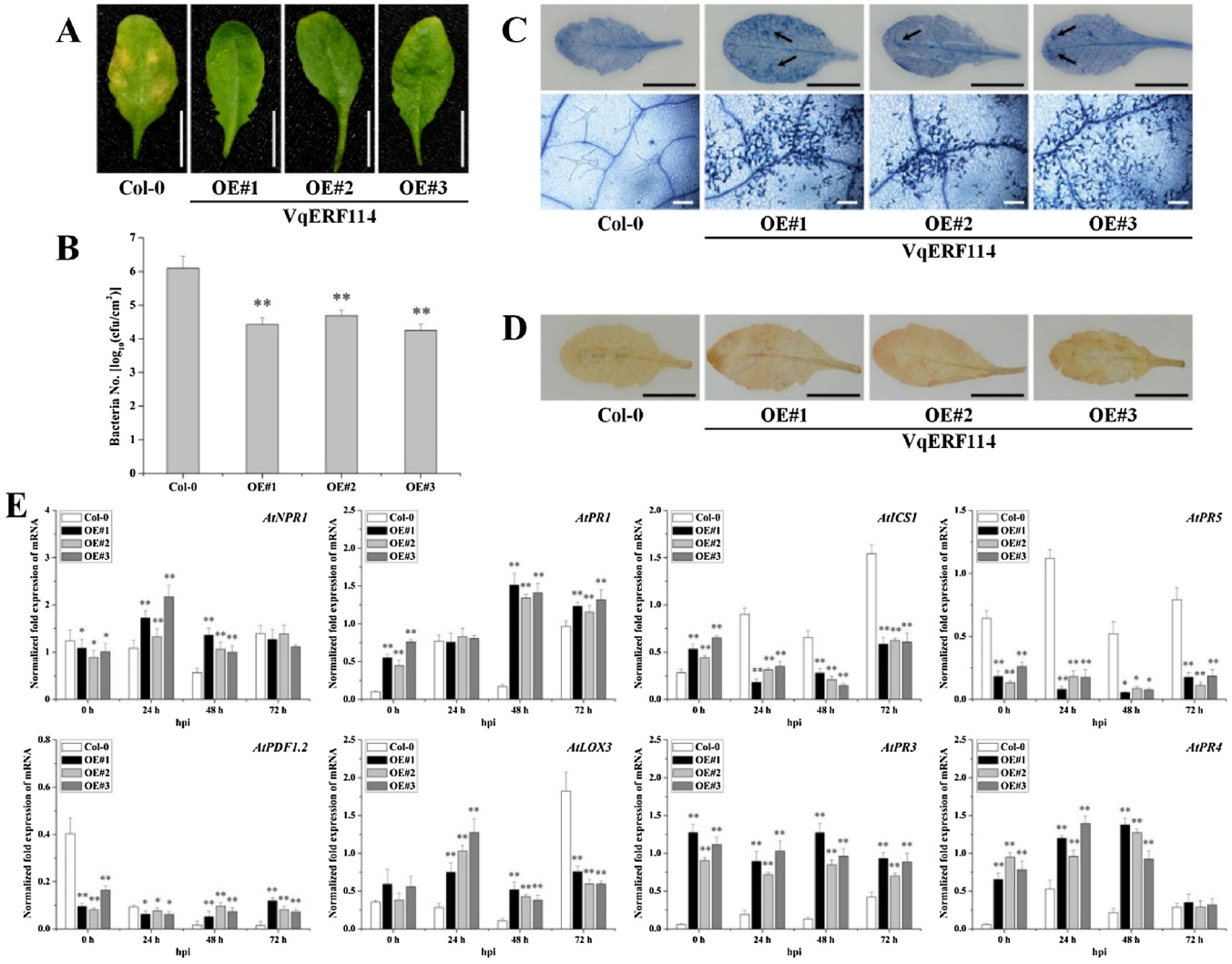

Fig. 5. Overexpression of *VqERF114* in *Arabidopsis thaliana* demonstrates enhanced resistance to *Pst* DC3000 inoculation. (A) The disease symptoms on *VqERF114*-OE transgenic lines and Col-0 leaves three days post-inoculation. Bars = 1 cm. (B) Bacterial population assays in inoculated *VqERF114*-OE transgenic lines and Col-0 leaves three days post-inoculation. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (**, $P < 0.01$). (C) Trypan blue straining for cell death. Bars = 1 cm (upper image). Bars = 200 μm (lower image). (D) DAB straining for H_2O_2 accumulation. Bars = 1 cm. (E) Expression analysis of SA- and JA/ET-responsive genes determined by qRT-PCR in *VqERF114*-OE transgenic lines and Col-0 plants at 0, 24, 48 and 72 h after *Pst* DC3000 inoculation. The *AtActin* gene was used as internal control. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$; **, $P < 0.01$).

solanacearum and *Phytophtora parasitica* var. *nicotianae* Tucker. However, transgenic tobacco overexpressing *VpERF1* became more susceptible to these two pathogens [27]. In *Arabidopsis*, overexpression of *V. amurensis VaERF20* enhanced resistance to *Pst* DC3000 and *B. cinerea* [34]. It has been reported that *RAP2.6 L* can respond to JA, ET, SA and ABA treatments [35]. Overexpression of *RAP2.6 L* in *Arabidopsis* improved tolerance to salt and drought stresses [36]. Furthermore, *RAP2.6 L* overexpression can increase stomatal closure and delay premature senescence induced by waterlogging [37]. In this study, phylogenetic analysis demonstrated that VqERF112 is closely related to AtRAP2.6 L (Fig. S1). As with *RAP2.6 L*, our qRT-PCR analysis results indicate that *VqERF112* can also be induced by ET, SA, MeJA, ABA and GA treatments (Fig. 2B). *Arabidopsis AtABR1* (*ABA REPRESSOR1*) was identified as the repressor in response to ABA and is involved in regulating the ABA mediated stress response. Mutations in *AtABR1* result in hypersensibility to ABA during seed germination [38–40]. *VqERF114*, the gene homologous to *AtABR1* in grapevine, can respond to ABA treatment (Figs. S1 and 2B). *Arabidopsis AtERF2* positively regulates the expression of JA-responsive defense genes and the resistance to *Fusarium oxysporum* [41,42]. In this study, *VqERF072* is identified as the gene homologous to *AtERF2* and it can be induced by MeJA treatment (Figs. S1 and 2B).

The Chinese wild *V. quinquangularis* accession 'Danfeng-2' is highly resistant to pathogens, such as powdery mildew [16]. However, *V. vinifera* L. cv. Thompson seedless is susceptible to pathogens. In this study, the expression profiles of ERF112, ERF114 and ERF072 in 'Danfeng-2' and Thompson seedless were analyzed by qRT-PCR. All these three ERF transcription factors can respond to powdery mildew, to *Pst* DC3000 and to *B. cinerea*. Moreover, the expressions of *Vq*ERF112, *Vq*ERF114 and *Vq*ERF072 increased more significantly than of *Vv*ERF112, *Vv*ERF114 or *Vv*ERF072 (Fig. 1). Powdery mildew and *B. cinerea* are major fungal diseases of grapevine, infecting the leaves, inflorescences and berries [43]. The results of tissue-specific expression analysis demonstrate that *Vq*ERF112, *Vq*ERF114 and *Vq*ERF072 are constitutively expressed in these tissues (Fig. 2A). The results indicate that *Vq*ERF112, *Vq*ERF114 and *Vq*ERF072 play a role in the high resistance to disease of 'Danfeng-2'.

The transcriptional expression of a gene is associated with its promoter activity. Therefore, a functional study of the promoter has high relevance to a functional study of a gene. China has abundant wild

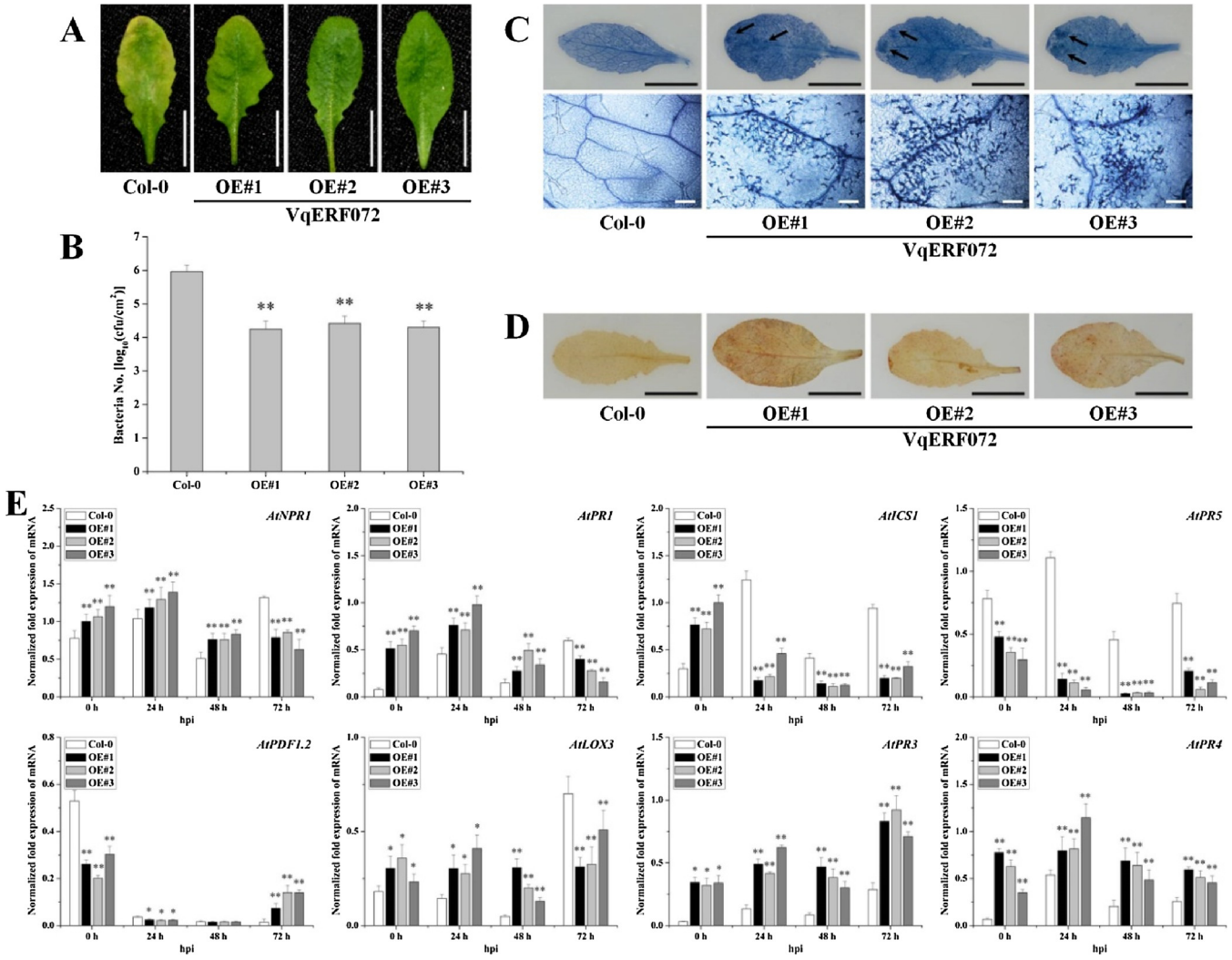

Fig. 6. Overexpression of *VqERF072* in *Arabidopsis thaliana* demonstrates enhanced resistance to *Pst* DC3000 inoculation. (A) The disease symptoms on *VqERF072*-OE transgenic lines and Col-0 leaves three days post-inoculation. Bars = 1 cm. (B) Bacterial population assays in inoculated *VqERF072*-OE transgenic lines and Col-0 leaves three days post-inoculation. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (**, $P < 0.01$). (C) Trypan blue straining for cell death. Bars = 1 cm (upper image). Bars = 200 μm (lower image). (D) DAB straining for H_2O_2 accumulation. Bars = 1 cm. (E) Expression analysis of SA- and JA/ET-responsive genes determined by qRT-PCR in *VqERF072*-OE transgenic lines and Col-0 plants at 0, 24, 48 and 72 h after *Pst* DC3000 inoculation. The *AtActin* gene was used as internal control. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$; **, $P < 0.01$).

grapevine germplasm resources, including *V. pseudoreticulata* 'Baihe-35-1', which is highly resistant to powdery mildew [44]. In our previous study, functional studies of disease-resistance related genes (the ubiquitin ligase gene and the stilbene synthase gene) and their promoters were carried out. The promoter of the ubiquitin ligase gene *VpRFP1* from 'Baihe-35-1' can be induced by SA, MeJA and powdery mildew. However, the promoter of *VvRFP1* from *V. vinifera* cv. Carignane (the gene homologous to *VpRFP1*) was slightly induced by powdery mildew inoculation. The core functional region of *VpRFP1* promoter was the -148 bp region, which contains one TGACG-motif, one TC-rich repeats and one HSE element. The core functional region of *VpRFP1* promoter results in differential expression between *VpRFP1* and *VvRFP1*, and plays an important role in the differing resistance phenotypes of these two grapevines [45]. The promoter of the stilbene synthase gene *VpSTS* can respond to SA, *U. necator* and *Alternaria alternata* [28]. *Cis*-element motifs were predicted in the promoters of *VpSTS* from 'Baihe-35-1', *VvcSTS* from Carignane and *VvtSTS* from Thompson seedless. The promoter of *VpSTS* contained one W-box (fungal elictior-responsive element) and one TC-rich repeats. The promoters of *VvcSTS* and *VvtSTS* contained two W-box, but no TC-rich repeats. The difference in *cis*-element motifs resulted in differential expression patterns of *STS* between 'Baihe-35-1' and the two *V. vinifera* genotypes [46]. In this study, the promoters of *VqERF112*, *VqERF114* and *VqERF072* from 'Danfeng-2' and their homologous genes *VvERF112*, *VvERF114* and *VvERF072* from Thompson seedless were cloned. The *cis*-element analysis demonstrates that the main differences in these two grapevine genotypes included defense- and stress-responsive TC-rich repeats, ET-responsive ERE, SA-responsive TCA-element and MeJA-responsive TGACG-motif (Fig. S2). Therefore, GUS activity analysis of promoters in response to powdery mildew inoculation and hormone treatments (ET, SA and MeJA) were carried out. As shown in Fig. 3A, the promoters of *VqERF112*, *VqERF114* and *VqERF072* significantly responded to powdery mildew inoculation. However, the promoters of *VvERF112*, *VvERF114* and *VvERF072* were not significantly induced by powdery mildew. The promoter activity of *VqERF112*, *VqERF114* and *VqERF072* was significantly enhanced by ET, SA and MeJA treatments. However, the promoters of *VvERF112* and *VvERF072* responded exclusively to SA treatment and *VvERF114* promoter responded only to MeJA treatment (Fig. 3B). These results indicate that the promoter activity of these three ERF transcription factors was different between 'Danfeng-2' and

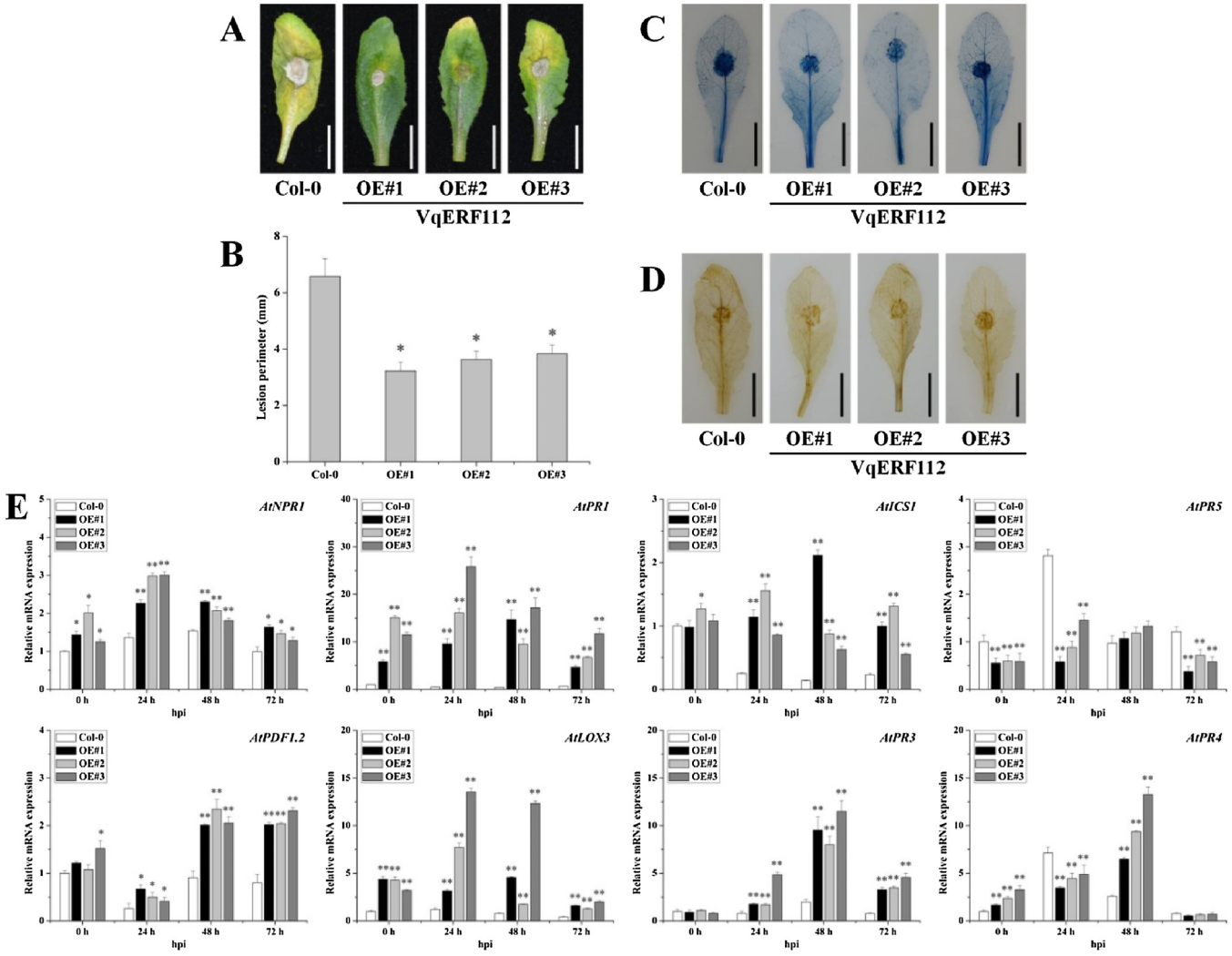

Fig. 7. Overexpression of *VqERF112* in *Arabidopsis thaliana* demonstrates enhanced resistance to *B. cinerea* inoculation. (A) The disease symptoms on *VqERF112*-OE transgenic lines and Col-0 leaves three days post-inoculation. Bars =1 cm. (B) Lesion perimeter of inoculated *VqERF112*-OE transgenic lines and Col-0 leaves three days post-inoculation. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$). (C) Trypan blue straining for cell death. Bars = 1 cm. (D) DAB straining for H_2O_2 accumulation. Bars =1 cm. (E) Expression analysis of SA- and JA/ET-responsive genes determined by qRT-PCR in *VqERF112*-OE transgenic lines and Col-0 plants at 0, 24, 48 and 72 h after *B. cinerea* inoculation. The *AtActin* gene was used as internal control. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$; **, $P < 0.01$).

Thompson seedless. *VqERF112*, *VqERF114* and *VqERF072* plays an important role in pathogen resistance in 'Danfeng-2'.

To explore the anti-disease function of the three ERF transcription factors, transgenic *Arabidopsis* lines overexpressing *VqERF112*, *VqERF114* and *VqERF072* were generated and inoculated with *Pst* DC3000 and *B. cinerea*. *VqERF112*-OE, *VqERF114*-OE and *VqERF072*-OE plants enhanced the resistance to *Pst* DC3000 compared to Col-0 plants (Figs. 4A, 4B, 5A, 5B, 6A and 6B). The immune responses were accompanied by PCD and ROS accumulation in the plants. Therefore, more intense cell death and a higher degree of H_2O_2 accumulation were observed in leaves of transgenic *Arabidopsis* lines (Figs. 4C,D, 5 C,D, 6 C,D). After *Pst* DC3000 inoculation, the expressions of two SA signaling-related genes (*AtNPR1* and *AtPR1*) and four JA/ET signaling-related genes (*AtPDF1.2*, *AtLOX3*, *AtPR3* and *AtPR4*) increased significantly in the transgenic *Arabidopsis* lines compared to the Col-0 plants (Figs. 4E, 5 E and 6 E). These results indicate that overexpression of *VqERF112*, *VqERF114* and *VqERF072* improve the resistance to *Pst* DC3000 via the JA/ET signaling pathway. When *Arabidopsis* leaves were inoculated with a *B. cinerea* conidial suspension, necrotic lesions could be observed. Transgenic *Arabidopsis* lines overexpressing *VqERF112*, *VqERF114* and *VqERF072* enhanced the resistance to *B. cinerea* (Figs. 7A,B, 8 A,B, 9 A,B). Therefore, we observed less intense cell death and lower H_2O_2 accumulation in leaves of *VqERF112*-OE, *VqERF114*-OE and *VqERF072*-OE plants than in Col-0 plants (Figs. 7C,D, 8 C,D, 9 C,D). After *B. cinerea* inoculation, the expression of three SA signaling-related genes (*AtNPR1*, *AtPR1* and *AtICS1*) and four JA/ET signaling-related genes (*AtPDF1.2*, *AtLOX3*, *AtPR3* and *AtPR4*) increased significantly in transgenic *Arabidopsis* lines compared to Col-0 plants (Figs. 7E, 8 E and 9 E). These results indicate that overexpression of *VqERF112*, *VqERF114* and *VqERF072* enhances the resistance to *B. cinerea* via the SA and JA/ET signaling pathways. Studies on ERF transcription factors regulating disease resistance have been widely reported in *Arabidopsis*. Many ERF transcription factors, including *ERF1*, *ORA59*, *ERF6* and *ERF96*, have recently been identified as participating in the immune response to pathogens [42]. Overexpression of *ERF1*, *ORA59* and *ERF6* enhanced the resistance to *B. cinerea* in *Arabidopsis*. Furthermore, *ERF96* positively regulates the resistance to necrotrophic pathogens by promoting the expression of JA/ET signaling-related PR genes, such as *PDF1.2*, *PR3* and *PR4* [47]. Transgenic *Arabidopsis* overexpressing *AtERF15* showed improved resistance to *Pst* DC3000 and *B. cinerea* and increased

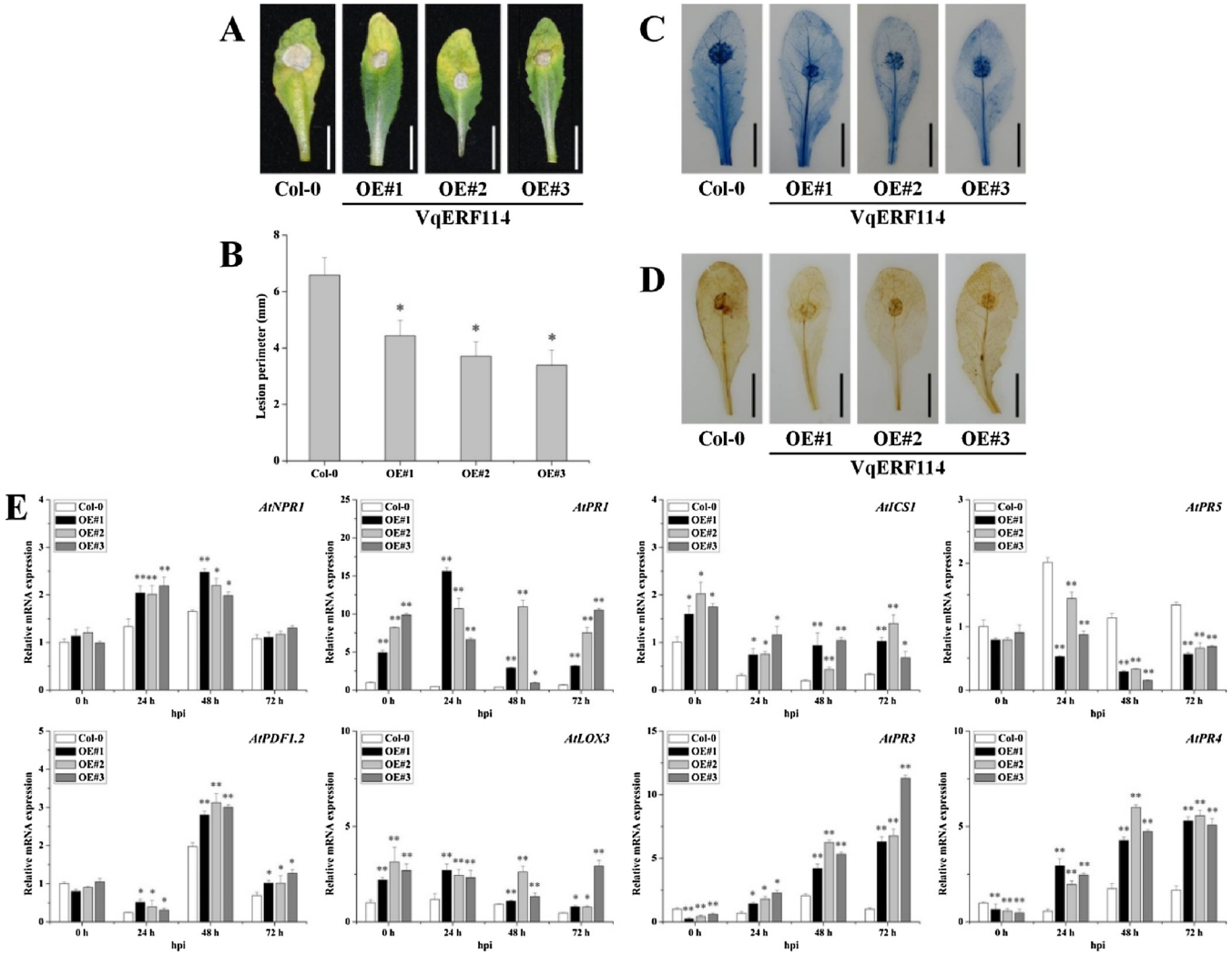

Fig. 8. Overexpression of *VqERF114* in *Arabidopsis thaliana* demonstrates enhanced resistance to *B. cinerea* inoculation. (A) The disease symptoms on *VqERF114*-OE transgenic lines and Col-0 leaves three days post-inoculation. Bars =1 cm. (B) Lesion perimeter of inoculated *VqERF114*-OE transgenic lines and Col-0 leaves three days post-inoculation. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$). (C) Trypan blue straining for cell death. Bars = 1 cm. (D) DAB straining for H_2O_2 accumulation. Bars =1 cm. (E) Expression analysis of SA- and JA/ET-responsive genes determined by qRT-PCR in *VqERF114*-OE transgenic lines and Col-0 plants at 0, 24, 48 and 72 h after *B. cinerea* inoculation. The *AtActin* gene was used as internal control. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$; **, $P < 0.01$).

expression of the defense genes. However, ROS accumulation decreased in transgenic *Arabidopsis* overexpressing *AtERF15* after *B. cinerea* inoculation [48]. In *Arabidopsis*, BT proteins regulate transcription, in which *BT4* enhances resistance to *Pst* DC3000. ERF11 can activate the expression of *BT4* by binding to its promoter and enhance SA/ET signaling mediated resistance to *Pst* DC3000 [49]. Overexpression of *AtERF014* improved the resistance to *Pst* DC3000 and the expressions of *AtPR1* and *AtPR5* [50]. Meanwhile, overexpression of *RAP2.2* enhanced the resistance to *B. cinerea* [51]. In addition to *Arabidopsis*, ERF transcription factors have also been reported to participate in pathogen resistance in other plants. When tomato *SlERF.A1*, *SlERF.A3*, *SlERF.B4* and *SlERF.C3* were silenced, resistance to *B. cinerea* and the expression of JA/ET signaling-related genes were decreased, but H_2O_2 accumulation was increased. Furthermore, resistance to *Pst* DC3000 also decreased when *SlERF.A3* was silenced [52]. Overexpression of *Atriplex canescens AcERF2* in *Arabidopsis* increased the expression of defense-related genes (*PR1*, *PR2*, *PR5*, *ERF1* and *ERF3*) and the resistance to *Pst* DC3000 and *B. cinerea* [53]. Expression analysis of ERF subfamily genes in response to *B. cinerea* inoculation was carried out in grapevine and ERF subfamily genes were found to be induced by *B. cinerea* [54]. Transgenic *Arabidopsis* overexpressing *V. amurensis VaERF20* showed enhanced resistances against *Pst* DC3000 and *B. cinerea* [34]. Similar to these results, the three ERF transcription factors in this study positively regulated resistance to *Pst* DC3000 and *B. cinerea* (Figs. 4–9). In contrast, some ERF transcription factors in *Arabidopsis* negatively regulate the resistance to *Pst* DC3000 and *B. cinerea*. For example, mutation in *RAP2.6 L* (the homologous gene to *VqERF112*) increased resistance to *Pst* DC3000 [55]. Knockout mutants of *ERF9* enhanced resistance to *B. cinerea* [56]. Overexpression of *AtERF014* decreased the resistance to *B. cinerea* and the expressions of *AtPR1* and *AtPR5* [50]. During *Arabidopsis* PTI, *ERF19* negatively regulated the resistances to *B. cinerea* and *Pst* DC3000 [57].

In conclusion, *VqERF112*, *VqERF114* and *VqERF072* enhance the resistance to *Pst* DC3000 and *B. cinerea* in *Arabidopsis* via the SA and JA/ET signaling pathways (Fig. 10). The functional study on these three ERF transcription factors provides new insights into the dynamic regulation of plant resistance to pathogen inoculation and novel evidence for grapevine breeding for disease resistance using Chinese wild *V. quinquangularis* accession 'Danfeng-2'. The disease resistance of *V. vinifera* can be increased by genetic transformation.

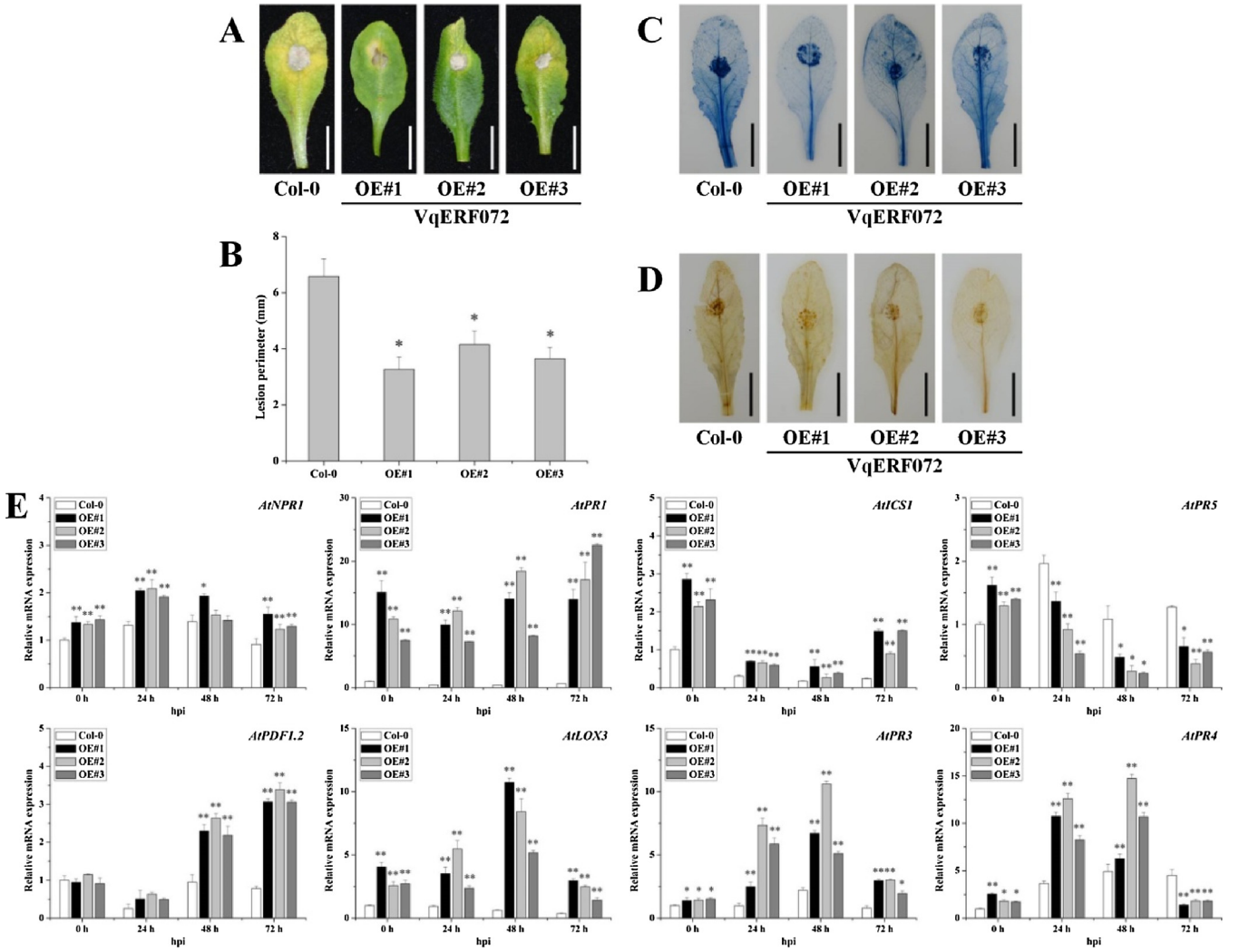

Fig. 9. Overexpression of *VqERF072* in *Arabidopsis thaliana* demonstrates enhanced resistance to *B. cinerea* inoculation. (A) The disease symptoms on *VqERF072*-OE transgenic lines and Col-0 leaves three days post-inoculation. Bars =1 cm. (B) Lesion perimeter of inoculated *VqERF072*-OE transgenic lines and Col-0 leaves three days post-inoculation. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$). (C) Trypan blue straining for cell death. Bars = 1 cm. (D) DAB straining for H_2O_2 accumulation. Bars =1 cm. (E) Expression analysis of SA- and JA/ET-responsive genes determined by qRT-PCR in *VqERF072*-OE transgenic lines and Col-0 plants at 0, 24, 48 and 72 h after *B. cinerea* inoculation. The *AtActin* gene was used as internal control. Error bars indicate the SD from three independent experiments. Statistical significance was determined by Student's two-tailed *t* test (*, $P < 0.05$; **, $P < 0.01$).

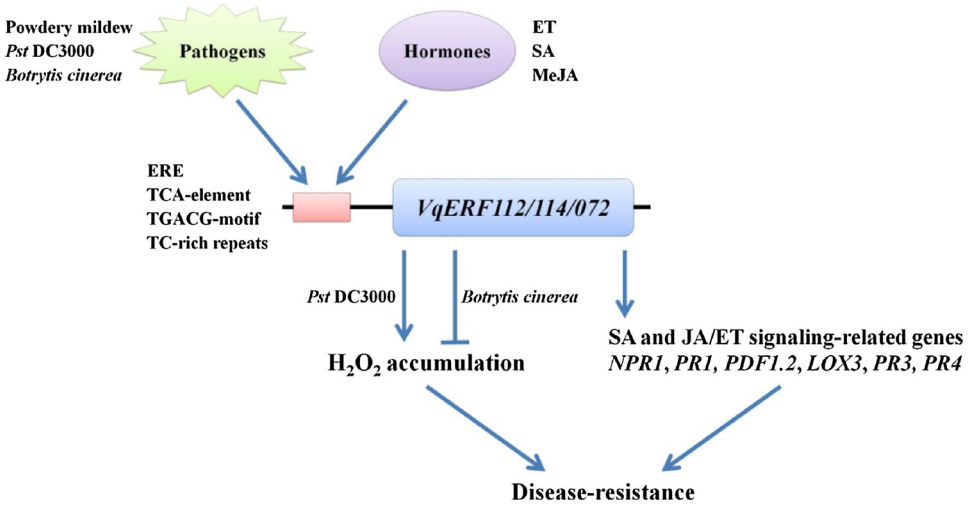

Fig. 10. Hypothetical model for disease-resistance regulation by the ERF transcription factors *VqERF112*, *VqERF114* and *VqERF072*. *VqERF112*, *VqERF114* and *VqERF072* respond to pathogen inoculation and hormone treatments. The promoters of *VqERF112*, *VqERF114* and *VqERF072* could be induced by powdery mildew inoculation and hormone treatments, including ET, SA and MeJA. Overexpression of *VqERF112*, *VqERF114* and *VqERF072* in transgenic *Arabidopsis* enhanced the resistance to *Pst* DC3000 and *B. cinerea*, and increased the expression of SA signaling-related genes *AtNPR1* and *AtPR1* and of JA/ET signaling-related genes *AtPDF1.2*, *AtLOX3*, *AtPR3* and *AtPR4*. Compared to Col-0 plants, the H_2O_2 accumulation in transgenic *Arabidopsis* increased after *Pst* DC3000 inoculation but decreased after *B. cinerea* inoculation. *VqERF112*, *VqERF114* and *VqERF072* positively regulate the resistance to *Pst* DC3000 and *B. cinerea*.

Author contribution statement

YW designed and initiated this study. LW carried out the experiments and analyzed the results. WL helped with the experimental works. LW wrote the manuscript and YW revised the manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2020.110421.

References

[1] J. Glazebrook, Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens, Annu. Rev. Phytopathol. 43 (2005) 205–227.
[2] T. Mengiste, Plant immunity to Necrotrophs, Annu. Rev. Phytopathol. 50 (2012) 267–294.
[3] D.M. Gadoury, L. Cadle-Davidson, W.F. Wilcox, I.B. Dry, R.C. Seem, M.G. Milgroom, Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph, Mol. Plant Pathol. 13 (2012) 1–16.
[4] J.L. Dangl, D.M. Horvath, B.J. Staskawicz, Pivoting the plant immune system from dissection to deployment, Science 341 (2013) 746–751.
[5] D. Couto, C. Zipfel, Regulation of pattern recognition receptor signalling in plants, Nat. Rev. Immunol. 16 (2016) 537–552.
[6] C. Zipfel, G.E.D. Oldroyd, Plant signalling in symbiosis and immunity, Nature 543 (2017) 328–336.
[7] M. Bernoux, J.G. Ellis, P.N. Dodds, New insights in plant immunity signaling activation, Curr. Opin. Plant Biol. 14 (2011) 512–518.
[8] H. Cui, K. Tsuda, J.E. Parker, Effector-Triggered Immunity: From Pathogen Perception to Robust Defense, Annu. Rev. Plant Biol. 66 (2015) 487–511.
[9] N. Coll, P. Epple, J. Dangl, Programmed cell death in the plant immune system, Cell Death Differ. 18 (2011) 1247–1256.
[10] M.A. Torres, J.D.G. Jones, J.L. Dangl, Reactive oxygen species signaling in response to pathogens, Plant Physiol. 141 (2006) 373–378.
[11] A. Baxter, R. Mittler, N. Suzuki, ROS as key players in plant stress signalling, J. Exp. Bot. 65 (2013) 1229–1240.
[12] C.M.J. Pieterse, D.V. Does, C. Zamioudis, A. Leon-Reyes, S.C.M.V. Wees, Hormonal modulation of plant immunity, Annu. Rev. Cell Dev. Biol. 28 (2012) 489–521.
[13] G.P. Bolwell, A. Daudi, Reactive Oxygen Species in Plant-Pathogen Interactions, (2009), pp. 113–133.
[14] B.P.H.J. Thomma, K. Eggermont, I.A.M.A. Penninckx, B. Mauch-Mani, R. Vogelsang, B.P.A. Cammue, W.F. Broekaert, Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 15107–15111.
[15] G. Bannenberg, M. Martínez, M. Hamberg, C. Castresana, Diversity of the enzymatic activity in the lipoxygenase gene family of *Arabidopsis thaliana*, Lipids 44 (2008) 85–95.
[16] Y. Wang, Y. Liu, P. He, J. Chen, O. Lamikanra, J. Lu, Evaluation of foliar resistance to *Uncinula necator* in Chinese wild *Vitis* species, Vitis 34 (1995) 159–164.
[17] J. Shi, M. He, J. Cao, H. Wang, J. Ding, Y. Jiao, R. Li, J. He, D. Wang, Y. Wang, The comparative analysis of the potential relationship between resveratrol and stilbene synthase gene family in the development stages of grapes (*Vitis quinquangularis* and *Vitis vinifera*), Plant Physiol. Biochem. 74 (2014) 24–32.
[18] S.R. Wessler, Homing into the origin of the AP2 DNA binding domain, Trends Plant Sci. 10 (2005) 54–56.
[19] M. Ohme-Takagi, H. Shinshi, Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element, Plant Cell 7 (1995) 173–182.
[20] Y. Sakuma, Q. Liu, J.G. Dubouzet, H. Abe, K. Shinozaki, K. Yamaguchi-Shinozaki, DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration-and cold-inducible gene expression, Biochem. Biophys. Res. Commun. 290 (2002) 998–1009.
[21] T. Nakano, K. Suzuki, T. Fujimura, H. Shinshi, Genome-wide analysis of the ERF gene family in Arabidopsis and rice, Plant Physiol. 140 (2006) 411–432.
[22] N. Gutterson, T.L. Reuber, Regulation of disease resistance pathways by AP2/ERF transcription factors, Curr. Opin. Plant Biol. 7 (2004) 465–471.
[23] K.C. McGrath, B. Dombrecht, J.M. Manners, P.M. Schenk, C.I. Edgar, D.J. Maclean, W.-R. Scheible, M.K. Udvardi, K. Kazan, Repressor- and activator-type ethylene response factors functioning in Jasmonate signaling and disease resistance identified via a genome-wide screen of Arabidopsis transcription factor gene expression, Plant Physiol. 139 (2005) 949–959.
[24] R. Li, X. Xie, F. Ma, D. Wang, L. Wang, J. Zhang, Y. Xu, X. Wang, C. Zhang, Y. Wang, Resveratrol accumulation and its involvement in stilbene synthetic pathway of Chinese wild grapes during berry development using quantitative proteome analysis, Sci. Rep. 7 (2017).
[25] R. Wan, X. Hou, X. Wang, J. Qu, S.D. Singer, Y. Wang, X. Wang, Resistance evaluation of Chinese wild *Vitis* genotypes against *Botrytis cinerea* and different responses of resistant and susceptible hosts to the infection, Front. Plant Sci. 6 (2015).
[26] Y. Jiao, D. Wang, L. Wang, C. Jiang, Y. Wang, *VqMAPKKK38* is essential for stilbene accumulation in grapevine, Hortic. Res. 4 (2017).
[27] Z. Zhu, J. Shi, W. Xu, H. Li, M. He, Y. Xu, T. Xu, Y. Yang, J. Cao, Y. Wang, Three ERF transcription factors from Chinese wild grapevine *Vitis pseudoreticulata* participate in different biotic and abiotic stress-responsive pathways, J. Plant Physiol. 170 (2013) 923–933.
[28] W. Xu, Y. Yu, J. Ding, Z. Hua, Y. Wang, Characterization of a novel stilbene synthase promoter involved in pathogen-and stress-inducible expression from Chinese wild *Vitis pseudoreticulata*, Planta 231 (2010) 475–487.
[29] S.J. Clough, A.F. Bent, Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*, Plant J. 16 (1998) 735–743.
[30] J. Zhuang, R.-H. Peng, Z.-M. Cheng, J. Zhang, B. Cai, Z. Zhang, F. Gao, B. Zhu, X.-Y. Fu, X.-F. Jin, J.-M. Chen, Y.-S. Qiao, A.-S. Xiong, Q.-H. Yao, Genome-wide analysis of the putative AP2/ERF family genes in *Vitis vinifera*, Sci. Hortic. 123 (2009) 73–81.
[31] F. Licausi, M. Ohme-Takagi, P. Perata, APETALA/Ethylene Responsive Factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs, New Phytol. 199 (2013) 639–649.
[32] M. Berrocal-Lobo, A. Molina, R. Solano, Constitutive expression of *ETHYLENE-RESPONSE-FACTOR1* in *Arabidopsis* confers resistance to several necrotrophic fungi, Plant J. 29 (2002) 23–32.
[33] D. Liu, X. Chen, J. Liu, J. Ye, Z. Guo, The rice ERF transcription factor OsERF922 negatively regulates resistance to *Magnaporthe oryzae* and salt tolerance, J. Exp. Bot. 63 (2012) 3899–3911.
[34] M. Wang, Y. Zhu, R. Han, W. Yin, C. Guo, Z. Li, X. Wang, Expression of *Vitis amurensis VaERF20* in *Arabidopsis thaliana* improves resistance to *Botrytis cinerea* and *Pseudomonas syringae* pv. *Tomato* DC3000, Int. J. Mol. Sci. 19 (2018).
[35] M. Asahina, K. Azuma, W. Pitaksaringkarn, T. Yamazaki, N. Mitsuda, M. Ohme-Takagi, S. Yamaguchi, Y. Kamiya, K. Okada, T. Nishimura, T. Koshiba, T. Yokota, H. Kamada, S. Satoh, Spatially selective hormonal control of RAP2.6L and ANAC071 transcription factors involved in tissue reunion in *Arabidopsis*, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 16128–16132.
[36] S. Krishnaswamy, S. Verma, M.H. Rahman, N.N.V. Kav, Functional characterization of four APETALA2-family genes (*RAP2.6*, *RAP2.6L*, *DREB19* and *DREB26*) in *Arabidopsis*, Plant Mol. Biol. 75 (2011) 107–127.
[37] P. Liu, F. Sun, R. Gao, H. Dong, *RAP2.6L* overexpression delays waterlogging induced premature senescence by increasing stomatal closure more than antioxidant enzyme activity, Plant Mol. Biol. 79 (2012) 609–622.
[38] G.K. Pandey, J.J. Grant, Y.H. Cheong, B.G. Kim, L. Li, S. Luan, ABR1, an APETALA2-domain transcription factor that functions as a repressor of ABA response in Arabidopsis, Plant Physiol. 139 (2005) 1185–1193.
[39] T. Li, X.-Y. Wu, H. Li, J.-H. Song, J.-Y. Liu, A dual-function transcription factor, AtYY1, is a novel negative regulator of the *Arabidopsis* aba response network, Mol. Plant 9 (2016) 650–661.
[40] S.K. Sanyal, P. Kanwar, A.K. Yadav, C. Sharma, A. Kumar, G.K. Pandey, Arabidopsis CBL interacting protein kinase 3 interacts with ABR1, an APETALA2 domain transcription factor, to regulate ABA responses, Plant Sci. 254 (2017) 48–59.
[41] M. Berrocal-Lobo, A. Molina, *Arabidopsis* defense response against *Fusarium oxysporum*, Trends Plant Sci. 13 (2008) 145–150.
[42] P.-Y. Huang, J. Catinot, L. Zimmerli, Ethylene response factors in Arabidopsis immunity, J. Exp. Bot. 67 (2015) 1231–1241.
[43] S. Coertze, G. Holz, A. Sadie, Germination and establishment of infection on grape berries by single airborne conidia of *Botrytis cinerea*, Plant Dis. 85 (2001) 668–677.
[44] Y. Wan, H. Schwaninger, P. He, Y. Wang, Comparison of resistance to powdery mildew and downy mildew in Chinese wild grapes, Vitis 46 (2007) 132–136.
[45] Y. Yu, W. Xu, J. Wang, L. Wang, W. Yao, Y. Xu, J. Ding, Y. Wang, A core functional region of the *RFP1* promoter from Chinese wild grapevine is activated by powdery mildew pathogen and heat stress, Planta 237 (2013) 293–303.
[46] W. Xu, Y. Yu, Q. Zhou, J. Ding, L. Dai, X. Xie, Y. Xu, C. Zhang, Y. Wang, Expression pattern, genomic structure, and promoter analysis of the gene encoding stilbene synthase from Chinese wild *Vitis pseudoreticulata*, J. Exp. Bot. 62 (2011) 2745–2761.
[47] J. Catinot, J.-B. Huang, P.-Y. Huang, M.-Y. Tseng, Y.-L. Chen, S.-Y. Gu, W.-S. Lo, L.-C. Wang, Y.-R. Chen, L. Zimmerli, ETHYLENE RESPONSE FACTOR 96 positively regulates *Arabidopsis* resistance to necrotrophic pathogens by direct binding to GCC elements of jasmonate - and ethylene-responsive defence genes, Plant Cell Environ. 38 (2015) 2721–2734.
[48] H. Zhang, L. Huang, Y. Dai, S. Liu, Y. Hong, L. Tian, L. Huang, Z. Cao, D. Li, F. Song, *Arabidopsis* AtERF15 positively regulates immunity against *Pseudomonas syringae* pv. *tomato* DC3000 and *Botrytis cinerea*, Front. Plant Sci. 6 (2015).

[49] X. Zheng, J. Xing, K. Zhang, X. Pang, Y. Zhao, G. Wang, J. Zang, R. Huang, J. Dong, Ethylene response factor ERF11 activates *BT4* transcription to regulate immunity to *Pseudomonas syringae*, Plant Physiol. 180 (2019) 1132–1151.
[50] H. Zhang, Y. Hong, L. Huang, D. Li, F. Song, Arabidopsis AtERF014 acts as a dual regulator that differentially modulates immunity against *Pseudomonas syringae* pv. *tomato* and *Botrytis cinerea*, Sci. Rep. 6 (2016).
[51] Y. Zhao, T. Wei, K.-Q. Yin, Z. Chen, H. Gu, L.-J. Qu, G. Qin, *Arabidopsis* RAP2.2 plays an important role in plant resistance to *Botrytis cinerea* and ethylene responses, New Phytol. 195 (2012) 450–460.
[52] Z. Ouyang, S. Liu, L. Huang, Y. Hong, X. Li, L. Huang, Y. Zhang, H. Zhang, D. Li, F. Song, Tomato SIERF.A1, SIERF.B4, SIERF.C3 and SIERF.A3, Members of B3 Group of ERF Family, Are Required for Resistance to *Botrytis cinerea*, Front. Plant Sci. 7 (2016).
[53] X. Sun, G. Yu, J. Li, J. Liu, X. Wang, G. Zhu, X. Zhang, H. Pan, AcERF2, an ethylene-responsive factor of *Atriplex canescens*, positively modulates osmotic and disease resistance in *Arabidopsis thaliana*, Plant Sci. 274 (2018) 32–43.
[54] Y. Zhu, Y. Li, S. Zhang, X. Zhang, J. Yao, Q. Luo, F. Sun, X. Wang, Genome-wide identification and expression analysis reveal the potential function of ethylene responsive factor gene family in response to *Botrytis cinerea* infection and ovule development in grapes (*Vitis vinifera* L.), Plant Biol. 21 (2019) 571–584.
[55] F. Sun, P. Liu, J. Xu, H. Dong, Mutation in RAP2.6L, a transactivator of the ERF transcription factor family, enhances *Arabidopsis* resistance to *Pseudomonas syringae*, Physiol. Mol. Plant Pathol. 74 (2010) 295–302.
[56] Y. Maruyama, N. Yamoto, Y. Suzuki, Y. Chiba, K. Yamazaki, T. Sato, J. Yamaguchi, The Arabidopsis transcriptional repressor ERF9 participates in resistance against necrotrophic fungi, Plant Sci. 213 (2013) 79–87.
[57] P.-Y. Huang, J. Zhang, B. Jiang, C. Chan, J.-H. Yu, Y.-P. Lu, K. Chung, L. Zimmerli, NINJA-associated ERF19 negatively regulates Arabidopsis pattern-triggered immunity, J. Exp. Bot. 70 (2019) 1033–1047.