

# Grapevine WRKY transcription factors

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## Abstract

Grape is one of the most economically important fruits and is cultivated worldwide, but the viticulture faces challenges of various biotic and abiotic stresses. WRKY transcription factors play important roles in regulating plant responses to these stresses, in addition to their roles in plant growth. Genome-wide identification and functional analysis of grape WRKY family genes have been conducted in recent years. However, different approaches were found in naming the grape WRKY family gene members among these reports, which causes a great deal of confusion and has become a barrier in the sharing of research findings in the research community. Here we attempt to comprehensively review the research progress on grape WRKY family transcription factors, and attempt to assign unified names for them.

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## Introduction

The WRKY family is one of the largest transcription factor families in plants, and is an important part of the signal network that modulate many plants processes<sup>[1]</sup>. WRKY family transcription factors are not only involved in the regulation of growth and development, but also in the regulation of plant response to biotic and abiotic stresses.

WRKY transcription factors contain a highly conserved WRKYGQK amino acid sequence and zinc finger motif (Cys(2)-His(2) or Cys(2)-His-Cys), and bind to the W box cis-acting element (TTGAC(C/T)) in the promoter of their target genes<sup>[2]</sup>. Some WRKY transcription factors can also bind to other cis-acting elements. For example, SUSIBA2 (HvWRKY46) binds to both W-box element and SURE element<sup>[3]</sup>, and OsWRKY13 binds to both W-box element and PRE4 element<sup>[4]</sup>. The WRKY domain and zinc finger motif of WRKY transcription factor play an indispensable role in the protein-DNA recognition processes<sup>[5,6]</sup>. The WRKY domain contains a conserved heptapeptide (WRKYGQK), whose amino acid substitutions could affect the binding properties of WRKY domain to W-box<sup>[7]</sup>. WRKYGEK and WRKYGKK are the most common variants<sup>[8]</sup>. In a few WRKY proteins, WRKY motif is also replaced by WIKY, WRMC, WSKY, WKRK, WVKY or WKKY<sup>[9]</sup>. The W-box core sequence is necessary for WRKY transcription factor proteins binding, and its adjacent sequence also affect the binding preference of WRKY protein<sup>[5]</sup>.

The WRKY proteins are generally divided into three groups according to the number of WRKY domains and the type of zinc finger motifs<sup>[2]</sup>. Group I contains two WRKY domains, and Group II and III contain one WRKY domain. Group II and Group III are distinguished according to their C-terminal zinc finger motifs, as Group II includes a C<sub>2</sub>H<sub>2</sub> zinc finger motif, while Group III includes a C<sub>2</sub>HC zinc finger motif. Group II members can be further divided into five subgroups (IIa, IIb, IIc, IId and IIe) based on their amino acid sequences. Besides the above three groups, some atypical proteins with WRKY domain and incomplete zinc finger motif are classified into Group IV<sup>[10]</sup>.

Grapes are one of the most economically important fruit crops in the world. Sustainable production of high-quality fruits in an ever-changing environment is the major goal of the grape industry. How to cope with biotic and abiotic stresses is the main focus of the grape biotechnology research community. WRKY transcription factors are important transcriptional regulators involved in plant growth, development and stress response. Their expression is affected by environment and growth status, which helps to find clues to their functions by transcription detection. At present, the functions of nearly 20 members of the grape WRKY transcription factor family have been identified. Here we attempt to summarize the research progress on biological functions, regulating networks and potential applications of the grape WRKY family transcription factors.

## Genome-wide Identification of Grape WRKYs

Grape WRKY family members have been identified and analyzed based on whole genome sequencing<sup>[11–15]</sup>. In two independent studies, a total of 59 putative WRKY family members were identified from the 12× *V. vinifera* cv. Pinot Noir (PN40024) genome sequences<sup>[11,12]</sup>. In another study, Wang et al.<sup>[13]</sup> also identified a total of 59 putative WRKY family members from three public databases (12× PN40024 grape genome sequence, NCBI GenBank database, and 8× coverage of the grape genome), but there are differences from the former two studies in the functional annotation of two candidate genes (*VIT\_02s0154g00210* and *XM\_002277347.4*). Zhang & Feng<sup>[14]</sup> obtained 80 putative WRKY family genes from the NCBI GenBank database, which may have resulted from the repeated calculation of genes in a heterozygous locus. Romero et al.<sup>[15]</sup> identified 61 putative WRKY family proteins based on the latest *V. vinifera* reference genome (PN40024 12×.v2, vcost. v3 annotation). It is noticeable that two putative WRKY family members, *VIT\_02s0154g00210* and *XP\_002277383.1*, varied among these studies. *VIT\_02s0154g00210* only has a partial zinc finger motif (CX<sub>4</sub>C) at the C-terminus, which is similar to OsWRKY33 and

OsWRKY38 in rice<sup>[10]</sup>. XM\_002277347.4, which encodes the XP\_002277383.1 protein in the NCBI GenBank database, is located on chromosome 1 (2348150 to 2350401), and is annotated as unknown protein (GSVIVT01020752001) due to different predictions of its translation starting site in the PN40024 grape genome (12x PN40024, v1 annotation). After removing the redundancy, a total of 61 putative WRKY family members were identified from the PN40024 grape genome (PN40024 12x.v2, vcost. v3 annotation) and NCBI database (*Vitis vinifera* Annotation Release 102) (Table 1).

## Characteristics of grape WRKY proteins

The grape WRKY family gene members are divided into different groups<sup>[11–14]</sup>. Group I and Group III have obvious structural features, and the number of their members are 12 and six, respectively. Two members with incomplete zinc finger structure belong to Group IV. The remaining 41 members are assigned to Group II and subdivided into five subgroups (IIa, IIb, IIc, II d, and II e) using the phylogenetic tree based on their amino acid sequences. Among them, five subgroups of Group II are slight differences among different studies.

In the grape WRKY family proteins, 56 members contain the conserved WRKYGQK sequence, and the other five members have variants in the heptapeptides, of which four members are replaced by WRKYGKK, and one member is WKKYGQK. Several grape WRKY transcription factors containing conserved WRKYGQK sequence can bind to the W-box element in the promoter of target genes<sup>[15–21]</sup>. It is worth noting that four out of five heptapeptide variants belonged to the subgroup IIc. Amino acid substitution of conserved hexapeptides may affect the binding activities of WRKY proteins to the W-box. For instance, tobacco NtWRKY12 containing WRKYGKK sequence could specifically bind to WK-box (TTTTCCAC) but not to W-box. When WRKYGKK of NtWRKY12 was replaced by WRKYGQK or WRKYGEK, the binding to WK box was abolished<sup>[7]</sup>. The five grape WRKY members that have various amino acid compositions in the heptapeptide might recognize and bind to other cis-acting elements in the promoters of target genes.

Since almost all grape WRKY transcription factors possess W-box elements on their own promoters, they could bind to themselves or to the promoters of other WRKY transcription factors to regulate self-expression or other WRKY transcription factors.

## Naming of grape WRKY members

In higher plants, the WRKY transcription factor family usually contains a dozen to a hundred members. A uniform nomenclature with consecutive numbering may help avoid confusion and facilitate scientific communication. Different nomenclature systems have been used among gene family members. For example, the nomenclature of WRKY family members in *Solanum lycopersicum*<sup>[22]</sup>, *S. tuberosum*<sup>[23]</sup>, and *Arabidopsis thaliana*<sup>[2]</sup> are based on their phylogenetic similarity to AtWRKY, grouping on the evolutionary tree, and direct assignment, respectively. It will be much simpler if a common method, such as naming family member genes according to their chromosome order or group order<sup>[24,25]</sup> is used. However, the reality is probably too complicated to have a uniform naming system. Even in the most studied plant genomes of *Arabidopsis* and rice, naming of family member genes does not

always follow a unique rationale, and in most cases, a unique number for each member was assigned based on the knowledge of the researchers<sup>[2,26,27]</sup>.

Due to the differences in putative members and naming methods, a WRKY number used for a grape WRKY member in a publication does not uniquely represent a certain protein<sup>[11–15]</sup>. In previous studies<sup>[11–13]</sup>, although the most common naming system for the grape WRKY family genes is based on their order in the *V. vinifera* genome, there are differences in predicted family members and the naming of members with uncertain positions, resulting in confusion in naming. For example, the grape WRKY family gene *VIT\_04s0008g01470* located on chromosome 4 has been named *VvWRKY8*<sup>[11]</sup>, *VvWRKY6*<sup>[13]</sup>, *VvWRKY53*<sup>[12]</sup>, and *VvWRKY50*<sup>[14]</sup> in different studies. When researchers identify and study a new WRKY family gene for a gene function purpose, this gene is often labeled with a number based on its homologous gene number identified in model plants<sup>[17,20,28–31]</sup>. For example, the grape WRKY family gene *VIT\_15s0046g01140*, homologous to *AtWRKY30*, was named *VvWRKY30* in a previous study<sup>[31]</sup>.

Since multiple naming systems are used for grape WRKY family genes, the same gene may be represented by different *VvWRKY* numbers, and vice versa. For example, the grape WRKY family gene *VIT\_01s0011g00220* are named *VvWRKY4*<sup>[11]</sup>, *VvWRKY58*<sup>[13]</sup>, and *VvWRKY3*<sup>[12]</sup> in different studies. Therefore, a uniform naming system for grape WRKY family genes is urgently needed for the grape research community. With this in mind, we here recommend that each WRKY member is re-assigned with a unique number on a continuous basis. The future discoveries of new *VvWRKY* members will be subsequently assigned a new number. In order to avoid confusion caused by the new numbering system, we recommend the use of the numbering system based on the study of Wang et al.<sup>[13]</sup>. All WRKY family members in this article are numbered in Table 1. We encourage the use of the names in Table 1 in future studies to avoid the confusion which often arises when multiple names are assigned to a given WRKY gene family member.

## Functional studies of grape WRKYs

The expression of WRKY transcription factors in various tissues and in response to different treatments had been extensively studied, in which the functions of about 20 grape WRKY family genes have been revealed. Due to the limitation of grape transgenic technology and growth cycle, most of the research on the functions of grape WRKY transcription factors were carried out in *Arabidopsis* or tobacco.

The expression of WRKY members in different environments provided important clues for studying their functions. Moreover, the functions of homologous proteins in model plants also shed light on the functional study of grape WRKY members.

## Plant growth and development

Plant WRKY proteins have been reported to participate in several plant growth and development processes, such as seed development, dormancy and germination, leaf senescence and trichome development<sup>[32]</sup>. Expression of grape WRKY family genes at different developmental stages and in different tissues have been examined<sup>[11–13]</sup>. The functions of some grape WRKY members in grape growth and development have been identified.

**Table 1.** Putative grape WRKY genes identified in the grape genome.

Gene name	Gene number	Location	Gene number	Former names
VvWRKY1	GSVIVT01012196001	chr1:628,681..633,595	VIT_01s0011g00720	
VvWRKY2	GSVIVT01020060001	chr1:10,977,206..10,982,627	VIT_01s0026g01730	
VvWRKY3	GSVIVT01010525001	chr1:21,460,152..21,461,423	VIT_01s0010g03930	VlWRKY3 <sup>[41]</sup> , VvWRKY8 <sup>[51]</sup> , VvWRKY3 <sup>[49]</sup>
VvWRKY4	GSVIVT01019419001	chr2:512,179..513,535	VIT_02s0025g00420	
VvWRKY5	GSVIVT01019511001	chr2:1,228,115..1,229,951	VIT_02s0025g01280	
VvWRKY6	GSVIVT01035426001	chr4:1,210,142..1,211,252	VIT_04s0008g01470	
VvWRKY7	GSVIVT01035884001	chr4:5,247,432..5,248,815	VIT_04s0008g05750	
VvWRKY8	GSVIVT01035885001	chr4:5,265,887..5,267,902	VIT_04s0008g05760	VpWRKY3 <sup>[59]</sup>
VvWRKY9	GSVIVT01035965001	chr4:6,569,931..6,576,445	VIT_04s0008g06600	
VvWRKY10	GSVIVT01033188001	chr4:9,363,044..9,365,026	VIT_04s0069g00920	VvWRKY11 <sup>[17]</sup>
VvWRKY11	GSVIVT01033194001	chr4:9,399,822..9,400,803	VIT_04s0069g00970	
VvWRKY12	GSVIVT01033195001	chr4:9,409,805..9,411,286	VIT_04s0069g00980	
VvWRKY13	GSVIVT01019109001	chr4:16,664,476..16,666,517	VIT_04s0023g00470	
VvWRKY14	GSVIVT01034968001	chr5:530,097..531,696	VIT_05s0007g00730	
VvWRKY15	GSVIVT01025491001	chr6:364,396..365,481	VIT_06s0004g00230	
VvWRKY16	GSVIVT01024624001	chr6:8,290,089..8,292,826	VIT_06s0004g07500	VaWRKY33 <sup>[57]</sup>
VvWRKY17	GSVIVT01000752001	chr7:381,054..383,675	VIT_07s0141g00680	
VvWRKY18	GSVIVT01028129001	chr7:4,044,185..4,045,807	VIT_07s0005g01520	
VvWRKY19	GSVIVT01028147001	chr7:4,200,461..4,202,183	VIT_07s0005g01710	
VvWRKY20	GSVIVT01028244001	chr7:4,899,940..4,903,113	VIT_07s0005g02570	
VvWRKY21	GSVIVT01022067001	chr7:16,322,554..16,324,062	VIT_07s0031g00080	
VvWRKY22	GSVIVT01022245001	chr7:17,794,621..17,797,164	VIT_07s0031g01710	
VvWRKY23	GSVIVT01022259001	chr7:17,958,339..17,960,800	VIT_07s0031g01840	VvWRKY13 <sup>[28,33,52,53]</sup>
VvWRKY24	GSVIVT01030258001	chr8:9,796,097..9,798,804	VIT_08s0058g00690	VvWRKY33 <sup>[29]</sup> , VvWRKY24 <sup>[49]</sup>
VvWRKY25	GSVIVT01030174001	chr8:10,843,800..10,846,082	VIT_08s0058g01390	VpWRKY1 <sup>[16]</sup>
VvWRKY26	GSVIVT01025562001	chr8:14,033,449..14,039,296	VIT_08s0040g03070	VvWRKY26 <sup>[34]</sup>
VvWRKY27	GSVIVT01034148001	chr8:14,828,036..14,830,056	VIT_08s0007g00570	
VvWRKY28	GSVIVT01015952001	chr9:16,094,245..16,096,198	VIT_09s0018g00240	VaWRKY14 <sup>[55]</sup> , VvWRKY40 <sup>[30]</sup>
VvWRKY29	GSVIVT01012682001	chr10:618,665..621,093	VIT_10s0116g01200	VaWRKY12 <sup>[20]</sup> , VpWRKY31 <sup>[45]</sup>
VvWRKY30	GSVIVT01021252001	chr10:3,008,699..3,010,258	VIT_10s0003g01600	
VvWRKY31	GSVIVT01021397001	chr10:4,894,494..4,896,041	VIT_10s0003g02810	
VvWRKY32	GSVIVT01021765001	chr10:10,756,001..10,759,241	VIT_10s0003g05740	
VvWRKY33	GSVIVT01023600001	chr11:7,838,041..7,845,641	VIT_11s0037g00150	VpWRKY2 <sup>[16]</sup>
VvWRKY34	GSVIVT01029265001	chr11:17,821,900..17,823,218	VIT_11s0052g00450	
VvWRKY35	GSVIVT01020864001	chr12:878,978..881,517	VIT_12s0028g00270	
VvWRKY36	GSVIVT01030453001	chr12:2,348,148..2,350,455	XP_002277383.1	
VvWRKY37	GSVIVT01030453001	chr12:5,678,804..5,681,082	VIT_12s0059g00880	
VvWRKY38	GSVIVT01030046001	chr12:9,116,720..9,122,740	VIT_12s0057g00550	
VvWRKY39	GSVIVT01029688001	chr12:13,065,135..13,067,116	VIT_12s0055g00340	
VvWRKY40	GSVIVT01032662001	chr13:1,716,836..1,718,877	VIT_13s0067g03130	
VvWRKY41	GSVIVT01032661001	chr13:1,719,393..1,720,803	VIT_13s0067g03140	
VvWRKY42	GSVIVT01036223001	chr14:8,753,748..8,755,749	VIT_14s0081g00560	
VvWRKY43	GSVIVT01033063001	chr14:25,479,103..25,481,917	VIT_14s0068g01770	VvWRKY43 <sup>[49]</sup>
VvWRKY44	GSVIVT01011356001	chr14:28,924,779..28,926,499	VIT_14s0108g00120	
VvWRKY45	GSVIVT01011472001	chr14:29,916,401..29,920,389	VIT_14s0108g01280	
VvWRKY46	GSVIVT01018300001	chr15:11,499,221..11,503,179	VIT_15s0021g01310	
VvWRKY47	GSVIVT01027069001	chr15:18,191,021..18,193,470	VIT_15s0046g01140	MrWRKY30 <sup>[31]</sup> , VlWRKY48 <sup>[42]</sup> , VvWRKY46 <sup>[19,44]</sup>
VvWRKY48	GSVIVT01026969001	chr15:18,940,954..18,942,146	VIT_15s0046g02150	
VvWRKY49	GSVIVT01026965001	chr15:18,957,235..18,958,812	VIT_15s0046g02190	VvWRKY22 <sup>[26]</sup>
VvWRKY50	GSVIVT01028823001	chr16:18,360,079..18,360,711	VIT_16s0050g01480	
VvWRKY51	GSVIVT01028718001	chr16:19,477,129..19,479,547	VIT_16s0050g02510	VqWRKY52 <sup>[43]</sup> , VvWRKY52 <sup>[44]</sup> , VvWRKY30 <sup>[21]</sup> , VdWRKY53 <sup>[56]</sup>
VvWRKY52	GSVIVT01008553001	chr17:922,644..925,087	VIT_17s0000g01280	VvWRKY1 <sup>[18,40]</sup>
VvWRKY53	GSVIVT01008046001	chr17:6,316,168..6,320,034	VIT_17s0000g05810	VvWRKY53 <sup>[49]</sup>
VvWRKY54	GSVIVT01009441001	chr18:8,392,053..8,393,721	VIT_18s0001g10030	
VvWRKY55	GSVIVT01037686001	chr19:6,882,399..6,884,987	VIT_19s0009g00840	
VvWRKY56	GSVIVT01037775001	chr19:7,760,183..7,767,013	VIT_19s0090g01720	
VvWRKY57	GSVIVT01014854001	chr19:10,665,189..10,669,055	VIT_19s0015g01870	
VvWRKY58	GSVIVT01001332001	chr1_random:297,588..311,938	VIT_01s0011g00220	VvWRKY2 <sup>[37,54,60]</sup>
VvWRKY59	GSVIVT01007006001	chrUn:29,694,084..29,699,952	VIT_00s0463g00010	
VvWRKY60	GSVIVT01001286001	chr2:4,989,461..4,989,778	VIT_02s0154g00210	
VvWRKY61	GSVIVT00037762001	chr16:18360079..18360711	VIT_16s0050g01480	

VvWRKY13 regulates the synthesis of Et (ethylene) and ABA, affecting the growth and development of grapevines. VvWRKY13, cloned from a grape cv. 'Zuoyouhong', was found to be ubiquitously expressed in various grapevine tissues<sup>[28]</sup>. Ectopic expression of the VvWRKY13 gene in Arabidopsis could also promote the synthesis of Et and ABA, showing constitutive triple responses, delayed seed germination, smaller stomatal aperture size, and several other phenotypes<sup>[28,33]</sup>.

Grape WRKYs also play important roles in regulating fruit development. VvWRKY26 might regulate grape fruit quality by participating in vacuolar transport and acidification, as well as flavonoid pathways<sup>[34]</sup>. VvWRKY26, which had the closest homology to petunia *PhPH3* and Arabidopsis *AtTTG2* (*AtWRKY44*), could fulfill the PH3 function in the regulation of vacuolar pH and restored the wild type pigmentation phenotype by up-regulating the expression level of the pH structural genes and the anthocyanin-related genes in the petunia *ph3* mutant<sup>[34]</sup>. In the early stage of berry development, VvWRKY26 was highly expressed in the inner integument of the seed coat and might be involved in the regulation of the expression of many proanthocyanidin related genes<sup>[34]</sup>. It was supported that VvWRKY26 function as a proanthocyanidin regulator and might also affect seed development and plant fertility in grapevine as its Arabidopsis homolog *AtTTG2*<sup>[35]</sup>.

VvWRKY22, cloned from *V. vinifera* 'Cabernet Sauvignon', was involved in the sugar metabolism of grape berries<sup>[36]</sup>. VvWRKY22 was co-expressed with 16 sugar-related genes in grape berries, and the expression of VvWRKY22 in grape suspension cells was inhibited by sucrose, but induced by fructose and ABA. Overexpression of VvWRKY22 in grape suspension cells reduced the content of sucrose, glucose, and fructose, while the silencing of VvWRKY22 gene in grape suspension cells increased the content of these sugars. The role of VvWRKY22 on sugar metabolism might be accomplished by regulating the expression of sugar and ABA-related genes, and the protein activity of VvWRKY22 might be directly regulated by VvSnRK1.1 (Sucrose non-fermenting-1-related protein kinase 1.1) and VvSnRK1.2.

VvWRKY2 played a role in lignin biosynthesis and xylem development<sup>[37]</sup>. VvWRKY2 was specifically and highly expressed in the lignified tissues of grapevine stems (sclerenchyma and xylem cells). Ectopic expression of VvWRKY2 in tobacco delayed

xylem formation and changed lignin composition in stems and petioles by regulating the expression of genes involved in lignin biosynthesis pathway and cell wall formation<sup>[37]</sup>. VvWRKY2 activated the expression of VvCH4 involved in the lignin biosynthesis pathway.

### Biotic and abiotic stresses

The expression profiles of grape WRKY family genes in response to biotic and abiotic stresses, or plant hormones have been studied to a certain extent. The transcription of most WRKY protein members can be altered by at least one stress treatment, which suggests that they are widely involved in plant response to biotic or abiotic stress<sup>[11–15]</sup>.

Plant defense responses to phytopathogen attack are regulated through a complex network of signaling pathways that involve SA (Salicylic acid), JA (Jasmonic acid), Et, and ABA<sup>[38,39]</sup>. JA and Et work synergistically in the inducing of defense-related genes against necrotrophs. SA works antagonistically to JA/Et, enhancing susceptibility to necrotrophic pathogens while promoting resistance to hemibiotrophs and biotrophs. ABA plays a positive or negative role in the plant defense response to necrotrophs, depending on the specific plant-pathogen interaction<sup>[39]</sup>. WRKY genes are often induced by these hormones and also involved in regulating downstream responses mediated by these hormones. Expression of some marker genes is commonly used to indicate the activation of these hormone signal transductions.

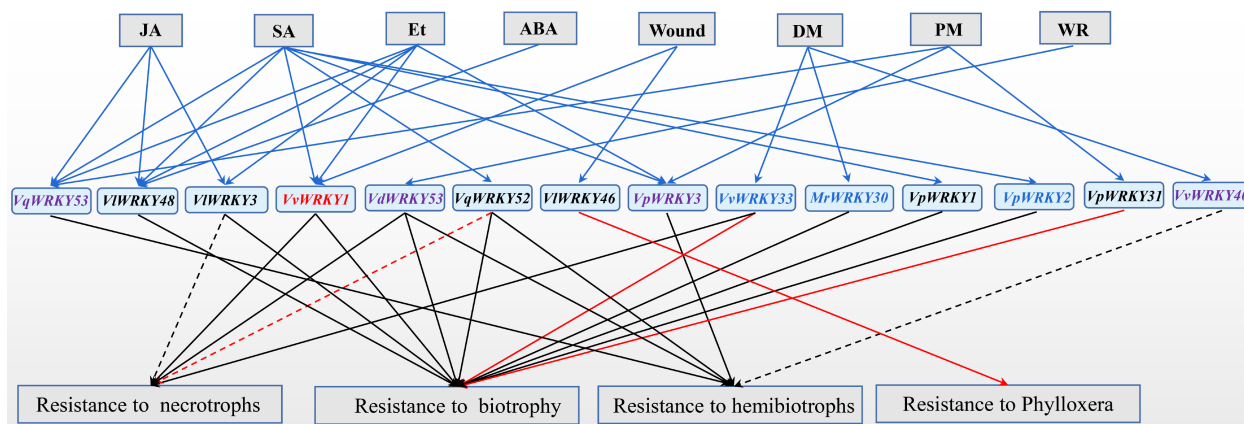
The functions of some grape WRKY members under biotic stress have been studied (listed in Table 2 and Fig. 1). However, most of their molecular mechanisms leading to the functions are still poorly understood. Besides VvWRKY40 and VvWRKY46, these grape WRKY members generally prompted resistance to biotrophs or hemibiotrophs (Fig. 1). VvWRKY1, VdWRKY53, and VvWRKY33 have been found to promote plant resistance to necrotrophs, and biotrophs or hemibiotrophs. While VvWRKY3 and VqWRKY52 can increase plant resistance to hemibiotrophs and semi-biotrophs, but increase susceptibility to necrotrophs. These results suggest that these WRKY transcription factors in grape manipulate various responses to pathogen attacks.

The expression of these grape WRKY transcription factors is affected by pathogen attack and plant hormones, and they also act as transcriptional regulators to regulate plant hormone-mediated defense response. The functions of these transcrip-

**Table 2.** Functional analysis of grape WRKY proteins under biotic stresses.

Former name	Gene function
VvWRKY3 <sup>[41]</sup>	Enhanced resistance to <i>Golovinomyces cichoracearum</i> , but increased susceptibility to <i>Botrytis cinerea</i> in Arabidopsis
VpWRKY3 <sup>[59]</sup>	Enhanced tolerance to <i>Ralstonia solanacearum</i> in tobacco
VvWRKY33 <sup>[29]</sup>	Enhanced resistance to <i>Plasmopara viticola</i> in grapevine; participate in resistance to <i>B. cinerea</i> in Arabidopsis
VpWRKY1 <sup>[16]</sup>	Enhanced resistance to <i>Erysiphe necator</i> in Arabidopsis
VvWRKY40 <sup>[30]</sup>	Increased susceptible to <i>Phytophthora capsica</i> in tobacco
VpWRKY31 <sup>[45]</sup>	Enhanced resistance to <i>E. necator</i> in susceptible <i>V. vinifera</i>
VpWRKY2 <sup>[16]</sup>	Enhanced resistance to <i>E. necator</i> in Arabidopsis
MrWRKY30 <sup>[31]</sup>	Enhanced resistance to <i>Hyaloperonospora parasitica</i> in Arabidopsis
VvWRKY48 <sup>[42]</sup>	Enhanced resistance to <i>G. cichoracearum</i> in Arabidopsis
VvWRKY46 <sup>[19]</sup>	Attenuated phylloxera attack and delayed nymph development in susceptible grape cultivars
VqWRKY52 <sup>[43,44]</sup>	Enhanced resistance to <i>G. cichoracearum</i> and <i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000, but increased susceptibility to <i>B. cinerea</i> in Arabidopsis. Enhanced resistance to <i>B. cinerea</i> in gene-edited grape
VdWRKY53 <sup>[56]</sup>	Enhanced resistance to <i>Coniella diplodiella</i> , <i>Pst</i> DC3000 and <i>G. cichoracearum</i> in Arabidopsis
VvWRKY1 <sup>[18,40]</sup>	Enhanced resistance to <i>Pythium</i> , <i>Peronospora tabacina</i> , and <i>E. cichoracearum</i> in tobacco. Enhanced resistance to <i>P. viticola</i> in grapevine
VqWRKY53 <sup>[50]</sup>	Enhanced resistance to <i>Pst</i> DC3000 in Arabidopsis





**Fig. 1** Functions of grape WRKY transcription factors in biotic stresses. The expression of grape WRKY members were induced (solid blue arrows) by pathogen attack (DM, *Plasmopara viticola*; PM, *Erysiphe ecichoraceum*; WR, *Coniella diplodiella*), hormone (JA, Methyl jasmonate; SA, Salicylic acid; Et, Ethylene), and wound. Grape WRKY members enhanced (solid arrows) or repressed (dotted lines) plant resistance to different types of pathogens. Grape WRKY members in red font were involved in regulating the expression of JA signaling pathway-related defense genes. Grape WRKY members in black font were involved in regulating the expression of SA signaling pathway-related defence genes. Grape WRKY members involved in regulating the expression of JA signaling pathway-related defence genes (red font), SA signaling pathway-related defence genes (black font), both SA and JA signaling pathway-related defence genes (blue font), or unknown (purple font). The function of WRKY members were identified in grapevines (red arrows) or other plants (black arrows).

tion factors on biotic stress is mostly attributed to the regulation of plant hormone-mediated defense response. VpWRKY2<sup>[16]</sup>, VvWRKY1<sup>[18,40]</sup>, VvWRKY33<sup>[29]</sup>, and MrWRKY30<sup>[31]</sup> are involved in regulating the expression of JA signaling pathway-related genes, while VIWRKY3<sup>[41]</sup>, VvWRKY33<sup>[29]</sup>, VpWRKY1<sup>[16]</sup>, VpWRKY2<sup>[16]</sup>, MrWRKY30<sup>[31]</sup>, VIWRKY48<sup>[42]</sup>, VIWRKY46<sup>[19]</sup>, and VqWRKY52<sup>[43,44]</sup>, and VpWRKY31<sup>[45]</sup> are involved in regulating the expression of SA signaling pathway-related genes. VpWRKY2 is also involved in regulating the expression of Et signaling pathway-related genes<sup>[16]</sup>.

To study the molecular functions and regulating networks, target genes of grape WRKY members have been identified. For example, VvWRKY52 enhanced grape resistance to *P. viticola* by activating the expression of JA pathway-related defense genes, including *JAZ1.1*, *JAZ1.2* and *LOX*<sup>[40]</sup>. VvWRKY33 specifically activated the expression of *VvPR10.1* gene, prompting the resistance to *P. viticola* in grapevine<sup>[29]</sup>. VIWRKY46 played a role in SA-mediated defense-regulatory network by directly binding to the downstream structural gene *VvCHIB*, and attenuated phylloxera attack and delayed nymph development in susceptible cultivars<sup>[19]</sup>.

Stilbene also plays an important role in protecting plants from pests, pathogens and abiotic stresses<sup>[46,47]</sup>. There was a high correlation between VvWRKY family genes and stilbene synthase genes (*STS*) expression in the microarray and RNA-Seq data<sup>[48–50]</sup>. Some grape WRKYs could directly bind to the promoters of stilbene synthase genes, and regulate their expression. Four VvWRKY genes, VvWRKY3, VvWRKY24, VvWRKY43, and VvWRKY53, were involved in regulating stilbene biosynthetic pathway in different hierarchies<sup>[49]</sup>. In particular, VvWRKY24 played a role in regulating the expression of *VvSTS29* gene alone, while VvWRKY3 and VvWRKY53 worked through the combined effect with VvMYB14. The VqWRKY53 involved in positively regulating the expression of stilbene synthase genes *VqSTS32* and *VqSTS41* to promote plant disease resistance<sup>[50]</sup>. VvWRKY8 interacted with VvMYB14 and prevented it from binding to the promoters of *VvSTS15* and *VvSTS21*, thereby

reducing the resveratrol accumulation<sup>[51]</sup>. VqWRKY31 could directly bind to the promoters of *VvSTS1* and *VvSTS2*, and promote their expression<sup>[45]</sup>. Over-expression of *VqWRKY31* conferred powdery mildew resistance in susceptible *V. vinifera* plants by prompting salicylic acid signaling, and specific stilbenoids and flavonoid synthesis<sup>[45]</sup>.

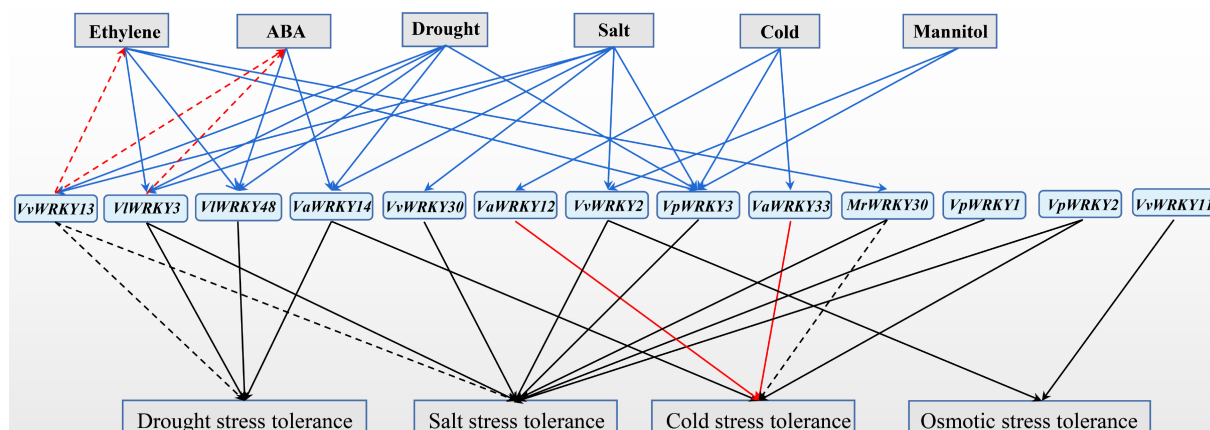
Grape WRKY transcription factors, as transcriptional activators or inhibitors, play roles in many processes of plant responses to abiotic stress (Table 3 and Fig. 2). Except for VvWRKY13 and MrWRKY30, other WRKY members enhance plant resistance to one or more abiotic stresses. VvWRKY13 negatively regulates plant resistance to drought and salt stress, while MrWRKY30 promotes plant resistance to salt stress but negatively regulates plant resistance to cold stress.

Under abiotic stress, grapevine WRKY family genes stimulate the synthesis of plant hormones ABA and Et, the accumulation of cell osmotic substances, the removal of ROS, and the expression of stress-related genes, etc.

Ectopic expression of VvWRKY13 in *Arabidopsis* promoted constitutive ABA and Et production, and impaired plant tolerance to drought and salt stress by decreasing the accumulation of cell osmotic substance, increasing the ROS level, and down-regulating the expression of stress-related genes<sup>[28,33,52,53]</sup>. VIWRKY3 promoted stress-induced ABA production by promoting the expression of ABA synthesis genes, thereby enhancing the resistance of *Arabidopsis* to drought and salt stress<sup>[41]</sup>.

VvWRKY13<sup>[52,53]</sup>, VvWRKY30<sup>[21]</sup>, and VvWRKY2<sup>[54]</sup> are found in regulating the accumulation of osmotic substances, and VIWRKY3<sup>[41]</sup>, VaWRKY14<sup>[55]</sup>, VvWRKY23<sup>[52,53]</sup>, VaWRKY12<sup>[56]</sup>, VIWRKY48<sup>[42]</sup>, VvWRKY30<sup>[21]</sup>, MrWRKY30<sup>[31]</sup> involved in regulating the level of ROS, affecting plant resistance to abiotic stress.

The expression of stress-responsive genes in transgenic materials reveal that these grape WRKY members have different functional mechanisms in response to abiotic stresses. VaWRKY14 could enhance the expression of stress-related genes including *COR15A*, *COR15B*, *COR413*, *KIN2* and *RD29A* in



**Fig. 2** Functions of grape WRKY transcription factors in abiotic stresses. The expression of grape WRKY members were induced (solid blue arrows) by abiotic stress, and hormones (ABA, Abscisic acid; Et, Ethylene). Grape WRKY members were involved in plant hormone synthesis (red dotted arrows). Grape WRKY members enhanced (solid arrows) or repressed (dotted arrows) plant abiotic stresses tolerance.

**Table 3.** Functional analysis of grape WRKY proteins under abiotic stresses.

Gene name	Gene function
<i>VvWRKY3</i> <sup>[41]</sup>	Enhanced tolerance to the salt and drought stress in Arabidopsis
<i>VpWRKY3</i> <sup>[59]</sup>	Enhanced tolerance to the salt stress in tobacco
<i>VvWRKY11</i> <sup>[17]</sup>	Enhanced tolerance to the osmotic stress in Arabidopsis
<i>VaWRKY33</i> <sup>[57]</sup>	Enhanced tolerance to the cold stress in Arabidopsis and grape calli
<i>VvWRKY13</i> <sup>[52,53]</sup>	Negatively regulated drought and salt stress tolerance of Arabidopsis
<i>VpWRKY7</i> <sup>[16]</sup>	Enhanced tolerance to the salt stress in Arabidopsis
<i>VaWRKY14</i> <sup>[55]</sup>	Enhanced tolerance to the drought stress in Arabidopsis
<i>VaWRKY12</i> <sup>[20]</sup>	Enhanced tolerance to the cold stress in Arabidopsis and grape calli
<i>VpWRKY2</i> <sup>[16]</sup>	Enhanced tolerance to the salt and cold stress in Arabidopsis
<i>VvWRKY48</i> <sup>[42]</sup>	Enhanced tolerance to the drought stress in Arabidopsis
<i>MrWRKY30</i> <sup>[31]</sup>	Enhanced tolerance to the cold stress in Arabidopsis, and negatively regulated salt stress tolerance of Arabidopsis
<i>VvWRKY30</i> <sup>[21]</sup>	Enhanced tolerance to the salt stress in Arabidopsis
<i>VvWRKY2</i> <sup>[54]</sup>	Enhanced tolerance to the salt stress and osmotic stress in tobacco

Arabidopsis under drought stress<sup>[55]</sup>. *VaWRKY12* prompted the expression of genes encoding antioxidant enzymes including peroxidases and glutathione S-transferases in Arabidopsis under drought stress<sup>[56]</sup>. Ectopic expression of *MrWRKY30* in Arabidopsis enhances cold stress tolerance by activating the AtCBF-mediated signaling pathway to induce the downstream *AtCOR47* gene, but impairs salt stress resistance by suppressing the expression of antioxidant genes<sup>[31]</sup>.

### Regulation of *VvWRKY* members

Only a few proteins involved in regulating the functions of grapes WRKY members have been identified. A cold-responsive ethylene response factor *VvERF92* directly regulated the response of *VaWRKY33* to low temperature and ethylene<sup>[57]</sup>. *VvSnRK1.1* and *VvSnRK1.2* interacted directly with *VvWRKY22*, and might be involved in regulating the role of *VvWRKY22* in sugar metabolism<sup>[36]</sup>. An E3 ubiquitin ligase *EIRP1* (*Erysiphe necator*-induced RING finger protein 1), which played a positive regulatory role in plant immunity, directly interacted with *VpWRKY11*, and promoted degradation of *VpWRKY11* by 26S proteasome<sup>[58]</sup>. The cytoplasmic effector protein *PvRXLR111* secreted by *P. viticola* interacted directly with *VvWRKY40* and increased the stability of *VvWRKY40* protein, thereby inhibiting plant immunity<sup>[30]</sup>.

The external environment affects not only the expression of WRKY members, but also the subcellular localization of the

grape WRKY members. For instance, *VaWRKY12* was located in the nucleus and cytoplasm under normal temperature, but it localized only in the nucleus at low temperature<sup>[20]</sup>.

### Conclusions

In this article, we summarized the research progress regarding functions of grape WRKY family members, and suggested future naming systems to avoid confusion in sharing scientific findings of grape WRKY family members.

Grape WRKY family transcription factors play important regulatory roles, not only in grapevine growth and development, but also in their responses to biotic and abiotic stress. Although functions of some WRKY proteins have already been identified, there are still many unanswered questions that require future investigation. Currently, most functional studies and regulating network of *VvWRKY* family genes were conducted using model plants (tobacco and Arabidopsis) or in vitro grape systems. Although some abiotic and biotic stresses can be well simulated in model plants, the growth characteristics of grape, as a perennial vine, are markedly different from the model plants, which may negatively impact the functional analysis of grape WRKY members in model plants. In addition, many grape pathogens are unable to infect these model plants, and some Arabidopsis pathogens are also unable to infect grapes. Therefore, it is necessary to verify the function of grape WRKY

## Grapevine WRKY transcription factors

transcription factors in an in vivo system of grapevines such as a stable grape transformation system.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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