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ORIGINAL ARTICLE

Comparative study of *DAM*, *Dof*, and *WRKY* gene families in fourteen species and their expression in *Vitis vinifera*

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Abstract

Bud dormancy is one of the most important defensive mechanisms through which plants resist cold stress during harsh winter weather. *DAM*, *Dof*, and *WRKY* have been reported to be involved in many biological processes, including bud dormancy. In the present study, grapevine (*Vitis vinifera*) and other thirteen plants (six woody plants and seven herbaceous plants) were analyzed for the quantity, sequence structure, and evolution patterns of their *DAM*, *Dof*, and *WRKY* gene family members. Moreover, the expression of *VvDAM*, *VvDof*, and *VvWRKY* genes was also investigated. Thus, 51 *DAM*, 1,205 *WRKY*, and 489 *Dof* genes were isolated from selected genomes, while 5 *DAM*, 114 *WRKY*, and 50 *Dof* duplicate gene pairs were identified in 10 genomes. Moreover, WGD and segmental duplication events were associated with the majority of the expansions of *Dof* and *WRKY* gene families. The *VvDAM*, *VvDof*, and *VvWRKY* genes significantly differentially expressed throughout bud dormancy outnumbered those significantly differentially expressed throughout fruit development or under abiotic stresses. Interestingly, multiple stress responsive genes were identified, such as *VvDAM* (VIT_00s0313g00070), two *VvDof* genes (VIT_18s0001g11310 and VIT_02s0025g02250), and two *VvWRKY* genes (VIT_07s0031g01710 and VIT_11s0052g00450). These data provide candidate genes for molecular biology research investigating bud dormancy and responses to abiotic stresses (namely salt, drought, copper, and waterlogging).

Keywords *Vitis vinifera* · Bud dormancy · *DAM* · *Dof* · *WRKY* · 14 species

Abbreviations

DAM	Dormancy associated MADS-box
Dof	DNA-binding one zinc finger
CDS	Coding sequence
MYA	Million years ago
WGD	Whole genome duplication

Introduction

Defense mechanisms have evolved in plants under various challenging conditions over deep evolutionary timescales. Dormancy mechanisms have evolved in plants as a mechanism of survival against the low temperatures and short photoperiods that occur during harsh winter seasons (Ríos et al. 2014). Plant growth and developmental cycles generally pause during dormancy periods and occur even in seeds and buds. Bud dormancy can be classified into three different categories (Lang 1987): (1) ecodormancy, when growth is prevented by environmental conditions, such as low temperatures, water shortage, and nutrient deficiency;

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(2) paradormancy, when growth is inhibited by distal organs, such as via apical dominance, and photoperiodic responses; (3) endodormancy, when growth is prevented by internal bud signaling, such as chilling responses, and photoperiodic responses. However, unfortunately, if there are too few accumulated chilling hours, flowering quality and uniformity can be affected, which causes a drastic reduction in fruit production in the subsequent season. Accordingly, endodormancy may play an important role in fruit production.

Much research has been conducted to investigate the mechanisms underlying dormancy period over recent decades, aside from investigations of its economic impacts. From the beginning of the twentieth century to the present, dormancy studies have gone through three stages: observation of phenomena, cellular modification research, and metabolic analysis (Beauvieux et al. 2018). In particular, this has been highlighted by genetic and genomics studies. Recent advancements in transcriptomic technology have led to the identification of molecular pathways controlling bud dormancy in perennial trees, including *Populus trichocarpa* (Rohde et al. 2007; Ruttink et al. 2007), *Prunus mume* (Yamane et al. 2008; Zhong et al. 2013), *Prunus persica* (Leida et al. 2010), *Pyrus bretschneideri* (Liu et al. 2012), *Pyrus pyrifolia* (Bai et al. 2013), *Prunus pseudocerasus* (Zhu et al. 2015), *Cunninghamia lanceolata* (Xu et al. 2016), *Quercus petraea* (Ueno et al. 2013), and *Vitis vinifera* (Fennell et al. 2015; Khalil-Ur-Rehman et al. 2017; Min et al. 2017; Sreekantan et al. 2010). Specific gene expression patterns suggest that bud dormancy is associated with stress responses, hormone signaling, chromatin modification, and carbon metabolism (Regier et al. 2010; Rios et al. 2014; Saito et al. 2015; Wen et al. 2016; Wisniewski et al. 2015).

Among the well-known pathways that have thus far been characterized in plants, *dormancy-associated MADS-box* (*DAM*), *DNA-binding one zinc finger* (*Dof*), and *WRKY* genes have been identified as being involved in dormancy regulation. In peach, six *PpDAM* genes show distinctive seasonal expression patterns in the top buds, and moreover, *PpDAM1*, *PpDAM2*, and *PpDAM4* appear to be closely linked with terminal bud formation (Bielenberg et al. 2008; Li et al. 2009). Pear *DAM1* could regulate bud dormancy transition under the direct activation of PpHB22 in 'Suli' pear (Yang et al. 2018). Overexpression of *PmDAM6* could inhibit growth, repress bud break competency of dormant buds and delays bud outgrowth in apple plants (Yamane et al. 2019). Similar regulation models have been demonstrated in *Euphorbia esula* (Horvath et al. 2010), *Pyrus pyrifolia* (Ito et al. 2015; Saito et al. 2015; Ubi et al. 2010), *Malus* × *domestica* (Mimida et al. 2015), *Pyrus bretschneideri* (Niu et al. 2015), and *Prunus mume* (Sasaki et al. 2011). *Dof* genes have been implicated in bud dormancy transitional stage development in *Prunus persica* and *Fagus sylvatica* (Chen et al. 2017; Lesur et al. 2015). In peach, five *PpDofs* were up-regulated in the transitional stage, may be involved in dormancy; and one *PpDof* was highly expressed at the end (5 February) of dormancy, may play a role in bud flush (Chen et al. 2017). Pear *Dof9.2* could repress flowering time by promoting the levels of *PbTFL1a* and *PbTFL1b* (Liu et al. 2019). The dormancy status in *Retama raetam* was accompanied by the transcription level of one *WRKY* (Pnueli et al. 2002). In *Arabidopsis*, WRKY41 controls seed dormancy via direct regulation the expression level of *ABI3* (Ding et al. 2014). Moreover, at least six peach *WRKY* genes have been proven to have higher expression levels in endodormancy and lower expression levels in ecodormancy, which indicates that they may play a key role in dormancy regulation (Chen et al. 2016).

In the present study, the number, sequence structure, and evolution of *DAM*, *Dof*, and *WRKY* gene families were analyzed by comparisons between these genes in *Vitis vinifera* (woody plant) and 13 other plant species, namely, 6 woody plant species (*Actinidia chinensis*, *Citrus sinensis*, *Malus domestica*, *Populus trichocarpa*, *Prunus persica*, and *Pyrus bretschneideri*) and 7 herbaceous plant species (*Ananas comosus*, *Arabidopsis thaliana*, *Carica papaya*, *Fragaria vesca*, *Musa acuminate*, *Oryza sativa*, and *Solanum lycopersicum*). Additionally, we analyzed the expression profiles of *VvDAM*, *VvDof*, and *VvWRKY* genes in *V. vinifera* during bud dormancy, fruit development, and different abiotic stresses to identify candidate genes for further study (Table 1).

Results

Identification of *DAM*, *WRKY*, and *Dof* gene family members in the selected genomes

A total number of 51 (*DAM*), 1205 (*WRKY*), and 489 (*Dof*) gene family members were identified from the selected genome (Table 2, detailed in Tables S1–S3). Most species had only two or three *DAMs*, while at least five *DAMs* were found in *Populus trichocarpa* (9), *Fragaria vesca* (6), *Malus domestica* (6), and *Prunus persica* (5). The total number of *WRKY* gene family members in the selected genomes ranged from 49 to 153, and the three species with the most *WRKY* genes were *Musa acuminate* (152), *Malus domestica* (141), and *Pyrus bretschneideri* (112). The total number of *Dof* gene family members in the selected genomes ranged from 20 to 73, and the three species with the most *Dof* TFs were *Musa acuminate* (73), *Malus domestica* (59), and *Pyrus bretschneideri* (46).

Table 1 The RT-qPCR primer sequences of *VvDAMs*, *VvDofs*, and *VvWRKYs*

Gene ID	Forward primer sequence	Reverse primer sequence
VIT_00s0313g00070	CAACAATCACTCCAGGTTGAGC	TTGGACACCCTTTGACTGAAGA
VIT_15s0107g00120	AGACTCAGAGGCTGAGGCAAAT	TCCTGCAGTAGGTCTCCCTTTC
VIT_18s0001g07460	GCTCAGCTGATGGAAGAGAACA	TGAGGAGGACCACTACTGCAAC
VIT_00s0218g00040	CCTTGTTTCAGCATCGATCAAC	TGCTGTAATTTCCAGTGCAGGT
VIT_00s0253g00060	TATGATTGGATCCCACATGACC	TTGTTGGTGCTGTTGAGTTTGA
VIT_00s0652g00010	CAGTTGGAGCCATTGAAGTGTC	GCAGATGTCTTGGGCCTCTTAT
VIT_01s0026g02580	CCAAAGACACTGAGGATTGACG	CACCGGAGACATTTCAGCTATG
VIT_02s0025g02250	CACAACTGGGTTTCCTATGCAG	CCCAGTTGAATCTCCTTGCTCT
VIT_03s0063g01350	GCCACTTCTGCAAATCATGC	GTTCAGCAACCTGGTGAAACTC
VIT_06s0004g03420	GGGTGCAGGAAGAACAAGAGAT	TACCCTAACTGCCCACCAGTTT
VIT_06s0004g04520	GCTCGATTAGACCTGGTTCGAT	ACCTCCTCTCGTCCAGTATCGT
VIT_07s0255g00020	CCACCAATCATGCCACATCTAT	CAAATCCCATGTCCTCGAAAC
VIT_08s0007g00180	GAGGTTTAGAGCAGTGGCGATT	AGCCACCTGACTTAACCCAGAC
VIT_08s0056g01230	ACCACGATTCAGACCCAGATTT	ACTTGAGTTTGCTGCTCCTCCT
VIT_08s0105g00170	ATATTTATGGGCTGCCACCACT	AATCAAATGGGCTCCAGTTACC
VIT_09s0002g02490	TCTACGTCGTCGGTGTCTTCTC	CGGATTCAGACTCTCCAACAAG
VIT_10s0003g00030	CCTTTCTCAGCCTCGCTACTTC	CGGAGATAAGTTGGCTGGTTCT
VIT_10s0003g00040	CTTCCGAGCTCAATCTTCCATT	TTCGGTAATCATCAGTGCGAGT
VIT_10s0003g01260	GGGTTCTTGGAGAGTTGCTTGT	CAAGTTTGACCTCCCGATGAG
VIT_13s0019g01410	AATTCCCGATTGGTAGCTGTTC	AACTCGGTGGCTCAGTACCTTC
VIT_14s0108g00980	ATGCGTTTCCTCCTCAGATACC	TAAGCAGCTGCAGGGTAGAATG
VIT_15s0046g00150	AGAAGCCAAGGACATGGAAGAG	TTGCAGAAGTACCTTGGCTGAG
VIT_16s0098g01420	TGCTCAAGAGTACTGGGATTGC	CTCCCATACCCACTTTGAAACC
VIT_17s0000g04850	GCAAAAGATGTCCGATGAGTTG	AACGTTTCCAGGAAGATCGAAG
VIT_17s0000g06310	AAAGCTGCCAGAGGTATTGGAC	CAATTCGAACAGTTTGGAGTGC
VIT_17s0000g08290	CTTCTTCACCTTCCGGAGACAT	TTGCAGAAGTACCTTGGCTGAG
VIT_18s0001g11310	TGGTACTCGCAAGAACTCCAAG	CAGACACATCCCCATTCAGACT
VIT_18s0001g15730	CTTGAAGGCCACACACAGTTG	TCACTGGCTGCCATGTAAAGTT
VIT_00s0463g00010	ATCCTGATTCAAAGCGAAGGAG	GGATTGCCTTTCACCACTTTCT
VIT_01s0010g03930	CTTTTCAAACCAGGAGCCAAGT	ATAGGCGTTGAACCTGCTTCTT
VIT_01s0011g00220	TAAGCCTGCTGATGATGGCTAC	TTGTTGGGTAGTGGTGCTTGAT
VIT_01s0011g00720	GCTATTACCGGTGCACAAACAG	AACTTCACGGTGGAACGACATA
VIT_01s0026g01730	AGAAAGCAGGTGCAAAGATGTG	GCAGTAGAGGAGGTAGCGAAGG
VIT_02s0025g00420	GTAACAGCCGAGGGTCTGTCTT	GGCTGTGTTCAGCTGTGTAGGT
VIT_02s0025g01280	TCCGATTCAAACTGCTTCTCAC	GGAAACTGGGATCAAAGTCCAC
VIT_02s0154g00210	AAAGGATCTCCACATCCAAGGT	GATGATCCAAGATGCAACAAGC
VIT_04s0008g01470	GAAACAGTGAGAGTGGGCAGAA	CCGTACTTCCTCCATTTGAACC
VIT_04s0008g05750	GAAGGAGCACACAACCATGAAC	TCCCGGAAAGAGTTAGATCGAG
VIT_04s0008g05760	ACCCTTCTCCCAGAGCCTACTT	AGCTCGGTTGAAGCCTAATGAC
VIT_04s0008g06600	AGGAGGAAATTGGTGATGTGGT	GGGAGCTGAACATGCTAAACCT
VIT_04s0023g00470	TCAGCTCTTGGATCCACACTTC	GAGCGCACTTACAGCAGATGTT
VIT_04s0069g00920	GCAGTGTTTCTAACGGAAAGCA	TTCTTCACCCGAGATCTCCTTC
VIT_04s0069g00970	TGGAGACTGCCAAGTGAAGAAG	TGGGAGGAAGCTTGTAAAGTCC
VIT_04s0069g00980	ATTGAGGACAAGATCGGAGGAG	CCTCTTCTTCACTTGGCATCCT
VIT_05s0077g00730	GGAGGAAGAGCAAGATCAGGAG	AAAGGGCTGTTCTTCACAGCTT
VIT_06s0004g00230	TTGCTGAGAAGCCAAAAGACAC	GAAGCTTCATTCATGGCTGCTA
VIT_06s0004g07500	CCTTCTCCGACAACAGGAACTT	TGTCCTCTCAAGGCTTGTTCAG
VIT_07s0005g01520	ATCAACCACAACAACCCATCAC	GCTGCTCATCAAATACGACACC
VIT_07s0005g01710	TGAAGAAACGAGTGGAGAGGTG	CATCGTTGAAAGAGGGATGTTG
VIT_07s0005g02570	TCTTCCACCTCGTTTCCTTCTC	GTGTTGCCATAACTGCTCCTTG
VIT_07s0031g00080	CCGGCAATAAGCATGAAAAT	ATCAGCATCTTTGGGTCGTC

Table 1 (continued)

Gene ID	Forward primer sequence	Reverse primer sequence
VIT_07s0031g01710	CAGTGAAGAACAGCCCCAAT	CATCAAAGGAACCTGGTCGT
VIT_07s0031g01840	TGCCAACAATCCCTAAAAGC	TGCTCAAGCACTCATTCACC
VIT_07s0141g00680	GTTTGGGAAACCCATCATTG	CTATTGCTCCGCCGATACAT
VIT_08s0007g00570	TCCCTTCACCATCCAAGAAG	ATCTTCCAGCAACCCTTGTG
VIT_08s0040g03070	ATGGCCAGAAGGTTGTGAAG	TTCAGAGGTTGCTGCATTTG
VIT_08s0058g00690	CCTTTTCCAGTCCCAAACAA	TTTGGATTGAAGCGATTTCC
VIT_08s0058g01390	GGTTATGCCTGGCGAAAATA	TGACTTCAGCATGCTTTTGC
VIT_09s0018g00240	GAACGCAGAAAACAGGAAGC	AGTTCCCAACGACTCCATTG
VIT_10s0003g01600	GCAATTTTCTCAAGCCAAGC	CGATTCATCCTCTTCCCTCA
VIT_10s0003g02810	CAGAAGTGCACGGTGAAGAA	CTCCCATCTGTCCTGGTGTT
VIT_10s0003g05740	GGAGATGGGAAACGTTGAGA	GCTGTTGTTCCCTGTCCAAT
VIT_10s0116g01200	CAATTGCAAGTGGAGCTTGA	TTGGGCCTAGATCGATGAAC
VIT_11s0037g00150	GGGATCTCAGGTGATGGCTA	AGGCATGTCATGGTCGTGTA
VIT_11s0052g00450	AGTCAAGCCCATTTCATTCG	GAGGTGCGAACAGAGGAGTC
VIT_12s0028g00270	GGCTTCCCATTCTTTTGTGA	CTGTACCCTTTCCCGAATCA
VIT_12s0055g00340	AGCGATGGCTTCAACAACTT	CTAATTGGGCGGATGAGTGT
VIT_12s0057g00550	CCAACCTGCTATTGCAGTCA	CGGTTCACCACTTGGCTTAT
VIT_12s0059g00880	CCAAGCCACCCACTACAGAT	GCTTGAAGAACCACCAGCTC
VIT_13s0067g03130	AAGCGCTCCAAGCACAGTAT	CCAATTCTAGGCGCTGCTAC
VIT_13s0067g03140	TGTCAGCTGAGGATCTGGTG	TTTTGCCCATATTTCCTCCA
VIT_14s0068g01770	TGGAGGCTTTGGTAGGTCTG	TGCCTTTTGACCGTACTTCC
VIT_14s0081g00560	TCCCACGATGTCATCCACTA	GCTCCCATCTTCTCCCCTAC
VIT_14s0108g00120	TGCCATTGATGAAGAATCCA	TTGGACGCAAACTCTGTCAC
VIT_14s0108g01280	CACCGATGCAACTACACACC	CTCCCTTTGACCTGCTTCTG
VIT_15s0021g01310	GAGGAGAACCAGGTGCTGAG	CATCGTTGGCATTACCAGTG
VIT_15s0046g01140	GGCCACATCTGAATCCAACT	TCCAGCGGGAAACTGTAGTC
VIT_15s0046g02150	CAGCGACCTTTACCCAAGAG	TTCCTCCAGCGATATCCATC
VIT_15s0046g02190	CCAAATTCAGCAGCAACAGA	AACCCCTCAGCTGGTATGTG
VIT_16s0050g01480	CAAGACAACCCCGAGGAGTA	CGTCTTCAAACATTGGCTCA
VIT_16s0050g02510	TGGGAAGTTGGGAACAAAAG	CGCTACACCTCAGCAATGAA
VIT_17s0000g01280	ATCAAAATCACCCGCAAATC	TGCTTTCTGCCCATATTTCC
VIT_17s0000g05810	TATCCTCCCCCAAGATTTCC	AGGATCTGGTAGGGGCTGTT
VIT_18s0001g10030	TCTGCTCCCAACTCTTTCGT	AAAAGATGATCCGCACTTGG
VIT_19s0015g01870	CATTCAAGCCTGTTGCAGAA	TGCCTGAACATGGAATGTGT
VIT_19s0090g00840	CATCACAACCATCCCCTACC	GCCTCTGGTGCTGTAAGAGG
VIT_19s0090g01720	TAATAGCCATGACGCAGCAG	TTAGTGCGGGTTTGGGTTAG

Sequence characteristics and genomic locations of *DAM*, *WRKY*, and *Dof* gene family members

First, the inferred protein sequences and coding sequences (CDS) of all three genes were characterized (Fig. 1). The average DAM protein sequences were shorter than those of WRKY and Dof protein sequences (Fig. 1a). The average Dof protein sequence in apple was longer than those in the others, while the average WRKY protein sequence in strawberry was longer than those in the other species (Fig. 1c, e). The average *DAM* gene CDS was significantly longer than those of *WRKY* and *Dof* genes (Fig. 1b). Most *Dof* genes contained two CDS regions in each species, while most *WRKY* genes contained three CDS regions in each species. Moreover, *Dof* genes in *Ananas comosus* and *WRKY* genes in *Arabidopsis thaliana* had a high level of agreement with each other, while *Dof* genes in *Musa acuminate* and *WRKY* genes in *Fragaria vesca* differed in the number of CDS regions (Fig. 1d, f).

The physical location of grapevine *DAM*, *WRKY*, and *Dof* gene family members were assigned to chromosomes 1–19, except for one *DAM* (VIT_00s0313g00070), two *WRKY* (VIT_00s0463g00010 and VIT_01s0011g00220), and three *Dof* (VIT_00s0218g00040, VIT_00s0253g00060, and VIT_00s0652g00010) genes, which were assigned to unmapped chromosomes (Fig. 2,

Table 2 Number of *DAM*, *Dof*, and *WRKY* in each species

	Actinidia chinensis	*Ananas comosus*	*Arabidopsis thaliana*	*Carica papaya*	*Fragaria vesca*	*Musa acuminata*	*Oryza sativa*	*Solanum lycopersicum*	*Citrus sinensis*	*Malus domestica*	*Populus trichocarpa*	*Prunus persica*	*Pyrus bretschneideri*	*Vitis vinifera*	Total
DAM	2	3	2	2	6	3	3	2	2	6	9	5	3	3	51
Dof	25	26	36	20	23	73	30	32	24	59	45	25	46	25	489
WRKY	106	56	72	49	58	152	101	81	53	140	105	61	112	59	1205

Tables S1–S3). No *WRKY* gene family member was assigned to grapevine chromosome 3, while no *Dof* gene family member was assigned to chromosomes 4, 5, 11, 12, or 19. The *WRKY* and *Dof* gene family members in the other plants shared a genomic distribution pattern similar to that observed in grapevine (Tables S2–S3).

Protein sequence alignment, phylogenetic tree, and domain analysis of *DAM*, *WRKY*, and *Dof* gene family members

All the protein sequences of *DAM* genes were aligned using MEGA, and woody species, such as *Vitis vinifera*, *Malus domestica*, and *Prunus persica* were separated from herbaceous species (e.g., *Arabidopsis thaliana*, *Oryza sativa*, and *Fragaria vesca* (Fig. 3a). Based on the large size of the *Dof* and *WRKY* families, Dof protein sequences from six plants and WRKY protein sequences from three plants were also aligned using MEGA, respectively. The NJ phylogenetic trees showed that Dof and WRKY protein sequences from woody plants were respectively separated from those from herbaceous plant species (Fig. 3b, c).

SMART analysis indicated that all the DAM members contained one MADS domain and one *K*-box domain, except Achn340131, LOC_Os06g11330.1, and MDP0000527190 (Fig. S1a). Achn340131 had two *K*-box domains and one MADS domain located between the two *K*-box domains. LOC_Os06g11330.1 and MDP0000527190 contained only one MADS domains. Fourteen domain models represented 489 *Dof* genes from 14 species (Fig. S1b). Four hundred and eighty-seven *Dof* members contained one zf-Dof domain, while two *Dof* genes contained two zf-Dof domains (Achn386081 and Achn321981). Fifteen other domains were also found in inferred Dof protein sequences. Some domains were annotated to have transcription functions (e.g., EF1_GNE and E2F_TDF), while others were annotated to be related to nucleic acid binding, DNA binding, and protein binding function (e.g., 35EXOc, AT_hook, and PHD) or signaling function (e.g., Efh and Jas). Only 9.5% of WRKY family members contained two WRKY domains, although most of them contained a single WRKY domain (Fig. S1c). Moreover, more than 30 other domains were also found in inferred WRKY protein sequences. WRKY proteins have been found to have extensive functions in plant development and stress responses. In this study, some domains were annotated to be involved in stress responses (e.g., LRR and LRR_CC), while others were annotated to have multiple binding functions (e.g., RRM, TIR, WD40, ZnF_BED, and PUA) or to be associated with proteins of unknown function (e.g., DUF1664, DUF4413, DUF863, and DUF2985).

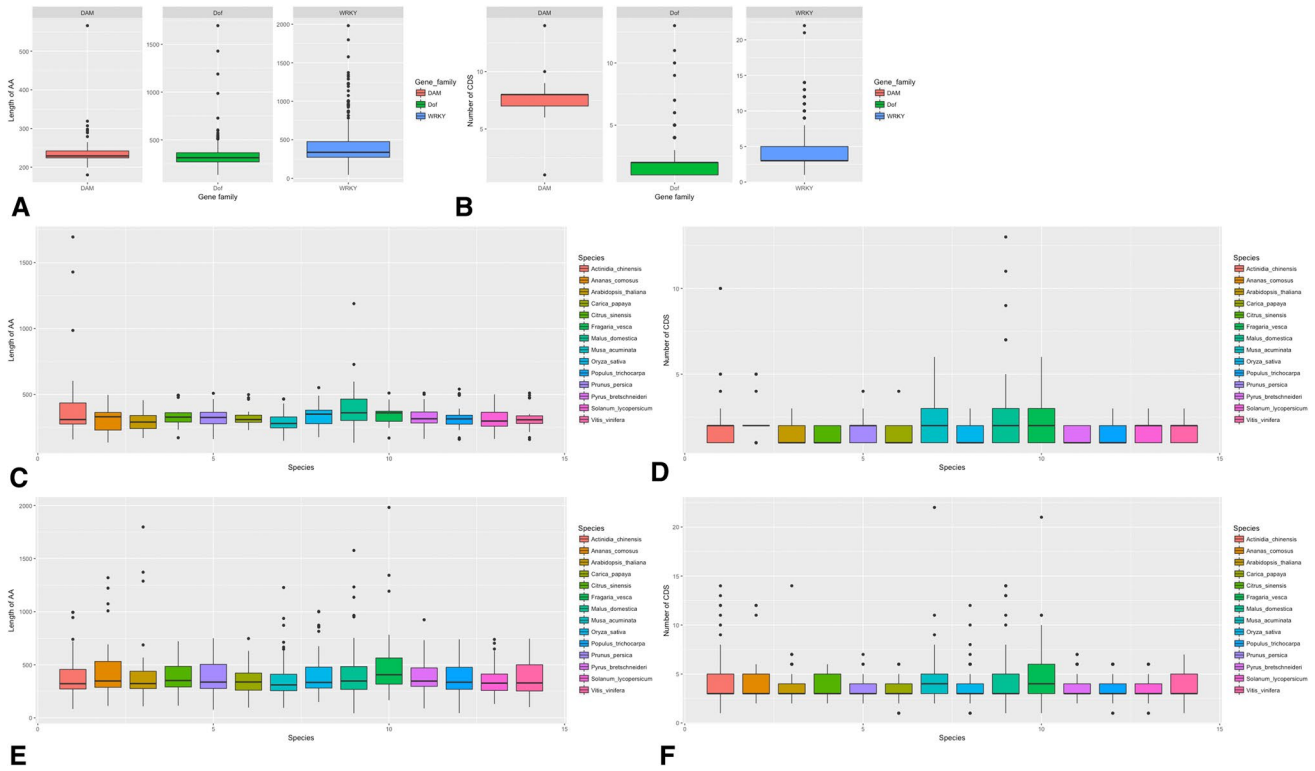

Fig. 1 The overview of length and CDS quantity of *DAM*, *Dof*, and *WRKY* in selected genomes. **a** The length of *DAM*, *Dof*, and *WRKY* in selected genomes. **b** The CDS quantity of *DAM*, *Dof*, and *WRKY* in selected genomes. **c** The boxplot of length of *Dof* in selected genomes. **d** The boxplot of CDS quantity of *Dof* in selected genomes. **e** The boxplot of length of *WRKY* in selected genomes. **f** The boxplot of CDS quantity of *WRKY* in selected genomes

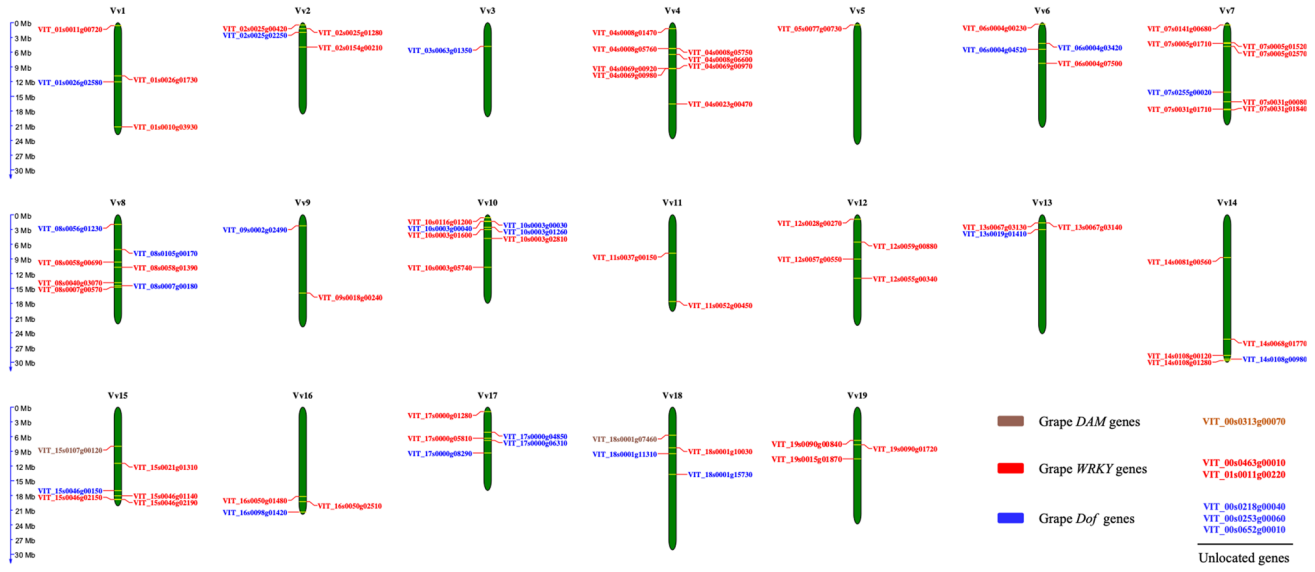

Fig. 2 The distribution of grapevine *DAMs*, *Dofs*, and *WRKYs* on chromosomes

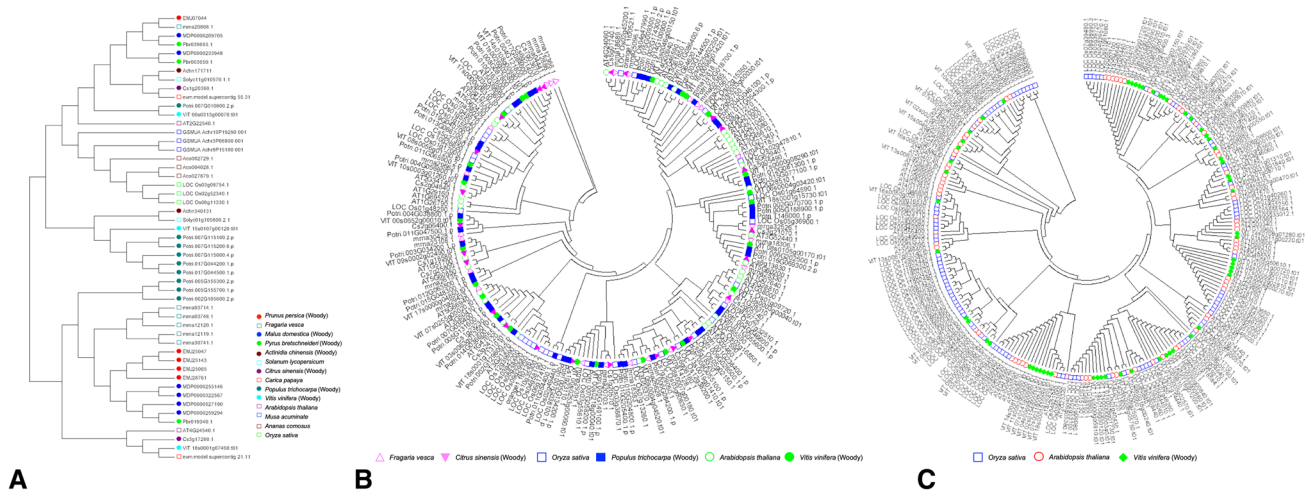

Fig. 3 Phylogenetic tree of *DAMs* (**a**), *Dofs* (**b**), and *WRKYs* (**c**) in selected genomes. The species were represented by different icons

Duplication events and divergence rates of *DAM*, *WRKY*, and *Dof* gene families

Owing to the inadequate annotations for pear, apple, peach, and papaya, these species were excluded from duplication event and divergence rate analyses, leaving the ten remaining species. Five duplicate gene pairs were found within the *DAM* gene family using MCScanX. Among these, one strawberry gene pair (mrna12119-mrna12120) and two *Populus* gene pairs (Potri.007G115000-Potri.007G115100, Potri.007G115100-Potri.007G115200) were identified as tandem repeats, while another two *DAM* gene pairs (LOC_Os06g11330-LOC_Os02g52340, Potri.017G044200-Potri.007G115000) were identified as segmental duplicate gene pairs (Table 3). The dates of three *Populus DAM* duplication events were projected to have occurred between 4.89 and 23.71 million years ago (MYA). One gene pair was under positive selection (Potri.007G115000-Potri.007G115100), while the others were found to be under purifying selection. Notably, the date of the rice DAM duplication event was predicted to have occurred 109.57 MYA, which was much earlier than that in poplar.

We also performed a synteny analysis with sequences from the remaining ten species (including grapevine), for which fourteen gene pairs were isolated (Table 4). Three of these gene pairs belonged to rice, one pair belonged to *Populus*, six pairs belonged to tomato, and four pairs belonged to grapevine. Notably, LOC_Os06g11330 and LOC_Os02g52340 were inferred to be segmental duplicate gene pairs in rice, which were inferred to be orthologs of a pineapple gene (Aco002729) and a banana gene (GSMUA_Achr3P06800_001), respectively. One tomato *DAM* gene (Solyc01g105800) was orthologous to two *Populus* genes (Potri.007G115200 and Potri.017G044200), while one tomato *DAM* gene (Solyc11g010570) was orthologous to one gene each in kiwifruit (Achn171711), *Arabidopsis* (AT2G22540), sweet orange (Cs1g20360), and *Populus* (Potri.007G010800), respectively. One grapevine *DAM* gene (VIT_18s0001g07460) was also orthologous to four *DAM* genes in four species. The K_a/K_s ratios of these fourteen duplicate gene pair were significantly lower than those among three *Populus* duplicate gene pairs (0.4927–1.1059). All the duplicate gene pairs, except tandem gene pairs, were also visualized using Circos software (Fig. 4a).

Table 3 K_a/K_s analysis and estimation of the absolute dates of the duplication events between the duplicated rice, *populus*, strawberry *DAM* homologues

Duplicated pair	Duplicate type	K_a	K_s	K_a/K_s	Purifying selection	Date (million years)
LOC_Os06g11330-LOC_Os02g52340	Segmental	0.0956	1.4244	0.0671	Yes	109.5715
mrna12119-mrna12120	Tandem	0.2459	0.7261	0.3387	Yes	NA
Potri.007G115000-Potri.007G115100	Tandem	0.1817	0.1643	1.1059	No	9.0266
Potri.007G115100-Potri.007G115200	Tandem	0.0724	0.0890	0.8128	Yes	4.8921
Potri.017G044200-Potri.007G115000	Segmental	0.2126	0.4314	0.4927	Yes	23.7055

Table 4 K_a/K_s analysis of duplicated *DAM* homologues in selected genomes

Duplicate pair	K_a	K_s	K_a/K_s	Purifying selection
LOC_Os02g52340-GSMUA_Achr3P06800_001	0.2525	1.5375	0.1642	Yes
LOC_Os03g08754-Aco004028	0.2825	2.0331	0.1390	Yes
LOC_Os06g11330-Aco002729	0.1990	0.7448	0.2672	Yes
Potri.002G105600-mrna12119	0.3443	1.4492	0.2376	Yes
Solyc01g105800-Potri.007G115200	0.3771	2.4953	0.1511	Yes
Solyc01g105800-Potri.017G044200	0.3659	1.4135	0.2589	Yes
Solyc11g010570-Achn171711	0.1392	3.7684	0.0369	Yes
Solyc11g010570-AT2G22540	0.2080	3.7418	0.0556	Yes
Solyc11g010570-Cs1g20360	0.1549	3.7352	0.0415	Yes
Solyc11g010570-Potri.007G010800	0.1355	1.5937	0.0850	Yes
VIT_18s0001g07460-AT4G24540	0.2373	3.6573	0.0649	Yes
VIT_18s0001g07460-Cs3g17260	0.1468	1.5855	0.0926	Yes
VIT_18s0001g07460-mrna12120	0.2717	1.1965	0.2271	Yes
VIT_18s0001g07460-Potri.002G105600	0.1548	1.9183	0.0807	Yes

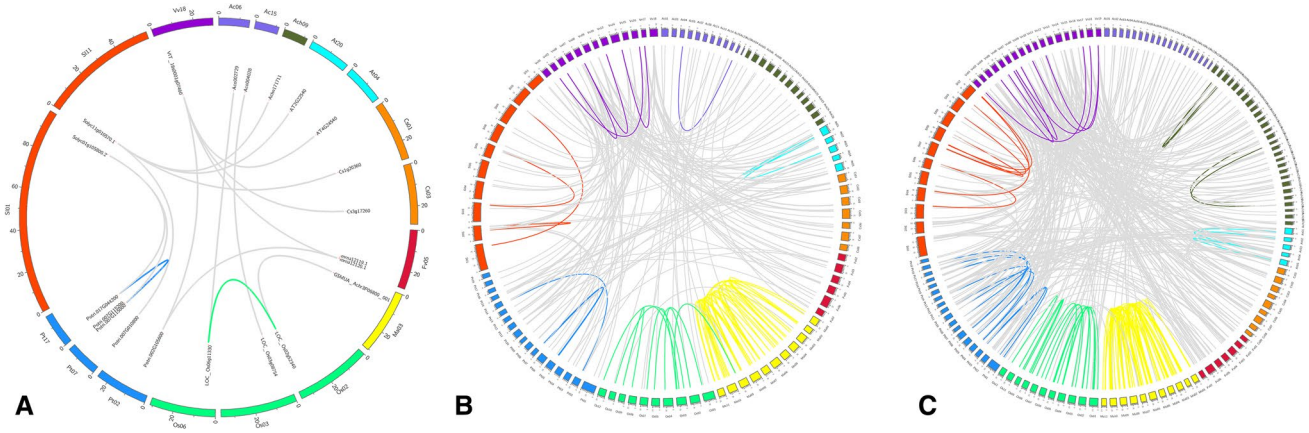

Fig. 4 Synteny analysis of *DAMs* (**a**), *Dofs* (**b**), and *WRKY* (*c*) among selected species

Fifty duplicate *Dof* gene pairs and 114 duplicate *WRKY* gene pairs were identified in each species, and all of them were under purifying selection. Moreover, 96% of *Dof* gene pairs and 89.47% *WRKY* gene pairs were inferred to have arisen through segmental duplication. Additionally, 72% and 68.42% of gene pairs belonged to banana and rice, respectively (Tables S4–S5).

The *Populus WRKY* duplication events were inferred to have occurred first, between 8.9075 and 102.8418 MYA (24.6086 MYA mean), followed by the duplication of *Arabidopsis WRKY* genes (6.7098–73.3367 MYA, 36.9943 MYA mean), tomato *WRKY* genes (3.0886–283.8233 MYA, 99.6510 MYA mean), grapevine *WRKY* genes (58.9438–376.3792 MYA, 173.1244 MYA mean), and rice *WRKY* genes (6.9985–297.8946 MYA, 185.9315 MYA mean). For *Dof* duplicate gene pairs, mean inferred duplication events within each species occurred in the following order: tomato (136.9214 MYA), grapevine (129.7212 MYA), rice (125.7269 MYA), *Arabidopsis* (29.9528 MYA), and *Populus* (14.7926 MYA).

One hundred and fifty-eight duplicate *Dof* gene pairs and 377 duplicate *WRKY* gene pairs were identified, with more than 70% of gene pairs having grapevine *WRKY* or *Dof* members (Tables S6–S7). All the duplicate gene pairs, except tandem gene pairs, were also visualized using Circos software (Fig. 4b, c).

The expression patterns of grapevine *DAM*, *WRKY*, and *Dof* gene family members during bud dormancy

The expression profiles of grapevine *DAM*, *WRKY*, and *Dof* gene family members were obtained from our unpublished RNA-seq data (Fig. 5 and Table S8) and RT-qPCR results (Fig. 6). Here, we also marked the differentially

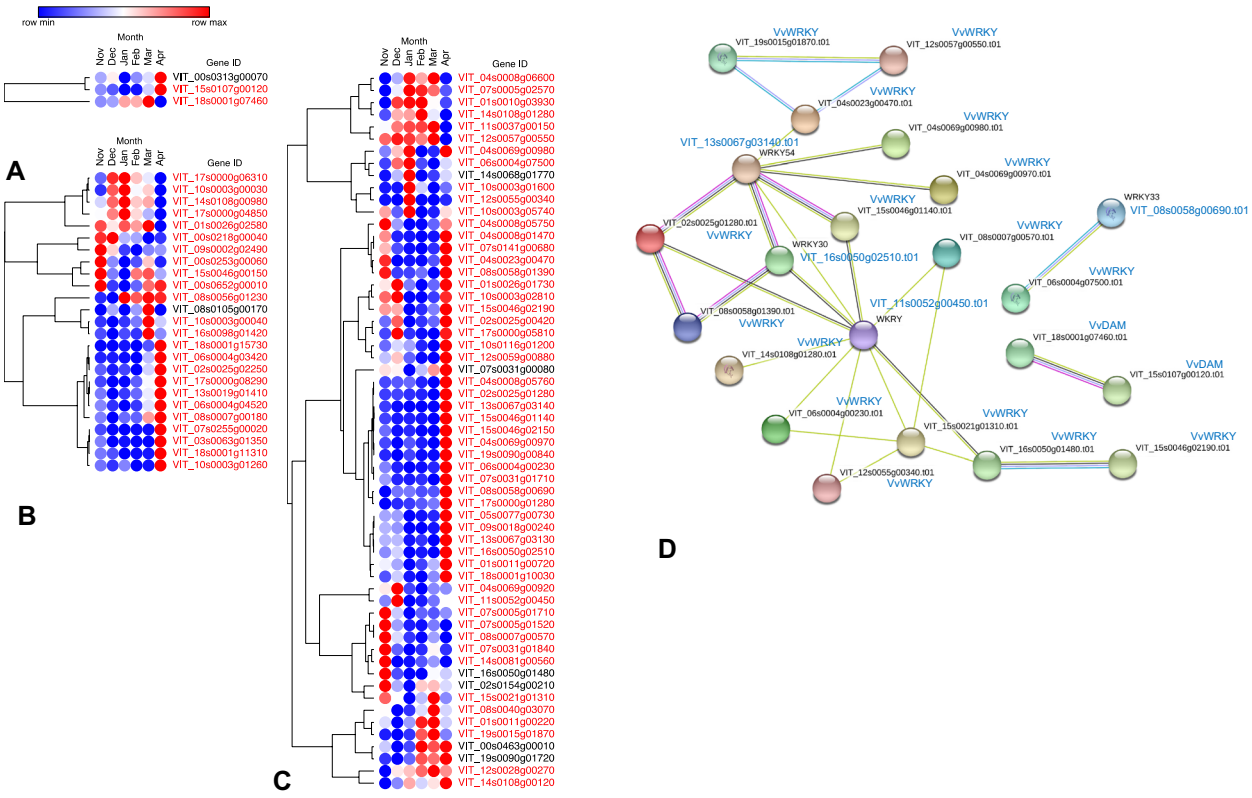

Fig. 5 Heatmaps and PPI analysis of *VvDAM*s, *VvDof*s, and *VvWRKY*s during grapevine bud dormancy. The expression value was represented by the colors, and the significantly expressed genes were marked as red font. **a** *VvDAMs* expression during grapevine bud dormancy. **b** *VvDofs* expression during grapevine bud dormancy. **c** *VvWRKYs* expression during grapevine bud dormancy. **d** Protein–protein interaction of VvDAMs, VvDofs, and VvWRKYs

expressed genes (DEGs) in red according their expression levels. Two *VvDAM* genes (VIT_15s0107g00120 and VIT_18s0001g07460) were significantly expressed during bud dormancy, and three *VvDAM* genes had their highest expression levels in April or March. Twenty-four out of 25 *VvDof* genes (except VIT_08S0105g00170) were significantly expressed during bud dormancy. These 24 *VvDof* genes were classified into 2 subgroups according to the highest expression level of each gene. Ten *VvDof* genes (VIT_17s0000g06310, VIT_10s0003g00030, VIT_14s0108g00980, VIT_17s0000g04850, VIT_01s0026g02580, VIT_00s0218g00040, VIT_09s0002g02490, VIT_00s0253g00060, VIT_15s0046g00150, VIT_00s0652g00010, and VIT_08s0056g01230) had their highest expression levels in November, December, or January. The remaining 14 *VvDof* genes had their highest expression levels in March or April, with relatively lower expression levels in November to January. Forty-nine out of 55 *VvWRKY* genes were significantly expressed during bud dormancy, and over half of them (33 *VvWRKY* genes) had their highest expression levels in April or March. The remaining *VvWRKY* genes had their highest expression levels in November or January. In total, nearly 90% of *VvDof* genes and *VvWRKY* genes were significantly differentially expressed during bud dormancy. Furthermore, we also performed a protein–protein interaction (PPI) analysis of the proteins encoded by differentially expressed *VvDAM*, *VvDof*, and *VvWRKY* genes in bud dormancy using the STRING database, inferring that 20 VvWRKY, 2 VvDAM, and 0 VvDof proteins interact. VvWRKY54 (VIT_13s0067g03140), VvWRKY30 (VIT_16s0050g02510), and VvWRKY (VIT_11s0052g00450) were also detected as part of the network core in the PPI analysis (Fig. 5d).

The expression patterns of *DAM*, *WRKY*, and *Dof* gene family members in *Arabidopsis* seed and potato tuber dormancy release

Three *AtDof* gene transcripts and one *AtWRKY* gene transcript were identified by Cadman et al. (2006) to be significantly expressed during *Arabidopsis* seed dormancy. In that study, among them, one *AtDof* gene (AT5G39660) had expression levels at least twofold higher in all five dormant states compared to both after-ripened states, while two *AtDof* genes (AT5G60200 and AT5G62940) and one *AtWRKY*

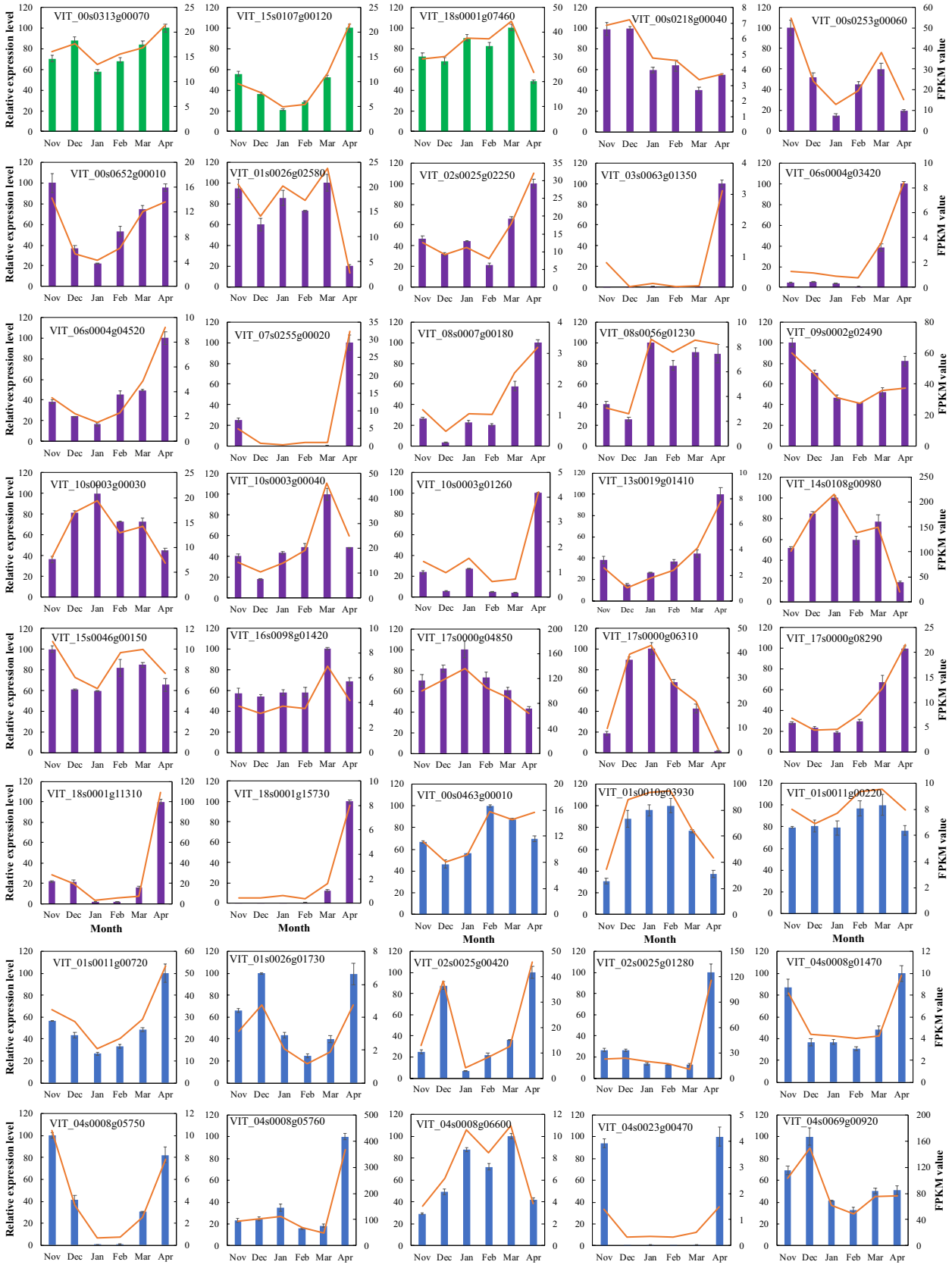

Fig. 6 RT-qPCR results of *VvDAMs* (green), *VvDofs* (purple), and *VvWRKYs* (blue) in grapevine bud dormancy

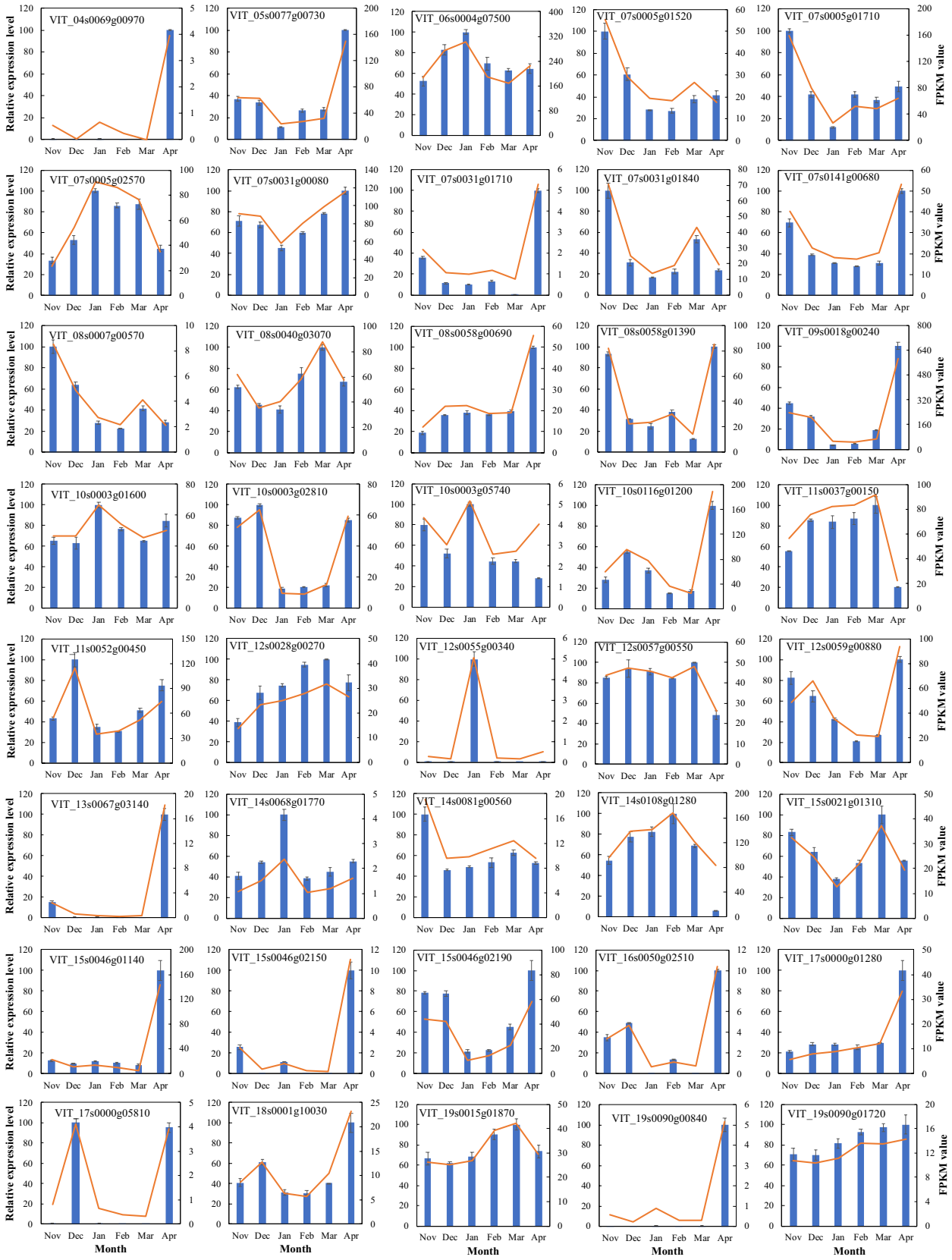

Fig. 6 (continued)

gene (AT1G13960) had showed higher expression levels that were at least twofold higher in both after-ripened states compared to all five dormant states (Table S9). Previous research revealed that 7 *StDof*, 35 *StWRKY*, and zero *StDAM* genes were identified as differentially expressed throughout potato tuber dormancy release (Liu et al. 2015). Notably, most *StWRKY* genes were down-regulated, whereas most *StDof* genes were up-regulated during potato tuber dormancy release (Table S9).

The expression patterns of grapevine *DAM*, *WRKY*, and *Dof* genes throughout fruit development

Fruit development is one of the most important metabolic processes in plants. One *VvDAM*, 16 *VvDof*, and 25 *VvWRKY* genes were identified as differentially expressed during fruit developmental stages (Fig. 7, Table S10). Remarkably, the number of differentially expressed genes (DEGs) across fruit development (1 *DAM*, 16 *Dof*, and 25 *WRKY* genes) was much lower than those across bud dormancy (2 *DAM*, 24 *Dof*, and 53 *WRKY* genes). Based on the expression profile data, all *VvDAM* DEGs, 62.5% of *VvDof* DEGs, and 68% of *VvWRKY* DEGs were highly expressed during the green stage, with expression thereafter reduced throughout the fruit maturation process.

The expression patterns of grapevine *DAM*, *WRKY*, and *Dof* genes under abiotic stress

To determine stress-responsive *VvDAM*, *VvDof*, and *VvWRKY* genes, the RNA-seq data from grapevine plants under drought, salt, waterlogging, and copper stresses were also used in this study. Under salt stress, two *VvDAM*, four *VvDof*, and nine *VvWRKY* genes were identified as differentially expressed. Except for three *VvDof* DEGs, the expression most of these DEGs were inhibited under salt stress. Two *VvDAM*, five *VvDof*, and eleven *VvWRKY* genes were identified as DEGs under copper stress, with most of these

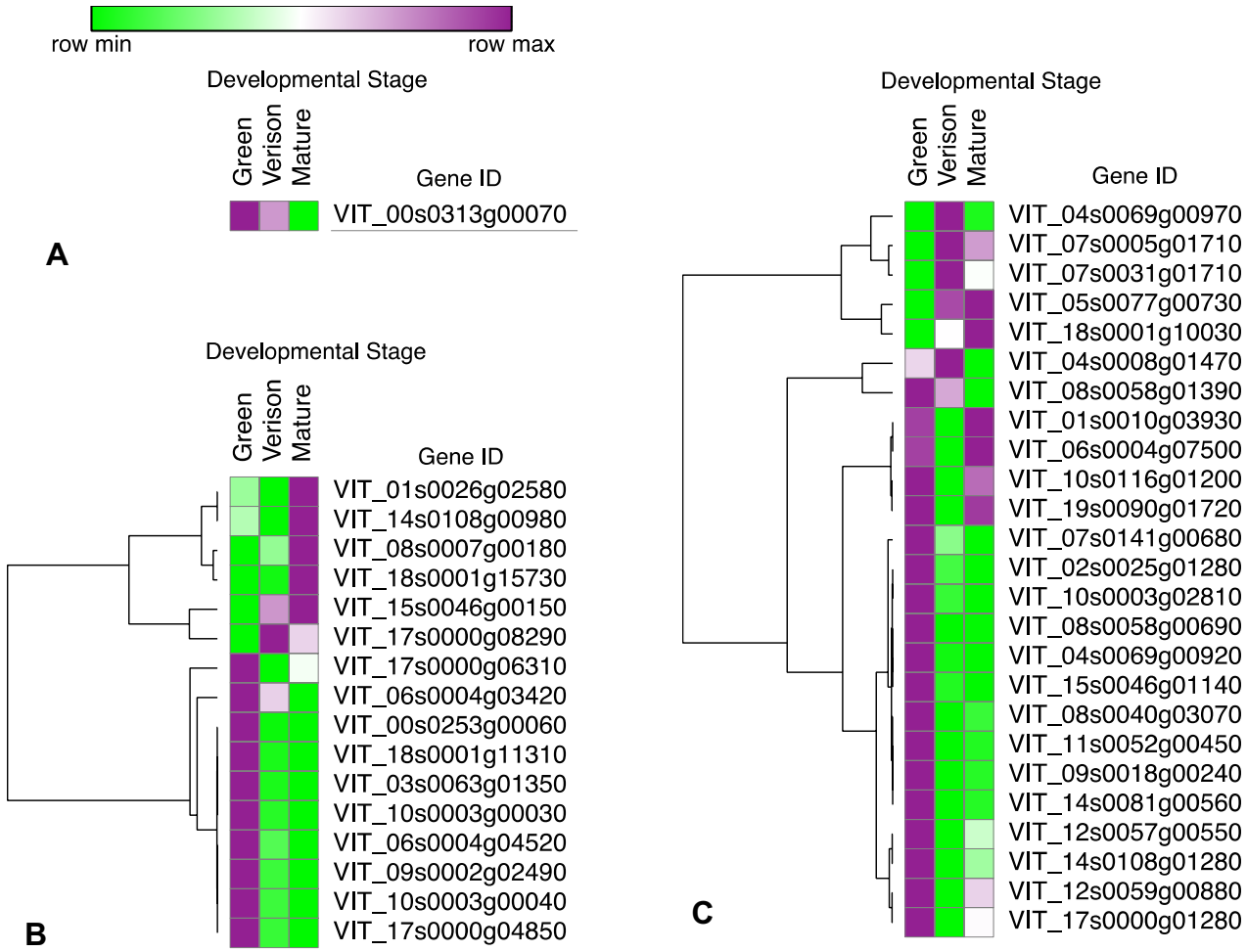

Fig. 7 Heatmaps of significantly differential expressed *VvDAM*s (**a**), *VvDofs* (**b**), and *VvWRKYs* (**c**) expression during grapevine fruit development. The expression value was represented by the colors

DEGs induced by copper stress. Four *VvDof* and twenty-three *VvWRKY* genes were identified as drought stress DEGs, with most of these DEGs repressed under drought stress. Two *VvDAM*, five *VvDof*, and twenty-three *VvWRKY* genes were identified as DEGs under waterlogging stress, and most of them were repressed under waterlogging stress. Some of the identified DEGs responded to multiple stresses. For example, one *VvDAM* (VIT_00s0313g00070) responded to copper, salt, and waterlogging stresses. One *VvDof* gene (VIT_18s0001g11310) responded to waterlogging and drought stresses, and two *VvDof* genes (VIT_02s0025g02250 and VIT_06s0004g03420) responded to salt, drought, and copper stresses. One *VvWRKY* gene (VIT_07s0031g01710) responded to salt, copper, drought, and waterlogging stresses, and six *VvWRKY* genes (VIT_01s0010g03930, VIT_07s0031g00080, VIT_10s0003g01600, VIT_10s0116g01200, VIT_11s0052g00450, and VIT_14s0108g01280) responded to at least three kinds of abiotic stress (Table S11, Fig. S2–S5).

Discussion

DAM, *Dof*, and *WRKY* gene family members were broadly distributed across the investigated plants species: *Arabidopsis*, rice, *Populus*, grapevine, and peach. Increasing evidence has indicated that these genes play an important role in the regulation of bud dormancy. However, these three gene families in woody plants and herbaceous plants had not been compared previously. In this study, seven woody plants and seven herbaceous plants were used as experimental materials to analyze the distribution of gene family members, sequence structure, and evolution of the *DAM*, *Dof*, and *WRKY* gene families.

The identification and evolution of *DAM*, *Dof*, and *WRKY* gene families in selected genomes

The abundance of duplicate genes in plant genomes was inferred to have mostly been caused by ancient duplication events, with a higher retention rate of extant pairs among duplicated genes (Panchy et al. 2016). As Panchy et al. (2016) reported, apple had the highest rate of duplication and retention (84.4%) among 41 sequenced land plant genomes. We also found that apple had the second most copies of *DAM*, *Dof*, and *WRKY* genes among the 14 species investigated (6 *DAM*, 59 *Dof*, and 140 *WRKY* gene family members, Table 2). Moreover, ten other species were also investigated in this work, and they were classified into two groups based on the inferred rounds of whole genome duplication (WGD) events that had produced them according to our results and work by Panchy et al. (2016). Among the ten species, three rounds of WGD events (two duplications and one triplication) took place in *Arabidopsis thaliana*, *Carica papaya, Citrus sinensis*, *Fragaria vesca*, *Prunus persica*, and *Vitis vinifera*, with the number of *DAM*, *Dof*, and *WRKY* gene copies ranging from 2 to 6, 20 to 36, and 49 to 72, respectively. Four or five rounds of WGD events took place in *Solanum lycopersicum* (two duplications and two triplications), *Oryza sativa* (five duplications), *Malus domestica* (three duplications and one triplication), and *Populus trichocarpa* (three duplications and one triplication), with the numbers of *DAM*, *Dof*, and *WRKY* gene copies ranging from 2 to 9, 30 to 59, and 81 to 140, respectively (Table 2). It is also clear that 40% of *DAM*, 96% of *Dof*, and 89.5% of *WRKY* duplicate gene pairs were associated with segmental duplications (Table 3, Table S4, and S5). Most of the *DAM*, *Dof*, and *WRKY* gene copies were derived from WGD or segmental duplication events, which has also been previously reported for soybean *WRKY* (Yin et al. 2013; Yu et al. 2016), peanut *WRKY* (Song et al. 2016), Chinese cabbage *WRKY* (Tang et al. 2014), Chinese white pear *WRKY* (Huang et al. 2015), *Musa WRKY*(Goel et al. 2016), *Populus WRKY* (He et al. 2012; Ma et al. 2015b), Chinese cabbage *Dof* (Ma et al. 2015a), and tomato *Dof* (Cai et al. 2013) gene families.

The average divergence times of *DAM*, *Dof*, and *WRKY* gene families across the species were also notable. The average divergence time of *Populus DAM* genes (12.5414 MYA), *Dof* genes (14.77926 MYA), and *WRKY* genes (24.6086 MYA) was substantially later than those in rice (109.5715 MYA in *DAM*, 125.7269 MYA in *Dof*, and 185.9315 MYA in *WRKY* genes), *Arabidopsis* (29.9528 MYA in *Dof* and 36.9943 MYA in *WRKY* genes), and tomato (136.9214 MYA in *Dof* and 99.6510 in *WRKY* genes). Otherwise, unlike *Populus*, grapevine (a woody plant) has an earlier divergence time among its *Dof* (129.7213 MYA) and *WRKY* (173.1244 MYA) gene families relative to those of *Populus* (Tables S4–S5). This may explain the high number of orthologs between grapevine and other species (Tables S6–S7).

VvDAM, *VvDof*, and *VvWRKY* expression profiles during fruit development and under abiotic stresses

A considerable body of research has indicated that *Dof* and *WRKY* genes are involved in fruit development, including in tomato (Cai et al. 2013; Rohrmann et al. 2011), banana (Feng et al. 2016; Wang et al. 2012a), and grapevine (Fernandez et al. 2007; Haider et al. 2017; Terrier et al. 2005). With the help of high-throughput sequencing data, *VvDAM*, *VvDof*, and *VvWRKY*, the expression profiles during fruit development were obtained. One *VvDAM* DEG, 10 out of 16 *VvDof* DEGs, and 17 out of 25 *VvWRKY* DEGs were highly expressed at the green stage (Fig. 7). Other *VvDof* and *VvWRKY* DEGs exhibited contrasting expression profiles, with higher expression levels at veraison (a key fruit development stage in grapevine indicating the onset of ripening)

or mature stages. This indicates that *VvDAM*, *VvDof*, and *VvWRKY* genes might play a significant role in grapevine fruit development through interactions with other proteins. Here, we also identified three *VvDof* (VIT_00s0652g00010, VIT_06s0004g03420, and VIT_02s0025g02250) genes and one *VvWRKY* gene (VIT_07s0031g01710) that were responsive to multiple stresses (Fig. S2–S5). In plants, various *Dof* and *WRKY* genes were determined to be responsive to abiotic stress. Overexpression of the homologs *SlCDF1* and *SlCDF3* in *Arabidopsis* increased salt and drought stresses tolerance, and various stress-responsive genes were activated in the overexpression lines (Corrales et al. 2014). *AtWRKY6* is involved in responses to low-phosphorus stress through regulating *PHOSPHATE* (*PHO1*) expression (Chen et al. 2009). In rice, 13 *OsWRKY* genes differentially responded to salt, drought, cold, or heat stresses (Qiu et al. 2004). Among 14 wheat *WRKY* genes, eight were responsive to low temperature, high temperature, salt, or drought stresses (Wu et al. 2008). However, overexpression of *VvWRKY30* in *Arabidopsis* increased resistance to salt stress (Zhu et al. 2019). Meanwhile the expression levels of *GmWRKY13*, *GmWRKY21*, and *GmWRKY54* were induced by salt and drought stresses in soybean (Zhou et al. 2008). Moreover, numerous studies have demonstrated that a single transcriptional factor may be responsive to multiple stresses. For example, the expression levels of *AtWRKY25* and *AtWRKY33* both respond to salt, cold, and heat stresses (Li et al. 2011; Jiang and Deyholos 2009). Interestingly, one *VvDAM* (VIT_00s0313g00070) was identified as a significantly expressed gene under multiple abiotic stresses, *e.g.*, copper, salt, and waterlogging stresses (Fig. S2, S3, and S5). Most recent reports on *DAM* gene family members have focused on dormancy (Li et al. 2009; Mimida et al. 2015; Niu et al. 2015). These data have isolated one *VvDAM*, two *VvDof*, and seven *VvWRKY* genes as major genes that respond to abiotic stress, which can function as regulators of several different processes and may also mediate the crosstalk between different signaling pathways.

VvDAM, *VvDof*, and *VvWRKY* expression during bud dormancy

Bud dormancy is an essential biological process for plant survival in cold temperatures. Many reports have revealed the involvement of *DAM*, *Dof*, and *WRKY* genes in metabolic activities. For example, *DAM1* and the ABA metabolism and signaling pathway (*NCED1* and *AREB1*) function through a feedback mechanism to regulate pear bud dormancy (Tuan et al. 2017). In hybrid aspen, the *SVP* ortholog *SVL* acts downstream of ABA in dormancy regulation and promotes dormancy by suppressing the growth-promoting gibberellic acid pathway (Singh et al. 2018). In addition to this, *Dof* and *WRKY* genes were found to be differentially expressed during bud dormancy in peach (Song et al. 2016), oak (Derory et al. 2006), and pear (Liu et al. 2012). The numbers of *VvDAM*, *VvDof*, and *VvWRKY* DEGs were 2, 24, and 49, respectively. In general, over 90% of *VvDof* and *VvWRKY* genes were differentially expressed throughout grapevine bud dormancy. Notably, there were substantially more *VvDAM*, *VvDof*, and *VvWRKY* genes associated with bud dormancy than with fruit development or abiotic stress. This suggests that bud dormancy was shaped by evolution of the *VvDof* and *VvWRKY* gene families, such that the *Dof* and *WRKY* DEGs associated with *Arabidopsis* seed and potato tuber dormancy release processes were much fewer in number. Intriguingly, multiple abiotic stress-responsive *VvDof* and *VvWRKY* genes were also differentially expressed across bud dormancy. For example, two *VvWRKY* genes (VIT_11s0052g00450 and VIT_14s0108g01280) responded to bud dormancy and multiple abiotic stresses, which should accordingly be treated as candidate genes for further study. However, the single multiple abiotic stress-responsive *VvDAM* gene (VIT_00s0313g00070) was not differentially expressed across bud dormancy. One *VvDAM* gene (VIT_18s0001g07460) is a possible candidate gene for grapevine bud dormancy regulation. This suggests that a differential regulation mechanism functions between bud dormancy and other biological processes.

The analysis of bud dormancy expression profiles of tandem duplicated gene*s* revealed high expression divergence between two *VvWRKY* pairs (VIT_07s0005g01710-VIT_05s0077g00730 and VIT_08s0058g00690-VIT_06s0004g07500). The highest expression value of VIT_07s0005g01710 was in November, while that of VIT_05s0077g00730 was in March. The highest expression value of VIT_08s0058g00690 was in March, while that of VIT_06s0004g07500 was in January. This also indicated that the gene family has evolved different functions across environmental stresses and throughout development, with evolutionary divergence occurring among gene families. Theses phenomena have been observed in the *glutathione S-transferase* (*GST*) family in sorghum, rice, and *Arabidopsis* (Chi et al. 2010). This study systematically analyzed *DAM*, *Dof*, and *WRKY* expression in grapevine under different environmental stresses and across different developmental stages, thus providing candidate genes for further functional analysis.

Conclusion

This study has investigated the distribution, sequence characteristics, evolution, and expression profile of grapevine *DAM*, *Dof*, and *WRKY* gene family members, with comparisons to those from other species. These three gene families in each species differed in number, but WGD and segmental duplication events had a predominant effect

on the expansion of *Dof* and *WRKY* gene families. More *DAM*, *Dof*, and *WRKY* genes were associated with bud dormancy than with fruit development or abiotic stresses. One, two, and seven candidate stress-response genes were identified among the *DAM*, *Dof*, and *WRKY* genes investigated. This study provides the foundation for further analyses of grapevine abiotic stress responses.

Methods

Plant materials

Three-year-old *Vitis vinifera* cv. 'Rosario Bianco' ('Rosaki' × 'Muscat of Alexandria') grapevines were grown in a greenhouse at Jiangsu Agricultural Expo Garden, Jiangsu, China (N32°0′41.99″, E119°15′7.11″) with permission from Jiangsu Vocational College of Agriculture and Forestry. The dormancy period began in November and ended by April, when bud break occurred. Grapevine buds were divided into three groups depending on their position along the stem: the bottom group (the third, fourth, and fifth buds from the cordon), the center group (the eighth, ninth, and tenth buds from the cordon), and the top group (the fourteenth, fifteenth, and sixteenth buds from the cordon). At least 30 buds from each group were sampled every month between November and April (of the following year). Samples were frozen in liquid nitrogen, and then stored at − 80 °C for further analysis.

Sequence retrieval

Protein, mRNA, and genome annotation files were downloaded from online databases. *Citrus sinensis* datasets were retrieved from the *Citrus sinensis* annotation project database (V2, https://citrus.hzau.edu.cn/orange/). *Actinidia chinensis* data were retrieved from the Kiwifruit Genome database (V1, https://bioinfo.bti.cornell.edu/kiwi). *Pyrus bretschneideri* data were retrieved from the Pear Genome Project archives (V1, https://peargenome.njau.edu.cn). Multiple datasets for *Arabidopsis thaliana* (TAIR10), *Musa acuminate* (MA1), *Prunus persica* (Prupe1.0), *Vitis vinifera*, and *Solanum lycopersicum* (SL2.50), (IGGP_12×) were downloaded from the Ensembl Plants database (https://plants.ensembl.org/). Multiple datasets for *Ananas comosus* (V3), *Carica papaya* (ASGPBv0.4), *Fragaria vesca* (V1.1), *Malus domestica* (V1.0), *Oryza sativa* (v7.0), and *Populus trichocarpa* (V3.1) were downloaded from JGI archives (https://genome.jgi.doe.gov/portal/).

Identification of *DAM*, *Dof*, and *WRKY* gene family members

To identify *DAM*, *Dof*, and *WRKY* gene family members from the 14 genomes, 2 search tools were employed: Blast and HMMER. (1) Blast searches were performed using the nucleotide sequences of target genes (9 *PpDAM*, 2 *AtDAM*, 2 *SlDAM,* 3 *OsDAM* genes; 36 *AtDof*, 30 *OsDof* genes*;* 72 *AtWRKY*, and 101 *OsWRKY* genes; detailed in Tables S1–S3) against the peptide databases of 14 species. (2) Protein sequences of target *DAM* genes (9 *PpDAM*, 2 *AtDAM*, 2 *SlDAM,* and 3 *OsDAM* genes, detailed in Table S1) were used to build the HMMER file. The Dof pfam file (PF02701) and WRKY pfam file (PF03106) were downloaded from the pfam database (https://pfam.xfam.org). DAM HMMER, Dof pfam, and WRKY pfam files were used to search the peptide databases of 14 species, respectively. (3) The results from the above two methods were combined, and redundant sequences were removed, leaving all the putative *DAM*, *Dof*, and *WRKY* gene family members from the 14 species.

Bioinformatic analysis

The protein sequence lengths and CDS numbers of *DAM*, *Dof*, and *WRKY* gene family members in each species were calculated using an in-house Perl script and visualized using the ggplot2 package in the R statistical computing environment. Protein sequence domain analysis, protein sequence alignment, phylogenetic analysis, gene distribution analysis, and gene structure visualization were conducted using the Simple Modular Architecture Research Tool (https://smart.embl-heidelberg.de/), the MEGA software, the MapGene2Chro online tool (https://mg2c.iask.in/mg2c_v2.0/), and the GSDS online tool (https://gsds.cbi.pku.edu.cn/index.php), respectively. Identical types and pairs were analyzed using MCScanX and visualized using Circos (Krzywinski et al. 2009; Wang et al. 2012b). ParaAT and K_aK_s_Calculator were used to estimate the number of non-synonymous substitutions per non-synonymous site (K_a) and the number of synonymous substitutions per synonymous site (K_s) (Zhang et al. 2006, 2012). The dates of duplication events were estimated using the equation $T = K_s/2\lambda$, where $\lambda = 6.5 \times 10^{-9}$ for grapes and rice (Gaut et al. 1996; Yu et al. 2005), 1.5×10^{-8} for *Arabidopsis*(Blanc and Wolfe 2004), 9.1×10^{-9} for *Populus* (Lynch and Conery 2000) and 6.96×10^{-9} for tomato (Cheng et al. 2009). Gene expression was analyzed using NCBI online datasets (GSE77218, SRP070475, SRP074162, and SRP159132) and published supplemental datasets (Leng et al. 2015). A heatmap of the data was drawn using Morpheus (https://software.broadinstitute.org/morpheus/). Protein–protein interaction (PPI) analysis was performed using the STRING database (https://string-db.org/).

RNA isolation and RT-qPCR

Extraction of total RNA was conducted using the SDS-phenol method (Zhang et al. 2010). The Revert Aid™ First-Strand cDNA Synthesis Kit was used for first-strand cDNA synthesis (Fermentas, Glen Burnie, MD, USA). The cDNAs were diluted in double-distilled water by 30-fold. Then, cDNA templates were pooled with EvaGreen 2 × qPCR MasterMix-ROX (ABM, Richmond, BC, Canada) to perform RT-qPCR (real time-quantitative PCR) using an Applied Biosystems® 7500 Real-Time PCR machine (Applied Biosystems, Foster City, CA, USA). The housekeeping gene *Actin* (AB073011, forward primer GGAAGCTGCGGGAATTCATGAG, reverse primer CCTTGATCTTCATGCTGCTGGG) was used as an internal control to quantify mRNA levels. The primer sequences used in the present study are given in Table 1. The 20-µl PCR volumes contained 500 ng of cDNA, 10 µl of EvaGreen 2 × qPCR MasterMix, 0.6 µl of forward primer, 0.6 µl of reverse primer, and 6.8 µl of nuclease-free H_2O. PCR was performed using the following cycling conditions: 10 min (95 °C), followed by 35 cycles of 15 s at 95 °C and 1 min at 62 °C, with a final cooling to 4 °C.

Authors' contributions Conceived and designed the experiments: LFSG and JGF. Analyzed the data: LFSG, XF, KKZ, and MXC. RT-qPCR: MXC, ZQX, and TZ. Wrote the paper: LFSG, XF, and JGF. Revised the paper: LFSG, JGF, YFP, and MXC.

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Availability of data and material Gene expression was analyzed using NCBI online datasets (GSE77218, SRP070475, SRP074162, and SRP159132) or Leng et al.'s supplemental datasets.

Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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