**VqMYB154** promotes polygene expression and enhances resistance to pathogens in Chinese wild grapevine

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**Abstract**

Resveratrol plays a crucial phytoalexin role in the grapevine and is beneficial to human health. However, the molecular mechanism of resveratrol accumulation in the enhancement of disease resistance is unclear. Here, we report that the transcription factor VqMYB154 from *Vitis quinquangularis* accession Danfeng-2 is strongly expressed under artificial inoculation with *Uncinula necator* and regulates resveratrol accumulation. Unlike its homolog, VqMYB154 has a pathogen-induced promoter and responds to stimulation by *U. necator*, *Pseudomonas syringae*, and other treatments. Yeast one-hybrid and GUS activity assays confirmed that VqMYB154 can activate the stilbene synthase genes VqSTS9, VqSTS32, and VqSTS42 by directly binding to their promoters. Overexpression of VqMYB154 in grape leaves resulted in activation of the stilbene pathway, upregulation of STS genes, and accumulation of stilbenoids. In addition, heterologous overexpression of VqMYB154 in *Arabidopsis* activated resistance-related genes and resulted in greater programmed cell death and accumulation of reactive oxygen species, which led to resistance against *P. syringae*. These results suggest that the transcription factor VqMYB154 from *V. quinquangularis* accession Danfeng-2 participates in the regulatory mechanism that improves the biosynthesis and accumulation of stilbenes and enhances resistance to disease in grapevine.

**Introduction**

Grapevine is one of the most prestigious economic fruit crops worldwide. According to data from the Food and Agriculture Organization, the total output of grapes ranked third among fruit crops in 2018. Of *Vitis vinifera* grown worldwide, few cultivars possess high resistance to phytopathogenic microorganisms, including *Uncinula necator*. If left unchecked, pathogen-triggered diseases will seriously affect the growth and quality of grapevine, ultimately leading to a decline in yield. Long-term use of pesticides pollutes the environment, and hazardous residues in grape berries threaten human health. Therefore, it is crucial to improve grapevine resistance to reduce the need for pesticide application. Some resistant grape species, such as *V. labrusca*, have an unacceptable foxy smell, which greatly limits their application in crossbreeding. China is a vital area of origin for wild grapevine germplasm, with many disease-resistant *Vitis* spp. These wild species do not have undesirable flavors, and can easily be crossed with *V. vinifera* cultivars; thus, they provide critical resources for resistance breeding of grapevine.

As a well-known stilbene-producing plant, the grapevine has high levels of resveratrol. This bioactive compound was first extracted from *Veratrum grandiflorum* and was determined to be present in grape tissues. Resveratrol has great research value. Furthermore, it is beneficial to human health due to its antioxidant activity.
and antiaging, neuroprotective, and cancer prevention properties. In addition, resveratrol acts as an important stilbene phytoalexin, which has been widely reported to possess antimicrobial ability against pathogen invasion. These positive effects of resveratrol regarding health benefits and phytopathology have led to worldwide research on its pharmacological properties. The biosynthesis of resveratrol involves numerous enzymatic reactions, with stilbene synthases (STSs) being the most closely related and essential enzymes. In the grapevine, reactions, with stilbene synthases (STSs) being the most closely related and essential enzymes.

In the grapevine, MYB14 and MYB15, activate STS41 and STS29 to promote stilbenoid accumulation, which suggests that specific TFs in plants closely participate in the regulation of STS genes. Genome sequencing provides extensive data to support in-depth research on the regulatory mechanisms of TFs in plants. In grapevine, 2004 TFs have been identified by sequencing of the Pinot Noir genome. Extensive research has shown that these TF genes are inextricably linked to the growth and development of grapevine, including cell expansion, seed morphogenesis, and berry ripening. Moreover, numerous regulators function in defense responses to exogenous stresses, such as drought, cold, high salinity, and pathogens. Members of the WRKY subfamily genes involved in defense regulation of various plant species, including 138 members in Arabidopsis, 126 in rice, and 134 in grape. The initial division into 25 subgroups in Arabidopsis based on the motif at the C-terminus, and a later study on grapevine increased the number of subgroups to 34. The numerous R2R3-MYB members may contain resistance-related TFs that are still unknown. Some resistance-related MYB proteins have been identified in Arabidopsis. Overexpression of AtMYB30 leads to hypersensitive cell death in Arabidopsis, thereby enhancing resistance to Pseudomonas syringae. Its homologous gene, MdMYB30, also has similar disease resistance properties. Furthermore, AtMYB96 enhances resistance to P. syringae by regulating salicylic acid (SA) biosynthesis and pathogenesis-related (PR) genes. Knockdown of AtMYB46 improves the resistance of Arabidopsis mutants to Botrytis cinerea. In wheat, the MYB gene TaPIMPI was shown to promote SA-related resistance genes PR1a and PR2, thereby enhancing resistance to Bipolaris sorokiniana. In addition to VvMYB14 and VvMYB15 in grapevine, VvMYB13 was shown to respond to infection by downy mildew and play a positive role in stilbenoid accumulation. VqMYB35 positively regulates the expression of STS genes by interacting with VqERF114. However, considering the complexity of the MYB superfamily, more potential factors still need to be determined to fully clarify the MYB-mediated phytoalexin metabolic network.

Based on our long-term observations in vineyards, the Chinese wild-growing grapevine accession Danfeng-2 has a higher content of resveratrol and disease resistance than V. vinifera. Therefore, this resistant germplasm has been used for in-depth analysis of grapevine-pathogen interactions. Recently, we determined the expression levels of 106 R2R3-MYB members in Danfeng-2 under artificial inoculation with U. necator, 27 of which showed greater than 4-fold upregulated expression (unpublished data). Coexpression analysis based on transcriptome data was performed to identify pathogen-induced MYB TFs that regulate STS genes. Here, a resistance-related MYB TF, VqMYB154, from the V. quinquangularis accession Danfeng-2, was screened and isolated. Our research indicated that VqMYB154 is a novel regulator that improves the accumulation of stilbene phytoalexins and enhances resistance to disease in transgenic Arabidopsis. These results are significant for elucidating the regulatory mechanisms involved in plant-pathogen interactions and provide valuable references for the long-term goal of disease-resistant grapevine breeding.

**Results**

**VqMYB154 is a resistance-related TF that participates in plant defense responses**

To screen out MYB genes involved in defense responses, we inoculated Danfeng-2 leaves with U. necator and performed qRT-PCR analysis. The results showed that the R2R3-type MYB gene VqMYB154 (this study) can be induced and significantly upregulated by U. necator (Fig. 1a, Supplementary Fig S1). To investigate whether MYB154...
exhibits different expression patterns in disease-resistant and disease-susceptible grapes under pathogen inoculation, we inoculated the leaves of Danfeng-2 and Cabernet Sauvignon with *U. necator* and *P. syringae* (*Pst* DC3000). After artificial inoculation, the expression level of *VqMYB154* from Danfeng-2 began to increase at 48 h and peaked at 72 h, increasing 8.2-fold, compared to the mock control at the same timepoint (Fig. 1b). In contrast to *VqMYB154*, *MYB154* from Cabernet Sauvignon showed no clear expression pattern (Fig. 1c). On the other hand, after *Pst* DC3000 infection, *VqMYB154* responded to induction at 24 h and then peaked at 48 h, increasing 5.0-fold (Fig. 1d). However, *VvMYB154* exhibited a downward trend and reached its lowest abundance at 24 h (Fig. 1e). Therefore, we speculate that *VqMYB154* is a resistance-related regulator in Danfeng-2.

To explore whether *MYB154* responds to exogenous phytohormones, we treated the leaves of Danfeng-2 and Cabernet Sauvignon by spraying them with phytohormones. Compared to the control at the same time, the transcript level of *VqMYB154* decreased at 0.5 h, 2 h, and 6 h but then increased 2.2-fold at 10 h after SA treatment. After MeJA treatment, the transcript level of *VqMYB154* increased at 1 h and peaked at 6 h by 2.9-fold. The transcript level of *VqMYB154* was upregulated at 2 h and peaked at 10 h by 4.4-fold after ABA treatment. Under Eth treatment, expression of *VqMYB154* decreased from 0.5 h to 2 h but then increased 2.8-fold at 10 h (Fig. 1f). On the other hand, compared to the mock control, transcript levels of *VvMYB154* were upregulated 2.4-fold at 10 h after SA treatment, 7.8-fold at 6 h after MeJA treatment, 3.3-fold at 2 h after ABA treatment, and 2.1-fold at 1 h after Eth treatment (Supplementary Fig. S3). These results demonstrate that the two *MYB154* genes respond to exogenous phytohormone induction and exhibit different expression patterns in Danfeng-2 and Cabernet Sauvignon. *VqMYB154* also responded to other exogenous signals, including H2O2 and CaCl2. After H2O2 treatment, expression of *VqMYB154* was significantly upregulated, reached its highest level at 2 h by 162.2-fold, and then gradually decreased. Under the induction of CaCl2, the expression level of *VqMYB154* was decreased at 0.5 h but increased at 1 h and 6 h and then reached its highest level at 10 h by 3.0-fold (Fig. 1f).

Expression profiles of *VqMYB154* in ‘Danfeng-2’ grapevine

After exploring the response patterns of *VqMYB154* under stress, we determined *VqMYB154* transcripts in various organs of Danfeng-2 under natural conditions (Fig. 2a). In nutritive organs, including stems, tendrils, and leaves, the expression level of *VqMYB154* in leaves was higher than that in other organs, and the expression level in young leaves was higher than that in mature leaves (Fig. 2b). The expression pattern of *VqMYB154* showed a downward trend during leaf development (Fig. 2c). In reproductive organs containing berries at the four developmental stages, expression of *VqMYB154* was higher in ripe berries than in other stages, showing an upward trend with the stage before veraison to the ripe stage (Fig. 2d). These observations indicate that young leaves and ripe berries in the Danfeng-2 grapevines are the primary sites where *VqMYB154* is expressed. Furthermore, we analyzed the expression of *VqSTS9*, *VqSTS32*, and *VqSTS42* in various organs of the Danfeng-2 grapevine. The results
demonstrated that in nutritive organs, the three VqSTS genes showed the highest expression levels in young leaves; in reproductive organs, the three VqSTS genes were mainly expressed in ripe berries, which was similar to the expression pattern of VqMYB154. This may reflect coexpression relationships between VqMYB154 and VqSTS9, VqSTS32 and VqSTS42 under natural conditions (Supplementary Fig. S4).

Structure analysis and characteristics of VqMYB154

To understand the function of the resistance-related gene VqMYB154, we first isolated VqMYB154 and performed sequence analysis. The length of the VqMYB154 gDNA is 1202 bp, containing two introns at positions 137–360 bp and 491–610 bp. A BLAST search in Grape Genome Browser indicated that VqMYB154 is located on chromosome 11 (Fig. 3a). The coding sequence (CDS) of VqMYB154 was cloned from Danfeng-2. The CDS of VqMYB154 is 858 bp and encodes a 285 amino acid protein (Fig. 3a, b). VqMYB154 shares 98.95% amino acid identity with VvMYB154. Compared to VvMYB154, three mutations were found in the VqMYB154 protein, from glutamine to proline, methionine to valine, and phenylalanine to isoleucine (Fig. 3b). The N-terminus of VqMYB154 contains highly conserved R2 and R3 MYB domains (residues 13–115 aa) (Fig. 3a, c). An NLS motif is present at the N-terminus of VqMYB154, indicating its nuclear localization, and phylogenetic analysis of VqMYB154 with homologous proteins from multiple species demonstrated that it exhibits high homology with VvMYB154 and MdMYB36 (Fig. 3d). Cluster analysis of VqMYB154 with MYB subfamily proteins from the grapevine, Arabidopsis, and rice indicated that VqMYB154 is a member of subgroup 14 (S14) (Fig. 3e). VvMYB148 in this subfamily is coexpressed with STS genes.

To determine the subcellular site where VqMYB154 functions, we performed a subcellular localization assay, in which the GFP signal of VqMYB154 overlapped with the mCherry signal of AtHY5, indicating that VqMYB154 is a nuclear protein (Fig. 3f). In addition, yeast cells harboring BD-VqMYB154 grew normally in all cultures and showed X-α-gal activation and AbA resistance (Fig. 3g). This indicates that VqMYB154 functions as a transcriptional activator.

The MYB154 promoter exhibits stronger pathogen-induced activity in the resistant grapevine Danfeng-2

Previous results have suggested that MYB154 from disease-resistant and disease-susceptible grapevines shows different pathogen-induced response patterns. To further explore the basis for these differences in expression, we separately cloned and obtained the promoters of MYB154 in Danfeng-2 and Cabernet Sauvignon. Compared with the 1014 bp promoter of VvMYB154, we detected six deletion mutations and three insertion mutations in the 1021 bp promoter of VqMYB154 (Fig. 4a). We further discovered numerous motifs in the VqMYB154 promoter, including an MBS element, ABRE element (ABA-responsive), CGTCA motif, TGACG motif (MeJA-responsive), ERE element (ethylene-responsive), and STRE element (stress-responsive). This suggests a potential role for VqMYB154 in multiple stress responses (Fig. 4b).
To investigate the effect of promoter differences on pathogen-induced response activity, we transiently transformed the two promoters into grape leaves and evaluated GUS activity in leaves inoculated with *U. necator* and *P. syringae* (*Pst DC3000*). Compared to the mock control, GUS activity driven by the *VqMYB154*
promoter was significantly enhanced after inoculation with *U. necator* and *Pst* DC3000, whereas GUS activity of the *VvMYB154* promoter showed no significant change after pathogen infection (Fig. 4c, d). These results indicate that *VqMYB154* possesses a pathogen-inducible promoter that responds to both fungal and bacterial pathogens, and the differential expression of *MYB154* genes in pathogen-inoculated leaves is closely associated with the activity of their promoters.

**VqMYB154 enhances the expression of STS genes and stilbenoid synthesis**

To identify the downstream target gene of VqMYB154, we conducted gene correlation analysis using Danfeng-2 transcriptome data. The Pearson correlation coefficient (PCC) was adopted as the key index for analyzing correlations. In particular, we noticed that *VqMYB154* was coexpressed with *VqSTS9* (VIT_16s0100g00770), *VqSTS32* (VIT_16s0100g01140), and *VqSTS42* (VIT_16s0100g01140) according to high PCC indexes of 0.94, 0.82, and 0.81 (Fig. 5a), respectively. Thus, VqMYB154 may act as a regulator of the stilbene pathway. To test this hypothesis, we amplified and obtained these three promoters using Danfeng-2 genomic DNA. By analyzing the sequences, we found that the *VqSTS* promoter contains an MYB binding motif L5-box and AC-box and that the *VqSTS32* promoter possesses two AC-boxes and an MYBCORE element. In addition, the *VqSTS42* promoter contains an AC box and L5 box element (Fig. 5b). Therefore, we deduced that these elements are binding sites for VqMYB154. We then used a yeast one-hybrid (Y1H) assay to examine the ability of VqMYB154 to bind to these motifs. VqMYB14 and VqMYB15 from Danfeng-2, which have been proven to promote the expression of *STS* genes, were used for comparison. The results indicated that VqMYB154 is able to bind to the L5-box and AC-box motifs but not the MYBCORE motif, which is consistent with the results for VqMYB14 but not VqMYB15 (Fig. 5c). A further Y1H assay demonstrated that VqMYB154 interacts with the promoters of *VqSTS9*, *VqSTS32*, and *VqSTS42* (Fig. 5d). To determine whether the impact of these interactions is positive or negative, we next performed a GUS activity determination assay in tobacco. The results demonstrate that transient overexpression of VqMYB154 activated the promoters of these *VqSTS* genes in vivo (Fig. 5e).

After determining the role of VqMYB154 in activating *STS* promoters, we investigated the effect of VqMYB154 on several node genes in the phenylalanine pathway and assessed the transcriptional levels of genes, including phenylalanine ammonia lyase (*PAL*), chalcone synthase (*CHS*), total *VqSTS*s (total expression level of *STS* genes), and resveratrol glycosyl transferase (*RSGT*), by performing transient overexpression assays in Danfeng-2 leaves. The results showed that overexpression of VqMYB154 led to activation of *PAL* and total *VqSTS*s but that the *CHS* gene was inhibited. Expression of *RSGT* was unaffected by VqMYB154 (Fig. 5f). Moreover, as expected, VqSTS9, VqSTS32, and VqSTS42 were activated in VqMYB154-overexpressing leaves (Fig. 5g). The HPLC assay indicated that the contents of trans-resveratrol and trans-piceid increased 2.9-fold and 1.6-fold, respectively, after VqMYB154 overexpression (Fig. 5h). Together, VqMYB154 can promote phenylalanine metabolism and the downstream STS pathway while inhibiting the flavonoid pathway.

We further analyzed the expression patterns of *VqSTS* genes in Danfeng-2 leaves under pathogen inoculation and found that VqSTS9, VqSTS32, and VqSTS42 can respond to induction by *U. necator*. Compared to the mock control, transcriptional levels of VqSTS9 and VqSTS42 were significantly upregulated at 24 h and peaked at 72 h by 3.4-fold and 2.8-fold, respectively. The transcriptional level of VqSTS32 was increased at 48 h and peaked at 72 h by 2.0-fold (Fig. 5i). Under inoculation with *Pst* DC3000, expression of the three *STS* genes was enhanced at 24 h and peaked at 48 h by 6.7-fold, 2.2-fold and 5.1-fold, respectively. Furthermore, three VqSTS...
genes shared the same upregulated periods with *VqMYB154* under the induction of *U. necator* (48 h and 72 h) and *Pst* DC3000 (24 h and 48 h), which indicates that *VqMYB154* is coexpressed with *VqSTS9*, *VqSTS32*, and *VqSTS42* under pathogen-induced conditions (Fig. 5).

**VqMYB154** is a positive regulator of resistance to *Pseudomonas syringae* in transgenic *Arabidopsis*.

*VqMYB154* and its promoter respond to induction by pathogens (Fig. 1, Fig. 4). To further explore its function in the defense response, we generated *VqMYB154*-
overexpressing *Arabidopsis* lines. Wild-type (Col-0) plants and *VqMYB154* transgenic lines (OE#3, OE#5, and OE#9) were used for disease assays (Fig. 6a). We first inoculated transgenic lines and Col-0 plants with *Golovinomyces cichoracearum*. At 168 h postinoculation, the leaves of three independent transgenic lines showed
fewer fungal spores and hyphae than did Col-0 plants, indicating the transgenic lines to be more resistant to G. cichoracearum (Fig. 6b). In addition, VqMYB154-overexpressing transgenic lines showed stronger resistance to Pst DC3000. At 72 h postinoculation, the transgenic lines exhibited less severe chlorosis symptoms than did Col-0 plants (Fig. 6c). To further evaluate stress tolerance under Pst DC3000 inoculation, resistance-related physiological indexes were measured. Notably, electrolyte leakage from transgenic lines was significantly increased, higher than that of Col-0 plants, within 24 h post-inoculation, which may indicate that a more intense hypersensitive reaction occurred in the transgenic lines. Furthermore, consistent with the phenotype of the inoculated plants, the transgenic lines had a lower malonaldehyde content and higher chlorophyll content and net photosynthetic rate than wild-type plants (Fig. 6d). We hypothesize that these phenotypic differences may be linked to the growth status of the pathogens in vivo. Therefore, we measured the abundance of bacteria in leaves at 72 h after Pst DC3000 inoculation and found them to be significantly lower than those of Col-0 plants (Fig. 6e, f). A trypsin blue assay was performed to visualize areas of cell death in transgenic lines and Col-0 plants, showing more intense cell death at 72 h after Pst DC3000 inoculation (Fig. 6g). By staining leaf samples with aniline blue, we observed more intensive callose deposition in the transgenic lines at 72 h after Pst DC3000 inoculation than in wild-type plants. The amount of callose in the transgenic lines was also significantly increased (Fig. 6h, i). These results indicate that VqMYB154 can enhance disease resistance against Pst DC3000.

**VqMYB154 stimulates the production of reactive oxygen species (ROS) in vivo and enhances resistance to P. syringae via the SA pathway**

To investigate whether ROS participate in the defense response, we performed DAB and NBT staining to visualize the contents of H2O2 and O2−. We observed that more ROS accumulated in VqMYB154-overexpressing transgenic lines than in wild-type plants at 72 h after Pst DC3000 inoculation (Fig. 7a). We also quantified endogenous H2O2 content. As expected, the H2O2 content in vivo was higher in transgenic plants, which is consistent with the above results (Fig. 7b). The further qRT-PCR analysis demonstrated that transcriptional levels of the NADPH oxidase genes AtrBOHD and AtrBOHF, which are defense-related genes involved in ROS production51,52, were upregulated in transgenic plants and higher than those in Col-0 plants at 24 h and 48 h postinoculation (Fig. 7c).

Our results indicate that VqMYB154 can respond to the defense-related phytohormones SA and MeJA (Fig. 1f). To explore whether VqMYB154 can activate resistance signaling pathways that involve SA or JA, we analyzed SA-independent and JA-independent defense genes in transgenic lines and Col-0 plants under Pst DC3000 inoculation. AtICS1 participates in SA biosynthesis for plant defense responses53, and AtPR5 is an important SA-independent resistance-related gene54. After pathogen inoculation, the expression level of AtICS1 in transgenic lines increased at 24 h and 48 h compared to wild-type. Expression of AtPR5 in transgenic lines was more intense than that in Col-0 plants at 72 h postinoculation (Fig. 7d). However, transcript levels of AtLOX3 and AtPDF1.2, which are JA-independent defense genes55,56, were
significantly lower than those of wild-type (Fig. 7d). These results demonstrate that overexpression of VqMYB154 stimulates ROS accumulation and enhances resistance to P. syringae via the SA signaling pathway.

**Discussion**

Grape diseases can cause yield loss and even depression in the grape industry. Disease-resistant grapevines possess unclarified grapevine-pathogen interaction mechanisms...
and elucidating transcription networks regulating plant resistance is vital for viticulture and vine breeding. In this study, we cloned and identified a novel resistance-related R2R3-MYB gene, VqMYB154, from the disease-resistant grapevine Danfeng-2. This research focused on elucidating its role in phytoalexin biosynthesis and defense responses. We found that pathogens are direct factors resulting in the activation of VqMYB154 and its promoter and further determined its involvement in the biosynthesis of stilbene phytoalexin by identifying its target STS genes. We then evaluated its resistance-related function using assays in VqMYB154-overexpressing Arabidopsis mutants. In summary, our results reveal that VqMYB154 positively contributes to plant defense responses.

VqMYB154 is a resistance-related transcription factor that participates in the plant defense response

The Chinese wild-growing V. quinquangularis accession Danfeng-2 shows excellent resistance to exogenous pathogens, including U. nectator. TFs involved in plant defense mechanisms can respond to pathogen induction. In our study, VqMYB154, an MYB TF isolated from resistant Danfeng-2, significantly responded to U. nectator and Pst DC3000 (Fig. 1a–c). However, its homolog in susceptible Cabernet Sauvignon is insensitive to pathogen invasion. Thus, VqMYB154 differs from VvMYB154, and there is a specific TF gene involved in the resistance mechanism of Danfeng-2. Furthermore, we found that various mutations, including insertions and deletions, exist in the VqMYB154 promoter (Fig. 4). Homologs may behave differently, even within the same species. For example, the ubiquitin ligase gene RH2 enhances resistance to powdery mildew in Chinese wild-growing grapevine Baihe-35-1 but not in susceptible cultivars because of mutations in the promoter sequence and differences in promoter elements. OsMYBS1 binds to the promoter of C2H2-type TF Bsr-d1 in disease-resistant rice Digu to improve resistance to rice blast, whereas, in susceptible rice, such binding does not occur because of a single-nucleotide variation ‘G’ to ‘A’ in the promoter. Therefore, we propose that the mutations between the two MYB154 genotypes are directly responsible for the different promoter activities. This is also the reason why the expression of VqMYB154 is more prominent in the disease response.

Previous studies have reported that P. syringae can trigger incompatible interactions and innate defense responses in grapevines. It is speculated that Pst DC3000 is a nonadapted pathogen for grapevine. Furthermore, interactions of Pst DC3000 with grapevine induce expression of resistance-related TFs, including ERF112, ERF114, and ERF072. Therefore, we combined U. nectator and Pst DC3000 together to investigate defense-related gene expression in the grapevine. The results showed that VqMYB154 was induced and exhibited upregulated expression under artificial inoculation.
with *Pst* DC3000. This may reflect that VqMYB154 also plays a role in incompatible grapevine-pathogen interactions, which deserves further research.

**VqMYB154 activates the STS pathway and enhances stilbenoid accumulation**

Resveratrol is an attention-attracting metabolite because of its antimicrobial activity in plants and positive pharmacological effects on human health\(^{11,62}\). It is particularly abundant in grape berries and gradually accumulates from veraison to the ripe period\(^{63,64}\). In recent years, several MYB proteins have been identified as resveratrol regulators\(^{20,30}\). *VviMYB14*, *VviMYB15*, and *VviMYB13*, which regulate the STS pathway, belong to MYB subgroup 2. Other identified MYB genes that are coexpressed with STS genes include *VviMYB139* (subgroup 3), *VviMYB148* (subgroup 14), and *VviMYB108A* (subgroup 20)\(^{48}\), and *VqMYB154* in this study belong to the same subfamily as *VviMYB148* (Fig. 3e). According to the grapevine expression atlas from *V. vinifera* L. cv. Corvina, *VviMYB14*, and *VviMYB15* are expressed during the developmental periods of berries, leaves, stems, and tendrils, while *VviMYB154* is only expressed in young leaves and tendrils\(^{65}\). In contrast to its homolog in Corvina, *VqMYB154* is expressed in multiple organs, and its mRNA accumulates significantly in veraison and ripe berries (Fig. 2). Expression levels of *VqSTS9*, *VqSTS32*, and *VqSTS42* in ripe berries were higher than those in young berries (Supplementary Fig. S5). These results indicate that *VqMYB154* is similar to *VviMYB14* and *VviMYB15* in the expression distribution in grapevine organs and provides evidence for the correlation between *VqMYB154* and the STS pathway under natural conditions.

Specific binding elements act as a “bridge” between transcription factors and their target genes. In grapevines, MYB14 recognizes and binds to the L5-box element (GAGTTGGTGAGA) in the STS promoter to regulate stilbene accumulation\(^{36}\). In addition, the AC box (ACCA/TAA/CT/C) and MYBCORE (CAGTTA and CTGTTG) serve as cis-acting sites for MYB protein\(^{34,35}\). In our study, the promoters of *VqSTS9* and *VqSTS42* were found to contain both an L5-box GAGTTGGTGAGA and AC-box ACCAACT. The promoter of *VqSTS32* harbors the AC-box ACCAACT and MYBCORE CAGTTA (Fig. 5b). The existence of these specific sites indicates that STS genes may be regulated by MYB TFs. As a control TF for comparing binding properties, VqMYB14 can bind to the L5-box element in Danfeng-2, which is consistent with a previous study on grapevine\(^{36}\). Moreover, we confirmed that the AC box acts as a novel binding element for VqMYB14. Furthermore, VqMYB15 can weakly interact with the L5 box but cannot bind to the other two elements, indicating that it may bind to other cis-elements with better affinity in STS promoters. Interestingly, VqMYB154 shares the same binding preferences as VqMYB14. This suggests that VqMYB154 performs transcriptional regulatory functions in a manner similar to that of VqMYB14.

Because of their common evolutionary origin, STS and CHS compete for the same substrates, such as *p*-coumaroyl-CoA\(^{15}\). As an upstream enzyme, PAL participates in *p*-coumaroyl-CoA biosynthesis. Therefore, activating the *PAL* gene can create more substrates to be used for resveratrol biosynthesis\(^{20}\). Our results show that VqMYB154 not only enhances expression of the three STS genes but also upregulates *VqPAL* expression and downregulates that of *VqCHS* (Fig. 5f, g). More substrates weakened CHS competitiveness, and higher STS activity promotes the accumulation of stilbene. As expected, we detected higher levels of trans-piceid and trans-resveratrol in *VqMYB154*-overexpressing grape leaves than in the control (Fig. 5h). Taken together, VqMYB154 is a transcriptional activator of resveratrol accumulation.

**VqMYB154 contributes to resistance against *P. syringae* in transgenic *Arabidopsis***

Plants have developed a series of emergency mechanisms to resist pathogens. Programmed cell death (PCD) leads to acute necrosis in infected cells, interrupting pathogen spread\(^{66}\). To date, only a few MYB TFs have been shown to directly regulate pathogen-triggered PCD\(^{67,68}\). Our results showed that heterologous expression of VqMYB154 in *Arabidopsis* leads to more intense PCD upon *Pst* DC3000 infection (Fig. 6f). Moreover, we noted that regional cell necrosis is accompanied by more reactive oxygen species (ROS) generation (Fig. 7a–c). ROS, such as H\(_2\)O\(_2\), can resist pathogen invasion by inducing resistance-related gene expression and further PCD\(^{69}\). In fact, it is commonly accepted that ROS and SA signals function synergistically in systemic acquired resistance\(^{70}\). Consistent with this viewpoint, we also detected upregulated expression of SA-dependent *AtICS1* and *AtPR5* following *Pst* DC3000 inoculation, indicating activation of the SA signaling pathway (Fig. 7d). Interestingly, VqMYB154 responded intensively to H\(_2\)O\(_2\) induction in grapevines (Fig. 1f), and the endogenous H\(_2\)O\(_2\) generated by pathogenic stimulation might further induce expression of VqMYB154, possibly constituting a positive feedback loop mediated by ROS. Furthermore, we observed that callose deposition was denser in transgenic lines upon *Pst* DC3000 inoculation (Fig. 6g). Overall, enhanced callose accumulation can more effectively protect cells against pathogen penetration\(^{11}\).

In summary, our research reveals that multiple resistance-related molecular mechanisms comprise the *VqMYB154*-mediated plant defense response. *VqMYB154* is activated by pathogens and promotes stilbenoid
biosynthesis by activating the promoters of STS genes. Moreover, VqMYB154 promotes the expression of PAL and inhibits that of CHS, further activating the resveratrol pathway. Thus, the accumulation of resveratrol contributes to plant disease resistance. In addition, VqMYB154 stimulates ROS accumulation and upregulates the SA signaling-related genes AtICS1 and AtPR5. These defense-related genes and ROS accumulation also contribute to disease resistance.

**Materials and methods**

**Plant materials**

Organs from Danfeng-2 and Cabernet Sauvignon were acquired from the Grape Germplasm Resources database at Northwest A&F University, Yangling, Shaanxi, China (34°20'N, 108°24'E). Grape berries were acquired during four developmental periods: green hard, before veraison, veraison, and ripe. The collection times were 25, 40, 50, and 80 days after flowering. The leaf collection periods were the same as those of berries. Based on the condition of the leaves, they were denoted Leaf-1, Leaf-2, Leaf-3, and Leaf-4. Tobacco (*Nicotiana benthamiana*) was cultivated in a climate chamber at 25 °C. *Arabidopsis* (Columbia ecotype) was cultivated in a plant incubator at 22 °C.

**Gene isolation and bioinformatics analysis**

cDNA from Danfeng-2 berries was used for gene amplification. With reference to the sequence of homologous genes in Pinot Noir, the VqMYB154 coding sequence (CDS) (VIT_11s0016g02780) was cloned using the primers VqMYB154-F/VqMYB154-R (Supplementary Table S1). Sequence alignment was analyzed using DNAMAN (Lynnon Biosoft, San Ramon, CA, USA). Cluster analysis was performed using MEGA 10.1.8 (Pennsylvania State University, University Park, USA) and FigTree (Andrew Rambaut, Institute of Evolutionary Biology, UK). Chromosomal localization was analyzed using Grape Genome Browser (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/). Conserved protein domains were determined on the website SMART (http://smart.embl-heidelberg.de/). The sequence of nuclear localization was analyzed using the SeqNLS website (http://mleg.cse.sc.edu/seqNLS/).

**Artificial inoculation of *U. necator* and *P. syringae* under field conditions**

*U. necator* was collected from the surface of susceptible grape leaves. Infection of Danfeng-2 and Cabernet Sauvignon leaves with *U. necator* was based on a previously described method4. Petioles of inoculated leaves were wrapped in moist, medical absorbent cotton and placed in flat trays with wet filter paper padded inside. The infected leaves were sampled at 0, 12, 24, 48, 72, 96, and 120 h, were wrapped in marked tin foil, immediately stored in liquid nitrogen, and placed in a cryogenic refrigerator for experiments. *P. syringae* (*Pst* DC3000) was cultivated in liquid medium72 with 25 mg/L rifampicin added in an orbital shaker (28 °C; 2 days). Healthy grape leaves were infiltrated with a suspension of *Pst* DC3000, and the inoculated leaves were collected at 0, 24, 48, and 72 h.

**Treatments using phytohormone and abiotic stress**

Leaves of Danfeng-2 and Cabernet Sauvignon were used for phytohormone treatment. SA, abscisic acid (ABA), methyl jasmonate (MeJA), and ethylene (Eth) were prepared with absolute ethanol and then diluted with double distilled water to 100 μM. Hydrogen peroxide (H2O2; 1% [w/v]) and 5 mM CaCl2 were used for the treatment of Danfeng-2 leaves. The leaves of the control group were
treated with double-distilled water. Treated leaves were collected after 0, 0.5, 1, 2, 6, and 10 h.

**Subcellular localization for VqMYB154 analysis**

The VqMYB154 CDS (stop codon excluded) was ligated to the pCAMBIA2300 vector\textsuperscript{75}, and the 35S-VqMYB154-GFP plasmid generated was used in this assay. The recombinant vector 35S-AtHY5-mCherry acted as a marker for the nucleus\textsuperscript{74}. The pCAMBIA2300 vector was used as the control. The plasmids (35S-AtHY5-mCherry +35S-VqMYB154-GFP and 35S-AtHY5-mCherry+35S-GFP) were transfected into Arabidopsis leaf protoplasts and cultivated for 22 h based on a previous protocol\textsuperscript{75}. GFP and mCherry signals were detected using a laser scanning confocal microscope (Olympus FV1000MPE, Tokyo, Japan). The color of the chloroplast signal in the figure is shown in blue to distinguish it from mCherry fluorescence.

**Yeast transactivation assay of VqMYB154**

The CDS of VqMYB154 was ligated to the pGBKT7 vector (Clontech, Mountain View, CA, USA). The BD-VqMYB154 plasmid generated was transferred into the Y2HGOLD strain (Clontech). The pGBKT7 vector served as the control. The transformed strains were cultured on SD/-Trp medium at 28–30 °C for 3 days, and transformants were grown on Petri dishes and cultivated at 28–30 °C for 3 days before observation. Three types of media were used: SD/-Trp, SD/-Trp with aureobasidin A (AbA), and SD/-Trp with AbA or X-α-Gal.

**Yeast one-hybrid for screening promoter assays**

Matchmaker™ Gold Yeast One-Hybrid System (Clontech, Palo Alto, USA) was adopted for experimental validation. The STS promoters of VqSTS9, VqSTS32, and VqSTS42 were integrated into the pAbAi vector to form pAbAi-ProVqSTS9, pAbAi-ProVqSTS32, and pAbAi-ProVqSTS42. Three tandem copy sequences of ACCACT (AC-box), GAGTTGGTGAGA (L5-box), and CAGTTA (MYBCORE) were also integrated into the pAbAi vector. Then, linearized vectors were digested with a single endonuclease and transfected into the Y1HGold strain; the strains generated were used as bait. The CDS of VqMYB154 was integrated into pGADT7 to form AD-VqMYB154. The fusion vector was transfected into baits separately; the pGADT7 vector was also transfected into baits as a control. Transformants were grown on medium with SD/-Leu with AbA.

**Agrobacterium-mediated transient overexpression in grape leaves**

The fusion vector 35S-VqMYB154-GFP and empty vector were ligated into Agrobacterium tumefaciens GV3101. The transformed strains were grown in a lysogeny broth (LB) liquid medium at 28 °C. After centrifugation, the pelleted bacteria were resuspended (OD\textsubscript{600} = 0.6). Leaves of Danfeng-2 were immersed in a jar containing an Agrobacterium suspension. After vacuum infiltration for 30 min using a previously described method\textsuperscript{76}, the samples were stored with the petioles wrapped in moist medical absorbent cotton in trays for 48 h before collection (Supplementary Fig. S2).

**GUS activity analysis**

The promoters of VqMYB154 and VvMYB154 were ligated into the pC0390-GUS vector, and the fusion vector was infused into GV3101 for transient expression in grape leaves\textsuperscript{77}. After vacuum infiltration, the grape leaves were cultivated for 2 days and then infected with U. necator for one d before collection. GUS activity experiments were performed as previously described\textsuperscript{72}. The CaMV35S-GUS vector was used as the positive control, and the negative control was the pC0390-GUS vector. For the stilbene regulation assay, the VqSTS promoters were integrated into the pC1391-GUS vector and then infused into the GV3101 strain. The vector 35S-VqMYB154-GFP was also infused into the GV3101 strain. Strains carrying various vector combinations were infiltrated into tobacco leaves based on Agrobacterium-mediated transient transformation\textsuperscript{78}. After 72 h of cultivation, GUS activity was detected. The empty vector pC1391-GUS was used as a negative control. A TECAN Infinite M200 PRO Absorbance Microplate Reader (TECAN, Switzerland) was used in the above assays.

**Stilbenoid extraction and analysis**

The fully ground powder of grape leaves was dried at \( -105 \) °C for 24 h; the samples were weighed and then extracted in methanol (Tedia, Fairfield, USA) away from light at 4 °C overnight. The insoluble solid was discarded by low-temperature centrifugation at 4000 \( \times g \) for 15 min. The clear methanol extracts were filtered through a 0.22 μm membrane filter and stored in sample bottles. HPLC determination was conducted using an Agilent 1260 Infinity HPLC system (Agilent, USA). Stilbene was separated from the filtered samples (10 μL) using a binary gradient of solvent A (acetonitrile) and solvent B (ultrapure water). The wavelength for fluorimetric determination was 306 nm. The gradient conditions were based on a previous study\textsuperscript{79}. The retention times were confirmed using standard samples of trans-resveratrol and trans-piceid (Sigma-Aldrich, USA). The stilbenoid concentration was determined based on the peak area.

**Arabidopsis transformation and disease assays**

The GV3101 strain carrying the 35S-VqMYB154-GFP construct was used for Arabidopsis transformation. T3 transgenic lines were adopted for disease assays. Four-
week-old Arabidopsis leaves were infected with a suspension containing Pst DC3000 following a previously described method. The samples were used for counting bacterial colonies after inoculation for 3 days. Leaves were acquired at 0, 24, 48, and 72 h postinfection and then used for quantitative RT-PCR. Callose deposition was observed using aniline blue. The transparent leaves decolorized with 95% ethanol were stained with aniline blue solution for 24 h and then observed under a fluorescence microscope (Olympus BX63, Tokyo, Japan) with UV irradiation. Cell death was detected using a trypan blue solution. The samples at 72 h postinfection were submersed in the solution and boiled for staining. The stained leaves were decolorized with chloral hydrate. DAB staining was performed to visualize levels of H2O2. Leaves to be observed were immersed in DAB, stained for 8 h, and then boiled in 95% ethanol for decolorization. NBT staining was used to visualize O2− levels in the leaves. Leaves at 72 h postinoculation were submersed in NBT solution for 2 h, soaked in 80% ethanol, and decolorized at 60 °C for 2 h.

To perform phenotypic analysis of Arabidopsis plants under artificial inoculation with Golovinomyces cichoracearum, the G. cichoracearum isolate UCSC1 was cultivated on pad4 Arabidopsis mutants. Four-week-old leaves were used for inoculation according to a previous method. After inoculation for 7 days, the plants were used for phenotypic analysis.

Measurement of plant physiological indexes
Relative electrolyte leakage (REL) was analyzed following a previous method. Approximately 0.1 g fresh leaves were immersed in 10 mL deionized water. After vacuum infiltration for 20 min, the leaves were allowed to stand for 3 h, and electrical conductivity (EC1) was recorded by a conductivity meter. Next, tubes containing leaf samples were boiled for 20 min and cooled down. The second electrical conductivity (EC2) was then recorded, and the REL ratio was determined (EC1/EC2 × 100%). The total chlorophyll content and malondialdehyde (MDA) content were analyzed as previously described. The assimilation rate (A), which represents the net photosynthetic rate, was determined using a portable photosynthetic apparatus CIRAS-3 (PP Systems, USA). A commercial detection kit was used to determine H2O2 content (Suzhou Keming Bioengineering Institute, China).

Gene expression analysis by qRT-PCR
RNA from grapes and Arabidopsis was extracted according to the manufacturer’s instructions (Omega, Norcross, GA, USA). cDNA was acquired using the FastKing RT kit (Tiangen, Beijing, China). The sample mixture consisted of 7 μL sterile water, 0.8 μL each primer, 1 μL template, and 10 μL SYBR Taq (Takara, Japan). The 2−ΔΔCt method was used for calculations. Grapevine GAPDH (XM_002278316.4) and Arabidopsis Actin (AT3G18780) were used as standard controls. The assays were performed using Applied Biosystems QuantStudio 6 Flex System (Applied Biosystems, Foster City, USA). Data are presented as the mean (±SD) from three biological replicates. For assays with multiple timepoint controls, the fold change was calculated based on the rate between the treatment group and the mock control at the same timepoint. For screening assays of resistance-related MYB TFs, the fold change was acquired by comparison with the expression in inoculated samples at 0 h. The fold change of gene expression in grapevine organs was calculated by comparison with the expression level in stems. Significance analysis was conducted with GraphPad Prism 7.0 (GraphPad Software, La Jolla, USA) using one-way ANOVA with Fisher’s LSD test (*P < 0.05; **P < 0.01). The qRT-PCR primers used are listed in Supplementary Tables S1 and S2.

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Author contributions
Y.J.W. designed the study. C.Y.J. conducted the related experiments and data analysis and wrote the manuscript. D.W. and J.Z. participated in the experiments. Y.X., C.Z., J.Z., and X.W. revised the manuscript. Y.J.W. reviewed and revised the manuscript.

Conflict of interest
The authors declare no competing interests.

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