Plant Mol Biol Rep (2014) 32:1085–1102
DOI 10.1007/s11105-014-0723-3

ORIGINAL PAPER

Evolutionary and Expression Analyses of Basic Zipper Transcription Factors in the Highly Homozygous Model Grape PN40024 (*Vitis vinifera* L.)

Min Gao · Hongjing Zhang · Chunlei Guo · Chenxia Cheng · Rongrong Guo · Linyong Mao · Zhangjun Fei · Xiping Wang

Published online: 10 April 2014

Abstract Basic leucine zipper (bZIP) proteins, which function as transcription factors and play important regulatory roles in all eukaryotic organisms, have been identified and classified in plants based on the sequenced genomes of model species such as *Arabidopsis thaliana* and rice (*Oryza sativa*). However, far less is currently known about the evolutionary relationships and expression patterns of bZIP genes in nonmodel plants. In this study, we performed a genome-wide analysis and identified a total of 47 bZIP transcription factors from grape (*Vitis vinifera* L., cv PN40024). Phylogenetic analysis of grape bZIP transcription factors along with their *Arabidopsis* and rice counterparts indicated that they can be classified into 13 different groups. Furthermore, evolutionary analysis of the grape bZIP transcription factors demonstrated that segmental duplications have contributed substantially to the expansion of this family in grape. In addition, synteny analysis between grape and *Arabidopsis* suggested that some of the bZIP members were present in their most recent common ancestor and that the major expansion occurred before the divergence of the two species. Gene expression analysis of the grape bZIP transcription factor-encoding genes revealed tissue-specific, biotic and abiotic stress and hormone-responsive expression profiles. Taken together, the genome-wide identification and characterization of grape bZIP transcription factors provide insights into their evolutionary history and a resource for further functional characterization in the context of crop improvement and stress tolerance.

Keywords Genome-wide · Evolution · bZIP transcription factor · Phylogenetic analysis · Expression · Grape

Introduction

Grapevine (*Vitis vinifera* L.) is globally the most economically important perennial fruit crop due to its diverse uses, including the production of wine, jam, juice and jelly, grape seed extracts, raisins, vinegar, and grape seed oil. However, grape production is limited by a range of biotic and abiotic stresses that cause significant losses in yield every year, as well as a reduction in berry quality (Ferreira et al. 2004). Consequently, there is great interest in developing grapes with enhanced tolerance to different stresses. Most biological responses in plants to abiotic and biotic stresses are finely controlled at the transcriptional level by a spectrum of transcription factors (TFs). These TFs show sequence-specific DNA-binding capacity and can be classified into families according to the conserved motifs that code for the characteristic DNA-binding domains (DBDs) (Yamasaki et al. 2012).

Electronic supplementary material The online version of this article (doi:10.1007/s11105-014-0723-3) contains supplementary material, which is available to authorized users.

M. Gao · H. Zhang · C. Guo · C. Cheng · R. Guo · X. Wang
State Key Laboratory of Crop Stress Biology in Arid Areas, College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100, China

M. Gao · H. Zhang · C. Guo · C. Cheng · R. Guo · X. Wang (✉)
Key Laboratory of Horticultural Plant Biology and Germplasm Innovation in Northwest China, Ministry of Agriculture, Northwest A&F University, Yangling, Shaanxi 712100, China
e-mail: wangxiping@nwsuaf.edu.cn

L. Mao · Z. Fei
Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 14853, USA

Z. Fei
USDA Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853, USA

Here, we focus on the basic leucine zipper (bZIP) TFs, named after a highly conserved bZIP domain (Hurst 1994), which contains 40 to 80 amino acids, with two functionally distinctive regions: the basic region (BR) and the Leu zipper (LZ) region (Corrêa et al. 2008). The BR is highly conserved, located at the N-terminus of the bZIP domain, consists of about 16 amino acids, and functions as a DNA-binding motif. The LZ region is less conserved than the BR and contains an amphipathic coiled coil domain that confers dimerization specificity (Nijhawan et al. 2008).

The bZIP proteins (bZIPs) are widely distributed in all eukaryotes and constitute one of the largest families of TFs (Amoutzias et al. 2007). In animals, they are involved in development, metabolism, circadian rhythm, learning, memory, and response to stress and radiation, as well as in sensing environmental signals (Deppmann et al. 2006). They also play numerous roles in plants and have been reported to contribute to a variety of developmental processes, such as cell elongation (Fukazawa et al. 2000), organ and tissue differentiation (Silveira et al. 2007), embryonic and floral development (Zou et al. 2008; Guan et al. 2009), seed maturation (Jakoby et al. 2002), plant senescence (Sohn et al. 2006), hormone signaling (Niggeweg et al. 2000; Fujita et al. 2005), and light signaling (Mallappa et al. 2006). Furthermore, there is growing evidence that bZIP TFs play important roles in plant resistance to pathogens and responses to biotic and abiotic stresses (Pontier et al. 2001; Wang et al. 2011a; Xiang et al. 2008). For example, TGA factors, a conserved family of plant bZIP TFs, serve both negative and positive regulatory roles in mediating defense responses (Pontier et al. 2001), and several genes in the sorghum bZIP TF family have been shown to regulate responses to various abiotic stresses (Wang et al. 2011a). In addition, overexpression of the rice *OsbZIP23* gene was found to significantly improve tolerance to drought and high salinity stresses in transgenic rice (Xiang et al. 2008). There is therefore considerable evidence that bZIP domain genes represent one of the key regulatory gene families involved in mediating plant growth and stress responses.

Functional diversification in gene families encoding TFs is emerging as a major source of morphological and physiological diversity in evolution (Carretero-Paulet et al. 2010; Riechmann et al. 2000). Several studies have provided genome-wide identification of bZIP domain genes from plants whose genome sequence is available. For example, the genomes of *Arabidopsis thaliana*, *Oryza sativa*, *Populus trichocarpa* (black cottonwood) and *Sorghum bicolor* are annotated as having 75, 89, 89, and 92 bZIP TFs, respectively (Jakoby et al. 2002; Nijhawan et al. 2008; Corrêa et al. 2008; Wang et al. 2011a). In one analysis, the 75 predicted *Arabidopsis* bZIP-encoding genes were divided into ten groups (Jakoby et al. 2002), while in another phylogenetic study, a total of 257 predicted bZIP TFs, including 76 from *Arabidopsis*, 92 from rice, and 89 from *P. trichocarpa*, were divided into 13 groups (Corrêa et al. 2008).

The release of the grape genome has provided an opportunity to identify protein families at the genome level, to analyze them, and to utilize the potential genes for grape crop improvement (Jaillon et al. 2007). Recently, 132 TF genes in the AP2/ERF family were identified in the grape genome (Zhuang et al. 2009), while in a separate study, Licausi et al. (2010) reported 149 AP2/ERF TF genes containing at least one ERF domain (Licausi et al. 2010). However, the grape bZIP TF family has not been analyzed in detail, and the phylogenetic relationship with other plant bZIP TFs remains poorly understood. In this study, we identified 47 bZIP TF genes from the grape genome sequence and carried out phylogenetic analyses to understand their interrelationships. Furthermore, we identified some of the duplication events that have likely contributed to the expansion of the grape bZIP family. Phylogenetic analysis of grape, *Arabidopsis*, and rice bZIP TFs allowed the identification of both shared and specific subgroups and an estimation of their number in their most recent common ancestor, as well as potential gene birth-and-death events. Moreover, analysis of gene and protein features and predicted location provided evidence for numerous independent intron loss events in the bZIP family. Finally, expression profile analyses during grape development and under different stress inductions revealed that bZIP proteins exhibit a variety of expression patterns, suggesting diverse functions. Through these analyses, we have increased the knowledge concerning the evolution and potential function of grape bZIP TF genes.

Materials and Methods

Identification and Annotation of Grape bZIP Transcription Factor Genes

A direct search of annotated bZIP transcription factor genes in the Grape Genome Database (12 X) (http://www.genoscope.cns.fr) was performed using the Hidden Markov Model (HMM) profile of the bZIP domain (PF00170) downloaded from Pfam (http://pfam.sanger.ac.uk/). We did not expand our study to other datasets beyond the scaffolds of the PN40024 genome. Information about the proteins, genes, and virtual complementary DNA (cDNA) sequences of all the grape bZIP TF genes was obtained from the Grape Genome Database (12 X) (www.cns.fr/externe/GenomeBrowser/Vitis). We then performed manual curation of the predicted gene structures of the identified 47 grape bZIP genes. We first extracted grape EST and messenger RNA (mRNA) sequences from GenBank, as well as Arabidopsis protein sequences (TAIR version 10), which have been under extensive manual curation. We aligned these sequences to the grape bZIP genes and manually checked the alignments. Of the 47 grape bZIP genes, the

predicted gene structures of 33 were accurate, supported by grape EST/mRNA and/or Arabidopsis protein sequences. Of the remaining 14 grape bZIP genes, we were able to correct the gene models of 13 of them, and the corrected gene models were also supported by grape EST/mRNA and/or Arabidopsis protein sequences. The predicted model of the last bZIP gene (GSVIVT01009846001) appeared to miss at least 400 bp at the 5′ end. However, we were not be able to correct this gene model as the corresponding genome region contains a lot of stop codons, thus cannot be translated through. The CDS and protein sequences of the 13 corrected gene models were provided in Supplementary Table 1. To confirm the obtained cDNA sequences, the nucleotide sequences were translated into amino acid sequences, which were then examined for the presence of a bZIP domain using the HMM of the SMART tool (http://smart.embl-heidelberg.de/) (Schultz et al. 1998; Letunic et al. 2012). Then we associated the 47 bZIP transcription factors, identified in the grape genome, to the accession numbers of the last annotation of the grape genome on the CRIBI website (http://genomes.cribi.unipd.it/grape/).

Sequence and Phylogenetic Analysis

The ClustalX (version 2.0) program was used with default parameters to perform multiple sequence alignments (Larkin et al. 2007). Phylogenetic trees were created using the MEGA 5.0 software and the neighbor-joining (NJ) method, and the bootstrap test was replicated 1,000 times (Tamura et al. 2011).

The MEME version 4.9.0 tool (http://meme.nbcr.net/meme/) was used to identify additional conserved motifs outside the bZIP domain shared among grape bZIP proteins. All 47 VvbZIP protein sequences were used as input, and a limit of 20 motifs was specified with all other parameters set to default. These motifs were analyzed manually based on *e*-value cutoff<$e-001$ and those considered significant that were shared by the majority of grape bZIP proteins were placed into the same group, according to their DNA-binding site specificity.

Exon/Intron Structure Analysis of Grape bZIP Transcription Factors

To obtain information about the intron/exon structure of the grape bZIP TFs, the coding sequences were aligned with the corresponding genomic sequences using the est2genome program (http://emboss.bioinformatics.nl) (Rice et al. 2000). The diagram of exon/intron structures was obtained using the online Gene Structure Display Server (GSDS: http://gsds.cbi.pku.edu.ch), which gives both exon position and gene length. Since the introns of several of the analyzed genes were relatively long, only the exons were drawn to scale.

Chromosome Localization and Synteny Analysis

Each of the grape bZIP TFs was mapped onto their corresponding chromosome using the grape genome browser at the Grape Genome Database (12 X). Tandem duplications of grape bZIP TF in the grape genome were predicted by identifying their physical locations on individual chromosomes. Tandem duplicated genes were defined as adjacent homologous bZIP TFs on the same chromosome, with no more than one intervening gene (Zhang et al. 2012).

For synteny analysis, synteny blocks within the grape genome and between grape and *Arabidopsis* genomes were downloaded from the Plant Genome Duplication Database (http://chibba.agtec.uga.edu/duplication) and those containing grape and *Arabidopsis* bZIP genes were identified and analyzed. The diagrams were generated by the program Circos (version 0.63) (http://circos.ca/).

Plant Materials

Grape tissues of young root, stem, leaf, tendril, flower at fully open stage, and fruit at 33 days after anthesis were harvested from an 8-year-old 'Kyoho' (*Vitis labrusca*×*V. vinifera*) grapevine with normal growth during the 2012 growing season. Two-year-old 'Kyoho' juvenile plants were used for high salt, drought stress, and exogenous hormone treatments. A Chinese wild *Vitis quinquangularis* clone 'Shang-24' was used for powdery mildew inoculation. Both grape species are present in the grape germplasm resource orchard of Northwest A&F University, Yangling, China (34° 20′ N, 108° 24′ E).

Abiotic, Hormone, and Biotic Stress Treatments

Two-year-old 'Kyoho' juvenile plants that had previously been planted in pots were used for all abiotic stress and hormone treatments. For high salinity stress, seedlings were irrigated with 2 L 250 mM NaCl solution (Upreti and Murti 2010; Boneh et al. 2012). After treatments for 1, 3, 6, 12, 24, and 48 h, fully unfolded young leaves were collected. For drought stress, a similar stage of seedlings was treated by withholding water for up to 7 days under field environment in June, until the leaves showed wilting (Peng et al. 2013). Young leaves of the seedlings were harvested at 24, 48, 72, 96, 120, 144, and 168 h post-treatment. Subsequently, the stressed plants were rewatered to soil saturation and leaves were collected at 48 h after rewatering (R48). For salt and drought stress, plants watered every 3 days were used as the control.

For hormone treatments, 100 μM salicylic acid (SA), 100 μM abscisic acid (ABA), 50 μM methyl jasmonate (MeJA), or 0.5 g/L ethylene (ET)-releasing ethephon was sprayed on the selected young leaves followed by sampling at 0.5, 1, 3, 6, 12, 24, and 48 h post-treatment as previously described (Li et al. 2010; Wang and Li 2006; Xiao and

Nassuth 2006; Boneh et al. 2012). Leaves sprayed with sterile water were used as the negative control.

Pathogen treatment was carried out by inoculating the young leaves of 'Shang-24' with *Erysiphe necator* (Schw.) Burr (powdery mildew) according to the method previously described (Wang et al. 1995). Leaves sprayed with sterile water were used as negative controls. At each inoculation period, leaves were sampled at 6, 12, 24, 48, 72, 96, and 120 h post-inoculation.

The third to fifth fully expanded young grapevine leaves beneath the apex for hormone treatments were chosen as the plant samples, at which time the shoots of the vines were 25–35 cm in length. At each time point of each treatment, six leaves from six separate plants were combined to form one sample then immediately frozen in liquid nitrogen and stored at −80 °C until use. The experiment was repeated to generate three biological replicates.

Semiquantitative RT-PCR Analysis

Total RNA from grapevine was extracted using the E.Z.N.A.® Plant RNA Kit (Omega Bio-tek, USA, R6827-01). The integrity of total RNA was assessed by electrophoresis on 1 % agarose gels and its quantity as well as purity was determined by a NanoDrop spectrophotometer (ND-7000, NanoDrop Technologies, USA). For each sample, 1 μg of total RNA was used to synthesize first-strand cDNA using a mixture of poly (dT) and random hexamer primers along with PrimeScript™ RTase (TaKaRa Biotechnology, Dalian, China). For subsequent experiments, the reverse transcription products were diluted six times.

Grape *Actin1* gene (GenBank accession number AY680701), amplified with primers F (5′-GAT TCT GGT GAT GGT GTG AGT-3′) and R (5′-GAC AAT TTC CCG TTC AGC AGT-3′), and grape *EF1-α* gene (GenBank accession number EC931777) with the primers F (5′-AGG AGG CAG CCA ACT TCA CC-3′) and R (5′-CAA ACC CTG CAT CAC CAT TC-3′) were used as the reference genes for normalizing the concentration of the cDNAs. Gene-specific primers were designed for the grape bZIP TF genes using Primer Premier 5.0 and optimized using oligo 7 (Supplementary Table 2). For semiquantitative reverse transcription-PCR (RT-PCR), a 20-μl reaction volume that included 1 μl of cDNA template, 1.6 μl of gene-specific primers (1.0 μM), 9.8 μl PCR Master Mix (Tiangen Biotech Co. Ltd., Beijing, China), and 7.6 μl sterile distilled water was used. The PCR parameters were 95 °C for 3 min, followed by 30–42 cycles of 95 °C for 30 s, 58–64 °C for 30 s, 72 °C for 25 s, and a final step at 72 °C for 2 min. PCR products were separated on a 1.5 % (*w*/*v*) agarose gel with ethidium bromide staining and imaging under UV light for further gene expression analysis. Each PCR reaction was replicated three times and the three independent analyses showed the same trends for each gene and treatment. The results of the semiquantitative RT-PCR reactions were quantified using the GeneSnap software. Grape *Actin1* was used as an internal control and fold changes were used to indicate expression levels in treated leaves compared to negative controls. The Log2-based fold changes of the relative expression levels of *VvbZIP* genes under abiotic, hormone, and biotic stress treatments compared to the control were used for hierarchical cluster analysis with Genesis software to create the heat map (Guo et al. 2013).

Real-Time Quantitative RT-PCR Analysis

To validate the results from the semiquantitative RT-PCR experiment, the real-time quantitative RT-PCR amplification of 20 selected genes was conducted using SYBR green (TaKaRa Biotechnology) on an IQ5 real time-PCR machine (Bio-Rad, Hercules, CA, USA). Gene-specific DNA primers were the same as those used for semi-qRT-PCR (Supplementary Table S2). Each reaction was performed in triplicate with a final volume of 20 μl mixture containing 10.0 μl SYBR Premix Ex *Taq* II (TaKaRa Biotechnology), 1.0 μl cDNA template, 0.8 μl each primer (1.0 μM), and 7.4 μl sterile distilled H_2O. Cycling parameters were 95 °C for 30 s, 40 cycles at 95 °C for 5 s, and 60 °C for 30 s. At the end of the PCR cycles, melt-curve analyses were carried out to examine the amplification specificity by the following program: 95 °C for 15 s, followed by a constant increase from 60 to 95 °C. The grape *Actin1* gene was used as the internal reference gene. The software IQ5 was used to analyze the relative expression levels using the normalized expression method (Gao et al. 2012). Asterisks indicate the corresponding gene significantly up- or downregulated under the differential treatment by *t* test (*$P<0.05$, **$P<0.01$).

Results and Discussion

Identification and Nomenclature of Grape bZIP Transcription Factors

To identify the putative bZIP proteins in the Grape (*V. vinifera* cv PN40024) Genome Database, an HMM search was utilized, with the HMM profile of the bZIP domain, which was extracted from Pfam (accession number PF00170). Based on the SMART tool, each matching sequence was then examined for the bZIP domain with an *e*-value cutoff of 1.0, resulting in a total of 47 potential genes that could be annotated as encoding putative as grape bZIP TFs. These *VvbZIP* genes were manually curated and subsequently named as *VvbZIP1* to *VvbZIP47* according to their location on chromosomes 1 to 19 and from top to bottom (Table 1). Similar criteria have previously been adopted for the nomenclature of bZIP proteins in rice and NAC proteins in potato (Nijhawan et al. 2008; Singh et al. 2013). The nomenclature and information related to all 47 *VvbZIP* genes, such as their

Table 1 List of transcription grape bZIP genes/proteins along with their gene locus ID, accession number, genomic location, CDS and protein length, and *A. thaliana* orthologs

Group	Gene	Gene locus ID	V1 gene locus ID in CRIBI	Accession no.	Chromosome	CDS length (bp)	Protein length (aa)	At ortholog locus	At locus description	Sore (bits)	*e*-value
D	VvbZIP1	GSVIVT01011929001	VIT_01s0011g03230	CBI27049.3	chr01	1,362	453	AT1G68640.1	AtbZIP46, PAN	429	e−120
S	VvbZIP2	GSVIVT01010152001	VIT_01s0010g00930	XP_002269495.1	chr01	588	195	AT3G30530.1	AtbZIP42	143	6.00e−35
G	VvbZIP3	GSVIVT01019481001	VIT_02s0025g01020	XP_003631394.1	chr02	1,197	398	AT2G46270.1	AtbZIP55, GBF3	180	2.00e−45
G	VvbZIP4	GSVIVT01013053001	VIT_02s0012g02250	XP_002276625.1	chr02	1,242	413	AT2G35530.1	AtbZIP16	446	e−125
I	VvbZIP5	GSVIVT01024160001	VIT_03s0038g00860	XP_002269363.1	chr03	1,623	540	AT4G38900.3	AtbZIP29	357	2.00e−98
S	VvbZIP6	GSVIVT01023817001	VIT_03s0038g04450	CBI37685.3	chr03	633	210	AT1G75390.1	AtbZIP44	152	1.00e−37
A	VvbZIP7	GSVIVT01031730001	VIT_03s0063g00310	XP_002285116.1	chr03	1,308	435	AT4G34000.2	AtbZIP37, ABF3, DPBF5	334	7.00e−92
C	VvbZIP8	GSVIVT01035557001	VIT_04s0008g02750	CBI20792.3	chr04	1,260	419	AT5G24800.1	AtbZIP9, BZO2H2	223	2.00e−58
H	VvbZIP9	GSVIVT01035829001	VIT_04s0008g05210	CAN83322.1	chr04	510	169	AT5G11260.1	AtbZIP56, HY5, TED 5	144	2.00e−35
A	VvbZIP10	GSVIVT01033216001	VIT_04s0069g01150	XP_003631846.1	chr04	606	201	AT3G56850.1	AtbZIP66, ABF3, DPBF3	111	3.00e−25
G	VvbZIP11	GSVIVT01019009001	VIT_04s0023g01360	XP_002272761.1	chr04	1,095	364	AT4G36730.1	AtbZIP41, GBF1	250	1.00e−66
H	VvbZIP12	GSVIVT01017714001	VIT_05s0020g01090	CBI26037.3	chr05	561	186	AT3G17609.2	AtbZIP64, HYH, HY5	151	3.00e−37
I	VvbZIP13	GSVIVT01010836001	VIT_05s0102g01120	XP_002270784.1	chr05	1,065	354	AT1G06070.1	AtbZIP69	399	e−111
I	VvbZIP14	GSVIVT01024562001	VIT_06s0004g08070	XP_002266792.1	chr06	1,029	342	AT2G40620.1	AtbZIP52	291	4.00e−79
A	VvbZIP15	GSVIVT01037434001	VIT_06s0009g01790	XP_002269808.1	chr06	747	248	AT3G56850.1	AtbZIP66, AREB3, DPBF3	223	1.00e−58
D	VvbZIP16	GSVIVT01036134001	VIT_06s0080g00360	CBI28327.3	chr06	1,503	500	AT1G08320.3	AtbZIP21, TGA9	572	e−163
A	VvbZIP17	GSVIVT01036137001	VIT_06s0080g00340	CBI28329.3	chr06	975	324	AT2G36270.1	AtbZIP39, ABI5, GIA1	144	8.00e−35
C	VvbZIP18	GSVIVT01000714001	VIT_07s0141g00170	XP_002281328.1	chr07	1,263	420	AT5G28770.2	AtbZIP63, BZO2H3	139	5.00e−33
D	VvbZIP19	GSVIVT01022200001	VIT_07s0031g01320	XP_002280782.1	chr07	1,050	349	AT5G65210.5	AtbZIP47, TGA1	516	e−146
B	VvbZIP20	GSVIVT01025788001	VIT_08s0040g00870	CBI32817.3	chr08	2,043	680	AT2G40950.1	AtbZIP17	489	e−138
A	VvbZIP21	GSVIVT01033832001	VIT_08s0007g03420	CBI30287.3	chr08	1,062	353	AT2G36270.1	AtbZIP39, ABI5, GIA1	324	4.00e−89
J	VvbZIP22	GSVIVT01033811001	VIT_08s0007g03640	XP_002277087.1	chr08	1,491	496	AT1G19490.1	AtbZIP62	138	1.00e−32
D	VvbZIP23	GSVIVT01033632001	VIT_08s0007g05170	XP_003632741.1	chr08	1,356	451	AT3G12250.2	AtbZIP45, TGA6	427	e−120
D	VvbZIP24	GSVIVT01033531001	VIT_08s0007g06160	XP_002275147.1	chr08	1,296	431	AT5G06839.3	AtbZIP65, TGA10	553	e−158
E	VvbZIP25	GSVIVT01020658001	VIT_12s0028g02590	XP_002275912.1	chr12	888	295	AT3G58120.1	AtbZIP61	317	8.00e−87
A	VvbZIP26	GSVIVT01029696001	VIT_12s0055g00420	CAN65151.1	chr12	846	281	AT5G44080.1	AtbZIP13	125	4.00e−29
A	VvbZIP27	GSVIVT01023089001	VIT_12s0034g00110	XP_003631846.1	chr12	660	219	AT3G56850.1	AtbZIP66, AREB3, DPBF3	121	3.00e−28
L	VvbZIP28	GSVIVT01023292001	VIT_12s0035g00620	XP_002266061.1	chr12	1,125	374	AT1G58110.2	AtbZIP76	310	1.00e−84
I	VvbZIP29	GSVIVT01032683001	VIT_13s0067g02900	XP_002280180.1	chr13	975	324	AT2G40620.1	AtbZIP18	330	8.00e−91
A	VvbZIP30	GSVIVT01001940001	VIT_13s0175g00120	CBI40894.3	chr13	753	250	AT3G56850.1	AtbZIP66, AREB3, DPBF3	229	2.00e−60
D	VvbZIP31	GSVIVT01036649001	VIT_13s0084g00660	XP_002263159.2	chr13	1,410	469	AT3G12250.2	AtbZIP45, TGA6	434	e−122
F	VvbZIP32	GSVIVT01001468001	VIT_13s0158g00380	CBI28617.3	chr13	1,092	363	AT2G16770.1	AtbZIP23	106	3.00e−23
S	VvbZIP33	GSVIVT01031238001	VIT_14s0060g01210	XP_002282195.1	chr14	438	145	AT3G62420.1	AtbZIP53	123	4.00e−29
C	VvbZIP34	GSVIVT01021790001	VIT_14s0030g02200	CBI34161.3	chr14	1,152	383	AT5G28770.2	AtbZIP63, BZO2H3	229	2.00e−60

Table 1 (continued)

Group	Gene	Gene locus ID	V1 gene locus ID in CRIBI	Accession no.	Chromosome	CDS length (bp)	Protein length (aa)	At ortholog locus	At locus description	Sore (bits)	*e*-value
G	VvbZIP35	GSVIVT01027040001	VIT_15s0046g01440	XP_002279966.2	chr15	1,293	430	AT2G46270.1	AtbZIP55, GBF3	280	1.00e−75
K	VvbZIP36	GSVIVT01013443001	VIT_18s0122g00500	CBI40780.3	chr18	579	192	AT1G42990.1	AtbZIP60	68	4.00e−12
D	VvbZIP37	GSVIVT01008960001	VIT_18s0001g04470	XP_002285820.1	chr18	1,086	361	AT5G10030.2	AtbZIP57, TGA4	464	e−131
A	VvbZIP38	GSVIVT01008961001	VIT_18s0001g04500	CBI19100.3	chr18	846	281	AT2G41070.2	AtbZIP12, EEL, DPBF4	148	4.00e−36
A	VvbZIP39	GSVIVT01009485001	VIT_18s0001g10450	CBI19512.3	chr18	792	263	AT1G45249.1	AtbZIP36, ABF2, AREB1	140	1.00e−33
G	VvbZIP40	GSVIVT01009665001	VIT_18s0001g12120	XP_002283244.1	chr18	1,197	398	AT4G36730.2	AtbZIP41, GBF1	231	7.00e−61
I	VvbZIP41	GSVIVT01009846001	VIT_18s0001g13740	CBI19815.3	chr18	396	131	AT4G38900.3	AtbZIP29	126	4.00e−30
A	VvbZIP42	GSVIVT01009970001	VIT_18s0001g14890	CBI19920.3	chr18	582	193	AT4G35900.1	AtbZIP14, FD, FD-1	119	2.00e−27
I	VvbZIP43	GSVIVT01034897001	VIT_18s0076g00330	XP_002266803.1	chr18	1,053	350	AT1G43700.1	AtbZIP51, VIP1, SUE3	246	2.00e−65
A	VvbZIP44	GSVIVT01034540001	VIT_18s0072g00470	XP_002266826.1	chr18	861	286	AT5G44080.1	AtbZIP13	178	4.00e−45
L	VvbZIP45	GSVIVT01001783001	VIT_18s0001g03010	CBI35539.3	chr18_random	1,065	354	AT1G58110.2	AtbZIP76	177	1.00e−44
E	VvbZIP46	GSVIVT01014246001	VIT_19s0014g01780	CBI20226.3	chr19	930	309	AT3G58120.1	AtbZIP61	259	1.00e−69
A	VvbZIP47	GSVIVT01014739001	VIT_19s0015g01020	XP_002272719.1	chr19	756	251	AT1G45249.1	AtbZIP36, ABF2, AREB1	59	3.00e−09

gene locus ID, chromosomal location, coding sequence (CDS), and protein length, are listed in Table 1 and Supplementary Table 3. The 47 grape bZIP proteins identified in this study shared a relatively low level of corresponding nucleotide sequence identity and varied in length from 131 to 680 amino acids (aa), with an average of about 336 aa. This high protein sequence variability among members of a bZIP TF family has also been reported in rice, where the size of predicted bZIP proteins ranges from 143 to 647 aa, with an average of 311 aa (Nijhawan et al. 2008). In grape, VvbZIP41 (131 aa) corresponded to the smallest bZIP protein.

Domain analysis performed using the protein BLAST search tool in the NCBI database (blast.ncbi.nlm.nih.gov) revealed that in most members, only one typical bZIP domain was present, but that 11 of the grape bZIP proteins contained an additional domain. Of these 11 proteins, in the case of five (VvbZIP3, VvbZIP4, VvbZIP11, VvbZIP35, and VvbZIP40), a multifunctional mosaic region (MFMR) was found at the N-terminus of the bZIP domain. This MFMR has previously been proposed to be involved in mediating protein-protein interactions (Meier and Gruissem 1994; Siberil et al. 2001). The remaining six VvbZIP proteins (VvbZIP1, VvbZIP16, VvbZIP23, VvbZIP24, VvbZIP31, and VvbZIP37) were predicted to contain a DELAY OF GERMINATION 1 (DOG1; PF14144) domain, which was reported as being present in *Arabidopsis* DOG1, a protein involved in the control of seed dormancy (Bentsink et al. 2006).

Given that the genome sequence of *Arabidopsis* is the best studied of any plants, and the functions of many Arabidopsis bZIP (*AtbZIP*) genes have been well characterized, we predicted the function of each *VvbZIP* gene based on the function of its *Arabidopsis* ortholog. For each of the 47 *VvbZIP* genes, the most similar gene in *Arabidopsis* was designated as the ortholog by a BLAST analysis. Interestingly, six of the *VvbZIP* genes (*VvbZIP16*, *VvbZIP24*, *VvbZIP19*, *VvbZIP37*, *VvbZIP31*, and *VvbZIP23*) were identified as orthologs of *Arabidopsis* TGA factors with strong *e*-value support. Previously, TGA factors have been found to regulate the expression of pathogenesis-related (PR) genes by binding to their promoters (Jakoby et al. 2002). In addition, the predicted ortholog of both *VvbZIP17* and *VvbZIP21* was identified as ABI5, which appears to function in ABA signal transduction both in late seed development and seed germination (Jakoby et al. 2002). Based on the functions of their *Arabidopsis* orthologs (Table 1), we propose that specific *VvbZIP* genes have roles in grape development and defense against pathogens and biotic stresses and will provide targets for future functional characterization.

All the Major Groups of Land Plants Have Large Numbers of bZIP Proteins

Phylogenetic analyses of plant bZIP proteins to date have provided a useful, but somewhat limited, phylogenetic

framework for their overall classification in green plants (Corrêa et al. 2008). Importantly, little is known about the evolutionary history of the bZIP family in woody species, such as grape. To provide a broader evolutionary context, we evaluated the number of bZIP TF genes of *V. vinifera* and nine other plant species (*Ostreococcus lucimarinus*, *Volvox carteri*, *Chlamydomonas reinhardtii*, *Physcomitrella patens*, *Selaginella moellendorffi*, *S. bicolor*, *O. sativa*, *P. trichocarpa*, and *A. thaliana*), one mammal (*Homo sapiens*), and one fungal species (*Saccharomyces cerevisiae*) according to previous studies (Feller et al. 2011; Wang et al. 2011a; Corrêa et al. 2008). This confirmed the presence of large numbers of bZIP proteins in a taxonomically diverse set of land plants whose genomes have been sequenced to date (Fig. 1). The results of this analysis suggest that most plant bZIP TFs were already present in early land plants, prior to the divergence of mosses and vascular plants, which is consistent with the observation that the plant bHLH TF family diversified before the divergence of lycophytes from other vascular plants (Pires and Dolan 2010). By contrast, chlorophytes encoded a few bZIP proteins, which might indicate that bZIP proteins have ancient origins and arose before plants transitioned from water to land. On the other hand, unicellular eukaryotic organism such as *S. cerevisiae* also has a small number of bZIP proteins, whereas *H. sapiens* has more than 50 bZIP TFs (Fig. 1). Due to the larger number of bZIP TFs in animals and land plants than other eukaryotes, we hypothesized that the size expansion of the bZIP family occurred independently during the evolution of plants and animals. This increase in number in both plants and animals, compared to other eukaryotes, has also been observed among members of the bHLH TF family (Pires and Dolan 2010). We noted that grape has fewer predicted bZIP TFs compared to other plants with fully sequenced genomes, since *Arabidopsis*, *P. trichocarpa*, *S. bicolor*, and *O. sativa* have 77, 89, 92, and 92 predicted bZIP TFs, respectively (Corrêa et al. 2008; Wang et al. 2011a). While this may reflect a true difference, a possible explanation is that some *VvbZIP* genes may be located in gaps in the current release of the grape genome and so have yet to be identified. Another explanation may be that there were potential limitations of our survey since we have not expanded our study to other datasets beyond the scaffolds of the PN40024 genome.

Phylogenetic Analysis of *VvbZIP* Genes from Three Plant Species

Genomic comparison can provide a highly efficient method for transferring genomic knowledge from one taxon in which the gene function is well established to a less studied taxon (Paterson et al. 2012). To investigate the phylogenetic relationship of VvbZIP proteins with eudicot (*Arabidopsis*) and monocot (rice, *O. sativa*) model plants (Corrêa et al. 2008), an unrooted neighbor-joining (NJ) tree was generated using a multiple alignment of the full-length bZIP protein sequences (Fig. 2). Among the 47 grape bZIP proteins, 64 % (30) grouped together with *Arabidopsis* bZIP proteins rather than rice proteins, suggesting that the majority of *V. vinifera* bZIP proteins are more closely related to those of *Arabidopsis* than to those of rice. This result is consistent with the fact that both grape and *Arabidopsis* are eudicots and diverged more recently from a common ancestor than from the lineage leading to monocots. Based on the topology, clade support values, and branch length, the phylogenic tree divided the 47 VvbZIP proteins into 13 distinct groups, which are shown with their *Arabidopsis* and rice orthologs (Fig. 2). These groups are in agreement with groups proposed by previous analyses in *Arabidopsis* and other green plants (Jakoby et al. 2002; Corrêa et al. 2008). We adopted the *Arabidopsis* bZIP group nomenclature (A, B, C, D, E, F, G, H, I, and S) proposed by

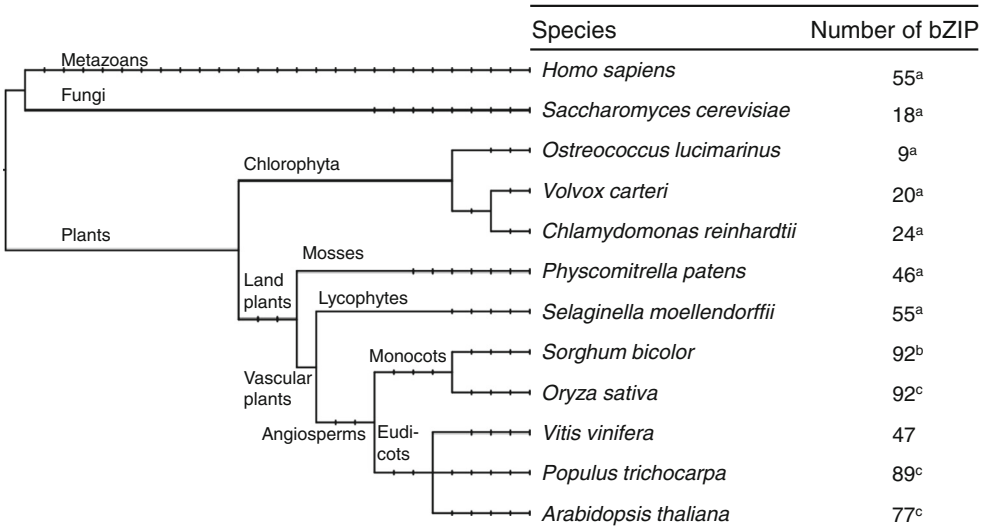

Fig. 1 Eukaryotic phylogeny showing the distribution of members of the bZIP family in 12 species. The total number of bZIP proteins found in the genome of each species is indicated on the right. *Superscript letter a*, Feller et al. (2011); *superscript letter b*, Wang et al. (2011a); *Superscript letter c*, Corrêa et al. (2008)

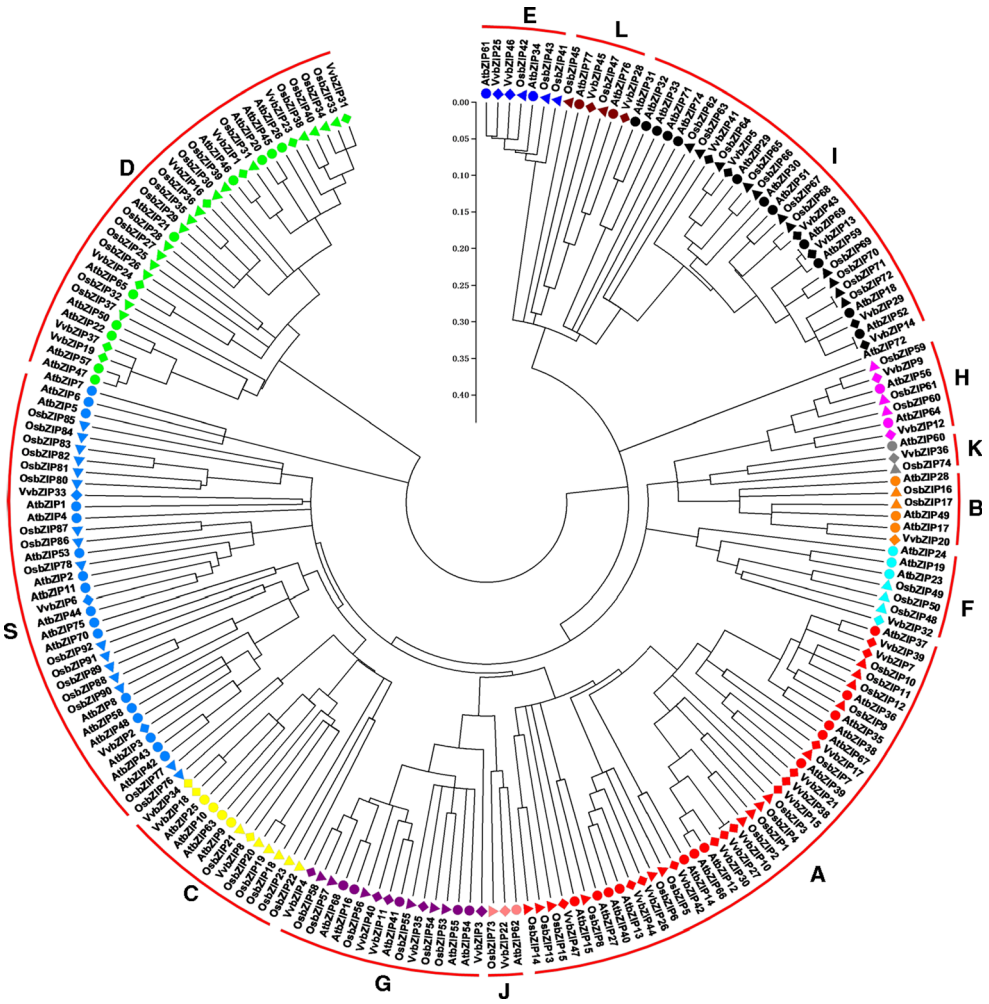

Fig. 2 Phylogenetic analysis of grape, *Arabidopsis*, and rice bZIP proteins. Multiple sequence alignment of full-length bZIP proteins from *V. vinifera* (Vv), *A. thaliana* (At), and *O. sativa* (Os) was performed using Clustal X 2.0, and the phylogenetic tree was constructed using MEGA 5.0 by the neighbor-joining method with 1,000 bootstrap replicates. The tree was divided into 13 groups, designated as A to S, as previously described. Members from grape, *Arabidopsis*, and rice were denoted by *diamond*, *circle*, and *triangle*, respectively

Jakoby et al. (2002) to label these groups, together with three groups (J, K, and L) classified by Corrêa et al. (2008).

In general, all these 13 groups were present in grape, *Arabidopsis*, and rice, indicating that most bZIP TFs in plants evolved prior to the monocot/eudicot divergence. Furthermore, no species-specific groups were observed, suggesting that bZIP TFs have been conserved throughout plant evolution. In addition, bZIP proteins from the same group tended to cluster together in the phylogenetic tree and were not equally represented within a given clade, suggesting that they experienced duplications after the lineages diverged. Remarkably, the grape, *Arabidopsis*, and rice bZIP proteins were distributed uniformly in seven groups (A, E, G, H, J, K, and L) and contained similar numbers of bZIP genes from each species, suggesting that major expansion/contraction in gene number of these groups has not occurred since the divergence of eudicots (*Arabidopsis* and grape) and monocots (rice). Conversely, in the remaining six groups, the number of genes from each of the three species differed considerably, indicating that expansion/contraction occurred after the separation of each lineage.

Recently, bZIP genes belonging to groups B, C, D, E, F, G, I, and J have been identified in the genome of bryophytes (*P. patens*), gymnosperms (*Pinus taeda* and *Picea glauca*), and angiosperms (*O. sativa*, *Arabidopsis*, and *P. trichocarpa*) (Corrêa et al. 2008), which suggests that they diverged from one another very early in land plant evolution. Moreover, groups D and I have been reported to be particularly important for the transition from early land plants to angiosperms (Corrêa et al. 2008), and both these groups were also present in the grape bZIP family. We note that group A, which probably first appeared in the most recent common ancestor (MRCA) of spermatophytes (Corrêa et al. 2008), is the largest group and contains 41 homologues (Fig. 2). Group A genes are absent from the genome of the moss, *P. patens* (Corrêa et al. 2008), and since members of this group are present in gymnosperms and angiosperms, it seems that bZIP genes of group A may have arisen more recently, following the divergence of vascular plants. Recently, members of the group A bZIPs have been studied in the context of ABA or abiotic stresses such as cold, drought, and high salinity signaling (Jakoby et al. 2002). The smallest groups were J and K, which

both contained only three members. Group J has been reported to be present in the MRCA of bryophytes and tracheophytes, while group K is angiosperm specific (Corrêa et al. 2008).

Structural and Phylogenetic Analysis of Grape bZIP Proteins

Multiple sequence alignment of the bZIP domain (51 aa) revealed that most of the 47 bZIP proteins contain a highly conserved bZIP domain with an invariant N-x_7-R/K motif in the basic region and a heptad repeat of Leu or other bulky hydrophobic amino acids, typically positioned nine amino acids upstream of R/K toward the C-terminus, as previously described (Fig. S1). Exceptions were provided by VvbZIP47, where the heptad repeat of Leu is 16 amino acids instead of the usual nine amino acids toward the C-terminus, and VvbZIP22, where the conserved Arg/Lys in the basic region is replaced by an Ile residue. This is similar to the previously reported rice bZIP TF OsZIP34, where the heptad repeat of Leu is positioned 16 amino acids toward the C-terminus, and OsZIP80 that also has the conserved Arg/Lys replaced by an Ile residue in the basic region (Nijhawan et al. 2008). In addition, there were two other VvbZIP TFs that were identified with unusual structures: VvbZIP28 and VvbZIP45, which both have a Lys in place of the conserved Asn in the basic region. This atypical basic domain has also been found in the rice bZIP TF OsZIP21 and OsZIP82 TF (Nijhawan et al. 2008).

Phylogenetic analysis was also performed using a multiple sequence alignment containing only the protein sequences of the 47 grape bZIP TFs identified here (Fig. 3a). The topology was similar to that constructed with all the bZIP protein sequences from the three plant species in Fig. 1, and as expected, bZIP proteins from the same group tended to cluster together, with bZIP proteins divided into 13 distinct groups. Group A contained the greatest number (13) of VvbZIP proteins, which is similar to the result of the multispecies analysis (Fig. 1), while groups B, F, J, and K each contained only one VvbZIP protein. In similar studies, phylogenetic analyses divided both sorghum bZIPs and rice bZIPs into ten groups (Wang et al. 2011a; Nijhawan et al. 2008). These observations may suggest that grape bZIP proteins have been more conserved during plant evolution.

To provide further confirmation of the evolutionary relationships among the grape bZIP genes, conserved motifs were predicted using the MEME program. Although the number of amino acids in the grape bZIP protein sequences varied from 131 to 680 (Table 1), proteins that clustered in the same group tended to contain the same number of amino acids and share a similar motif composition, which further supported the group definitions, indicating that the molecular structures of the members in the same group were highly conserved during evolution (Fig. 3b). A total of 20 motifs, including the bZIP domain, were analyzed and the multilevel consensus sequence and the amino acid length of these conserved motifs are given in Supplementary Table 4. The existence of highly conserved, group-specific motifs outside of the bZIP domain also indicates a common origin, despite variability among the different groups (Du et al. 2013). A part of the conserved TLED/E sequence and [TN][LV][DE][ED] in motif 6 represent potential casein kinase II (CKII)-like phosphorylation sites (S/TxxD/E, where x represents any amino acid), which have been identified in members of bZIP transcription factors in *Arabidopsis* and rice (Jakoby et al. 2002; Nijhawan et al. 2008). The identification of conserved motifs outside the conserved domains is in agreement with sequence analysis of other TF families, such as ERF and NAC (Nakano et al. 2006; Singh et al. 2013).

Since exon/intron structure can also provide important evidence to support phylogenic relationships in a gene family (Li et al. 2006), we investigated the exon/intron organization of the 47 *VvbZIP* genes to obtain further insight into their possible structural evolution. Among the 47 genes, the number of introns ranged from 0 to 11 and most introns showed conserved positions and phases (Fig. 3c). Two *VvbZIP* genes had no introns, accounting for 4 % of the total *VvbZIP* genes, which was less than the total percentages of bZIP transcription factor genes predicted to be without introns based on studies in rice and sorghum where in each case they accounted for 19 % (Nijhawan et al. 2008; Wang et al. 2011a). Our analysis proved a strong correlation between the phylogenetic relationships of the genes and the exon/intron structure (Fig. 3) and *VvbZIP* genes that clustered together possessed a similar gene structure. As a result, five sets of genes (*VvbZIP11*/*VvbZIP40*, *VvbZIP9*/*VvbZIP12*, *VvbZIP25*/*VvbZIP46*, *VvbZIP19*/*VvbZIP37*, and *VvbZIP23*/*VvbZIP31*) contained the same number of exons with nearly identical exon length (Fig. 3c), indicating that they may be the products of segmental or tandem duplication events. These results were confirmed with the conserved motifs analysis since *VvbZIP* genes that fell into different groups showed a large degree of sequence divergence, whereas those in the same group bore a closer relationship. However, intron/exon loss/gain within the same *VvbZIP* gene clade was also observed. For example, *VvbZIP21* is comprised of six exons, whereas its paralog *VvbZIP17* has only three, indicating that it may have acquired three more exons during evolution. In addition, compared to its paralog *VvbZIP34*, *VvbZIP18* has lost its final intron in the course of its evolutionary history.

Chromosomal Distribution and Duplication Events Among *VvbZIP* Genes

The *VvbZIP* genes were assigned to the 19 grape chromosomes based on the annotation of the grapevine genome, revealing that they are distributed unevenly and that not all

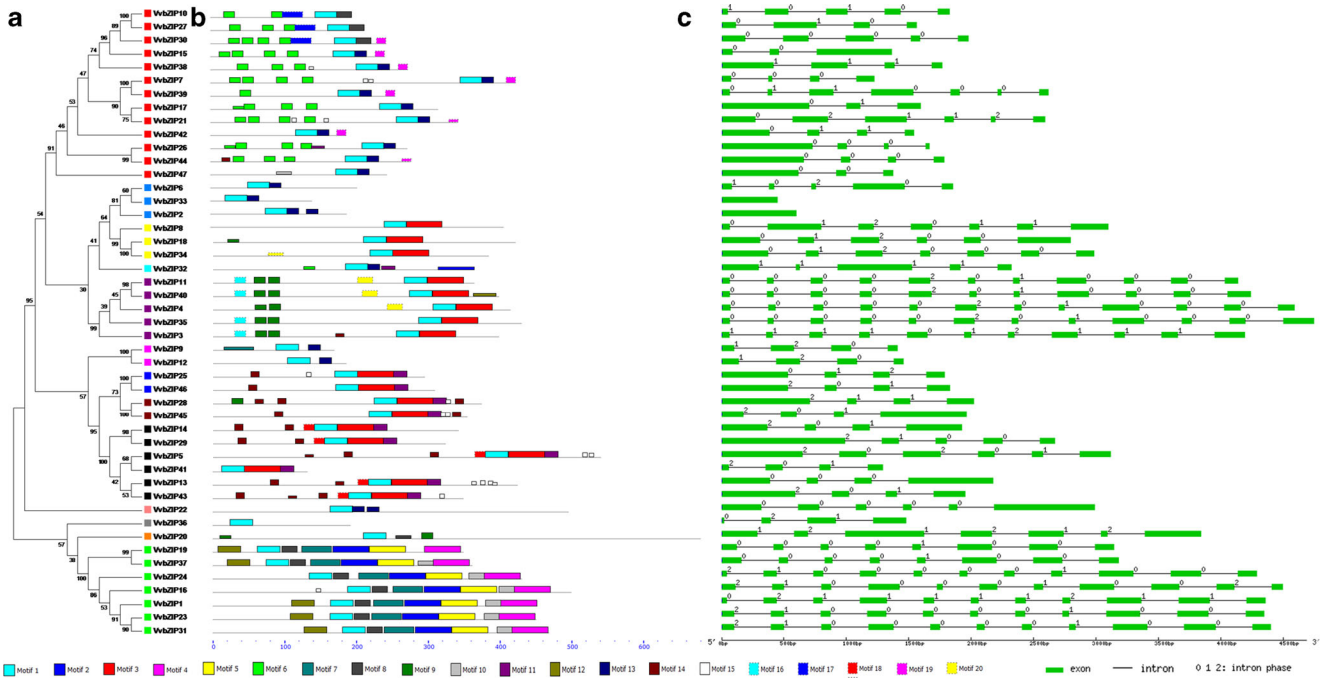

Fig. 3 The VvbZIP genes. **a** Phylogenetic analysis of grape bZIP proteins. *Numbers above or below the branches of the tree* indicate bootstrap values. **b** Schematic representation of the conserved motifs in the grape bZIP proteins as revealed by MEME analysis. *Gray lines* represent the nonconserved sequences, and each motif is represented by a *box numbered at the bottom*. The length of each protein can be estimated using the scale at the bottom. **c** Exon/intron structures of VvbZIP genes. Only the exons, represented by *green boxes*, were drawn to scale. *Black lines* connecting two exons represent introns

19 chromosomes contain a *VvbZIP* gene. Chromosomes 9, 10, 11, 16, and 17 have no *VvbZIP* gene, whereas relatively high densities are present in certain chromosomes and chromosomal regions, including the top of chromosomes 3 and 4, the bottom of chromosomes 6, 8, 12, and 13, and the central sections of chromosome 18 (Fig. 4). Notably, nine bZIP members are located on chromosome 18, the largest number on any chromosome, while one gene, *VvbZIP45*, is not located on any of the defined chromosomes, but rather the random chromosome 18.

Genome duplications, among which tandem and segmental duplication events are thought to be two of the main processes, have contributed to gene family expansions throughout plant evolution (Cannon et al. 2004; Li et al. 2006). Grapevine, one of the oldest land plants, appearing approximately 65 million years ago (This et al. 2006), has undergone abundant gene duplication throughout its evolutionary history and 17,922 duplicated genes in the grapevine genome have been identified. Of these, 2,039 and 628 were identified as being produced by genome-wide and tandem duplications, respectively (Wang et al. 2013). To detect a possible relationship between the *VvbZIP* genes and potential genome duplications, both segmental and tandem duplication events were examined. We identified only two pairs of tandem duplications (*VvbZIP16*/*VvbZIP17* and *VvbZIP37*/*VvbZIP38*) on grape chromosomes 6 and 18, respectively (Fig. 4), indicating the limited contribution of tandem duplication to this gene family expansion. Similar results were obtained from the analysis of the sorghum genome, where only three pairs of tandem duplicates were identified (Wang et al. 2011a). We then examined the segmentally duplicated genes within the grapevine genome and determined that there are ten pairs of *VvbZIP* genes (*VvbZIP18*/*VvbZIP34*, *VvbZIP37*/*VvbZIP19*, *VvbZIP38*/*VvbZIP27*, *VvbZIP39*/*VvbZIP7*, *VvbZIP40*/*VvbZIP11*, *VvbZIP41*/*VvbZIP5*, *VvbZIP46*/*VvbZIP25*, *VvbZIP3*/*VvbZIP35*, *VvbZIP14*/*VvbZIP29*, *VvbZIP21*/*VvbZIP17*) involved in segmental duplications, accounting for 43 % of the total *VvbZIP* genes (Supplementary Table 5). It is worth noting that the *VvbZIP40* paralog, GSVIVT01031924001, does not belong to the bZIP transcription factor gene family, but is predicted to encode a protein with a coiled coil region. This domain was also detected in VvbZIP40, thus providing evidence of a segmental duplication. Interestingly, all *VvbZIP* genes that located to the duplicated segments of chromosomes belong to the same group and the majority have highly similar exon/intron structures (Fig. 3), such as *VvbZIP19* and *VvbZIP37*, which both belong to group C (Fig. 3a). These results are consistent with a study that showed that all rice *OsbZIP* genes that located to the duplicated segments of chromosomes belong to the same group (Nijhawan et al. 2008). In summary, 20 out of the 47 *VvbZIP* genes were associated with segmental duplication events, indicating that such duplications have played an important role in the expansion of the grape bZIP family. Moreover, similar contribution

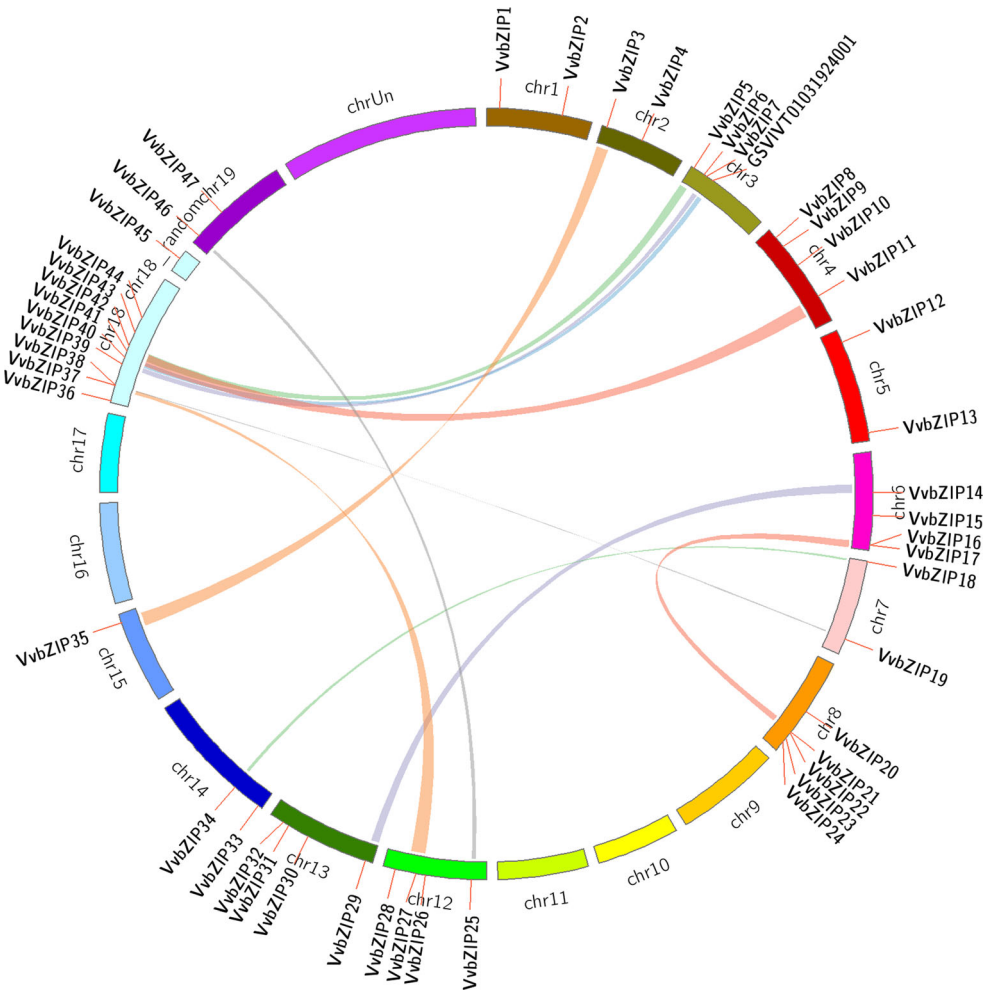

Fig. 4 Distribution and synteny analysis of VvbZIP genes on the grape chromosomes. bZIP genes are indicated by *vertical orange lines*. *Colored bars* connecting two chromosomal regions denote syntenic regions of the grape genome

rates of segmental duplication to the bZIP family expansion have been reported in *Arabidopsis*, rice, and sorghum, suggesting that segmental duplication can be regarded as a major mechanism contributing to the expansion of plant bZIP families (Nijhawan et al. 2008; Jakoby et al. 2002; Wang et al. 2011a).

Evolutionary Relationships Between Grape and *Arabidopsis* of bZIP Genes

It is now possible to reconstruct the evolutionary history of a gene in its entirety by comparing the sequences of all genes between genomes from different taxa and within each genome (Koonin 2005). As described above, *Arabidopsis* is considered to be the best studied model plant species and the functions of many AtbZIP TFs have been well characterized. Thus, we analyzed the comparative synteny map between the grape and *Arabidopsis* genomes to gain further insight into the origin and evolutionary history of the *VvbZIP* genes. Large-scale synteny data revealed that 26 *VvbZIP* genes have 31 *Arabidopsis* corresponding orthologs (Fig. 5), suggesting that the large-scale expansion might have occurred before the divergence of grape and *Arabidopsis*. These *Arabidopsis* 31 orthologs could also be classified within the bZIP TF family and are listed in Supplementary Table 5, using the previously proposed *Arabidopsis* bZIP nomenclature. With regard to single grape-to-*Arabidopsis* bZIP TF gene orthology, the syntenies were unambiguous and included 17 pairs of orthologs as follows: *VvbZIP16-AtbZIP21*, *VvbZIP4-AtbZIP68*, *VvbZIP36-AtbZIP60*, *VvbZIP28-AtbZIP76*, *VvbZIP1-AtbZIP46*, *VvbZIP2-AtbZIP8*, *VvbZIP42-AtbZIP27*, *VvbZIP12-AtbZIP64*, *VvbZIP11-AtbZIP41*, *VvbZIP31-AtbZIP20*, *VvbZIP9-AtbZIP56*, and *VvbZIP8-AtbZIP9* (Supplementary Table 6), indicating that these VvbZIP genes and their *Arabidopsis* bZIP counterparts share a common ancestor. More challenging aspects of the syntenic interpretation were cases where grape segmental duplications corresponded to single *Arabidopsis* genes, or where single grape genes corresponded to multiple *Arabidopsis* genes. The former situation included *VvbZIP37*/*VvbZIP19-AtbZIP57*, *VvbZIP39*/*VvbZIP7-AtbZIP38*, *VvbZIP3*/*VvbZIP35-AtbZIP55*, and *VvbZIP14*/*VvbZIP29-AtbZIP18*, whereas the latter included *VvbZIP14-AtbZIP52*/*AtbZIP18*, *VvbZIP5-AtbZIP30*/*AtbZIP29*, *VvbZIP20-AtbZIP17*/*AtbZIP49*, *VvbZIP24-AtbZIP45*/*AtbZIP65*, *VvbZIP33-AtbZIP53*/*AtbZIP1*, *VvbZIP35-AtbZIP54*/*AtbZIP55*,

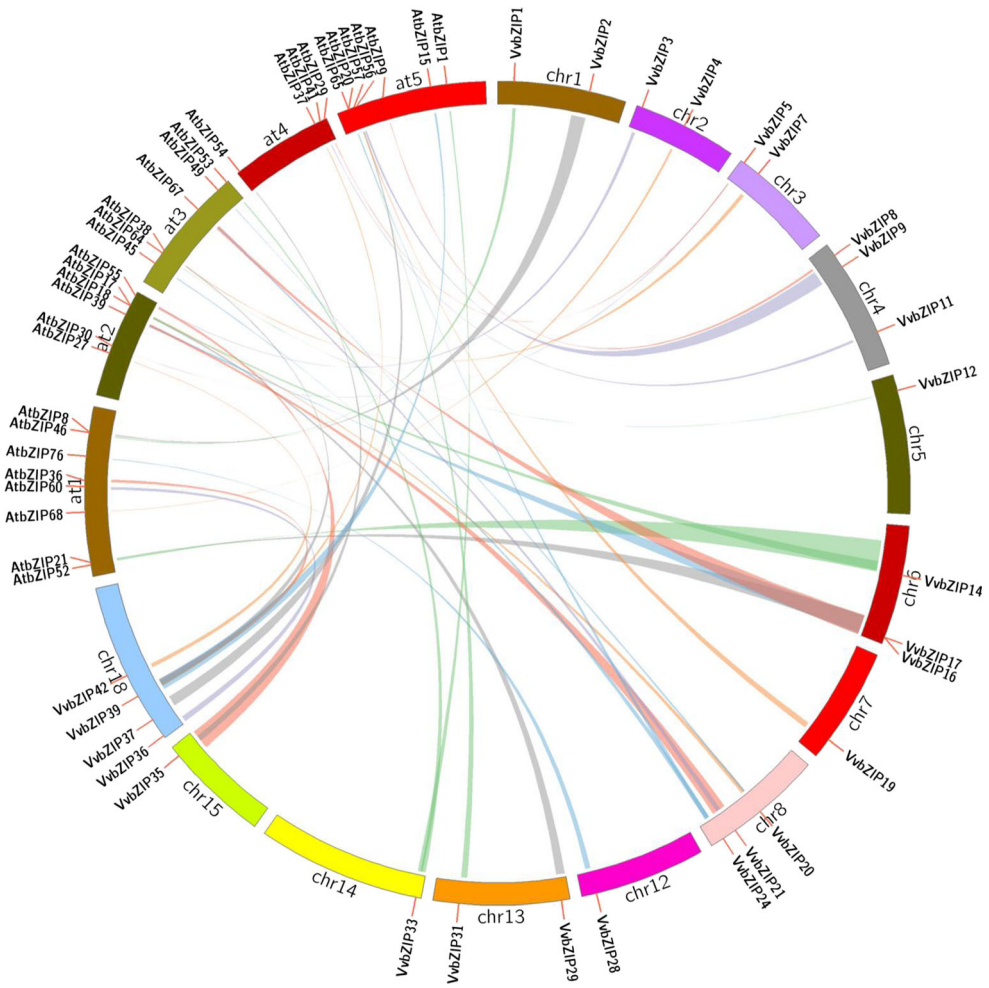

Fig. 5 Synteny analysis of bZIP transcription factor genes between grape and *Arabidopsis*. Grape and *Arabidopsis* bZIP genes are indicated by *vertical orange lines*. *Colored bars* denote syntenic regions between grape and *Arabidopsis* chromosomes

and *VvbZIP39-AtbZIP36*/*AtbZIP38*/*AtbZIP37*/*AtbZIP15* (Fig. 5). Finally, a third case was identified where two duplicated grape genes corresponded to two *Arabidopsis* genes (*VvbZIP21*/*VvbZIP17-AtbZIP39*/*AtbZIP67*) (Fig. 5). In this instance, it was difficult to determine whether the segmental duplications appeared before or after the divergence from a common ancestor. Although the remaining 21 *VvbZIP* genes could not be mapped to any synteny blocks, it cannot be concluded that they did not share a common ancestor with *Arabidopsis* bZIP TF genes since, after the speciation of grape and *Arabidopsis*, their genomes have undergone multiple rounds of significant chromosomal rearrangement and fusions, followed by selective gene loss, which can severely obscure chromosomal syntenies (Paterson et al. 2012; Zhang et al. 2012).

Expression Profiles of *VvbZIP* Genes in Different Organs

Semiquantitative RT-PCR was performed to detect conserved and tissue-specific expression profiles of VvbZIP genes under normal growth conditions in six different grapevine organs: roots, stems, leaves, flowers, fruits, and tendrils. For this analysis, two grapevine genes, *Actin 1* and *EF-α*, were used as references. In total, the expression profile of 44 VvbZIP genes (~94 %) in six different organs was evaluated, while the remaining three VvbZIP genes (*VvbZIP41*, *VvbZIP45*, and *VvbZIP46*) had no detectable expression. Of the 44 VvbZIP genes, 37 (~84 %) were ubiquitously expressed in all six organs and might therefore regulate the transcription of a broad set of genes, while three (~7 %) were expressed in one to three organs, and four (~9 %) were expressed in four to five organs (Fig. 6). The expression levels for different VvbZIP genes varied significantly, such as *VvbZIP1*, *VvbZIP16*, *VvbZIP24*, and *VvbZIP38* were expressed at higher levels in flowers (Fig. 6), suggesting organ-specific functions, similar to their *Arabidopsis* or rice orthologs. For example, *AtbZIP46* (Perianthia/PAN; ortholog of *VvbZIP1*) regulates the number and their relative positions of floral organs (Chuang et al. 1999). Consistent with a role in flower development, *OsbZIP31* and *OsbZIP30*, orthologs of *VvbZIP1* and *VvbZIP16*, respectively (Fig. 2), were predominantly expressed in early/late panicle stages in rice (Nijhawan et al. 2008) and so the grape bZIP TF homologs may have a similar role. Similarly, *VvbZIP8* and *VvbZIP36* showed particularly high expression levels in fruits compared to other

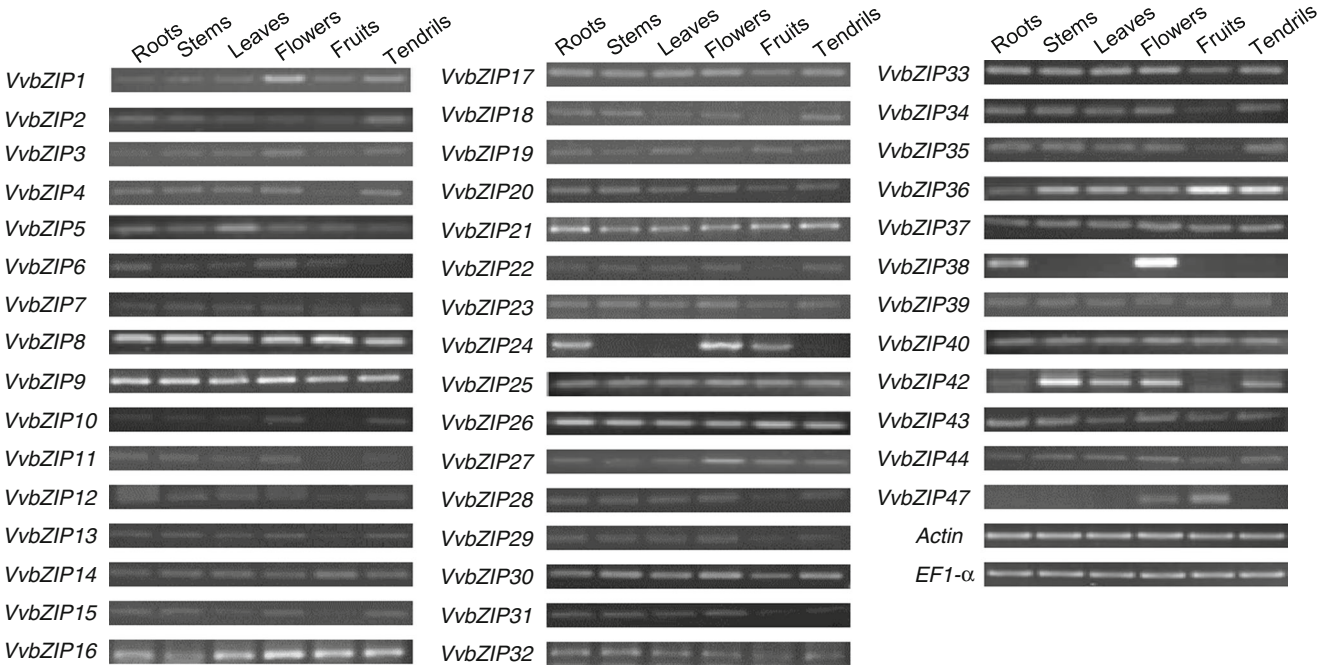

Fig. 6 Expression profiles of 44 VvbZIP genes in various organs, as determined by semiquantitative RT-PCR analyses. The experiments were repeated three times and the results were consistent

tissues (Fig. 6). Interestingly, one of these, *VvbZIP8*, represents the closely related ortholog of *OsbZIP19* and *OsbZIP21* (Fig. 2), which have been considered to have a specific role in regulating the expression of genes involved in different stages of seed development, from embryo and endosperm development to seed maturation. In addition, further analysis of the expression profiles presented in Fig. 6 demonstrated that all organs expressed at least one *VvbZIP* gene, indicating that the bZIP TF family plays extensive roles in grapevine development.

Three genes (*VvbZIP41*, *VvbZIP45*, and *VvbZIP46*) for which no signal could be detected in any organ also gave no detectable expression in leaves following drought and high salinity stress treatments, or following methyl jasmonate (MeJA), abscisic acid (ABA), salicylic acid (SA), and ethylene (ET) application, even when using multiple sets of gene-specific PCR primers. There are several possible reasons why no expression signal was detected: these genes may have been silenced, they are responsive to other abiotic or biotic stresses, or their expression is too weak to be detected in this study. We note that a similar study in sorghum reported the absence of expression of six bZIP TF genes under abiotic stress conditions (Wang et al. 2011a).

Expression Profiles of VvbZIP Genes Following Different Stresses and Hormone Treatments

In addition to regulating plant development, some members of the bZIP TF family have been shown to be involved in responding to stress conditions, including abiotic and biotic factors and hormone treatments (Liu et al. 2012). Since the expression pattern of a gene can sometimes be used to infer function, the expression profiles of 41 VvbZIP genes were assessed in plants exposed to various abiotic and biotic stress conditions and hormone treatments, by semi-qRT-PCR analysis. The remaining six VvbZIP genes were not analyzed further because their expression was not detectable in leaves. Detailed expression profiles of these genes are listed in Supplementary Table 7 and a heat map representation of their expression profiles is shown in Fig. 7.

Plants are often exposed to abiotic stresses, such as salinity, drought, and extreme temperatures which can seriously affect growth, crop production, and a range of specific developmental processes. bZIP TF gene induction by cold, high salinity, and drought stresses has been reported for many plants, such as *Arabidopsis*, rice, grape, tomato, soybean, and banana, suggesting roles in adaptation to these stresses. For example, rice *OsABI5* was reported to be induced in seedlings by high salinity but downregulated by drought and cold (Zou et al. 2008), while *OsbZIP52* and grape *VvbZIP23* were both induced by cold and drought stress (Liu et al. 2012; Tak and Mhatre 2013), and tomato *StABF1* was induced by drought, salt, and cold stress (García et al. 2012). Functional evidence for a role in stress tolerance was also provided by studies of *Arabidopsis* lines overexpressing the bZIP TF ABP9, which showed enhanced tolerance of heat, water, and drought stresses (Zhang et al. 2008), and of different soybean bZIP TF genes, which exhibited increased tolerance of salt and cold stresses (Liao et al. 2008).

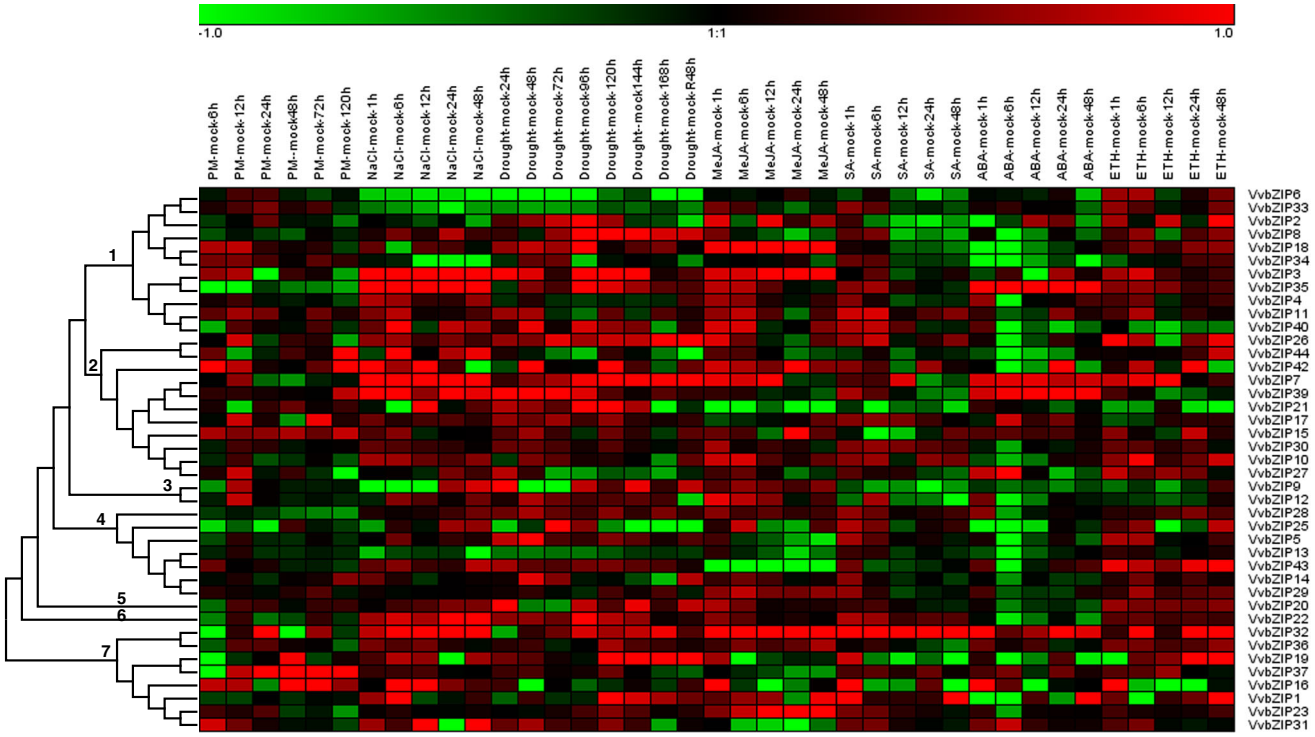

Fig. 7 Hierarchical clustering of 41 VvbZIP genes. The results of semi-quantitative RT-PCR were quantified using the Gene Tools software, and the log-transformed values of the relative expression levels of VvbZIP genes under various conditions compared to the controls were used for hierarchical cluster analysis with the Genesis software (original results shown in Fig. S2, S3, S4, S5, S6, S7, and S8). The color scale represents relative expression levels, with *red* as increased transcript abundance and *green* as decreased transcript abundance. *Gray* represents not detected under the corresponding treatments. Sampling times are indicated at the *top of the figure*: R48 represents sampling 48 h after recovery from 1 week (168 h) of drought treatment. The experiments were repeated three times and the results were consistent

In the present study, we investigated the bZIP expression dynamics in responses to osmotic stresses, including those induced by high salinity and drought. At least 23 VvbZIP genes showed differential expression associated with at least one abiotic stress (Fig. 7). Among the genes affected, 19 genes were upregulated, while four (*VvbZIP6*, *VvbZIP13*, *VvbZIP34*, and *VvbZIP33*) were downregulated. Six *VvbZIP* genes (*VvbZIP7*, *VvbZIP39*, *VvbZIP22*, *VvbZIP3*, *VvbZIP35*, and *VvbZIP32*) were upregulated under both stress conditions. Interestingly, expression of the *VvbZIP7* and *VvbZIP39*, orthologs of *Arabidopsis AtbZIP37*/ABF3/DPBF5 and *AtbZIP36*/ABF2/AREB1, respectively, were highly induced by both salinity and drought stress treatments (Fig. 7). Previously, bZIP TFs of the ABF/AREB family have been reported to be involved in many stress responses in plants (He et al. 2013). The phytohormone abscisic acid (ABA) plays an important role in abiotic stress-related signaling (Liao et al. 2008), and it has been reported that most of the bZIP genes that respond to drought, high salinity, and cold stress can be induced by exogenous ABA through *cis* elements that include the ABA response element (ABRE) (Jakoby et al. 2002). In *Arabidopsis*, many group A bZIP genes are involved in ABA responses (Jakoby et al. 2002), and overexpression of the group A bZIP TFs ABF2/AREB1, ABF3, and ABF4/AREB2 in transgenic plants conferred hypersensitivity to ABA, as well as tolerance of drought and water deficit, suggesting that these proteins are positive regulators of ABA signaling (Fujita et al. 2005; Kang et al. 2002; Barbosa et al. 2013). In addition, overexpression of a wheat AREB/ABF gene, *HvABI5*, in transgenic tobacco plants enhanced tolerance of freezing and osmotic and salt stresses (Kobayashi et al. 2008). Our findings are largely consistent with these studies and further suggest that the majority of bZIP genes in group A are induced by osmotic stress (Fujita et al. 2005; Kang et al. 2002; Barbosa et al. 2013; Kobayashi et al. 2008). We propose that *VvbZIP7* and *VvbZIP39* from group A act as positive regulators of ABA signaling, and functional characterization of the AREB/ABF orthologs identified here may provide opportunities to enhance abiotic stress tolerance in grapevine.

It has previously been shown that bZIP proteins, and especially TGA factors from group D, participate in plant defense against pathogens, not only because they bind to the *as-1 cis* elements in the promoters of pathogenesis-related (PR) genes, but also because they interact with the NPR protein, which is necessary for PR gene induction (Jakoby et al. 2002). It has been reported that rice *OsbZIP1* was strongly induced in response to infection by the rice blast fungus *Magnaporthe grisea*, suggesting a potential role of bZIP proteins in plant defense against pathogens (Meng et al. 2005). To determine

whether the VvbZIP genes identified here are responsive to biotic stress, we used powdery mildew infection to induce biotic stress. Powdery mildew, caused by the fungus *Uncinula necator* (Schw.) Burr, is one of the most devastating diseases of grapevine. Our analysis indicated that while the expression levels of most *VvbZIP* genes were not significantly altered upon *U. necator* infection, those of *VvbZIP37* and *VvbZIP16* (Fig. 7) were induced. Interestingly, the *Arabidopsis* ortholog (*AtbZIP57*/TGA4) of one of these genes (*VvbZIP37*) has been shown to interact with AtEBP, which binds the ethylene response element present in many PR gene promoters (Büttner and Singh 1997). In conclusion, our analysis supports the potential involvement of VvbZIP genes in responses to pathogen infection, but their roles and functional significance remain to be determined.

The roles of plant hormones such as SA, jasmonic acid (JA), ET, and ABA in the adaptation of plants to a wide range of biotic and abiotic stresses have been well characterized (Bari and Jones 2009; Pieterse et al. 2012; Yang et al. 2013). Among these, ABA is extensively involved in responses to abiotic stresses, such as drought, low temperature, and osmotic stress, whereas SA, JA, and ET play central roles in biotic stress signaling upon pathogen infection. It has been shown that bZIP proteins regulate a variety of plant processes by mediating hormone signaling. Our analysis indicated that 36 out of 41 VvbZIP genes showed differential expression following treatment with one or more hormones (Fig. 7). Interestingly, the expression of *VvbZIP32* was induced by all the phytohormone treatments that were analyzed in this study, suggesting a central role in plant stress tolerance. It should be noted that *VvbZIP32*, which was upregulated by all the abiotic stresses and hormone treatments, corresponds to the previously described grape *VvbZIP23* gene, the expression of which was induced by a wide spectrum of abiotic stresses including drought, salt, and cold, as well as exogenous MeJA, SA, ABA, and ET (Tak and Mhatre 2013), thus supporting our expression data. A total of 18 VvbZIP genes showed differential expression (11 upregulated and seven downregulated) following MeJA treatment, 13 VvbZIP genes were affected (four upregulated and nine downregulated) by SA treatment, and the expression of 24 VvbZIP genes was altered (four upregulated and 21 downregulated) by ABA treatment, and 12 VvbZIP genes showed differential expression (seven upregulated and five downregulated) following ET treatment alone (Fig. 7). It has been suggested that both ABA-dependent and ABA-independent regulatory systems are involved in osmotic stress tolerance in plants (Chinnusamy et al. 2004) and the majority of the VvbZIP genes showing significantly altered expression in response to salinity or drought stress were also ABA-responsive. For instance, the expression of *VvbZIP35* was significantly upregulated by salinity and drought stresses, and also by ABA, suggesting the involvement of the ABA-dependent regulatory system. This observation is consistent with a previous study of the rice ABA-inducible bZIP TF *OsbZIP05* (referred to as *OsbZIP53* in this study), which represents a closely related ortholog of *VvbZIP35* (Nijhawan et al. 2008), further indicating that the function of some bZIP proteins is conserved among plant species.

In addition, a total of 42 VvbZIP genes that could be associated with different stresses and hormone treatments, based on semiquantitative RT-PCR analysis (Fig. 7 and Supplementary Figs. S2–S8), were selected for further analysis and validation using real-time quantitative RT-PCR (Fig. 8 and Supplementary Fig. S9). The qRT-PCR analysis results mostly corroborated the expression profiles indicated by semi-qRT-PCR analysis. For example, expression of the *Arabidopsis* ABF/AREB orthologs *VvbZIP7* and *VvbZIP39* was highly induced by salt and drought stress, respectively, which is in agreement with the semi-qRT-PCR expression data (Figs. 7 and 8a). In most cases, a similar result was seen, including one gene (*VvbZIP32*) that was induced by all four hormone treatments, and four genes (*VvbZIP6*, *VvbZIP31*, *VvbZIP37*, and *VvbZIP34*) showed no significant difference in the real-time quantitative PCR under different hormone treatments, respectively. These results further confirm the reliability of our expression data (Fig. 8b).

We also analyzed the correlation between expression of different VvbZIP genes with their phylogenetic placement or gene duplication history. The 41 VvbZIP genes were clustered into seven main clades (one to seven), which contain 11, 11, 2, 7, 1, 1, and 8 *VvbZIP* genes, respectively (Fig. 7). By comparing the cluster of expression data with phylogeny, no correlation between gene evolution and expression profiles was apparent. In addition, we determined that some VvbZIP genes within the same segmental duplicated pair, and which grouped closely in the phylogenetic tree, usually had similar expression patterns. For example, we identified genes within two pairs *VvbZIP7*/*VvbZIP39* and *VvbZIP3*/*VvbZIP35* that were all significantly induced by salt and drought stresses, while the pair *VvbZIP14*/*VvbZIP29* shared similar expression patterns in response to hormone treatments (Fig. 7). Some VvbZIP genes predicted to have same conserved motifs and those possessing the same exon/intron structures were also found to exhibit similar expression profiles, such as *VvbZIP9* and *VvbZIP12*, which were both upregulated by MeJA treatment (Fig. 7). In contrast, it was found that the *VvbZIP* genes (*VvbZIP16*/*VvbZIP17*), established by tandem duplication, not only grouped in different phylogenetic clades but also exhibited distinctly different expression patterns. Since expression profiles are highly correlated with gene function, this may mean that grape bZIP genes produced by segmental duplication rarely diverge with respect to their original function, whereas the function of genes produced by tandem duplication has been altered much due to long-term evolution. Similar phenomena have

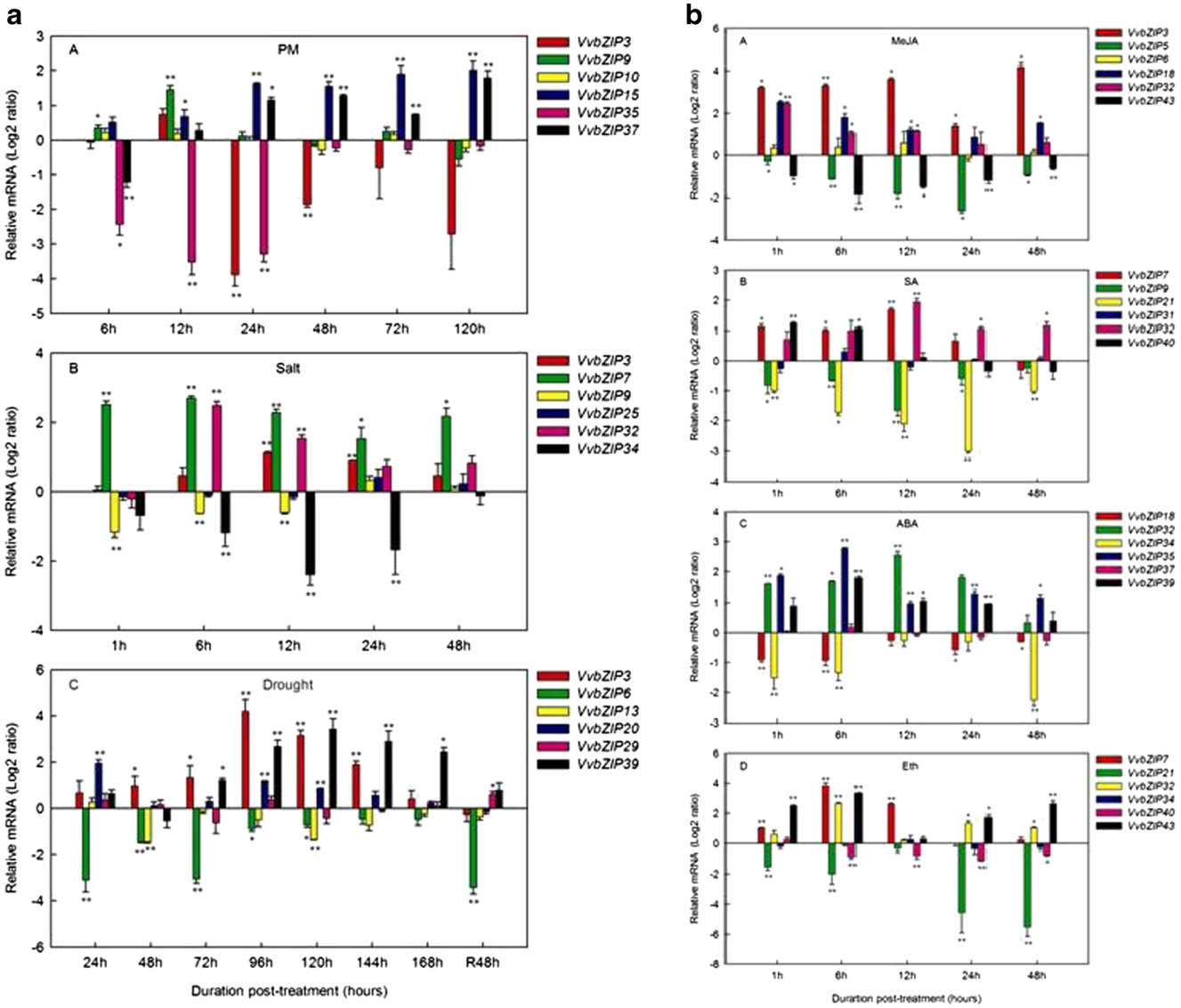

Fig. 8 Real-time quantitative PCR expression levels of selected VvbZIP genes following powdery mildew inoculation, salt stress, drought stress treatments, and various hormone treatments. **a** Expression levels of selected VvbZIP genes under biotic stress (powdery mildew) and abiotic stresses (salt and drought). **b** Expression levels of selected *VvbZIP* gene under hormone (MeJA, SA, ABA Eth) treatments. The expression levels were normalized to 6 h (powdery mildew inoculation), 1 h (salt stress treatment), 24 h (drought stress treatment), and 1 h (hormone treatments) CK sample, respectively (original results shown in Fig. S9). Mean values and SDs were obtained from three biological and three technical replicates. *Asterisks* indicate the corresponding gene significantly up- or downregulated under the differential treatment by *t* test (**P*<0.05, ***P*<0.01)

also been observed in previous studies (Wang et al. 2011b; Flagel and Wendel 2009).

Conclusion

We performed extensive analyses to identify and describe key attributes of uncharacterized bZIP TFs from the grapevine genome and compared them with those of *Arabidopsis* and rice. We identified 47 grape bZIP TFs and present here a unified nomenclature according to their chromosome location. Given that the functions of many *Arabidopsis* bZIP TFs have been well characterized, we then assigned *Arabidopsis* orthologs to each grape bZIP protein, in order to infer their functions. Subsequently, we classified the 47 VvbZIP genes into 13 distinct groups by comparative phylogenetic analysis with *Arabidopsis* and rice bZIP TFs and showed that the groups are supported by the predicted phylogeny, additional protein motifs, and intron/exon structures. This phylogenetic analysis supported previous results and indicated that the grape bZIPs are more closely related to those of *Arabidopsis* than to those of rice. Furthermore, our data suggest that segmental duplications have contributed more than tandem duplications to the expansion of the grape bZIP gene family.

In addition, synteny analysis between grape and *Arabidopsis* showed that the majority of grape and *Arabidopsis* bZIP genes are located in syntenic regions, indicating common ancestry. Finally, we carried out an expression analysis to reveal organ-specific, biotic and abiotic stress and hormone-responsive grape bZIP genes, and the extensive expression data supported the hypothesis that VvbZIP genes perform a variety of functions in different organs and are involved in tolerance to environmental and biotic stresses.

Acknowledgments The authors thank Dr. Yi Zheng for providing help with bioinformatics analysis. This work was supported by the National Natural Science Foundation of China grant no. 31272136 and the Program for Innovative Research Team of Grape Germplasm Resources and Breeding grant no. 2013KCT-25 to X.W.

References

Amoutzias G, Veron A, Weiner J, Robinson-Rechavi M, Bornberg-Bauer E, Oliver S, Robertson D (2007) One billion years of bZIP transcription factor evolution: conservation and change in dimerization and DNA-binding site specificity. Mol Biol Evol 24(3):827–835

Büttner M, Singh KB (1997) *Arabidopsis thaliana* ethylene-responsive element binding protein (AtEBP), an ethylene-inducible, GCC box DNA-binding protein interacts with an *ocs* element binding protein. Proc Natl Acad Sci U S A 94(11):5961–5966

Barbosa EGG, Leite JP, Marin SRR, Marinho JP, Carvalho JFC, Fuganti-Pagliarini R, Farias JRB, Neumaier N, Marcelino-Guimarães FC, de Oliveira MCN (2013) Overexpression of the ABA-dependent AREB1 transcription factor from *Arabidopsis thaliana* improves soybean tolerance to water deficit. Plant Mol Biol Rep 31(3):719–730

Bari R, Jones JD (2009) Role of plant hormones in plant defence responses. Plant Mol Biol 69(4):473–488

Bentsink L, Jowett J, Hanhart CJ, Koornneef M (2006) Cloning of DOG1, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. Proc Natl Acad Sci U S A 103(45):17042–17047

Boneh U, Biton I, Zheng C, Schwartz A, Ben-Ari G (2012) Characterization of potential ABA receptors in *Vitis vinifera*. Plant Cell Rep 31(2):311–321

Cannon SB, Mitra A, Baumgarten A, Young ND, May G (2004) The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. BMC Plant Biol 4(1):10

Carretero-Paulet L, Galstyan A, Roig-Villanova I, Martínez-García JF, Bilbao-Castro JR, Robertson DL (2010) Genome-wide classification and evolutionary analysis of the bHLH family of transcription factors in Arabidopsis, poplar, rice, moss, and algae. Plant Physiol 153(3):1398–1412

Chinnusamy V, Schumaker K, Zhu JK (2004) Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. J Exp Bot 55(395):225–236

Chuang C-F, Running MP, Williams RW, Meyerowitz EM (1999) The PERIANTHIA gene encodes a bZIP protein involved in the determination of floral organ number in *Arabidopsis thaliana*. Genes Dev 13(3):334–344

Corrêa LGG, Riaño-Pachón DM, Schrago CG, Dos Santos RV, Mueller-Roeber B, Vincentz M (2008) The role of bZIP transcription factors in green plant evolution: adaptive features emerging from four founder genes. PLoS One 3(8):e2944

Deppmann CD, Alvania RS, Taparowsky EJ (2006) Cross-species annotation of basic leucine zipper factor interactions: insight into the evolution of closed interaction networks. Mol Biol Evol 23(8):1480–1492

Du H, Wang Y-B, Xie Y, Liang Z, Jiang S-J, Zhang S-S, Huang Y-B, Tang Y-X (2013) Genome-wide identification and evolutionary and expression analyses of MYB-related genes in land plants. DNA Res 20(5):437–448

Feller A, Machemer K, Braun EL, Grotewold E (2011) Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. Plant J 66(1):94–116

Ferreira RB, Monteiro SS, Piçarra-Pereira MA, Teixeira AR (2004) Engineering grapevine for increased resistance to fungal pathogens without compromising wine stability. Trends Biotechnol 22(4):168–173

Flagel LE, Wendel JF (2009) Gene duplication and evolutionary novelty in plants. New Phytol 183(3):557–564

Fujita Y, Fujita M, Satoh R, Maruyama K, Parvez MM, Seki M, Hiratsu K, Ohme-Takagi M, Shinozaki K, Yamaguchi-Shinozaki K (2005) AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in *Arabidopsis*. Plant Cell 17(12):3470–3488

Fukazawa J, Sakai T, Ishida S, Yamaguchi I, Kamiya Y, Takahashi Y (2000) Repression of shoot growth, a bZIP transcriptional activator, regulates cell elongation by controlling the level of gibberellins. Plant Cell 12(6):901–915

Gao M, Wang Q, Wan R, Fei Z, Wang X (2012) Identification of genes differentially expressed in grapevine associated with resistance to *Elsinoe ampelina* through suppressive subtraction hybridization. Plant Physiol Biochem 58:253–268

García MNM, Giammaria V, Grandellis C, Téllez-Iñón MT, Ulloa RM, Capiati DA (2012) Characterization of StABF1, a stress-responsive bZIP transcription factor from *Solanum tuberosum* L. that is phosphorylated by StCDPK2 in vitro. Planta 235(4):761–778

Guan Y, Ren H, Xie H, Ma Z, Chen F (2009) Identification and characterization of bZIP-type transcription factors involved in carrot (*Daucus carota* L.) somatic embryogenesis. Plant J 60(2):207–217

Guo R, Xu X, Carole B, Li X, Gao M, Zheng Y, Wang X (2013) Genome-wide identification, evolutionary and expression analysis of the aspartic protease gene superfamily in grape. BMC Genomics 14:554

He S, Shan W, Kuang J-f, Xie H, Xiao Y-y, Lu W-j, Chen J-y (2013) Molecular characterization of a stress-response bZIP transcription factor in banana. Plant Cell Tiss Org Cul 113(2):173–187

Hurst HC (1994) Transcription factors 1: bZIP proteins. Protein Profile 2(2):101–168

Jaillon O, Aury J-M, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449(7161):463–467

Jakoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in *Arabidopsis*. Trends Plant Sci 7(3):106–111

Kang J-y, Choi H-i, Im M-y, Kim SY (2002) Arabidopsis basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. Plant Cell 14(2):343–357

Kobayashi F, Maeta E, Terashima A, Takumi S (2008) Positive role of a wheat *HvABI5* ortholog in abiotic stress response of seedlings. Physiol Plant 134(1):74–86

Koonin EV (2005) Orthologs, paralogs, and evolutionary genomics. Annu Rev Genet 39:309–338

Larkin M, Blackshields G, Brown N, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23(21):2947–2948

Letunic I, Doerks T, Bork P (2012) SMART 7: recent updates to the protein domain annotation resource. Nucleic Acid Res 40(D1):D302–D305

Li HE, Xu Y, Xiao Y, Zhu ZG, Xie XQ, Zhao HQ, Wang YJ (2010) Expression and functional analysis of two genes encoding

transcription factors, *VpWRKY1* and *VpWRKY2*, isolated from Chinese wild *Vitis pseudoreticulata*. Planta 232(6):1325–1337

Li X, Duan X, Jiang H, Sun Y, Tang Y, Yuan Z, Guo J, Liang W, Chen L, Yin J (2006) Genome-wide analysis of basic/helix-loop-helix transcription factor family in rice and Arabidopsis. Plant Physiol 141(4): 1167–1184

Liao Y, Zou H-F, Wei W, Hao Y-J, Tian A-G, Huang J, Liu Y-F, Zhang J-S, Chen S-Y (2008) Soybean *GmbZIP44*, *GmbZIP62* and *GmbZIP78* genes function as negative regulator of ABA signaling and confer salt and freezing tolerance in transgenic *Arabidopsis*. Planta 228(2):225–240

Licausi F, Giorgi F, Zenoni S, Osti F, Pezzotti M, Perata P (2010) Genomic and transcriptomic analysis of the AP2/ERF superfamily in *Vitis vinifera*. BMC Genomics 11(1):719

Liu C, Wu Y, Wang X (2012) bZIP transcription factor *OsbZIP52*/*RISBZ5*: a potential negative regulator of cold and drought stress response in rice. Planta 235(6):1157–1169

Mallappa C, Yadav V, Negi P, Chattopadhyay S (2006) A basic leucine zipper transcription factor, G-box-binding factor 1, regulates blue light-mediated photomorphogenic growth in Arabidopsis. J Biol Chem 281(31):22190–22199

Meier I, Gruissem W (1994) Novel conserved sequence motifs in plant G-box binding proteins and implications for interactive domains. Nucleic Acid Res 22(3):470–478

Meng X, Zhao W, Lin R, Wang M, Peng Y (2005) Identification of a novel rice bZIP-type transcription factor gene, *OsbZIP1*, involved in response to infection of *Magnaporthe grisea*. Plant Mol Biol Rep 23(3):301–302

Nakano T, Suzuki K, Fujimura T, Shinshi H (2006) Genome-wide analysis of the ERF gene family in Arabidopsis and rice. Plant Physiol 140(2):411–432

Niggeweg R, Thurow C, Kegler C, Gatz C (2000) Tobacco transcription factor TGA2.2 is the main component of as-1-binding factor ASF-1 and is involved in salicylic acid- and auxin-inducible expression of as-1-containing target promoters. J Biol Chem 275(26):19897–19905

Nijhawan A, Jain M, Tyagi AK, Khurana JP (2008) Genomic survey and gene expression analysis of the basic leucine zipper transcription factor family in rice. Plant Physiol 146(2):333–350

Paterson AH, Wang X, Tang H, Lee TH (2012) Synteny and genomic rearrangements. Plant Gen Diver 1:195–207

Peng S, Zhu Z, Zhao K, Shi J, Yang Y, He M, Wang Y (2013) A novel heat shock transcription factor, *VpHsf1*, from Chinese wild *Vitis pseudoreticulata* is involved in biotic and abiotic stresses. Plant Mol Biol Rep 31(1):240–247

Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521

Pires N, Dolan L (2010) Origin and diversification of basic-helix-loop-helix proteins in plants. Mol Biol Evol 27(4):862–874

Pontier D, Miao ZH, Lam E (2001) Trans-dominant suppression of plant TGA factors reveals their negative and positive roles in plant defense responses. Plant J 27(6):529–538

Rice P, Longden I, Bleasby A (2000) EMBOSS: the European molecular biology open software suite. Trends Genet 16(6):276–277

Riechmann J, Heard J, Martin G, Reuber L, Keddie J, Adam L, Pineda O, Ratcliffe O, Samaha R, Creelman R (2000) Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science 290(5499):2105–2110

Schultz J, Milpetz F, Bork P, Ponting CP (1998) SMART, a simple modular architecture research tool: identification of signaling domains. Proc Natl Acad Sci U S A 95(11):5857–5864

Siberil Y, Doireau P, Gantet P (2001) Plant bZIP G-box binding factors. Eur J Biochem 268(22):5655–5666

Silveira AB, Gauer L, Tomaz JP, Cardoso PR, Carmello-Guerreiro S, Vincentz M (2007) The Arabidopsis AtbZIP9 protein fused to the VP16 transcriptional activation domain alters leaf and vascular development. Plant Sci 172(6):1148–1156

Singh AK, Sharma V, Pal AK, Acharya V, Ahuja PS (2013) Genome-wide organization and expression profiling of the NAC transcription factor family in potato (*Solanum tuberosum* L.). DNA Res 20(4):403–423

Sohn KH, Lee SC, Jung HW, Hong JK, Hwang BK (2006) Expression and functional roles of the pepper pathogen-induced transcription factor RAV1 in bacterial disease resistance, and drought and salt stress tolerance. Plant Mol Biol 61(6):897–915

Tak H, Mhatre M (2013) Cloning and molecular characterization of a putative bZIP transcription factor VvbZIP23 from *Vitis vinifera*. Protoplasma 250(1):333–345

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10):2731–2739

This P, Lacombe T, Thomas MR (2006) Historical origins and genetic diversity of wine grapes. Trends Genet 22(9):511–519

Upreti K, Murti G (2010) Response of grape rootstocks to salinity: changes in root growth, polyamines and abscisic acid. Biol Plant 54(4):730–734

Wang J, Zhou J, Zhang B, Vanitha J, Ramachandran S, Jiang SY (2011a) Genome-wide expansion and expression divergence of the basic leucine zipper transcription factors in higher plants with an emphasis on sorghum. J Integr Plant Biol 53(3):212–231

Wang LJ, Li SH (2006) Thermotolerance and related antioxidant enzyme activities induced by heat acclimation and salicylic acid in grape (*Vitis vinifera* L.) leaves. Plant Grow Reg 48(2):137–144

Wang N, Xiang Y, Fang L, Wang Y, Xin H, Li S (2013) Patterns of gene duplication and their contribution to expansion of gene families in grapevine. Plant Mol Biol Rep 31:852–861

Wang Y, Liu Y, He P, Chen J (1995) Evaluation of foliar resistance to *Uncinula necator* in Chinese wild *Vitis* species. Vitis 34(3):159–164

Wang Y, Wang X, Tang H, Tan X, Ficklin SP, Feltus FA, Paterson AH (2011b) Modes of gene duplication contribute differently to genetic novelty and redundancy, but show parallels across divergent angiosperms. PLoS One 6(12):e28150

Xiang Y, Tang N, Du H, Ye H, Xiong L (2008) Characterization of *OsbZIP23* as a key player of the basic leucine zipper transcription factor family for conferring abscisic acid sensitivity and salinity and drought tolerance in rice. Plant Physiol 148(4):1938–1952

Xiao HG, Nassuth A (2006) Stress- and development-induced expression of spliced and unspliced transcripts from two highly similar dehydrin 1 genes in *V. riparia* and *V. vinifera*. Plant Cell Rep 25(9):968–977

Yamasaki K, Kigawa T, Seki M, Shinozaki K, Yokoyama S (2012) DNA-binding domains of plant-specific transcription factors: structure, function, and evolution. Trends Plant Sci 18:267–276

Yang D-L, Yang Y, He Z (2013) Role of plant hormones and their cross-talks in rice immunity. Mol Plant 6:675–685

Zhang X, Wollenweber B, Jiang D, Liu F, Zhao J (2008) Water deficits and heat shock effects on photosynthesis of a transgenic *Arabidopsis thaliana* constitutively expressing ABP9, a bZIP transcription factor. J Exp Bot 59(4):839–848

Zhang Y, Mao L, Wang H, Brocker C, Yin X, Vasiliou V, Fei Z, Wang X (2012) Genome-wide identification and analysis of grape aldehyde dehydrogenase (ALDH) gene superfamily. PloS One 7(2):e32153

Zhuang J, Peng R, Cheng Z, Zhang J, Cai B, Zhang Z, Gao F, Zhu B, Fu X, Jin X (2009) Genome-wide analysis of the putative AP2/ERF family genes in *Vitis vinifera*. Sci Hortic 123(1):73–81

Zou M, Guan Y, Ren H, Zhang F, Chen F (2008) A bZIP transcription factor, *OsABI5*, is involved in rice fertility and stress tolerance. Plant Mol Biol 66(6):675–683