



Transcription factor VqERF114 regulates stilbene synthesis in Chinese wild *Vitis quinquangularis* by interacting with VqMYB35

Lan Wang^{1,2,3} · Yuejin Wang^{1,2,3}

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Abstract

Key message VqERF114 regulates stilbene synthesis by interacting with VqMYB35.

Abstract Resveratrol is a stilbene, an important class of secondary metabolites that accumulates in some plant species, including grapevine. In the plant, these are involved in the response to attack by plant pathogens and, as a component of the human diet, they offer a range of significant health benefits. Stilbene synthase (STS), the key enzyme responsible for resveratrol synthesis, has been characterised in a small number of plant species. However, the regulatory mechanisms for stilbene synthesis are uncertain. Here, an ERF family transcription factor from Chinese wild *Vitis quinquangularis*, VqERF114, was characterised as an indirect regulator of stilbene synthesis. A transient overexpression assay of VqERF114 in grapevine leaves led to increased STS expression and stilbene accumulation. However, VqERF114 did not bind to the promoters of VqSTSs but the MYB transcription factor, VqMYB35, did interact with VqERF114. This interaction was confirmed by a yeast two-hybrid assay and bimolecular fluorescence complementation. Furthermore, VqMYB35 showed activation effects on the expressions of VqSTS15, VqSTS28, VqSTS42 and VqSTS46 by binding directly to the MBS elements in their promoters. Co-overexpression of VqERF114 and VqMYB35 resulted in higher VqSTSs expression and more stilbene synthesis. These results demonstrate that VqERF114 regulates stilbene synthesis by interacting with VqMYB35.

Keywords Chinese wild *Vitis* · VqERF114 · VqMYB35 · Stilbene synthase · Resveratrol

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✉ Yuejin Wang
wangyj@nwsuaf.edu.cn

Lan Wang
wanglan0427@163.com

- ¹ College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China
- ² Key Laboratory of Horticultural Plant Biology and Germplasm Innovation in Northwest China, Ministry of Agriculture, Yangling 712100, Shaanxi, China
- ³ State Key Laboratory of Crop Stress Biology in Arid Areas, Northwest A&F University, Yangling 712100, Shaanxi, China

Introduction

Resveratrol is one of a number of natural polyphenols produced by secondary metabolism in some plant species. Resveratrol has so far been found in approximately 72 species, including in grapevine (Langcake and Pryce 1976), peanuts (Jennifer et al. 2002), sorghum (Yu et al. 2005), Scots pine (Rosemann et al. 1991), cranberry, blueberry and knotweed (Vastano et al. 2000). Resveratrol is a major stilbene-type phytoalexin that plays a role as an antimicrobial metabolite in plants (Hart 1981). In human nutrition, resveratrol offers a range of health benefits, having properties that include: anti-inflammatory, anticancer and anti-oxidative (Jang et al. 1997). Increasing attention is recently being paid to resveratrol, not only because its functions in plant defence but also because of its medical properties. In most stilbene-producing plants including grapevine, resveratrol is synthesised via the phenylpropanoid pathway. The phenylpropanoid pathway is a fully characterised metabolic pathway in plants. It produces all kinds of secondary metabolites including lignins, flavonoids and taxon-specific compounds, such as

the stilbenes in grapevine (Chen et al. 2006). The full phenylpropanoid pathway contains two branches, the flavonoid branch and the stilbene branch. These two branches have three same upstream enzymes—phenylalanine ammonia-lyase (*PAL*), cinnamate 4-hydroxylase (*C4H*) and 4-coumarate: CoA ligase (*4CL*). They are distinguished by the last step catalytic enzymes, chalcone synthases (*CHSs*) and stilbene synthases (*STSs*) (Vannozzi et al. 2012). For the *CHSs*, the substrates are *p*-coumaroyl-CoA and malonyl-CoA which result in the synthesis of the flavonoids, while *STSs* catalyse the same substrates to synthesise resveratrol. The key enzyme responsible for the synthesis of resveratrol is *STS*, which belongs to the chalcone synthase superfamily of type III polyketide synthases (Chong et al. 2009). In grapevine, an exhaustive characterisation of the *STS* multigenic family has been carried out with the PN40024 and PN ENTAV 115 genomes (Jaillon et al. 2007; Velasco et al. 2007), resulting in the identification of 48 putative *STS* gene sequences (Vannozzi et al. 2012). Of the two geometric isomers of resveratrol, *trans*-resveratrol is thought to be the more biologically active (Orallo 2006). In addition to resveratrol, more complex derivatives have also been found in grapevine, including *trans*-piceid (Waterhouse and Lamuela-Raventós 1994; Teguo et al. 1996; Romero-Pérez et al. 2001; Gatto et al. 2008), viniferins, pterostilbene (Langcake 1981), and piceatannol (Bavaresco et al. 2002). Resveratrol glucosyltransferase (*RSGT*) is the enzyme that synthesises stilbenoid glucoside from resveratrol (Hall and De Luca 2010).

China is one of the worlds' major biodiversity centres for *Vitis*, so Chinese wild grapevines are very important germplasm resources. They also exhibit high levels of resistance to a number of key pathogens. The Chinese wild species *Vitis quinquangularis*, accession 'Danfeng-2' has attracted considerable interest because of its high resistance to pathogen invasion and the high levels of resveratrol found in its ripe berries (Wan et al. 2007; Shi et al. 2014; Zhou et al. 2015).

The AP2/ERF superfamily is a large group of transcription factors (TFs) in plants (Wessler 2005). This superfamily is identified by the existence of the AP2/ERF domain, which is composed of about 60–70 conserved amino acid residues and participates in DNA binding (Jofuku et al. 1994; Ohme-Takagi and Shinshi 1995). Based on the number of AP2 domains and on the presence of other domains, such as the B3 DNA-binding domain, the AP2/ERF superfamily is classified into three families: AP2, ERF and RAV (Sakuma et al. 2002). Thus, the AP2 family contains proteins with a tandem repetition of two AP2 domains (Okamuro et al. 1997), the ERF family proteins contain one AP2 domain (Nakano et al. 2006) and the RAV family proteins possess a single AP2 domain and an additional B3 domain (Kagaya et al. 1999). Within the ERF family, the AP2/ERF DNA-binding domain can be divided into DREB and ERF domains, based

on the identity of residues at specific positions (Sakuma et al. 2002). The DREB subfamily (group A) proteins interact with an A/GCCGAC element (Stockinger et al. 1997), while the proteins of the ERF subfamily (group B) typically bind to an AGCCGCC element, called the GCC-box. GCC-box element is usually found in the promoters of genes in response to ethylene, pathogens and wounding (Ohme-Takagi and Shinshi 1995). These two subfamilies are further subdivided into six groups, respectively. Members of the ERF family are characterised and involved in many different functions, including the regulation of metabolism, response to biotic and abiotic stresses, hormonal signal transduction and developmental processes in numerous plant species (Nakano et al. 2006). The ERF transcription factor regulating the expression of *STS* has not yet been identified.

The MYB proteins are key factors in regulatory networks participating in metabolism, in development and in response to biotic and abiotic stresses (Dubos et al. 2010). The R2R3-MYB family members from different species possess DNA-binding specificities, with three main MYB binding types so far identified—type I: CNGTTR, type II: TNGTTR and type IIG/AC-elements: CCWAMC (Romero et al. 2010; Prouse and Campbell 2012). Several other variants have also been reported (Kelemen et al. 2015). In grapevine, the first survey of the R2R3-MYB family proteins characterised 108 genes in the $\times 8.4$ genome draft sequence (Matus et al. 2008). Subsequently, a total of 134 MYB sequences have been retrieved in the CRIBI 12 \times v1 genome accession (Wong et al. 2016). Recently, R2R3-MYB TFs have been reported to be involved in the regulation of the *STS* pathway in grapevine. VvMYB14 and VvMYB15 can enhance promoter activity of Vv*STS29* and Vv*STS41* (Höll et al. 2013), and VvMYB14 can bind to the Vv*STS48* promoter (Fang et al. 2014). Moreover, another MYB transcription factor, VvMYB13, is characterised as playing a key role in regulating stilbene accumulation (Wong et al. 2016).

In our previous study, transcriptome analysis was performed on 'Danfeng-2' during the various stages of berry development to obtain specific TFs involved in stilbene synthetic pathway (PRJNA306731; GEO: GSE76256). Eighteen ERF TFs were found to be strongly co-expressed with *STS* genes. Expression analyses of 18 *ERF* genes co-expressed with stilbene synthases in *V. quinquangularis* accession 'Danfeng-2' in response to *Uncinula necator* were carried out, of which, Vq*ERF114* (VIT_18s0072g00260) was found to be strongly co-expressed with *STS* genes and in response to *U. necator*. The functional study of Vq*ERF114* involved in disease resistance will be explored in our further study. In this study, Vq*ERF114* is identified as an activator of *STS* expression. However, it cannot directly bind to the promoters of Vq*STSs*. A yeast two-hybrid assay and a BiFC assay demonstrate that Vq*ERF114* interacts physically with VqMYB35. VqMYB35 can promote the expressions

of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46* by directly binding to their promoters. Furthermore, *VqERF114* co-expresses with *VqMYB35*, resulting in higher expression of *VqSTSs* and in the enhanced accumulation of stilbene. These results demonstrate that *VqERF114* is an indirect positive regulator of stilbene synthesis by interacting with *VqMYB35* in grapevine.

Materials and methods

Plant materials

The Chinese wild grapevine species *V. quinquangularis* accession ‘Danfeng-2’ used in this study was cultivated in the grape germplasm collection at Northwest A&F University, Yangling, Shaanxi, China. The tobacco plants (*Nicotiana benthamiana*) used for subcellular localisation analysis and dual-luciferase assays were grown in the phytotron with a light/dark cycle of 16/8 h at 25 °C.

Gene isolation and sequence analysis

Total RNA was extracted from grape berries or leaves using a Plant RNA Kit (OMEGA). The PrimeScript™ RT reagent Kit (TaKaRa) was used to reverse transcribe the purified RNA into complementary DNA (cDNA) following the manufacturer’s protocol. Full-length coding sequences (CDSs) of *VqERF114*, *VqMYB35*, *VqMYB14* and *VqMYB15* were cloned using the special primers listed in Table S1, using the *V. vinifera* cv. ‘Pinot Noir’ genome database P40024 as a reference. DNAMAN software was used to carry out amino-acid sequence alignment analyses of three ERF proteins, including *VqERF114*, *VvERF114* (Gene ID: VIT_18s0072g00260) and *AtABR1* (Gene ID: AT5G64750). The phylogenetic tree was constructed using MEGA 5.0 software to analyse the evolutionary relationships among the ERF subfamily proteins from grapevine and *Arabidopsis*.

Subcellular localisation

The *VqERF114* and *VqMYB35* CDSs without the stop codon were amplified and cloned into the *KpnI*–*XbaI* sites of the CaMV35S-GFP vector. The fusion construct was mobilised into the GV3101 strain of *Agrobacterium tumefaciens* and then transferred into tobacco leaves. Overnight grown bacterial cells were pelleted by centrifugation, resuspended with infiltration buffer (10 mM MES pH 5.8, 10 mM MgCl₂, 200 μM acetosyringone) and incubated at 28 °C for 3 h. Tobacco leaves were transiently infiltrated with GV3101 carrying either the fusion construct or the control (CaMV35S-GFP), and then cultured in a growth chamber for 48 h. GFP

fluorescence was visualised under a Zeiss confocal microscope LSM510 (Xie and Wang 2016).

Transcriptional activation assay in yeast

The full length of *VqERF114* was amplified and inserted into the pGBKT7 vector (Clontech). The recombinant vector and the empty control vector (pGBKT7) were transferred into the yeast strain Y2HGold. In this assay, pGBKT7-53 vector co-transformed with pGADT7-T vector acted as positive control and pGBKT7-Lam vector co-transformed with pGADT7-T vector acted as negative control. The transformants were grown on SD/-Trp medium at 30 °C for 3–5 days. The yeast transformants were spotted on plates containing two media types: SD/-Trp + X-α-Gal and SD/-Trp + X-α-Gal + Aureobasidin A (200 ng mL⁻¹). The trans-activation activity was assessed by observing the growth of the yeast cells 2 days later.

Transient overexpression assay of *VqERF114* and *VqMYB35* in grapevine leaves

The *A. tumefaciens* GV3101-containing vector expressing *VqERF114-GFP* and *VqMYB35-GFP* fusion genes under the CaMV35S promoter was applied in the *Agrobacterium*-mediated transient assay. This assay was carried out as previously described by Xu et al. (2010) with minor modification. Leaves of *V. quinquangularis* accession ‘Danfeng-2’ were harvested 2 days after the treatment for quantitative RT-PCR and HPLC analysis.

qRT-PCR analysis

Agro-infiltrated leaves were collected to extract total RNA and 1 μg purified RNA was reversely transcribed into cDNA. Synthetic cDNA was diluted tenfold and used as template for qRT-PCR analysis. The PCR mix (20 μL) contained 10 μL of SYBR (novoprotein), 10 μM each primer, 1 μL of cDNA and sterile water. Three-step qRT-PCRs were carried out using the following procedure: 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 15 s, extension at 72 °C for 15 s and a melt cycle with 0.5 °C increments (5 s) from 60 to 95 °C. Each experiment was carried out with three biological replicates, and three technical replicates were analysed for each biological sample. The expression of grapevine *GAPDH* was used to normalise gene transcript levels. The qRT-PCR primers of *VqERF114*, *VqMYB35*, *PAL*, *STS*, *RSGT*, *VqSTS15*, *VqSTS28*, *VqSTS42/46* and *GAPDH* are described in Table S1.

Analysis of stilbene in grapevine leaves using HPLC

HPLC analysis of stilbene was carried out on an HPLC device (Waters) equipped with an Agilent Zorbax SB-C₁₈ column (5 μm , 4.6 \times 250 mm). Grapevine leaves harvested from transient expression assay were dried under vacuum for 24 h and then extracted by adding methanol (HPLC grade, 50 mL g⁻¹) at 4 °C overnight. The extracts were filtered through a 0.22 μm membrane filter and analysed in an HPLC system. Chromatographic separation was carried out using a solvent system of ultrapure water and acetonitrile according to the procedure described in Xu et al. (2011). Stilbene level, expressed as $\mu\text{g g}^{-1}$ dry weight, is the average of three replicates for each independent sample.

GUS activity assay

Three tandem copies of GCC-box were inserted into pC0380-GUS vector and fused with GUS reporter. pGCC-GUS was co-expressed with CaMV35S-VqERF114 in leaves of *V. vinifera* L. cv. Thompson seedless using *Agrobacterium*-mediated transient transformation. pMini35S-GUS, pGCC-GUS and pMini35S-GUS co-expressed with CaMV35S-VqERF114 served as controls. Grapevine leaves were collected for GUS staining and protein determination after culture for 2 days. The GUS activity assay was carried out according to the method described in Xu et al. (2010).

Yeast two-hybrid assay

The interaction between VqERF114 and VqMYB35 was investigated using the Matchmaker™ Gold Yeast Two-Hybrid System (Clontech). The *VqERF114* sequence was cloned into the pGBKT7 (BD) vector and the *VqMYB35* sequence was cloned into the pGADT7 (AD) vector. BD and AD vectors were co-transferred into the yeast strain Y2HGold. Transformed cells were spread onto the following plates: SD/-Leu/-Trp medium and SD/-Ade/-His/-Leu/-Trp + X- α -Gal + AbA (400 ng mL⁻¹) medium. The positive control and the negative control were the same as in the transcriptional activation assay.

Bimolecular fluorescence complementation assays

For the bimolecular fluorescence complementation (BiFC) assays, the coding sequences of *VqERF114* and *VqMYB35* without the stop codon were cloned into the pSPYNE and pSPYCE vectors, respectively. Tobacco protoplasts were co-transformed with the various plasmids using the polyethylene glycol (PEG) method (Yu et al. 2013). Protoplasts were cultured at room temperature for 24 h in the dark and observed using a Zeiss confocal microscope LSM510.

Promoter analysis

Genomic DNA of grape berries or leaves was isolated using the method of Xu et al. (2010). The promoters of the stilbene synthase genes (*VqSTSs*) were amplified using primers (Table S1) according to the homologous sequences. The Plant-CARE database was used to predict conserved *cis*-element motifs presented in each promoter (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

Dual-luciferase assays

Dual-luciferase assays were carried out to measure transactivation activities of VqERF114 and VqMYB35 on the target promoter. The full-length cDNA of *VqERF114* or *VqMYB35* was inserted into pGreenII 62-SK vector and the promoters of *VqSTSs* were cloned into pGreenII 0800-LUC vector. All constructs were individually electroporated into *Agrobacterium* GV3101 and expressed transiently in tobacco leaves by *A. tumefaciens*-mediated infiltration. *Agrobacterium* cultures were resuspended with infiltration buffer to an OD₆₀₀ of 0.4. The binding activity of the TFs on the promoter was assayed 3 days after infiltration and assessed by the ratio of enzyme activities of Firefly luciferase (LUC) and Renilla luciferase (REN) using Dual Luciferase Reporter Gene Assay Kit (Beyotime). The *A. tumefaciens* culture of TF (900 μL) was mixed with promoter (100 μL) to test the activity of a special TF on the promoter. The LUC/REN value of the empty vector 62-SK on the promoter was set as 1, as a calibrator. To determine the combined effect of two TFs on the promoter, 450 μL of each TF was mixed with 100 μL of promoter. The effect of the mixtures which contained each TF (450 μL) and empty vector 62-SK (450 μL) was also tested on the promoter as control. For each interaction between TF and promoter, three independent experiments were carried out, with three replicates in each experiment.

Yeast one-hybrid assay

The Matchmaker™ Gold Yeast One-Hybrid System (Clontech) was used to carry out the Y1H assay. Three tandem copies of GCC-box and the promoters of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46* were inserted into pAbAi vector to build the pAbAi-bait and the constructs were integrated separately into the genome of the Y1HGold yeast strain. The core sequence of MBS element in the promoters of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46* was mutated from CAGTTA to AAAAAA. The full length of VqMYB35 was cloned into pGADT7 to build the AD-prey vector. The minimal inhibitory concentrations of Aureobasidin A (AbA) for the bait strains were tested according to the system user manual. The AD-prey vectors were transferred into the bait

strain and grown on SD/-Leu/AbA plate to confirm the interaction between TF and promoter.

Results

Identification and molecular characterisation of VqERF114

The cDNA clone of VqERF114 consists of a 1266 bp ORF predicted to encode a protein of 421 amino acids. Its

predicted molecular weight and isoelectric point (pI) are 44.48 kDa and 6.526, respectively. There are 73 predicted ERF subfamily proteins in grapevine and 65 in *Arabidopsis* (Zhuang et al. 2009). Amino acid alignments show that VqERF114 contains a single AP2 DNA-binding domain, containing 58 amino acids from 201 to 258 aa. VqERF114 shows very high similarity to VvERF114 in *V. vinifera* (99% identity) (Fig. 1a). To determine the classification of VqERF114, a phylogenetic tree was built using the neighbour-joining method. This shows that VqERF114 is closely related to VvERF114 and that both belong to the

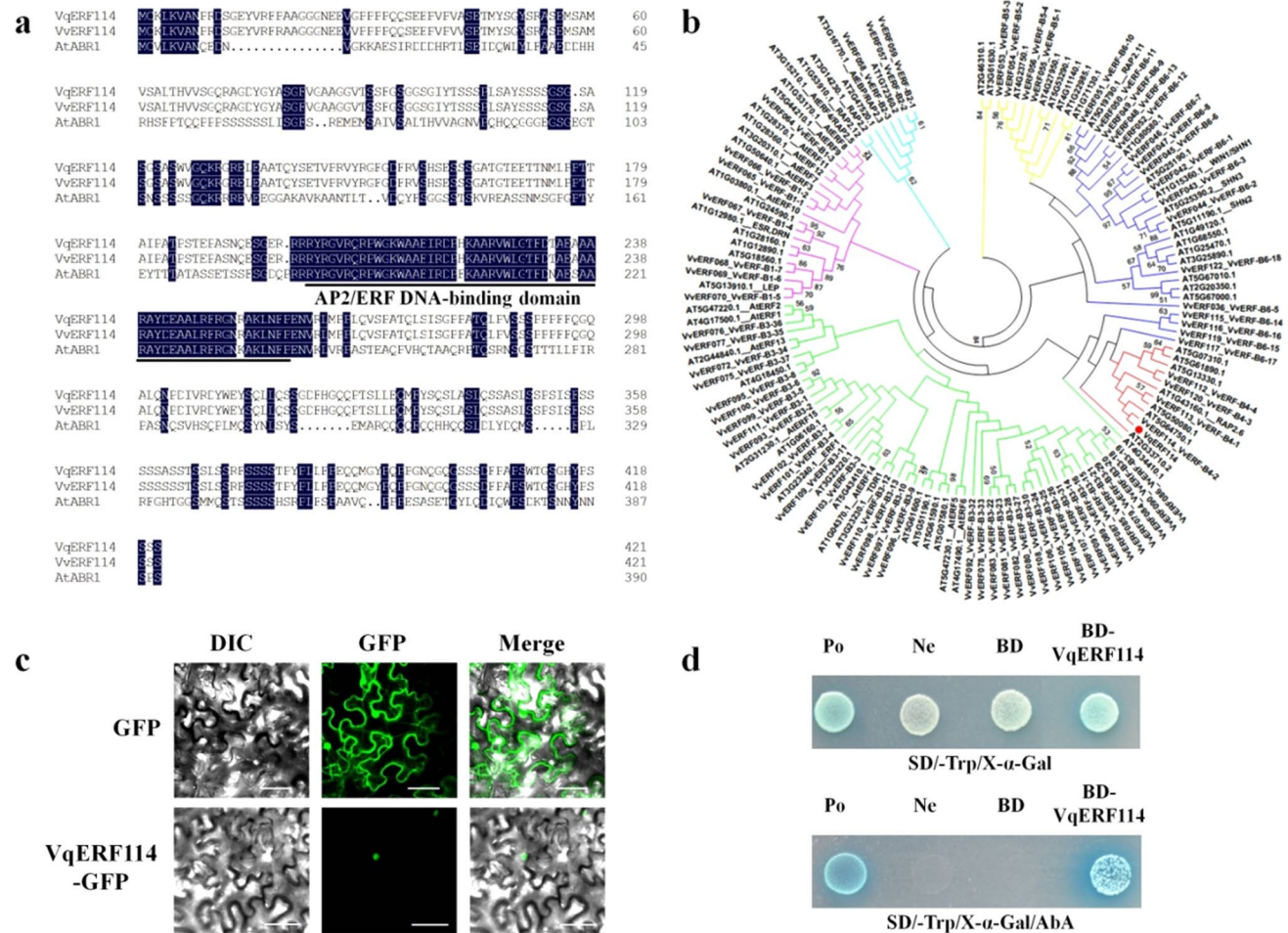


Fig. 1 Sequence alignment analysis, phylogenetic analysis and molecular characterisation of VqERF114. **a** Sequence alignment of the deduced VqERF114 protein with other ERF proteins including VvERF114 (Gene ID: VIT_18s0072g00260) and AtABR1 (Gene ID: AT5G64750). The consensus sequence is shown in black and the solid black line indicates the conserved AP2/ERF DNA-binding domain. Dashes show gaps in the alignment. **b** The phylogenetic analysis of VqERF114 and ERF subfamily proteins from *Vitis vinifera* and *Arabidopsis thaliana*. Multiple sequence alignment was carried out using ClustalW and the phylogenetic tree was constructed with MEGA 5.0 using a bootstrap test of phylogeny with the neighbour-joining method. **c** Subcellular localisation of VqERF114 in tobacco

leaves. The fusion protein (VqERF114-GFP) and GFP control were transiently expressed in *Nicotiana benthamiana* leaves by *Agrobacterium tumefaciens* strain GV3101. GFP fluorescence was observed with a confocal microscope. Bars 50 μm. **d** Transcriptional activation of VqERF114 in yeast cells. The coding region of VqERF114 was cloned into the pGBKT7 (BD) vector to build the BD-VqERF114 construct. BD-VqERF114 together with the positive control (p53 + T-antigen), negative control (Lam + T-antigen) and empty vector (BD) were transferred into yeast cells. Yeast clones transformed with different constructs were grown on SD/-Trp plates at 30 °C for 3–5 days. Transcription activation was monitored according to the growth status of yeast cells and the X-α-Gal assay

ERF subfamily group B-4 (Fig. 1b). VqERF114 and green fluorescent protein (GFP) fusion construct were transiently expressed in tobacco leaves to determine the subcellular localisation of VqERF114. The signal of VqERF114–GFP was found exclusively in the nuclei of the epidermal cells whereas the GFP fluorescence in the control was observed throughout the cells (Fig. 1c). To test the transcriptional activity of VqERF114, pGBKT7-VqERF114 construct was produced and transferred into Y2HGold yeast cells. As with the positive control, yeast cells expressing VqERF114 grew well on SD/-Trp plates and showed AbA resistance (Fig. 1d), indicating that VqERF114 possesses transactivation ability in yeast cells. These results demonstrate that VqERF114 protein is nuclear localised, consistent with its putative role as a transcription factor.

Transient overexpression of *VqERF114* in grapevine leaves

The expression of *VqERF114* in infiltrated leaves was analysed using qRT-PCR 2 days after infiltration. The results show that the transcript levels were very much higher (8.9-fold) in *VqERF114* overexpressed leaves than in the empty vector (EV) controls (Fig. 2a), confirming that *VqERF114* was successfully overexpressed. The expressions of *PAL* and *STSs* were examined in the same leaves, and the transcript abundances were also increased by 5.6-fold and 10.9-fold, respectively (Fig. 2a). However, the transcript level of *RSGT* in the *VqERF114*-overexpressing leaves showed no significant difference from in the control EV (Fig. 2a). The stilbene

levels in the transformed grapevine leaves were measured by HPLC. As shown in Fig. 2b, the contents of *trans*-resveratrol and *trans*-piceid increased after *VqERF114* was overexpressed. Taken together, these results indicate that *VqERF114* promotes the transcription of *STSs* and stilbene synthesis.

VqERF114 binds to GCC-box element

Proteins of the ERF subfamily specifically bind to an AGCCGCC element, called GCC-box, present in the promoters of genes (Ohme-Takagi and Shinshi 1995). Three tandem copies of GCC-box were inserted into pC0380-GUS vector and fused with GUS reporter. pGCC-GUS was co-expressed with CaMV35S-VqERF114 in grapevine leaves using *Agrobacterium*-mediated transient transformation. In GUS histochemical staining, no GUS activity was observed in pMini35S-GUS, pGCC-GUS or in pMini35S-GUS + CaMV35S-VqERF114. However, obvious GUS activity was observed in leaves co-infiltrated with pGCC-GUS and CaMV35S-GUS. The same result was obtained in the GUS protein determination assay. GUS activity appeared higher when pGCC-GUS co-expressed with CaMV35S-VqERF114 than in the control (Fig. 3a). Three tandem copies of GCC-box were inserted into pAbAi vector and transferred into Y1HGOLD. A yeast one-hybrid assay was carried out to confirm that VqERF114 binds specifically to the GCC-box element (Fig. 3b). It has been reported that the STS multigenic family contains 48 members and 33 members encoding

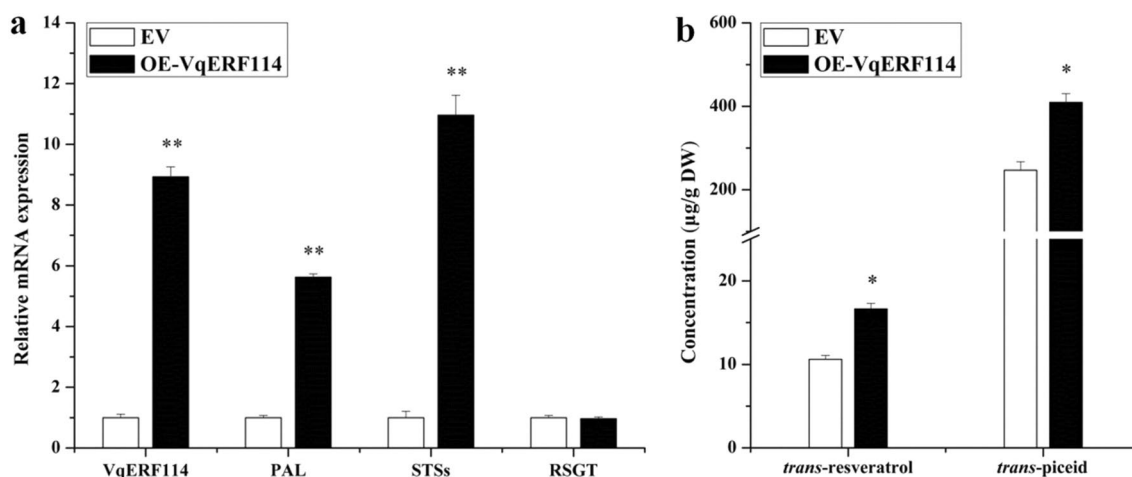


Fig. 2 Transient overexpression of *VqERF114* in grapevine leaves. **a** Relative expressions of *VqERF114*, *PAL*, *STSs* and *RSGT* in *VqERF114* overexpressed grapevine leaves 2 days after infiltration. The expression was calculated relative to the average value in empty vector (EV) control leaves (set as 1). EV represents grapevine leaves treated with bacterial suspension carrying empty vector (CaMV35S-GFP). The *GAPDH* gene was used as internal control. Error bars indi-

cate the SD from three independent experiments. **b** Concentrations of *trans*-resveratrol and *trans*-piceid in *VqERF114* overexpressed grapevine leaves. The stilbene level, expressed as $\mu\text{g g}^{-1}$ dry weight, is the average of three replicates for each independent sample. Statistical significance was determined by Student's two-tailed *t* test (* $P < 0.05$; ** $P < 0.01$)

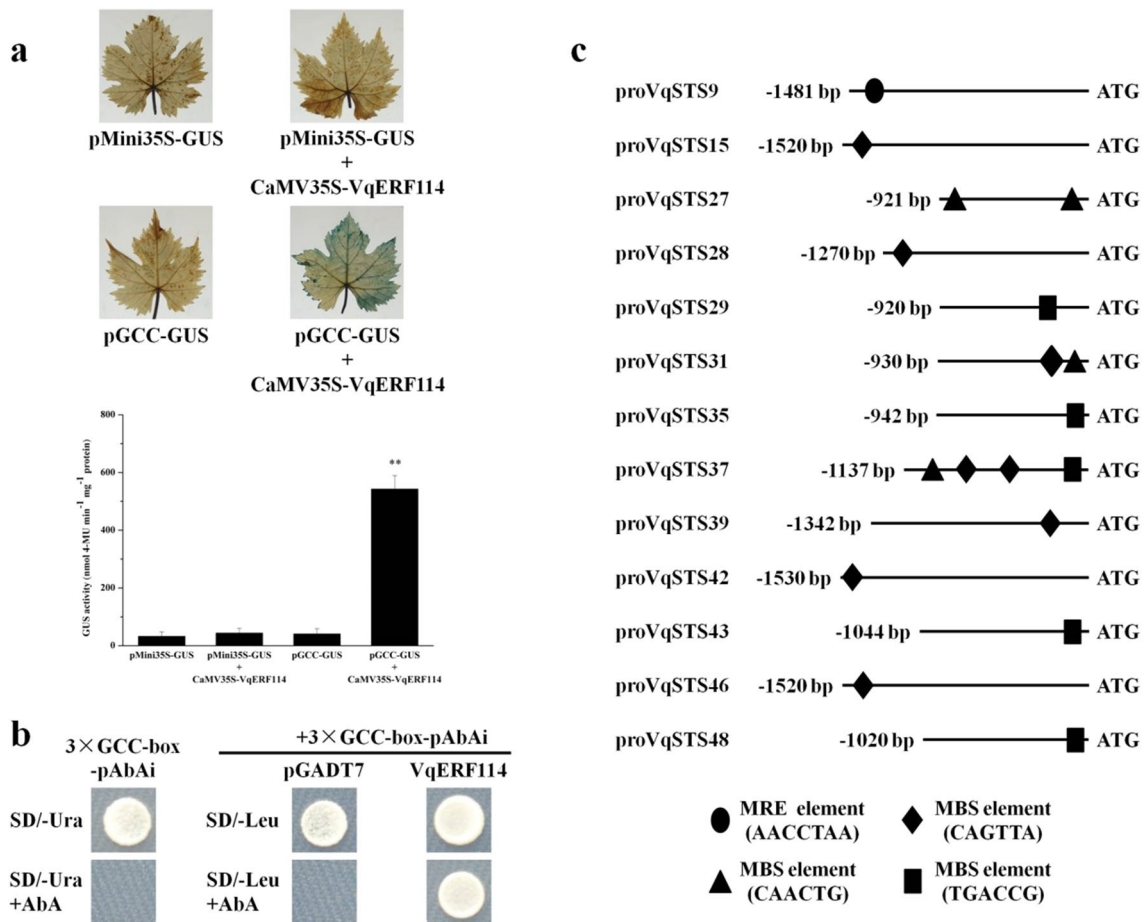


Fig. 3 Transcription factor VqERF114 binds directly to GCC-box element. **a** GUS activity assay in transiently transformed grapevine leaves. Three tandem copies of GCC-box were inserted into pC0380-GUS vector and fused with GUS reporter. pGCC-GUS was co-expressed with CaMV35S-VqERF114 using *Agrobacterium*-mediated transient transformation. pMini35S-GUS, pGCC-GUS and pMini35S-GUS co-expressed with CaMV35S-VqERF114 served as controls. Grapevine leaves were collected for GUS staining and protein determination after culture for 2 days. Statistical significance was

determined by Student’s two-tailed *t* test (***P* < 0.01). **b** Yeast one-hybrid assay of the interaction between VqERF114 and GCC-box. Three tandem copies of GCC-box were inserted into pAbAi vector. 3×GCC-box-pAbAi vector was transferred into yeast Y1HGold strain and interaction was confirmed on an SD/-Leu + AbA medium. **c** *Cis*-element motifs presented in each *STS* promoter are shown by different shapes. None of the promoters of *VqSTS*s contain the GCC-box element and 13 *STS* promoters contain MRE or MBS element which can be recognised by the MYB transcription factor

complete ORFs (Vannozzi et al. 2012). Promoters of 13 full-length coding *VqSTS*s were amplified based on the homologous sequences, and the Plant-CARE database was used to predict the conserved *cis*-element motifs in each promoter. However, analysis of *cis*-element motifs indicates that promoters of these 13 *VqSTS*s had no GCC-box, but contained the MRE or MBS element, which can be recognised by MYB TFs (Fig. 3c). This led us to speculate that VqERF114 activates the expression of *STS*s which may occur via a transcription complex involving an additional (as yet unknown) regulator that can bind directly to the promoters of *VqSTS*s, such as MYB TFs.

Identification of VqMYB35 as a VqERF114 interacting protein

It has been reported that the R2R3-MYB TFs VvMYB14 and VvMYB15 can regulate the expression of *STS* in grapevine (Höll et al. 2013). All MYB TFs co-expressed with *STS* according to our previous study were amplified and cloned into pGADT7 vector. The yeast two-hybrid system was carried out to confirm the potential interactions between VqERF114 and VqMYBs co-expressed with *STS* (data not shown). Among all VqMYBs, one MYB was found to interact with VqERF114, showing 98% amino acid

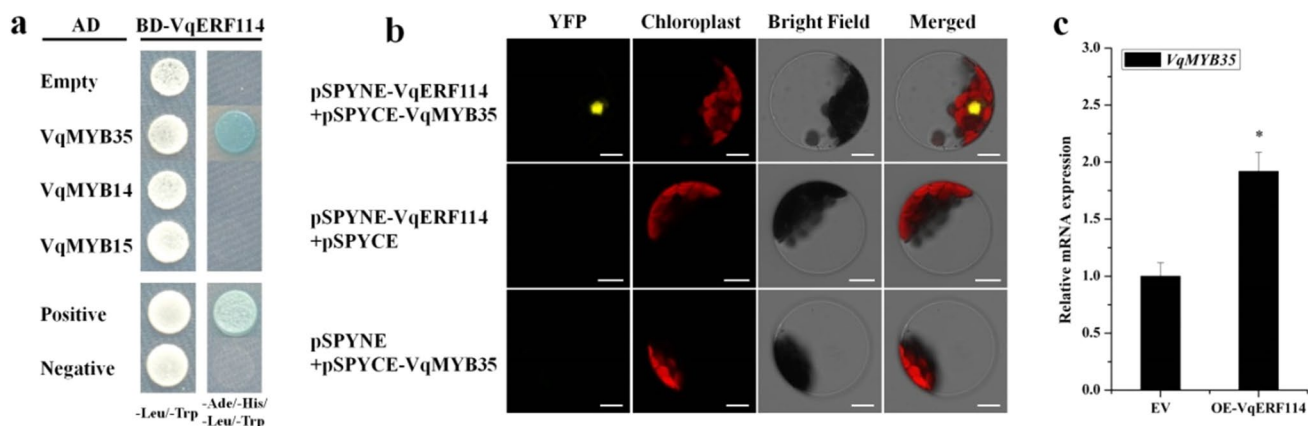


Fig. 4 VqERF114 interacts with the R2R3-MYB transcription factor VqMYB35 and positively regulates its expression. **a** Interaction of VqERF114 with VqMYB35 in yeast two-hybrid assay. pGADT7-VqMYB35 and pGBKT7-VqERF114 plasmids were co-transferred into yeast Y2H Gold cells. The empty pGADT7 vector co-transformed with pGBKT7-VqERF114 was used as control. Transformed cells were spread onto the SD/-Ade/-His/-Leu/-Trp + X- α -Gal + AbA medium for the confirmation assay. **b** BiFC assays were carried out to confirm the interaction between VqERF114 and VqMYB35 in

tobacco protoplasts. Plasmids carrying pSPYNE-VqERF114 and pSPYCE-VqMYB35 were transferred into protoplasts with the combinations indicated. Bars 10 μ m. **c** Overexpression of VqERF114 increased the transcript of VqMYB35. The expression of VqMYB35 was calculated using qRT-PCR. The GAPDH 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 (* $P < 0.05$)

identity with VvMYB35 (Gene ID: VIT_14s0066g02180). This was designated VqMYB35. However, VqERF114 could not interact with VqMYB14 or VqMYB15 (Fig. 4a). A BiFC assay was carried out using a tobacco protoplast transient expression system to further confirm the interaction between VqERF114 and VqMYB35. Fluorescence was observed in the nuclei only when pSPYNE-VqERF114 was co-expressed with pSPYCE-VqMYB35, while negative controls produced no detectable fluorescence (Fig. 4b). Overexpression of VqERF114 could enhance the transcription of VqMYB35 as it has been proven via qRT-PCR analysis (Fig. 4c). These results suggest that VqERF114 interacts with VqMYB35 *in planta* and positively regulates the transcription of VqMYB35.

VqMYB35 activates the expressions of VqSTSs by directly targeting their promoters

Analysis of *cis*-element motifs indicates that there were 13 promoters of VqSTSs containing MRE or MBS elements, which can be recognised by MYB transcription factor (Fig. 3c). Dual-luciferase assays were carried out in tobacco leaves to detect the effects of VqMYB35 on the promoter expression of 13 VqSTSs. VqSTSs promoters were inserted into the pGreenII 0800-LUC vector and co-infiltrated into tobacco leaves with VqMYB35 (Fig. 5a). As illustrated in Fig. 5b, VqMYB35 showed a significant activation effect on the VqSTS15, VqSTS28, VqSTS42 and VqSTS46 promoters, while the remaining members showed very limited effects. In qRT-PCR analyses, the transcripts of VqMYB35,

VqERF114, VqSTSs, VqSTS15, VqSTS28 and VqSTS42/46 were increased in VqMYB35-overexpressing grapevine leaves compared with the EV control (Fig. 5c). This confirms that VqMYB35 positively regulates the transcription of VqERF114. Subcellular localisation analysis revealed that VqMYB35 is also localised in the nuclei (Fig. 5d). Analysis of conserved *cis*-element motifs demonstrates that these four promoters contain single MBS element (Fig. 6a). The yeast one-hybrid assay confirmed that VqMYB35 can bind directly to the VqSTS15, VqSTS28, VqSTS42 and VqSTS46 promoters (Fig. 6b). However, the binding activity disappeared when the MBS core sequence CAGTTA was mutated to AAAAAA (mMBS) (Fig. 6c). These results illustrate that the MBS element in the VqSTS15, VqSTS28, VqSTS42 and VqSTS46 promoters can be recognised by VqMYB35. This indicates that VqMYB35 activates the expressions of VqSTSs by directly targeting their promoters via the MBS element.

VqERF114 is an indirect regulator of VqSTSs by cooperating with VqMYB35

When the combined effects of VqERF114 and VqMYB35 were analysed in the dual-luciferase assays, at least a synergistic 3.5-fold activation of the VqSTS15, VqSTS28, VqSTS42 and VqSTS46 promoters was observed, compared with the activation by either VqERF114 or VqMYB35, which alone promoted *trans*-activation at most 1.3- and 2-fold, respectively (Fig. 7a). Additionally, transient overexpression of both genes (VqERF114/VqMYB35) in grapevine

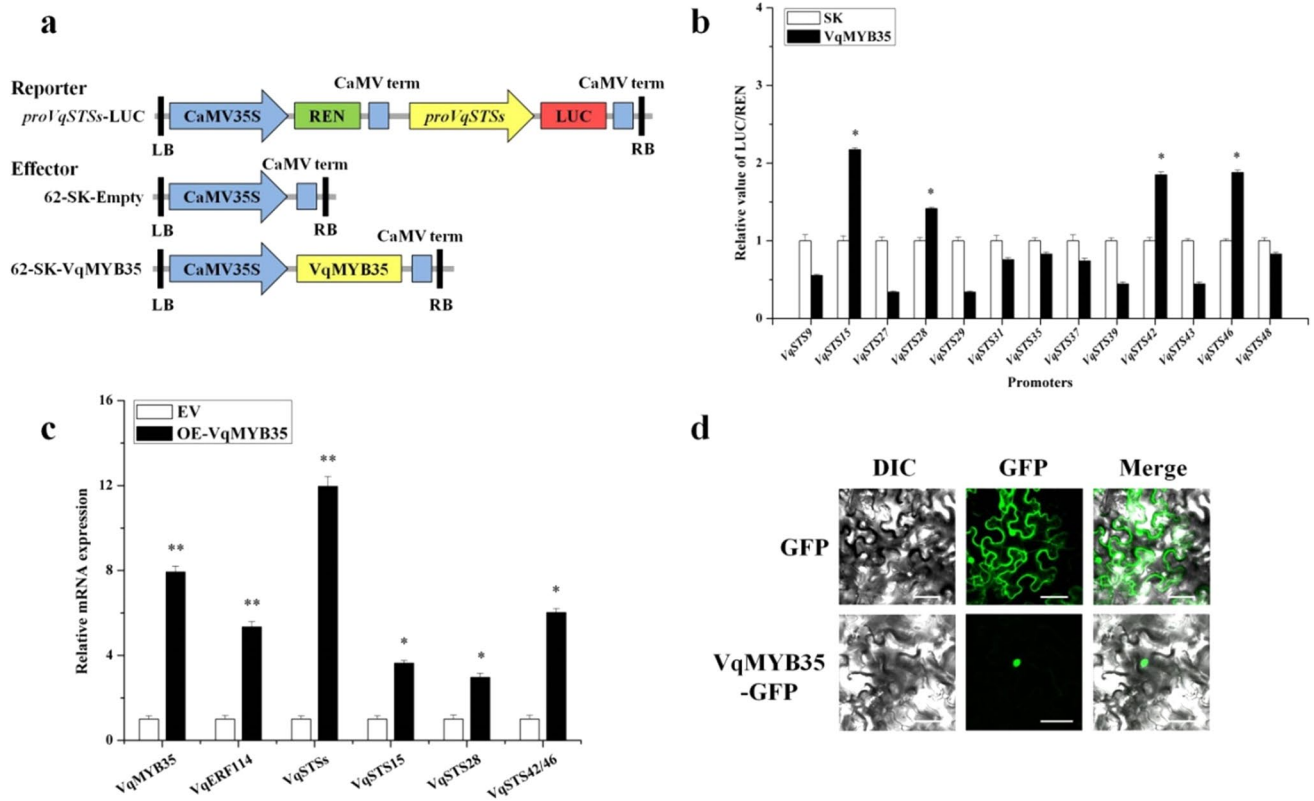


Fig. 5 VqMYB35 is localised in the nucleus and activates the expressions of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46*. **a** Diagrams of the reporter and effector vectors used in dual-luciferase assays. **b** VqMYB35 activates the promoters of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46* in dual-luciferase assays. The LUC/REN value of the empty vector 62-SK on each promoter was set as 1, as a calibrator. Each value represents the mean \pm SD of three independent experiments, with three replicates in each experiment. Statistical significance was determined by Student's two-tailed *t* test (* $P < 0.05$). **c** Overexpression of VqMYB35 promotes the expressions of *VqERF114*, *VqSTS8*, *VqSTS15*, *VqSTS28* and *VqSTS42/46*.

leaves resulted in higher expressions of *VqSTSs*, *VqSTS15*, *VqSTS28* and *VqSTS42/46* transcripts (Fig. 7b) and higher stilbene synthesis (Fig. 7c) than overexpression of either *VqERF114* or *VqMYB35*. These results indicate that the VqERF114–VqMYB35 transcription complex regulates stilbene synthesis by activating the expressions of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46*.

Discussion

AP2/ERF TFs exist widely in the plant kingdom. These proteins play role in the regulation of primary and secondary metabolism, responses to environmental stimuli and in the processes of growth and development. The participation of ERF TFs in the control of secondary metabolism derives mostly from plants producing metabolites for pharmaceutical

The expression was calculated relative to the average value in empty vector (EV) control leaves (set as 1). EV represents grapevine leaves treated with bacterial suspension carrying empty vector (CaMV35S-GFP). The *GAPDH* 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 (* $P < 0.05$; ** $P < 0.01$). **d** Subcellular localisation of VqMYB35 in tobacco leaves. The fusion protein (VqMYB35-GFP) and GFP control were transiently expressed in *Nicotiana benthamiana* leaves by *Agrobacterium tumefaciens* strain GV3101. GFP fluorescence was observed with a confocal microscope. Bars 50 μ m

application (Licausi et al. 2013). For example, ORCA2 and ORCA3 in *Catharanthus roseus* regulate the expression of strictosidine synthase, and participate in the synthesis of terpenoid indole alkaloids (Van Der Fits and Memelink 2001). In tobacco, the AP2/ERF factor ORC1 is characterised as regulating the nicotine synthesis (De Boer et al. 2011). The expression of artemisin synthetic genes is controlled by AaERF1 and AaERF2 in *Artemisia annua* (Yu et al. 2012). Moreover, TcERF12 and TcERF15 regulate taxol synthesis in *Taxus chinensis*, by acting as repressor and activator, respectively, of the *tasy* gene (Zhang et al. 2015). In Newhall sweet orange (*Citrus sinensis* Osbeck), CitERF71 can regulate the synthesis of *E*-geraniol by binding directly with the *CitTPS16* promoter (Li et al. 2017). The production of phenolic acids is positively regulated by SmERF115 in *Salvia miltiorrhiza* (Sun et al. 2019), while MdERF1B controls anthocyanin and proanthocyanidin synthesis in apple

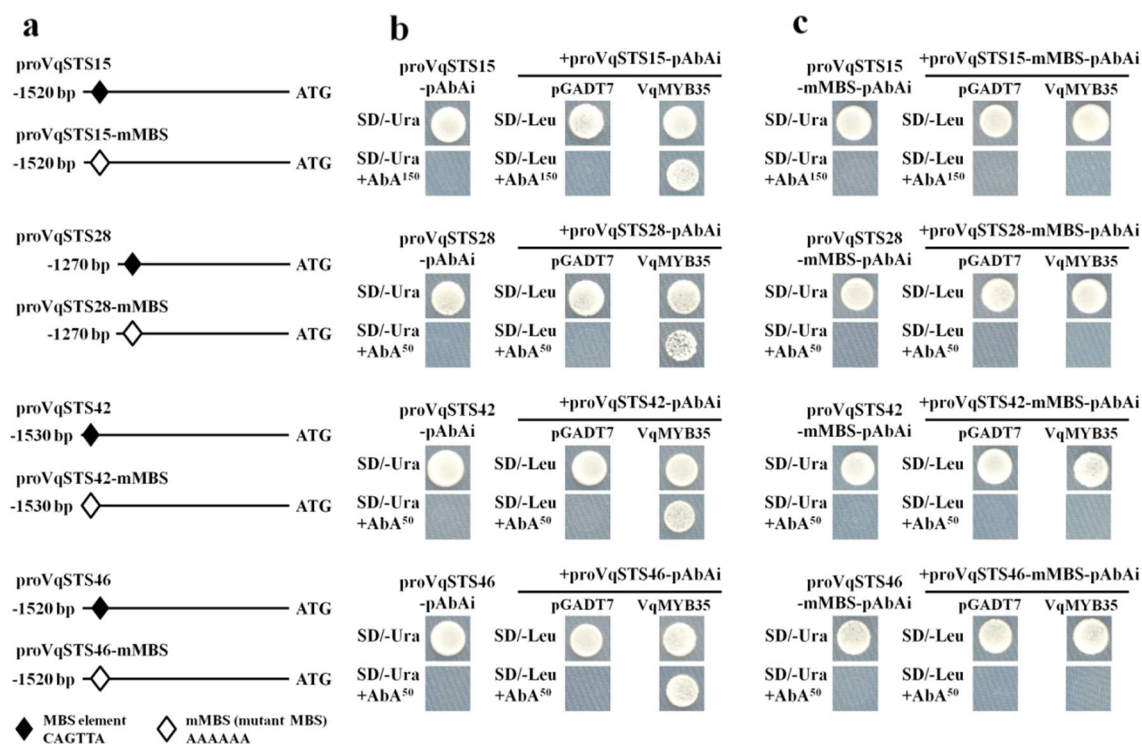


Fig. 6 VqMYB35 directly targets the promoters of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46* by binding to the MBS element. **a** Schematic representation of promoter structure of four *STS* genes. Black diamonds indicate MBS element (CAGTTA) and white diamonds indicate mutant MBS element (AAAAAA). **b** Yeast one-hybrid assay of the interaction between VqMYB35 and four *STS*

promoters. **c** Yeast one-hybrid assay of the interaction between VqMYB35 and four mutant *STS* promoters. The minimal inhibitory concentrations of Aureobasidin A (AbA) for the bait strains were tested on SD/-Ura medium in the presence of AbA and interaction was confirmed on SD/-Leu medium in the presence of AbA

(Zhang et al. 2018a). In grapevine, resveratrol is a major secondary metabolite that plays important role in pathogen resistance. To identify new regulators of secondary metabolism in grapevine, multi-omics studies incorporating systems biological approaches have been carried out by Wong and Matus (2017). The phenylpropanoid regulatory network in the grape berry was generated, and positive co-expression correlations between AP2/ERF TFs and *STS* genes were demonstrated (Wong and Matus 2017). Furthermore, VvERF114, the homologue of VqERF114 in *V. vinifera*, is identified as part of the grapevine *STS* regulatory network (Wong et al. 2016). Vannozi et al. (2018) integrated two subnetworks built from RNA-Seq data and microarray, and obtained the gene co-expression network (GCN) of grapevine TFs and *STS*. In this GCN, VvERF114 was also highly represented in the microarray interaction module (Vannozi et al. 2018). As shown in Fig. 2, VqERF114 positively co-expressed with *STS* and facilitated the accumulation of stilbene. Our own experimental results further confirm the GCN of grapevine *STS* and VqERF114. Proteins of the ERF subfamily specifically bind to the GCC-box element, which is often found in the promoters of genes (Ohme-Takagi and Shinshi 1995). However, none of the promoters of *VqSTSs*

contain the GCC-box element (Fig. 3c). This result led us to speculate that VqERF114 may regulate the expression of *VqSTSs* in an indirect manner, rather than directly binding to the promoters of *VqSTSs*.

Recently, an increasing number of TFs have been identified as being involved in the regulation of *STS* expression in grapevine. Members of R2R3-MYB TFs, such as VvMYB13, VvMYB14 and VvMYB15, have been characterised as regulators of stilbene synthesis (Höll et al. 2013; Fang et al. 2014; Wong et al. 2016). Besides MYB TFs, some WRKY TFs have been identified as involved in control of the stilbene synthetic pathway. For example, VvWRKY24 seems to be the sole activator of the *VvSTS29* promoter and VvWRKY3 functions through a combined effect with VvMYB14 (Vannozi et al. 2018). Moreover, VvWRKY8 represses the expressions of *VvSTS15* and *VvSTS21* through direct interaction with VvMYB14 (Jiang et al. 2018). With the increasing numbers of TFs identified as regulators of *STS* genes, the regulatory network of stilbene synthesis in the grapevine is becoming increasingly clear. Here, VqMYB35 is demonstrated to activate the expressions of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46* by directly targeting the MBS element in their promoters (Figs. 5, 6). In *Arabidopsis*,

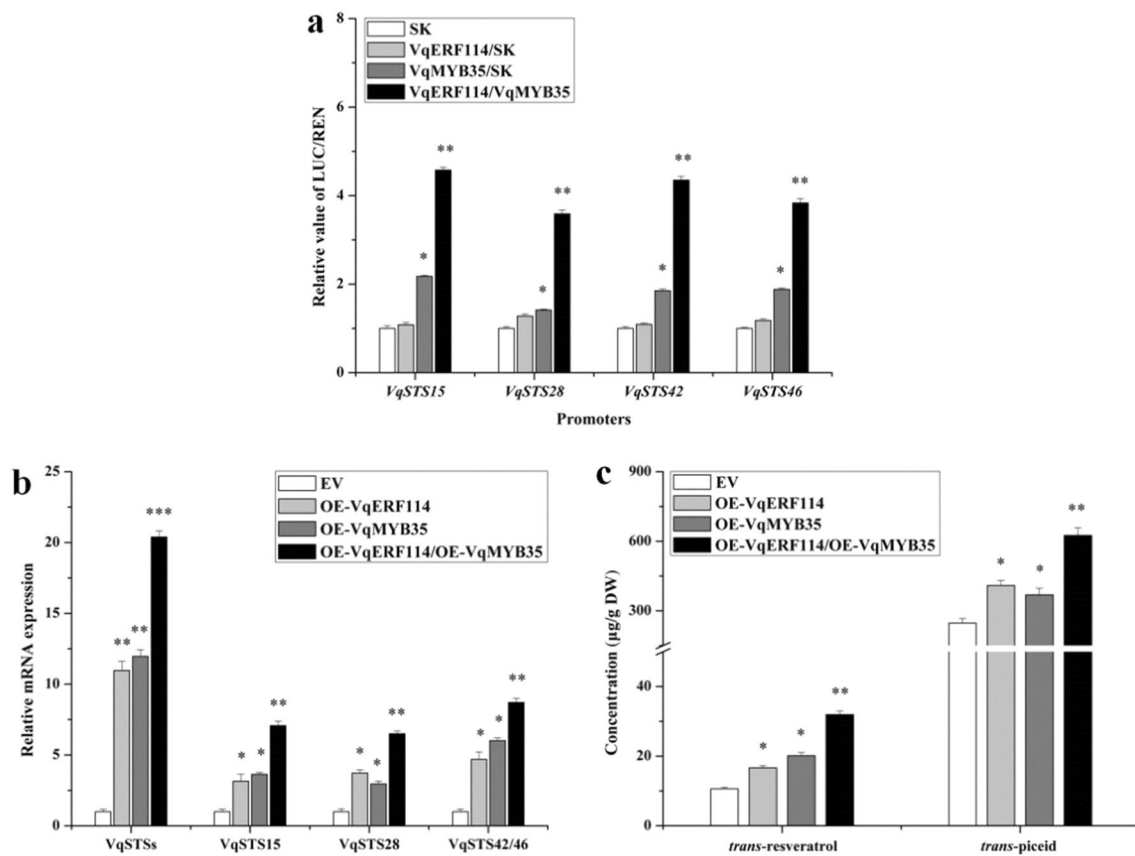


Fig. 7 Combined effects of VqERF114/VqMYB35 on the promoter activation of *VqSTSs*, the *STS* expression and stilbene synthesis. **a** VqERF114 co-expressed with VqMYB35 in dual-luciferase assays shows more significant activation of four *STS* promoters. The LUC/REN value of the empty vector 62-SK on each promoter was set as 1, as a calibrator. Each value represents the mean \pm SD of three independent experiments, with three replicates in each experiment. **b** qRT-PCR analysis of combined effects of VqERF114/VqMYB35

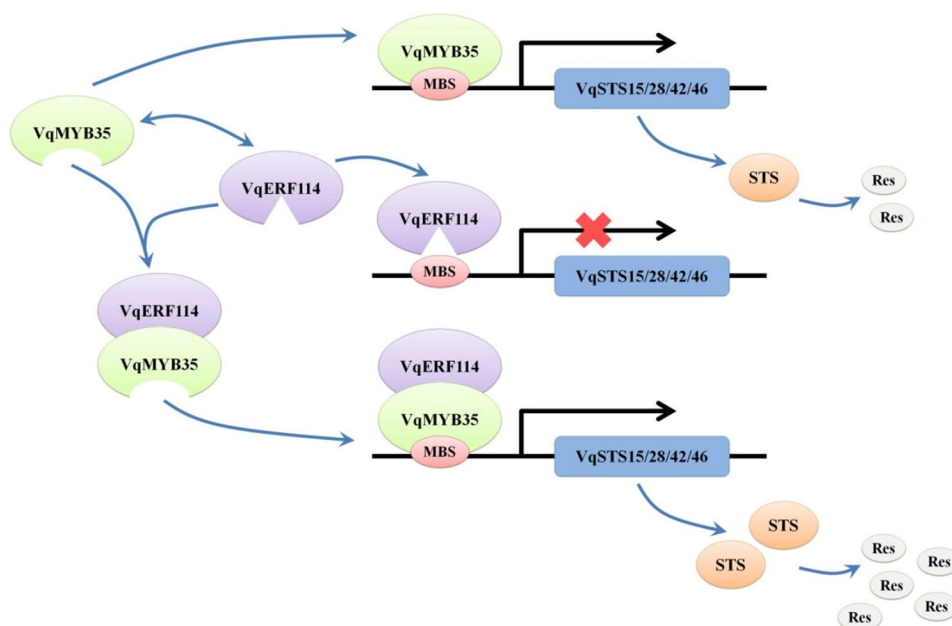
on the *STS* expression. The *GAPDH* gene was used as internal control. Error bars indicate the SD from three independent experiments. **c** Combined effects of VqERF114/VqMYB35 on the synthesis of *trans-resveratrol* and *trans-piceid*. The stilbene level, expressed as $\mu\text{g g}^{-1}$ dry weight, was the average of three replicates for each independent sample. Statistical significance was determined by Student's two-tailed *t* test (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

AtMYB35 (*TDF1*) is identified as playing a key role in anther development and in tapetal function for microspore maturation (Dubos et al. 2010; Zhu et al. 2010). Meanwhile, *AoMYB35* is a candidate for the sex-determining genes in *Asparagus officinalis* (Tsugama et al. 2017). Our study discovers a novel function for VqMYB35 in regulating the expression of *STS* genes in grapevine.

Transcriptional regulation by TFs is a complex process and most plant TFs play a role in a combined manner. The TFs can bind to DNA and regulate the expression of the corresponding gene or cooperate by protein–protein interactions by forming protein complexes (Bemer et al. 2017). A meaningful number of interfamily TF interactions have recently been recognised. These help us to explore specific and dynamic gene expression. Cross-family TF interactions between AP2/ERF and MYB have been identified by employing the publicly available BioGRID database (Bemer et al. 2017). In Chinese wild *V. quinquangularis*

accession ‘Danfeng-2’, the interaction between VqERF114 and VqMYB35 was confirmed using the yeast two-hybrid assay and the BiFC assay (Fig. 4). Moreover, VqERF114 and VqMYB35 can positively regulate the transcription of one another (Figs. 4c, 5c). Recent research has provided evidence for interactions between ERF and MYB TFs in regulating secondary metabolism. In pear, PyERF3 is reported to interact with PyMYB114 to regulate fruit anthocyanin synthesis (Yao et al. 2017), while Pp4ERF24 and Pp12ERF96 can interact with PpMYB114 to control fruit coloration (Ni et al. 2019). Besides fruit colour, the ERF-MYB transcription complex can also affect fruit fragrance by controlling the synthesis of volatile compounds. For example, FaERF9 and FaMYB98 can form transcription complex to activate the expression of *quinone oxidoreductase* and enhance furaneol synthesis in strawberry (Zhang et al. 2018b). It has been demonstrated that VqERF114 can interact with VqMYB35 (Fig. 4a, b)

Fig. 8 Hypothetical model for resveratrol synthesis regulated by VqERF114/VqMYB35 transcription complex. VqERF114 interacts with VqMYB35 and activates *VqMYB35* transcription. Meanwhile, VqMYB35 increases the expression of *VqERF114*. VqMYB35 also promotes the expressions of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46* by binding to the MBS element in their promoters and increases resveratrol synthesis. Hence, VqERF114 indirectly regulates *STS* expression and resveratrol synthesis by interacting with VqMYB35. Co-overexpression of *VqERF114* and *VqMYB35* results in much higher accumulations of *STS* transcripts and stilbene



and VqMYB35 can activate the expressions of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46* by directly binding to the MBS element in their promoters (Figs. 5, 6). These findings allow us to speculate a regulatory mechanism in which VqERF114 regulates *STS* expression and resveratrol production as part of a complex with VqMYB35. As shown in Fig. 7, co-overexpression of *VqERF114* and *VqMYB35* resulted in much higher accumulations of *STS* transcripts and stilbene. These results provide evidence that VqERF114 controls the expression of *STS* indirectly by interacting with VqMYB35.

All the results presented here indicate that VqERF114 participates indirectly in stilbene synthesis by interacting with VqMYB35, and VqMYB35 then enhances the expressions of *VqSTS15*, *VqSTS28*, *VqSTS42* and *VqSTS46* by targeting their promoters. These results confirm this, previously uncharacterised, regulatory model of *STS* expression which is regulated by ERF transcription factor (Fig. 8). This work offers new ideas regarding the complex regulatory network controlling the synthesis of stilbene.

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Author contribution statement YW designed and initiated this study. LW carried out the experiments and analysed the results. LW wrote the manuscript and YW revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Bavaresco L, Fregoni M, Trevisan M, Mattivi F, Vrhovsek U, Falchetti R (2002) The occurrence of the stilbene piceatannol in grapes. *Vitis* 41:133–136
- Bemer M, van Dijk AD, Immink RG, Angenot GC (2017) Cross-family transcription factor interactions: an additional layer of gene regulation. *Trends Plant Sci* 22:66–80
- Chen JY, Wen PF, Kong WF, Pan QH, Zhan JC, Li JM, Wan SB, Huang WD (2006) Effect of salicylic acid on phenylpropanoids and phenylalanine ammonia-lyase in harvested grape berries. *Postharvest Biol Technol* 40:64–72
- Chong J, Poutaraud A, Huguency P (2009) Metabolism and roles of stilbenes in plants. *Plant Sci* 177:143–155
- De Boer K, Tilleman S, Pauwels L, Vanden Bossche R, De Sutter V, Vanderhaeghen R, Hilson P, Hamill JD, Goossens A (2011) APETALA2/ETHYLENE RESPONSE FACTOR and basic helix-loop-helix tobacco transcription factors cooperatively mediate jasmonate-elicited nicotine biosynthesis. *Plant J* 66:1053–1065
- Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L (2010) MYB transcription factors in *Arabidopsis*. *Trends Plant Sci* 15:573–581
- Fang L, Hou Y, Wang L, Xin H, Wang N, Li S (2014) *Myb14*, a direct activator of *STS*, is associated with resveratrol content variation in berry skin in two grape cultivars. *Plant Cell Rep* 33:1629–1640
- Gatto P, Vrhovsek U, Muth J, Segala C, Romualdi C, Fontana P, Pruefer D, Stefanini M, Moser C, Mattivi F (2008) Ripening and genotype control stilbene accumulation in healthy grapes. *J Agric Food Chem* 56:11773–11785

- Hall D, De Luca V (2010) Mesocarp localization of a bi-functional resveratrol/hydroxycinnamic acid glucosyltransferase of Concord grape (*Vitis labrusca*). *Plant J* 49:579–591
- Hart JH (1981) Role of phytostilbenes in decay and disease resistance. *Annu Rev Phytopathol* 19:437–458
- Höll J, Vannozzi A, Czemplak S, D'Onofrio C, Walker AR, Rausch T, Lucchin M, Boss PK, Dry IB, Bogs J (2013) The R2R3-MYB transcription factors MYB14 and MYB15 regulate stilbene biosynthesis in *Vitis vinifera*. *Plant Cell* 25:4135–4149
- Jaillon O, Aury J-M, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449:463–467
- Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 275:218–220
- Jennifer B, Takao Y, Hiroshi A, Lean MEJ, Alan C (2002) Plant foods and herbal sources of resveratrol. *J Agric Food Chem* 50:3337–3340
- Jiang J, Xi H, Dai Z, Lecourieux F, Yuan L, Liu X, Patra B, Wei Y, Li S, Wang L (2018) VvWRKY8 represses stilbene synthase gene through direct interaction with VvMYB14 to control resveratrol biosynthesis in grapevine. *J Exp Bot* 70:715–729
- Jofuku KD, Den Boer B, Van Montagu M, Okamoto JK (1994) Control of Arabidopsis flower and seed development by the homeotic gene *APETALA2*. *Plant Cell* 6:1211–1225
- Kagaya Y, Ohmiya K, Hattori T (1999) RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants. *Nucleic Acids Res* 27:470–478
- Kelemen Z, Sebastian A, Xu W, Grain D, Salsac F, Avon A, Berger N, Tran J, Dubreucq B, Lurin C (2015) Analysis of the DNA-binding activities of the Arabidopsis R2R3-MYB transcription factor family by one-hybrid experiments in yeast. *PLoS One* 10:e0141044
- Langcake P (1981) Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ϵ -viniferin, α -viniferin and pterostilbene. *Physiol Plant Pathol* 18:213–226
- Langcake P, Pryce R (1976) The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. *Physiol Plant Pathol* 9:77–86
- Li X, Xu Y, Shen S, Yin X, Klee H, Zhang B, Chen K (2017) Transcription factor CitERF71 activates the terpene synthase gene *CitTPS16* involved in the synthesis of *E*-geraniol in sweet orange fruit. *J Exp Bot* 68:4929–4938
- Licausi F, Ohme-Takagi M, Perata P (2013) APETALA/ethylene responsive factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs. *New Phytol* 199:639–649
- Matus JT, Aquea F, Arce-Johnson P (2008) Analysis of the grape *MYB R2R3* subfamily reveals expanded wine quality-related clades and conserved gene structure organization across *Vitis* and *Arabidopsis* genomes. *BMC Plant Biol* 8:83
- Nakano T, Suzuki K, Fujimura T, Shinshi H (2006) Genome-wide analysis of the ERF gene family in Arabidopsis and rice. *Plant Physiol* 140:411–432
- Ni J, Bai S, Zhao Y, Qian M, Tao R, Yin L, Gao L, Teng Y (2019) Ethylene response factors Pp4ERF24 and Pp12ERF96 regulate blue light-induced anthocyanin biosynthesis in 'Red Zasu' pear fruits by interacting with MYB114. *Plant Mol Biol* 99:67–78
- Ohme-Takagi M, Shinshi H (1995) Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. *Plant Cell* 7:173–182
- Okamoto JK, Caster B, Villarreal R, Van Montagu M, Jofuku KD (1997) The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in Arabidopsis. *Proc Natl Acad Sci* 94:7076–7081
- Orallo F (2006) Comparative studies of the antioxidant effects of *cis*- and *trans*-resveratrol. *Curr Med Chem* 13:87–98
- Prouse MB, Campbell MM (2012) The interaction between MYB proteins and their target DNA binding sites. *BBA Gene Regul Mech* 1819:67–77
- Romero I, Fuertes A, Benito MJ, Malpica JM, Leyva A, Paz AJ (2010) More than 80R2R3-MYB regulatory genes in the genome of *Arabidopsis thaliana*. *Plant J* 14:273–284
- Romero-Pérez AI, Lamuela-Raventós RM, Andrés-Lacueva C, Torrer-Boronat MC, La De (2001) Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *J Agric Food Chem* 49:210–215
- Rosemann D, Heller W, Sandermann H (1991) Biochemical plant responses to ozone: II. Induction of stilbene biosynthesis in Scots Pine (*Pinus sylvestris* L.) seedlings. *Plant Physiol* 97:1280–1286
- Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K (2002) 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* 290:998–1009
- Shi J, He M, Cao J, Wang H, Ding J, Jiao Y, Li R, He J, Wang D, Wang Y (2014) The comparative analysis of the potential relationship between resveratrol and stilbene synthase gene family in the development stages of grapes (*Vitis quinqueangularis* and *Vitis vinifera*). *Plant Physiol Biochem* 74:24–32
- Stockinger EJ, Gilmour SJ, Thomashow MF (1997) Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. *Proc Natl Acad Sci USA* 94:1035–1040
- Sun M, Shi M, Wang Y, Huang Q, Yuan T, Wang Q, Wang C, Zhou W, Kai G (2019) The AP2/ERF transcription factor SmERF115 positively regulates the biosynthesis of phenolic acids in *Salvia miltiorrhiza*. *J Exp Bot* 70:243–254
- Teguo PW, Decendit A, Vercauteren JG, Merillon JM (1996) Trans-resveratrol-3-O- β -glucoside (piceid) in cell suspension cultures of *Vitis Vinifera*. *Phytochemistry* 42:1591–1593
- Tsugama D, Matsuyama K, Ide M, Hayashi M, Fujino K, Masuda K (2017) A putative MYB35 ortholog is a candidate for the sex-determining genes in *Asparagus officinalis*. *Sci Rep* 7:41497. <https://doi.org/10.1038/srep41497>
- Van Der Fits L, Memelink J (2001) The jasmonate-inducible AP2/ERF-domain transcription factor ORCA3 activates gene expression via interaction with a jasmonate-responsive promoter element. *Plant J* 25:43–53
- Vannozzi A, Dry IB, Fasoli M, Zenoni S, Lucchin M (2012) Genome-wide analysis of the grapevine stilbene synthase multigenic family: genomic organization and expression profiles upon biotic and abiotic stresses. *BMC Plant Biol* 12:130. <https://doi.org/10.1186/1471-2229-12-130>
- Vannozzi A, Wong DCJ, Höll J, Hmam I, Matus JT, Bogs J, Ziegler T, Dry I, Barcaccia G, Lucchin M (2018) Combinatorial regulation of stilbene synthase genes by WRKY and MYB transcription factors in grapevine (*Vitis vinifera* L.). *Plant Cell Physiol* 59:1043–1059
- Vastano BC, Chen Y, Zhu N, Ho C-T, Zhou Z, Rosen RT (2000) Isolation and identification of stilbenes in two varieties of *Polygonum cuspidatum*. *J Agric Food Chem* 48:253–256
- Velasco R, Zharkikh A, Troglio M, Cartwright DA, Cestaro A, Pruss D, Pindo M, FitzGerald LM, Vezzulli S, Reid J (2007) A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS One* 2:e1326

- Wan Y, Schwaninger H, He P, Wang Y (2007) Comparison of resistance to powdery mildew and downy mildew in Chinese wild grapes. *Vitis* 46:132–136
- Waterhouse AL, Lamuela-Raventós RM (1994) The occurrence of piceid, a stilbene glucoside, in grape berries. *Phytochemistry* 37:571–573
- Wessler SR (2005) Homing into the origin of the AP2 DNA binding domain. *Trends Plant Sci* 10:54–56
- Wong DCJ, Matus JT (2017) Constructing integrated networks for identifying new secondary metabolic pathway regulators in grapevine: recent applications and future opportunities. *Front Plant Sci* 8:505. <https://doi.org/10.3389/fpls.2017.00505>
- Wong DCJ, Schlechter R, Vannozzi A, Höll J, Himmam I, Bogs J, Tornielli GB, Castellarin SD, Matus JT (2016) A systems-oriented analysis of the grapevine R2R3-MYB transcription factor family uncovers new insights into the regulation of stilbene accumulation. *DNA Res* 23:451–466
- Xie X, Wang Y (2016) *VqDUF642*, a gene isolated from the Chinese grape *Vitis quinquangularis*, is involved in berry development and pathogen resistance. *Planta* 244:1075–1094
- Xu W, Yu Y, Ding J, Hua Z, Wang Y (2010) Characterization of a novel stilbene synthase promoter involved in pathogen- and stress-inducible expression from Chinese wild *Vitis pseudoreticulata*. *Planta* 231:475–487
- Xu W, Yu Y, Zhou Q, Ding J, Dai L, Xie X, Xu Y, Zhang C, Wang Y (2011) Expression pattern, genomic structure, and promoter analysis of the gene encoding stilbene synthase from Chinese wild *Vitis pseudoreticulata*. *J Exp Bot* 62:2745–2761
- Yao G, Ming M, Allan AC, Gu C, Li L, Wu X, Wang R, Chang Y, Qi K, Zhang S, Wu J (2017) Map-based cloning of the pear gene *MYB114* identifies an interaction with other transcription factors to coordinately regulate fruit anthocyanin biosynthesis. *Plant J Cell Mol Biol* 92:437–451
- Yu CKY, Springob K, Schmidt J, Nicholson RL, Chu IK, Yip WK, Lo C (2005) A stilbene synthase gene (*SbSTS1*) is involved in host and nonhost defense responses in sorghum. *Plant Physiol* 138:393–401
- Yu Z, Li J, Yang C, Hu W, Wang L, Chen X (2012) The jasmonate-responsive AP2/ERF transcription factors AaERF1 and AaERF2 positively regulate artemisinin biosynthesis in *Artemisia annua* L. *Mol Plant* 5:353–365
- Yu Y, Xu W, Wang J, Wang L, Yao W, Yang Y, Xu Y, Ma F, Du Y, Wang Y (2013) The Chinese wild grapevine (*Vitis pseudoreticulata*) E3 ubiquitin ligase *Erysiphe necator*-induced RING finger protein 1 (EIRP1) activates plant defense responses by inducing proteolysis of the VpWRKY11 transcription factor. *New Phytol* 200:834–846
- Zhang M, Li S, Nie L, Chen Q, Xu X, Yu L, Fu C (2015) Two jasmonate-responsive factors, TcERF12 and TcERF15, respectively act as repressor and activator of *tasy* gene of taxol biosynthesis in *Taxus chinensis*. *Plant Mol Biol* 89:463–473
- Zhang J, Xu H, Wang N, Jiang S, Fang H, Zhang Z, Yang G, Wang Y, Su M, Xu L, Chen X (2018a) The ethylene response factor MdERF1B regulates anthocyanin and proanthocyanidin biosynthesis in apple. *Plant Mol Biol* 98:205–218
- Zhang Y, Yin X, Xiao Y, Zhang Z, Li S, Liu X, Zhang B, Yang X, Grierson D, Jiang G, Klee HJ, Chen K (2018b) An ETHYLENE RESPONSE FACTOR-MYB transcription complex regulates furaneol biosynthesis by activating *QUINONE OXIDOREDUCTASE* expression in strawberry. *Plant Physiol* 178:189–201
- Zhou Q, Du Y, Cheng S, Li R, Zhang J, Wang Y (2015) Resveratrol derivatives in four tissues of six wild Chinese grapevine species. *N Z J Crop Hortic Sci* 43:204–213
- Zhu J, Chen H, Li H, Gao J-F, Jiang H, Wang C, Guan Y-F, Yang Z-N (2010) *Defective in Tapetal development and function 1* is essential for anther development and tapetal function for microspore maturation in Arabidopsis. *Plant J* 55:266–277
- Zhuang J, Peng R-H, Cheng Z-M, Zhang J, Cai B, Zhang Z, Gao F, Zhu B, Fu X-Y, Jin X-F, Chen J-M, Qiao Y-S, Xiong A-S, Yao Q-H (2009) Genome-wide analysis of the putative AP2/ERF family genes in *Vitis vinifera*. *Sci Hortic* 123:73–81

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