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ORIGINAL ARTICLE

Genome-wide identification and expression analysis of the bZIP gene family in apple (*Malus domestica*)

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Abstract The basic leucine zipper (bZIP) family is one of the largest transcription factor (TF) families in plants, which play crucial roles in plant growth and development. bZIP proteins are involved in multiple biological processes, as well as responses to various biotic/abiotic stresses. Although genome-wide analysis of the *bZIP* gene family has been conducted in several plant species, only few comprehensive characterization of this gene family has been reported in apple (*Malus domestica*), an important tree fruit in the *Rosaceae* family. In this study, we identified 114 *bZIP* genes from the apple genome, which were divided into 10 subgroups based on their sequences. We further characterized these *MdbZIP* genes in terms of gene structure, protein model, and chromosomal distribution. Genome-wide expression profile of *MdbZIP* genes indicated that 14 *MdbZIP*s were highly expressed during apple fruit development, and 17 *MdbZIP*s showed differential expression in leaves and mature apple fruit. Analysis of the expression of 16 *MdbZIP*s under drought and salt stresses in apple leaves and roots using quantitative real-time PCR (qRT-PCR) indicated that they all exhibited differential transcript levels in both treatments, suggesting that *MdbZIP* genes are involved in abiotic stress responses. The genome-wide identification, characterization, and expression analysis of apple *bZIP* genes provide new insights into the roles of the bZIP TF family and lay a solid foundation for future cloning and functional analysis of this gene family, which may be used to manipulate apple growth and development and improve its stress tolerance.

Keywords Apple · bZIP transcription factor · Expression analysis · Drought stress · Salt stress

Yuan-Yuan Li and Dong Meng contributed equally to this work.

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Introduction

Transcription factors play crucial roles in regulating gene networks in plants by binding to the promoters of the corresponding genes, thereby activating and/or repressing their expression. Typically, a transcription factor has the following four regions working together to regulate a wide range of biological processes: a DNA-binding motif, a transcript-activation motif, a nuclear localization signal, and an oligomerization site (Du et al. 2012).

Transcription factors can be classified into different families based on their DNA binding domains. Currently, 64 transcription factor (TF) families have been found in the plant kingdom (Perez-Rodriguez et al. 2010). Among them, the basic leucine zipper (bZIP) TF family is one of the largest and most diverse families in eukaryotes (Nijhawan et al. 2008). The bZIP TFs are named based on their common feature, the conserved bZIP domain, which is 60–80 amino acids in length and composed of two structural regions, a basic region and a leucine zipper region (Talanian et al. 1990). The basic region of 16 amino acids with an invariant motif

N-×7-R/K-×9 is primarily responsible for nuclear localization and DNA binding, whereas the leucine zipper region mediates the homo- and/or hetero-dimerization of bZIP proteins. The leucine zipper consists of several heptad repeats of Leu or other bulky hydrophobic amino acids, such as lle, Val, Phe, or Met, positioned exactly nine amino acids toward the C-terminus (Landschulz et al. 1988; Ellenberger et al. 1992).

Many members of the bZIP TF family have been identified or predicted in multiple eukaryotic genomes, including plants. It has been reported that there are 75 *bZIP*s in *Arabidopsis* (Jakoby et al. 2002), 89 in rice (*Oryza sativa*) (Nijhawan et al. 2008), 92 in sorghum (Wang et al. 2011), 131 in soybean (*Glycine max*) (Liao et al. 2008), 170 in maize (*Zea mays*) (Wei et al. 2012), 64 in cucumber (Baloglu et al. 2014), 96 in *Brachypodium distachyon* (Liu and Chu 2015), 69 in tomato (*Solanum lycopersicum* L.) (Li et al. 2015), and 55 in grapevine (*Vitis vinifera*) (Liu et al. 2014a, b), but only a small number of *bZIP*s have been functionally characterized in plants. bZIP TFs are involved in diverse biological and physiological processes in plant growth and development, as well as responses to biotic/abiotic stress. bZIP proteins have been found to participate in organ/tissue differentiation processes, such as seed maturation and germination, floral transition and initiation, embryogenesis, and vascular development (Toh et al. 2012; Izawa et al. 1994; Walsh and Freeling 1999; Wigge et al. 2005; Guan et al. 2009; Yin et al. 1997). In addition, the bZIP TFs play important roles in regulating various stress responses, including drought, salinity, osmotic, cold, and heat stress, ABA signaling, and pathogen defense (Chen et al. 2012; Hsieh et al. 2012; Rook et al. 1998; Shimizu et al. 2005; Liu et al. 2012; Lopez-Molina et al. 2002; Thurow et al. 2005). For example, the transcript level of *OsbZIP16* was dramatically induced in rice plants under drought and exogenous ABA treatment (Chen et al. 2012). In tomato, a bZIP transcription factor, *SlAREB*, is involved in responses to water deficit and salt stress (Hsieh et al. 2012). In rice, bZIP proteins LIP19 and OsOBF1 interact together to bind to the C/G hybrid sequence, but their expression patterns were opposite in response to low temperatures (Shimizu et al. 2005). In grapevine, some of the bZIP transcription factors respond to heat based on microarray analysis (Liu et al. 2012).

Apple is one of the most important fruit crops worldwide. Sequencing of the apple genome has found that a relatively recent genome-wide duplication event contributed to its large genome size and expansion of gene families (Velasco et al. 2010). However, only few comprehensive characterization of the bZIP gene family has been reported in apple (Wang et al. 2015). In addition, because apple trees are often exposed to multiple environmental stresses, it is necessary to understand the roles of the *bZIP* family in stress responses. In this study, we identified all the *bZIP* genes in apple genome and analyzed the gene structure, protein modeling, and chromosomal distribution of the *bZIP*s in apple. Furthermore, we determined the differential expression profiles of *MdbZIP* genes at different fruit development stages and between mature apple fruit and leaves based on our RNA-seq data. We also quantified the transcript levels of 16 selected *MdbZIP* genes in an apple rootstock G41 in response to salt and drought stresses using quantitative real-time PCR (qRT-PCR).

Materials and methods

Database search and identification of *MdbZIP* genes

Based on the features of the *bZIP* genes in *Arabidopsis thaliana*, we used two different approaches to identify the candidate *bZIP* genes in *Malus domestica*. First, the sequences of the *Arabidopsis* bZIP proteins were downloaded from the TAIR website (http://www.Arabidopsis.org/). A BLASTP search was performed through the Genome Database for *Rosaceae* (GDR; https://www.rosaceae.org/) using *Arabidopsis* bZIP proteins as query sequences. Second, a bZIP domain (cd14703) was used as query sequence to search the apple genome. After the batch BLAST searches, we also checked the MdbZIP TFs in the PlantTFDB database (http://planttfdb.cbi.pku.edu.cn/) to retrieve any additional *MdbZIP* genes. Each candidate *MdbZIP* sequence was confirmed as a member of MdbZIP family through the following two databases: the SMART database (http://smart.embl-heidelberg.de/) and the NCBI Conserved Domains (CD) search tools (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Redundant sequences or sequences lacking the *bZIP* domain were removed.

Phylogenetic classification, gene and protein structure, and chromosomal locations of the *MdbZIP*s

Twenty-one AtbZIP proteins (subgroup A, AtbZIP12, 35, and 67; subgroup B, AtbZIP28 and 49; subgroup C, AtbZIP9 and 10; subgroup D, AtbZIP45 and 50; subgroup E, AtbZIP34 and 61; subgroup F, AtbZIP19 and 23; subgroup G, AtbZIP16 and 41; subgroup H, AtbZIP56 and 64; subgroup I, AtbZIP18 and 29; subgroup S, AtbZIP1 and 2) were selected from ten different subgroups of AtbZIP proteins (Marc Jakoby et al. 2002). The protein sequences were downloaded from the TAIR website (http://www.Arabidopsis.org/) and used as references to categorize the MdbZIP proteins using the MUSCLE algorithm integrated into the MEGA6.0 package (Tamura et al. 2013). Phylogenetic analysis was conducted with MEGA version 6.0 (http://www.megasoftware.net/) using the neighbor-joining method, and bootstrap values were calculated using 1000 iterations. All of the 114 MdbZIP protein structures were analyzed using the MEME website (http://meme-suite.org/), resulting in the identification of five potential protein motifs with sizes ranging from 6 to 50 amino

acids. The physical distribution of the *MdbZIP* genes on the apple chromosomes was drawn using MapChart based on the gene's position in the GDR (https://www.rosaceae.org/) (Velasco et al. 2010). The markers of apple genome were also chosen from the GDR website.

Analysis of gene expression patterns

RNA-seq data were generated from "Greensleeves" apple fruit taken at five developmental stages (16, 41, 70, 94, and 128 days after bloom (DAB)) and recently fully expanded leaves during active shoot growth, with three biological replicates, to determine the expression patterns of the apple *bZIP* genes. The methods of RNA-seq library construction and data analysis were previously described by Wang et al. (2011). Total RNA was isolated using a modified cetyltrimethylammonium bromide (CTAB) method (Gasic et al. 2004). The total RNA was dissolved in EB buffer (Qiagen, Germantown, MD). Then, the RNA samples were treated with DNase I (amplification grade, Invitrogen) at 37 °C for 30 min, followed by heat inactivation at 65 °C for 15 min. Two-percent agarose gel was used to evaluate the RNA quality, and NanoDrop 1000 (Thermo Scientific, Waltham, MA) was also used. After that, the NEB Next Poly (A) mRNA Magnetic Isolation Module and NEB Next Ultra Directional RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA) were applied for messenger RNA (mRNA) isolation and strand-specific RNA-seq library preparation. Cluster 3.0 software (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm) was applied to analyze the RNA-seq data. Centroid-linkage hierarchical clustering was performed with log2-based fluorescence intensity values to obtain a better estimation of small differences in gene transcription. Then, all the RPKM values of *MdbZIP* genes were selected through its GDR ID number in the RNA-seq database. Heat maps for the *MdbZIP* genes were generated by MeV (Multiple Experiment Viewer) v4.8 software (http://www.tm4.org/).

RNA isolation and quantitative real-time PCR

Total RNA was isolated from young leaves and roots of an apple rootstock "G41" using a modified CTAB method (Gasic et al. 2004). After treatment with RQ1 DNase (Promega, WI, USA), cDNA was reverse-transcribed from 1-μg total RNA using the iScript cDNA Synthesis Kit (Bio-Rad, CA, USA). The specific quantitative primers for the *MdbZIP* genes are listed in the supplemental materials (Table S1). qRT-PCR was performed on an Icycler iQ5 system (Bio-Rad) using the SYBR Green Supermix Kit (Bio-Rad), following the manufacturer's instruction. *Md18S* was used as loading controls (Table S1). Each reaction was replicated three times. The relative expression level of each *MdbZIP* gene was calculated as $2^{-\Delta CT}$ values compared to that of untreated control plants (Udrardi et al. 2008).

Analysis of the interaction network of MdbZIP2

To better understand the interaction between MdbZIP2 and other proteins, we used the STRING website version 10.0 (http://string-db.org/cgi/input.pl? UserId=HiT0uy0jZlny&sessionId=kUKdHCtpMK5M&input_page_active_form=single_sequence) to search the protein interaction network followed by Szklarczyk et al. (2014). Briefly, we used the "single protein by sequence" and "*A. thaliana*" as method and organism, respectively. The apple MdbZIP2 amino acid sequence was applied to search the STRING software database by the BLASTP method with default parameters. Then, a list of proteins in the database which are similar to MdbZIP2 sequence was found. We chose the most similar one, *A. thaliana* bZIP2, according to the bit score, E-value, and the conserved domain analysis (Fig. 2) to establish the protein interaction network chart.

Plant materials, growth conditions, and stress treatment

Plants of G41, an apple rootstock, were grown in 10-cm-diameter × 9-cm-tall pots with a 3:1 ratio mixture of Cornell mix and sand in a greenhouse with a day/night temperature of 25/15 °C and a photoperiod of 14 h with supplemental lights at Cornell University in Ithaca, NY, USA. Control pots were watered to dripping each day. For the drought stress treatment and recovery, water was withheld for 5 days, and then, the plants were re-watered for 2 days the same way as the controls. For the salt stress treatment, each pot was watered with 200 ml, 100 mM NaCl for 7 days. There were five replicates for each treatment with three plants per replicate in a completely randomized design. Root and leaf samples were collected at 5 h into the photoperiod on days 0, 1, 3, 5, and 7 after the initiation of the treatments, frozen in liquid nitrogen, and stored at −80 °C for further analysis.

Results and discussion

Genome-wide identification of the apple bZIP transcriptional factor family

To identify the apple *bZIP* gene family, the entire apple genome was searched for genes that encode proteins containing the bZIP domain. BLASTP searches were performed using the conserved bZIP domain (cd14703) sequence of *Arabidopsis* as a query with the default E-value. One-hundred-fourteen sequences were initially obtained using an

Table 1 List of the identified *bZIP* genes in apple genome

Name	GDR number	Chromosome	Number of amino acids	Molecular weight	Theoretical pI
MdbZIP1	MDP0000197219	chr1:10,520,899.10,523,983	451	48,472.4	8.47
MdbZIP2	MDP0000249561	chr2:12,516,047.12,516,520	157	17,825.9	5.19
MdbZIP3	MDP0000265875	chr2:12,713,645.12,714,118	157	17,825.1	5.73
MdbZIP4	MDP0000251332	chr2:13,279,945.13,285,109	386	40,506.4	5.78
MdbZIP5	MDP0000273211	chr2:30,554,835.30,560,990	528	59,249.7	9.44
MdbZIP6	MDP0000300532	chr2:32,162,650.32,171,692	285	32,201.5	7.03
MdbZIP7	MDP0000174930	chr2:32,167,138.32,171,496	300	34,060.7	8.61
MdbZIP8	MDP0000898701	chr2:34,429,393.34,430,130	242	26,820.8	6.79
MdbZIP9	MDP0000893802	chr2:34,458,341.34,459,078	242	26,820.8	6.79
MdbZIP10	MDP0000190186	chr2:34,462,771.34,463,508	242	26,820.8	6.79
MdbZIP11	MDP0000270677	chr2:35,004,195.35,008,282	754	81,263.8	6.13
MdbZIP12	MDP0000180785	chr2:35,007,856.35,011,943	753	81,150.7	6.13
MdbZIP13	MDP0000293847	chr3:4,682,921.4,686,053	451	48,229.2	5.92
MdbZIP14	MDP0000120158	chr3:4,782,005.4,784,925	469	50,848	6.66
MdbZIP15	MDP0000295681	chr3:4,782,814.4,784,704	320	34,305.3	5.62
MdbZIP16	MDP0000222114	chr3:5,620,437.5,623,701	280	31,965.4	9.76
MdbZIP17	MDP0000301399	chr3:5,626,357.5,631,201	340	37,276.5	6.87
MdbZIP18	MDP0000488746	chr3:5,626,620.5,631,464	335	36,778	7.75
MdbZIP19	MDP0000772665	chr3:5,637,807.5,639,294	281	31,955.9	5.46
MdbZIP20	MDP0000134936	chr3:27,871,861.27,873,934	351	39,666.7	6.5
MdbZIP21	MDP0000437680	chr3:33,166,561.33,166,989	141	16,220.2	9.09
MdbZIP22	MDP0000247372	chr4:3,234,880.3,237,744	420	46,849.3	5.86
MdbZIP23	MDP0000834642	chr4:6,813,691.6,815,518	213	23,784.6	9.09
MdbZIP24	MDP0000231542	chr4:8,917,835.8,924,220	1000	112,419.5	8.83
MdbZIP25	MDP0000275309	chr5:14,238,089.14,245,492	794	85,591	7.7
MdbZIP26	MDP0000248567	chr5:27,744,402.27,746,779	431	46,567.5	9.62
MdbZIP27	MDP0000185553	chr6:3,270,543.3,277,761	660	71,613.3	9.1
MdbZIP28	MDP0000917315	chr6:22,353,119.22,353,583	154	17,582.8	6.98
MdbZIP29	MDP0000299504	chr7:1,372,519.1,376,449	735	79,183.1	6.16
MdbZIP30	MDP0000138811	chr7:1,379,763.1,383,691	735	79,183.1	6.16
MdbZIP31	MDP0000378041	chr7:1,391,809.1,395,238	99	11,410.5	9.29
MdbZIP32	MDP0000772633	chr7:1,913,688.1,914,425	241	26,639.7	6.74
MdbZIP33	MDP0000320524	chr7:3,433,485.3,446,567	526	59,310.2	9.6
MdbZIP34	MDP0000215106	chr7:4,115,354.4,119,277	408	45,853.3	8.8
MdbZIP35	MDP0000138052	chr7:15,981,497.15,984,608	438	47,155.5	6.12
MdbZIP36	MDP0000493795	chr7:15,982,839.15,984,782	278	29,814.5	6.67
MdbZIP37	MDP0000282828	chr8:1,209,801.1,216,496	1162	127,556.3	6.43
MdbZIP38	MDP0000300820	chr8:1,214,016.1,216,474	508	55,523.3	5.94
MdbZIP39	MDP0000521934	chr8:1,641,214.1,641,684	152	17,148	6.58
MdbZIP40	MDP0000205823	chr8:2,303,356.2,303,835	159	17,718.6	5.93
MdbZIP41	MDP0000891108	chr8:6,132,321.6,132,899	192	22,518.9	6.34
MdbZIP42	MDP0000319187	chr8:8,925,905.8,927,336	356	39,426.9	7.92
MdbZIP43	MDP0000140166	chr8:11,589,213.11,589,767	183	21,311.3	9.12
MdbZIP44	MDP0000200822	chr8:11,591,288.11,591,746	152	17,657	9.09
MdbZIP45	MDP0000701734	chr8:13,223,842.13,234,772	637	69,192.2	8.94
MdbZIP46	MDP0000250947	chr8:22,548,807.22,554,714	526	57,578.2	4.44
MdbZIP47	MDP0000706379	chr8:27,911,843.27,914,522	296	31,746.1	6.27
MdbZIP48	MDP0000738631	chr8:28,097,624.28,098,310	225	25,843.3	10.62
MdbZIP49	MDP0000407755	chr9:4,760,296.4,760,904	202	23,310.7	5.63
MdbZIP50	MDP0000178326	chr9:32,583,190.32,585,878	328	36,548.5	6.46
MdbZIP51	MDP0000306302	chr9:32,586,980.32,588,028	272	30,181.4	6.37
MdbZIP52	MDP0000141948	chr10:1,902,910.1,910,401	909	101,407.7	9.13
MdbZIP53	MDP0000208334	chr10:2,137,715.2,141,599	310	34,240.2	6.07
MdbZIP54	MDP0000159670	chr10:7,691,211.7,692,609	280	30,882.6	5.82
MdbZIP55	MDP0000176747	chr10:19,745,441.19,751,773	869	94,874.4	8.08
MdbZIP56	MDP0000740787	chr10:29,978,607.29,983,162	517	56,473.8	8.39
MdbZIP57	MDP0000234166	chr11:1,063,026.1,065,436	345	37,766.4	5.83
MdbZIP58	MDP0000297791	chr11:4,673,815.4,676,378	448	47,881.4	5.97
MdbZIP59	MDP0000305387	chr11:4,683,517.4,686,081	447	47,768.3	5.97
MdbZIP60	MDP0000239026	chr11:24,590,229.24,590,711	160	17,801.7	6.51
MdbZIP61	MDP0000133698	chr11:29,559,299.29,562,177	356	40,177.4	6.22
MdbZIP62	MDP0000234798	chr11:34,951,292.34,951,717	141	16,235.3	6.84
MdbZIP63	MDP0000949327	chr11:34,954,027.34,954,452	141	16,235.3	6.84
MdbZIP64	MDP0000177486	chr12:1,946,497.1,948,984	469	51,281.5	9.15
MdbZIP65	MDP0000144105	chr12:1,963,619.1,966,105	469	51,281.5	9.15

Table 1 (continued)

Name	GDR number	Chromosome	Number of amino acids	Molecular weight	Theoretical pI
MdbZIP66	MDP0000262210	chr12:11,304,636.11,311,688	534	59,899.6	9.67
MdbZIP67	MDP0000586302	chr12:17,051,287.17,053,624	163	17,860.6	8.01
MdbZIP68	MDP0000264514	chr12:17,054,996.17,059,849	227	24,940.7	9.35
MdbZIP69	MDP0000636541	chr12:20,963,304.20,970,848	484	53,568.8	9.49
MdbZIP70	MDP0000280559	chr12:21,390,957.21,393,913	580	63,173.7	6.86
MdbZIP71	MDP0000479652	chr12:21,395,646.21,398,279	527	57,466.2	6.83
MdbZIP72	MDP0000448715	chr12:21,746,621.21,747,094	157	17,683.7	7.03
MdbZIP73	MDP0000210251	chr12:26,145,161.26,147,824	359	39,925.2	6.08
MdbZIP74	MDP0000270365	chr12:30,191,422.30,194,800	432	47,325.9	5.8
MdbZIP75	MDP0000145555	chr13:2,703,862.2,707,043	463	51,595.5	5.85
MdbZIP76	MDP0000301884	chr13:2,710,089.2,713,271	464	51,758.7	5.85
MdbZIP77	MDP0000891899	chr13:3,386,464.3,387,078	203	23,302.8	5.93
MdbZIP78	MDP0000279891	chr13:10,470,937.10,474,463	361	41,605	5.13
MdbZIP79	MDP0000219041	chr13:10,499,732.10,501,144	241	27,260.4	9.02
MdbZIP80	MDP0000431572	chr13:31,289,060.31,292,057	342	37,146.2	4.92
MdbZIP81	MDP0000441891	chr13:31,289,069.31,292,136	342	37,146.2	4.92
MdbZIP82	MDP0000121603	chr14:1,336,067.1,339,625	506	55,645.4	7.2
MdbZIP83	MDP0000147745	chr14:1,336,237.1,339,584	582	63,666.4	8.54
MdbZIP84	MDP0000320322	chr14:7,564,687.7,571,368	540	60,317.3	6.81
MdbZIP85	MDP0000239688	chr14:14,476,695.14,489,099	854	95,687.7	7.94
MdbZIP86	MDP0000307943	chr14:24,064,421.24,072,646	489	54,771.4	6.64
MdbZIP87	MDP0000905135	chr14:26,993,073.26,993,594	173	19,765.1	7.07
MdbZIP88	MDP0000231274	chr14:28,607,551.28,610,272	351	36,636.1	5.66
MdbZIP89	MDP0000190277	chr15:1,339,936.1,340,394	152	17,760.2	8.52
MdbZIP90	MDP0000296303	chr15:2,474,633.2,485,694	823	91,869.2	8.79
MdbZIP91	MDP0000680042	chr15:4,062,791.4,065,343	326	35,775.5	5.76
MdbZIP92	MDP0000435971	chr15:11,167,017.11,170,907	441	48,315.2	5.85
MdbZIP93	MDP0000169473	chr15:17,793,553.17,794,938	286	31,000.1	9.03
MdbZIP94	MDP0000261154	chr15:20,032,093.20,041,062	852	97,230.5	9.03
MdbZIP95	MDP0000121258	chr15:30,826,162.30,830,577	518	59,001.7	5.7
MdbZIP96	MDP0000277999	chr15:36,408,113.36,420,573	688	77,528.5	8.69
MdbZIP97	MDP0000129112	chr15:40,776,203.40,779,181	488	53,421.3	6.45
MdbZIP98	MDP0000602946	chr15:41,411,070.41,414,903	420	46,439.1	9.13
MdbZIP99	MDP0000198495	chr15:44,193,653.44,196,263	385	42,041	5.84
MdbZIP100	MDP0000250967	chr16:1,376,596.1,386,527	1011	112,449.1	8.51
MdbZIP101	MDP0000183562	chr16:1,860,154.1,861,757	357	40,301.1	6.6
MdbZIP102	MDP0000286846	chr16:17,102,379.17,109,216	448	48,076.6	6.85
MdbZIP103	MDP0000863909	chr17:5,338,915.5,339,523	199	23,011.3	5.81
MdbZIP104	MDP0000555457	chr17:19,165,120.19,167,848	322	36,020.8	6.81
MdbZIP105	MDP0000120802	unanchored:7,122,677.7,124,802	439	48,304.3	5.98
MdbZIP106	MDP0000386314	unanchored:9,106,458.9,114,241	433	47,720	5.2
MdbZIP107	MDP0000374836	unanchored:14,508,562.14,511,180	441	49,194	7.56
MdbZIP108	MDP0000203904	unanchored:14,534,461.14,537,997	539	59,268.4	8.66
MdbZIP109	MDP0000536881	unanchored:15,746,255.15,752,194	450	49,693.6	7.16
MdbZIP110	MDP0000137206	unanchored:50,581,196.50,585,348	425	47,976.4	8.78
MdbZIP111	MDP0000545420	unanchored:73,542,720.73,544,664	277	29,717.4	6.67
MdbZIP112	MDP0000129203	unanchored:79,664,124.79,666,821	359	39,955.2	6.08
MdbZIP113	MDP0000123107	unanchored:97,931,795.97,932,619	229	25,293.7	6.06
MdbZIP114	MDP0000267964	unanchored:117,559,360.117,560,488	325	37,180.5	10.14

E-value <0.1 from the apple genome database (GDR; https://www.rosaceae.org/), and then, the SMART website (http://smart.embl-heidelberg.de/) was used to confirm the result. The 114 apple *bZIP* genes are named from *MdbZIP1* to *MdbZIP114* according to the gene coordinate order on apple chromosomes from top to bottom. The deduced molecular weights of the 114 MdbZIPs range from 11.41 to 127.56 kDa. The length of the 114 *bZIP* genes range from 99 to 1162 amino acids (AAs) with an average of 411 AAs. The nomenclature and related information are listed in Table 1. Compared to *Arabidopsis*, which has 75 bZIP TF members with an average length of 321 AAs, the bZIP family in apple is larger with longer proteins (Jakoby et al. 2002). This could be related to the larger genome size of apple.

Phylogenetic classification of the apple bZIP TF family

To investigate the evolutionary relationships within the *bZIP* gene family in apple, a combined phylogenetic analysis of apple bZIP proteins was performed using MEGA 6.0 software

(http://www.megasoftware.net/) to obtain a neighbor-joining (NJ) tree. The 114 bZIP domain protein sequences of the MdbZIPs were used to construct the phylogenetic tree (Fig. 1). Meanwhile, 21 bZIP sequences from different subgroups of the *Arabidopsis* bZIP family were added to the tree and used as grouping markers. Furthermore, the maximum parsimony (MP) method was also applied to generate a MP tree (data not shown), which is almost identical to the NJ one, indicating that the two analysis methods were in strong agreement. Therefore, the NJ tree was used for further analysis.

Based on the phylogenetic tree, the 114 MdbZIPs were divided into the same 10 subgroups as in *Arabidopsis*. Group S, which contains 21 MdbZIPs, is the largest group. Groups I and A, which have 18 and 16 members, respectively, are the second and third largest groups. Twelve and 14 MdbZIPs were categorized into groups D and G, respectively. Groups F, C, B, and E have 6, 6, 8, and 8 MdbZIPs, respectively. Group H is containing just five MdbZIPs. Two or three *Arabidopsis* bZIPs are distributed in each group, suggesting similar evolutionary trajectories in *Arabidopsis* and apple.

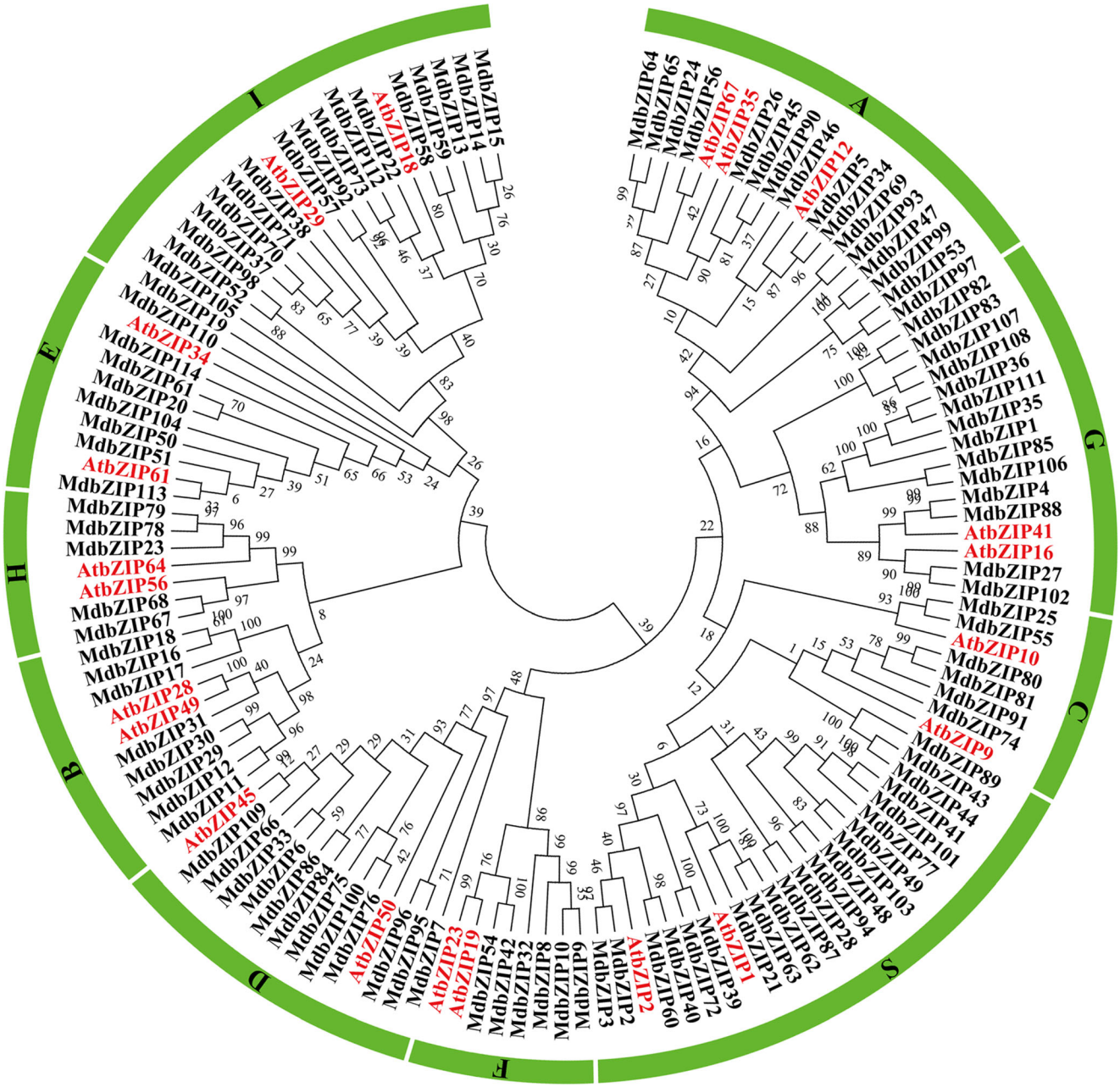


Fig. 1 Phylogenetic relationship of apple and *Arabidopsis* bZIP proteins. The bZIP domain protein sequences of the bZIPs, 114 from apple (MdbZIPs) and 21 from *Arabidopsis* (AtbZIPs, *red color*), were aligned by ClustalW. The phylogenetic tree was constructed using the neighbor-joining method by MEGA 6.0. The bootstrap values of 1000 replicates were calculated at each node. The proteins were classified into 10 distinct subgroups, A–I and S

There also have another report for bZIP family in apple (Wang et al. 2015). One-hundred-sixteen bZIP genes were identified from apple genome in that study. There are six different bZIP genes between the two studies, two genes only found in this study (MdbZIP5, MDP0000123107; MdbZIP20, MDP0000174930) and four bZIP genes only found in that work (MDP0000136654, MDP0000169112, MDP0000274723, MDP0000470928). It suggests that the different analysis methods have different advantages, but they also exist some limitations. To better understand the bZIP family in apple, a phylogenetic tree containing those four bZIPs is also build and shown in supplemental Fig. Fig. S2.

Structure of *MdbZIP* genes and homology modeling of MdbZIP proteins

The bZIP domain, which binds to the promoters of downstream genes, is the key to the function of the *bZIP* gene family. However, the function of the *MdbZIP* genes may also be attributed to other motifs in the MdbZIP proteins. In our study, five major conserved sequences, including the MdbZIP domain, were found in the MdbZIP proteins using the MEME website (http://meme-suite.org/). The consensus amino acid sequences of the five motifs were created in Logo and are listed from 1 to 5 in Fig. 2a. The distribution of the motifs in different types of the MdbZIP members is shown in Fig. 2b. The results indicate that most members classified into the same group shared the same or similar conserved motifs. Motif 1, the bZIP domain, was present in all 114 MdbZIP proteins. Motifs 1 and 4 occurred in all 10 types of MdbZIPs, motif 2 was present in the type I and some of the type E MdbZIP proteins, and motifs 3 and 5 specifically appeared only in the type D MdbZIPs. Additionally, we found that all types F, H, A, C, S, G, and B MdbZIPs only contain motifs 1 and 4; most type D MdbZIPs possess motifs 1, 3, 4, and 5; and all members of type I share motifs 1, 2, and 4 with seven members of type E. The gene structure with the corresponding conserved motifs in each subgroup was consistent with the classification of the MdbZIP genes in Fig. 1.

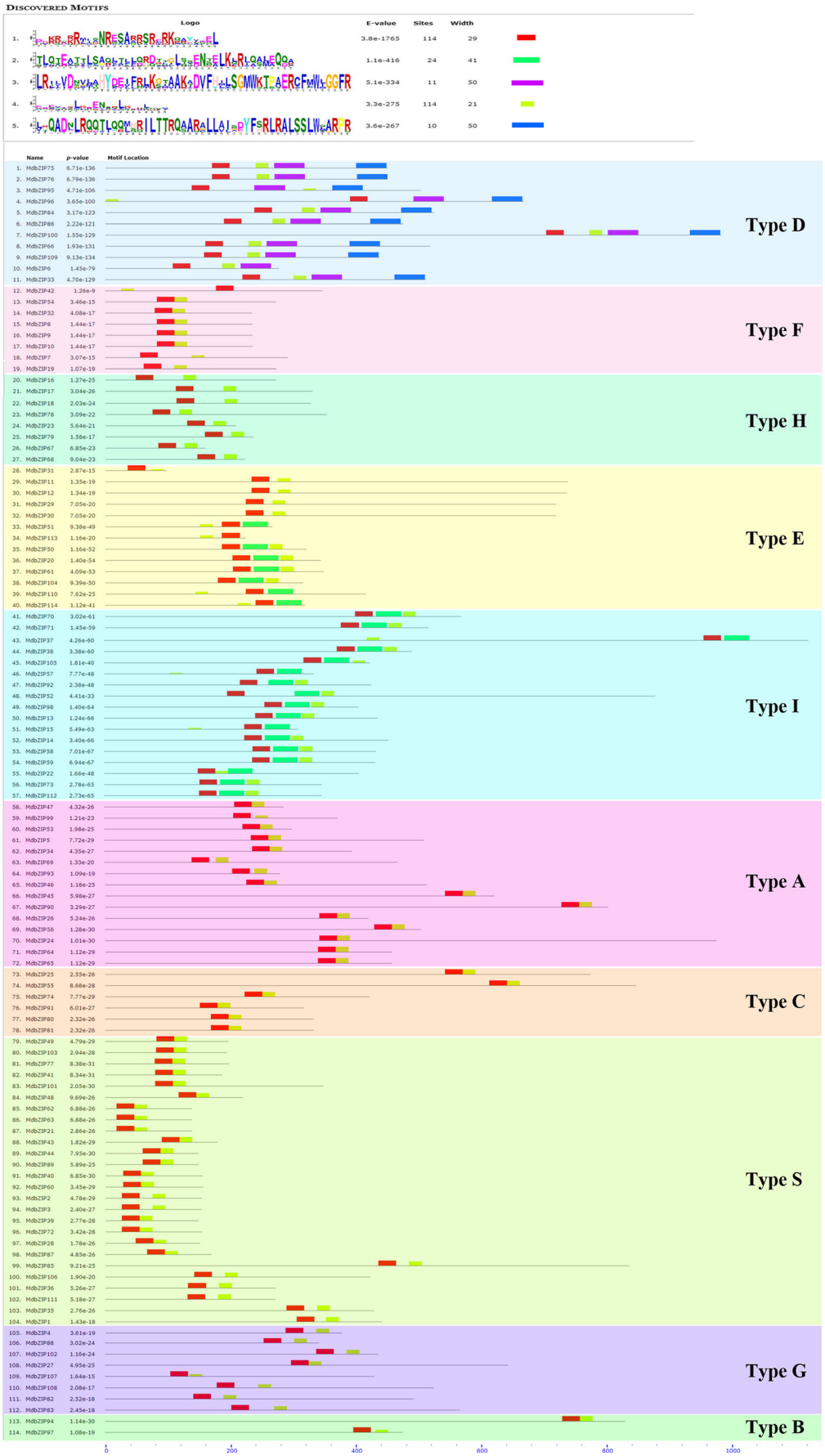
To obtain the homology model of the MdbZIP proteins, the BLASTP search method was re-run against the Protein Data Bank (PDB; http://www.rcsb.org/pdb/home/home.do). As shown in Fig. 3, 15 MdbZIP proteins, MdbZIP 2, 4, 8, 10, 46, 60, 69, 74, 78, 79, 94, 96, 98, 104, and 109, had the highest homology among the 114 MdbZIPs. To increase the alignment accuracy, we used detection rate to predict the homology modeling in PHYRE2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index), and hidden Markov models were applied to the alignment using the HMM-HMM search (Soding 2005). The structures of the 15 most highly homologous MdbZIP proteins were modeled at >90 % confidence (Fig. 3). The secondary structure contained both α helices and β sheets. This model creates a preliminary foundation for further exploration of the function of the MdbZIP proteins.

Chromosomal distribution of the MdbZIP TF family

To investigate the genomic distribution of the *MdbZIP*s, the location of each *MdbZIP* gene on the chromosomes was marked within the graphical representation in Fig. 4 based on the SNP markers in the apple genome. The DNA sequence of each apple *bZIP* gene was used in a BLASTN search of the apple genome database (GDR; https://www.rosaceae.org/). Chromosomal location analysis revealed that a total of 104 apple *bZIP* gene members were mapped to the 17 chromosomes of apple; however, 10 bZIP genes were not found on any specific apple chromosome and therefore are marked as located on chromosome 0. Although each of the 17 apple chromosomes included several *bZIP* genes, the distribution was uneven. Chromosome 8 contained the largest number of *MdbZIP* genes with a total of 12 genes, *MdbZIP37* to *MdbZIP48*. The first *bZIP* TF number, *MdbZIP1*, was mapped onto chromosome 1. Among the other 91 *MdbZIP* genes, 11 *MdbZIP* genes were located on each of the chromosomes 2, 12, and 15; 9 *MdbZIP*s were mapped to chromosome 3; 8 *MdbZIP* genes were situated on chromosome 7; 7 *MdbZIP* genes were found on each of the chromosomes 11, 13, and 14; 5 *MdbZIP*s were located on chromosome 10; 3 *MdbZIP* genes were mapped to each of the chromosomes 4, 9, and 16; and 2 *MdbZIP*s were mapped to each of the chromosomes 5, 6, and 17. The gene density per chromosome ranged from 0.1 to 10.5 %. Most of the *MdbZIP* genes congregated on the upper and lower parts of each apple chromosome, but the central regions of all the apple chromosomes lacked *MdbZIP* genes.

Expression analysis of *MdbZIP* genes at different development stages in apple fruit

Transcriptional patterns of genes provide important clues toward the understanding of gene function. To identify the expression profiles of bZIP TFs in apple during fruit growth and development, we analyzed the transcript levels of all 114 *MdbZIP* genes using an Illumina RNA-seq approach. The mRNAs in the RNA-seq data were isolated from the following five developmental stages of apple fruit: 16, 41, 70, 94, and 128 DAB. A heat map transcription pattern of the 114 *MdbZIP* genes at these five development stages was made based on the RNA-seq data (Fig. 5). According to the hierarchical clustering (HCL), the *MdbZIP* gene family has a broad transcript level during apple fruit development. Among the 114 *MdbZIP* genes, 14 (*MdbZIP* 2, 21, 39, 40, 42, 54, 55, 58, 62, 63, 70, 71, 72, and 97) were highly expressed at all five fruit developmental stages, suggesting that some of the


DISCOVERED MOTIFS
Logo
E-value
Sites
Width
1. 3.8e-1765 114 29
2. 1.1e-416 24 41
3. 5.1e-334 11 50
4. 3.3e-275 114 21
5. 3.6e-267 10 50
Name
p-value
Motif Location
Type D
Type F
Type H
Type E
Type I
Type A
Type C
Type S
Type G
Type B
0
200
400
600
800
1000

◀ **Fig. 2** Conserved domains of the bZIP proteins in apple. **a** The motif details of five highly conserved domains identified in MdbZIP proteins and the logos of these domains created using the MEME program. **b** The protein structure of each MdbZIP displaying the predicted conserved domains for the 10 subgroups in Fig. 1. Each motif is represented by a *colored box*

MdbZIP genes play regulatory roles throughout apple fruit development. In contrast, the transcript level of 29 *MdbZIP* genes (*MdbZIP* 6, 7, 16, 17, 18, 19, 24, 28, 31, 32, 50, 51, 56, 68, 69, 74, 75, 76, 82, 84, 86, 93, 96, 100, 101, 104, 105, 113, and 114) was relatively low during fruit development. Moreover, expression analysis also revealed that many *MdbZIP* genes exhibited decreased expression with fruit development (Fig. 5a, c). For example, *MdbZIP* 3 and 46 were expressed abundantly only at the early stage of fruit development; the expressions of *MdbZIP* 4, 8, 9, 10, 61, 78, 79, and 88 declined with fruit development, indicating that these *MdbZIP*s might function as negative regulators in apple fruit development. Among the 114 *MdbZIP* genes, only 2 genes, *MdbZIP* 39 and 72, showed dramatically increased transcript levels with fruit development (Fig. 5b). Interestingly, these

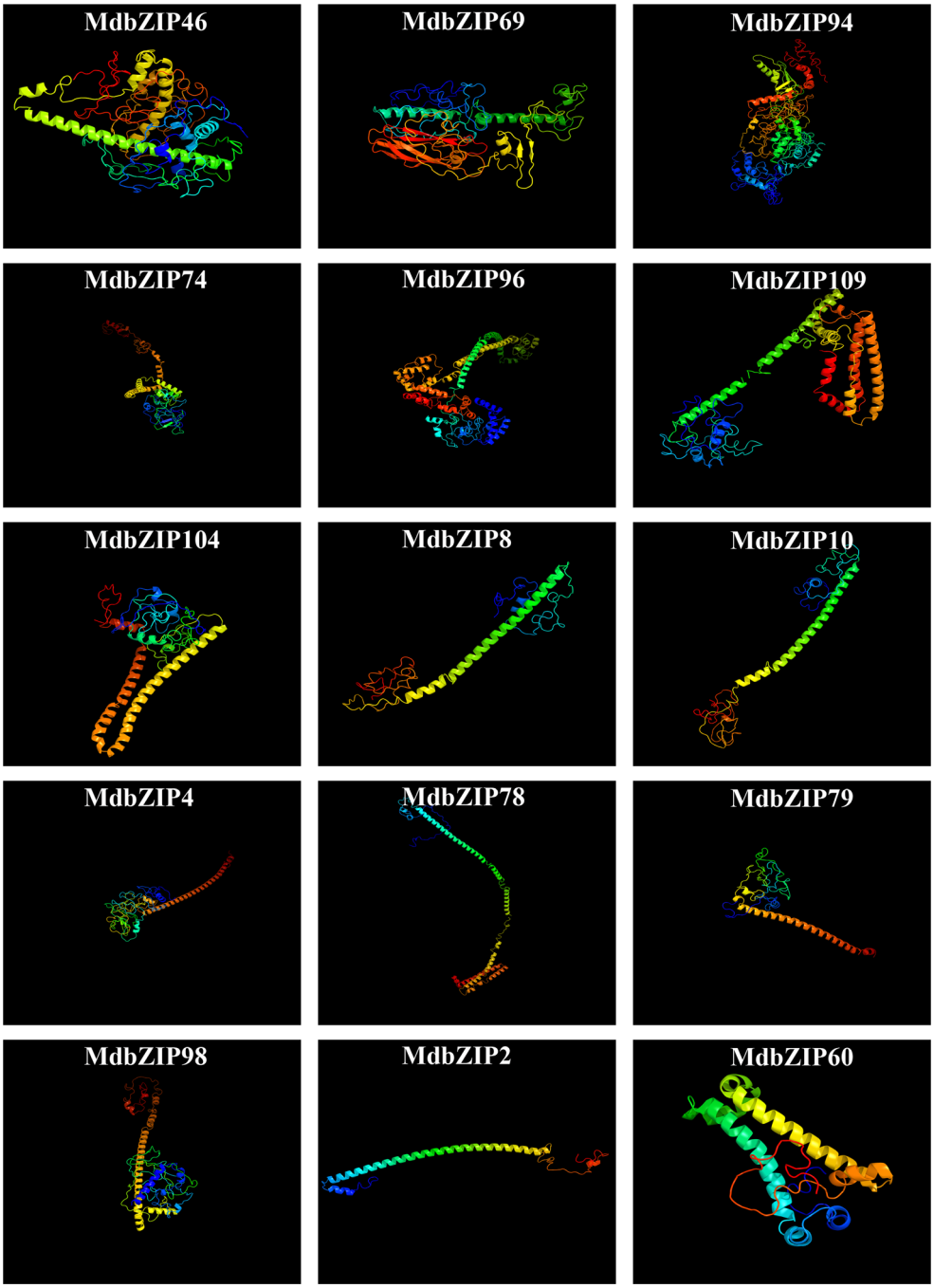


Fig. 3 Predicted structures of MdbZIP proteins. The structures of 15 MdbZIP proteins were predicted to a >90 % confidence interval

two genes both belong to group S and showed the highest similarity in the phylogenetic tree (Fig. 1). This suggests that they may participate in some specific biological processes during fruit development similar to the tomato genes *bZIP1* and *bZIP61*, which respond to ethylene and exhibit differential expression levels during tomato fruit development (Alba et al. 2005).

There was a few reports of bZIP gene play function during fruit development in plants. In tomato, a bZIP gene (SIAP2a) as a negative regulator impacted pigment accumulation during fruit ripening (Chung et al. 2010). A peach bZIP gene was highly expressed during the peach fruit ripening and might regulate some ripening metabolic pathway (Lovisetto et al. 2013). In rice, OsbZIP58 was directly binding to the promoters of starch-synthesizing genes and regulate their expression (Wang et al. 2013). All those studies show that bZIP family should play an important regulation role during fruit ripening and development. At this point, the function of most of the *bZIP* genes in apple is still unknown, especially in apple fruit. Our expression analysis shows that *MdbZIP* gene family has a variety of transcript profiles in apple fruit growth and development. The distinct expression patterns of the 114

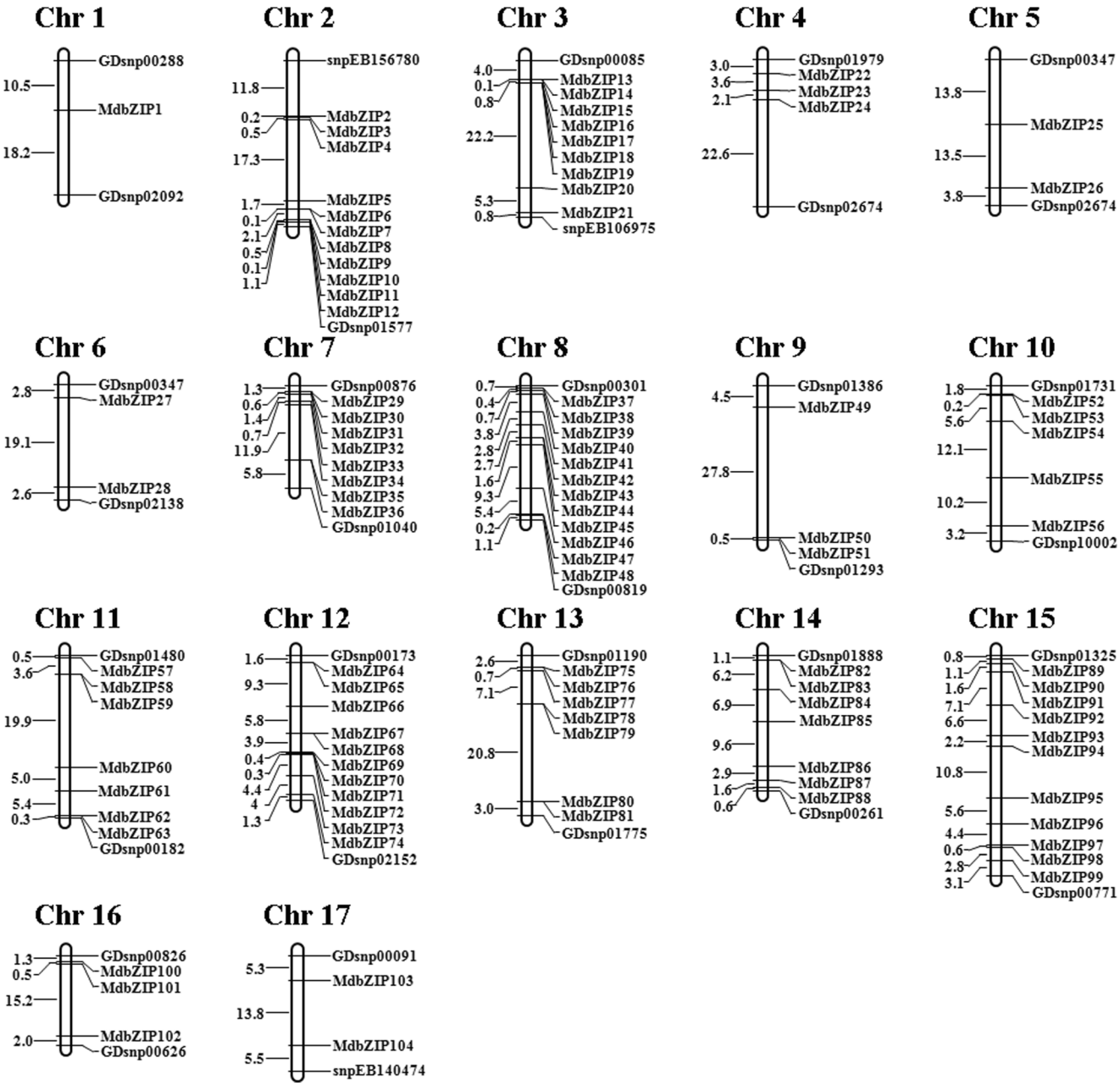


Fig. 4 Distribution of *MdbZIP* genes on 17 apple chromosomes. Chromosomal distances are given in Mbp

MdbZIP genes during apple fruit development help to elucidate the specific functions of the *MdbZIP* genes in apple developmental biology.

Expression analysis between leaves and mature apple fruit

bZIP TFs are widely involved in the development of many plant organs and tissues. Evidence shows tissue-specific expression profiles of bZIP TFs in cucumber (Baloglu et al. 2014), castor bean (Jin et al. 2014), and grapevine (Liu et al. 2014a, b). To understand the transcription pattern of the *MdbZIP* gene family in source and sink organs of apple, we conducted an Illumina RNA-seq analysis on apple leaves and mature fruit. We found distinct expression profile of *MdbZIP* genes in apple leaves and fruit (Fig. 6). Approximately 10 % of the 114 *MdbZIP* genes have relatively high expression levels in both leaves and fruit. In contrast, more than 70 % of the 114 *MdbZIP*s were expressed at relatively low levels in both organs, suggesting that functions of these MdbZIP proteins may not be involved in leaf and fruit differentiation and development under normal growth conditions. Other *MdbZIP*s show distinct expression patterns between leaves and fruit. For example, *MdbZIP1*, *58*, and *59* were highly expressed specifically in apple fruit, whereas *MdbZIP98* was

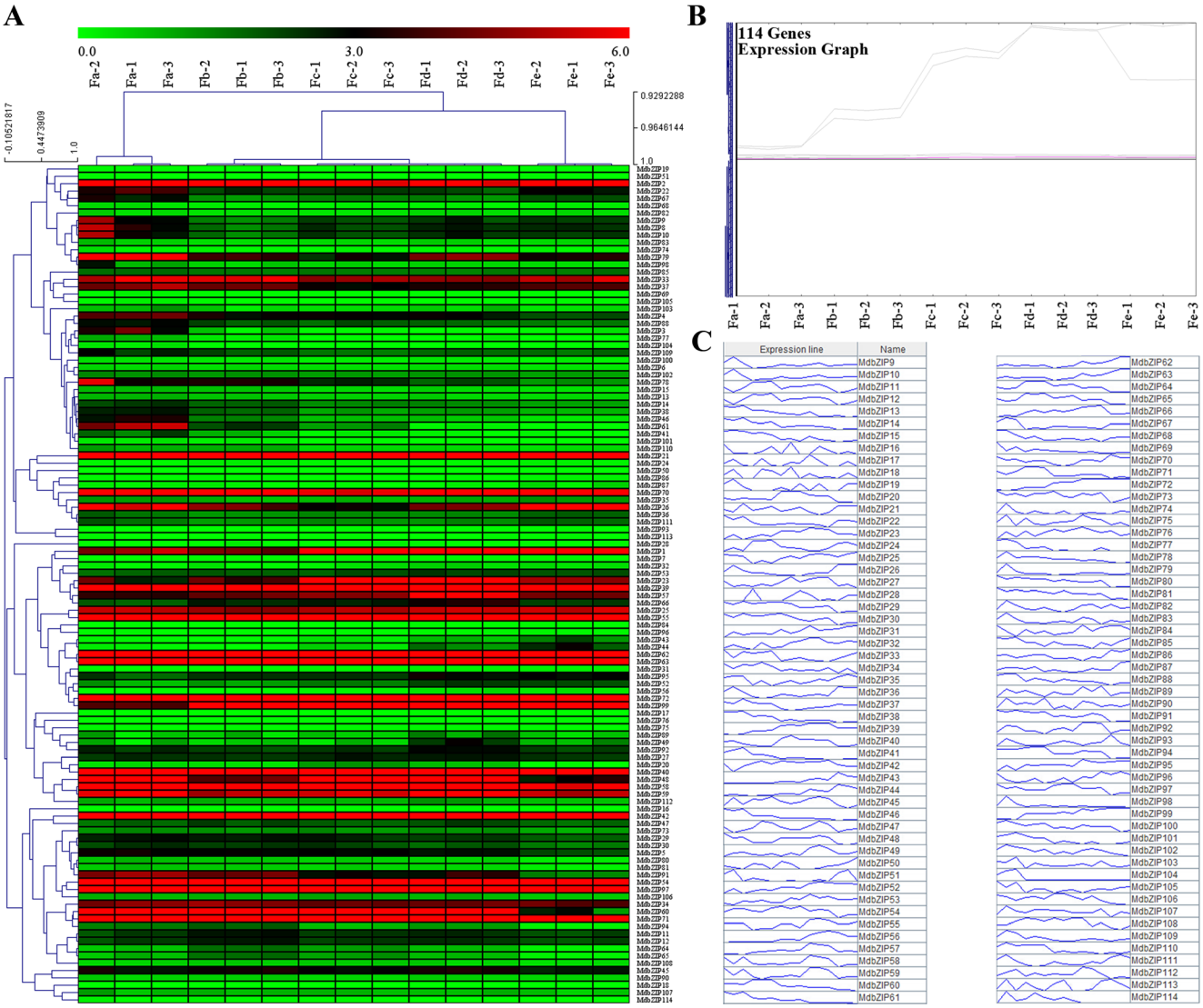
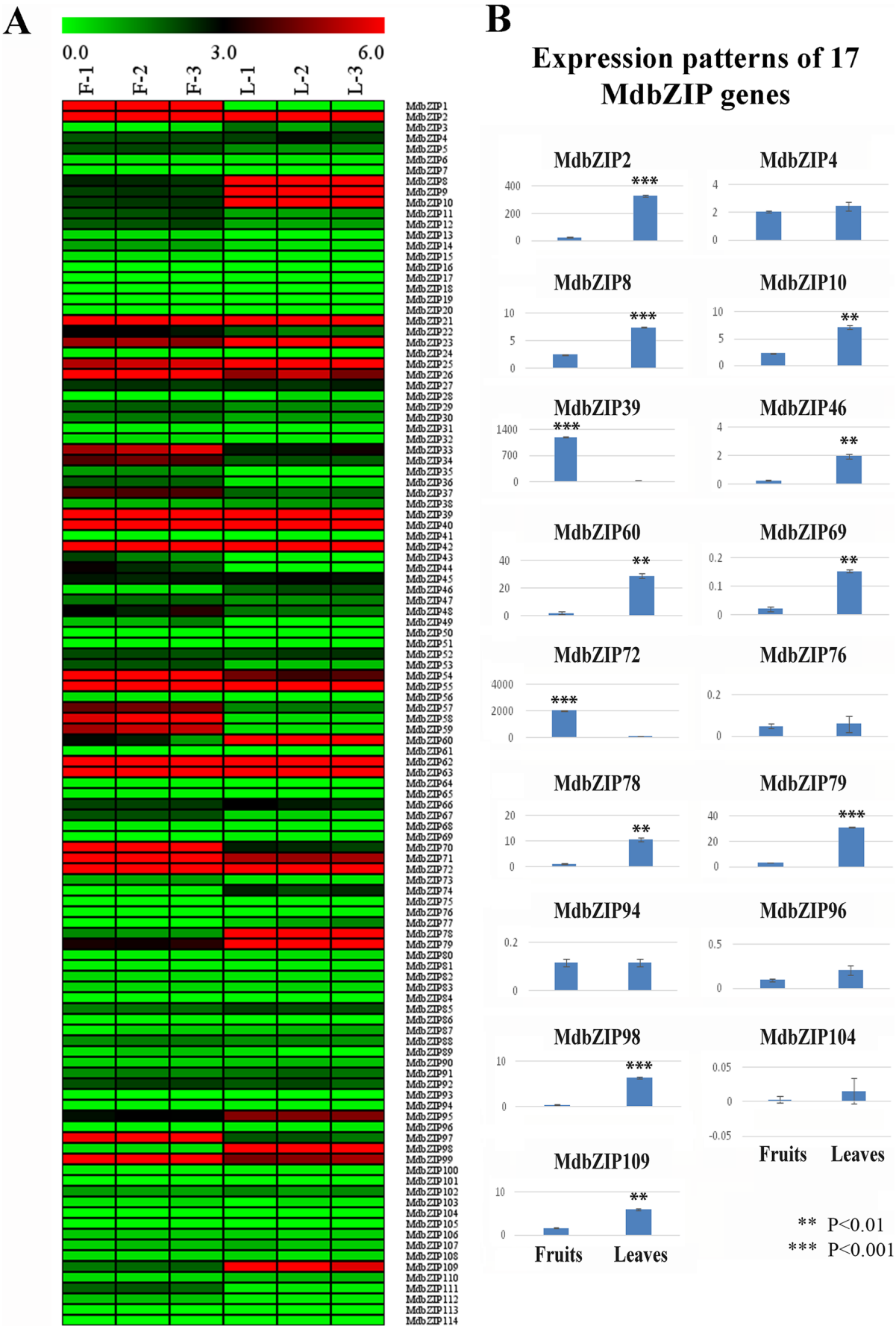


Fig. 5 Heat map of *MdbZIP* genes expressed during apple fruit growth and development. **a** The image summarizes the expression profiles of the 114 *MdbZIP* genes at five stages of fruit development. Fa-Fe indicates the apple fruit harvested at 18, 37, 67, 90, and 132 days after bloom. Three replicates (1–3) were performed for each stage. The *bar at the top* represents the relative expression value. **b** The expression of the 114 *MdbZIP* genes during apple fruit development shown in one chart. **c** The expression of each *MdbZIP* gene during fruit development shown separately


A
0.0
3.0
6.0
F-1
F-2
F-3
L-1
L-2
L-3
B
Expression patterns of 17 MdbZIP genes
MdbZIP2
MdbZIP4
MdbZIP8
MdbZIP10
MdbZIP39
MdbZIP46
MdbZIP60
MdbZIP69
MdbZIP72
MdbZIP76
MdbZIP78
MdbZIP79
MdbZIP94
MdbZIP96
MdbZIP98
MdbZIP104
MdbZIP109
Fruits
Leaves
** P<0.01
*** P<0.001

◀ **Fig. 6** Heat map of *MdbZIP* genes expressed in leaves and fruit. **a** The image summarizes the expression profiles of the 114 *MdbZIP* genes in mature apple fruit (F) and leaves (L). Three replicates (1–3) were performed for each organ. The *bar on the top* represents the relative expression values. **b** Expression of the 17 *MdbZIP* genes with highly differential transcript levels between leaves and fruit. The *symbols* ** and *** are significant at 1 and 0.1 % levels, respectively

only expressed in leaves (Fig. 6a). Furthermore, we found that 17 *MdbZIP* genes, *MdbZIP* 2, 4, 8, 10, 39, 46, 60, 69, 72, 76, 78, 79, 94, 96, 98, 104, and 109, exhibited differential and relatively high expression levels in leaves and fruit (Fig. 6b). For example, expression levels of *MdbZIP39* and *MdbZIP72* were higher in fruit than in leaves, and transcript abundance of *MdbZIP94* was very high in both leaves and fruit. The other 14 *MdbZIP*s exhibited a higher expression level in leaves than in fruit. These data illustrate that some *MdbZIP* genes may participate in regulating the differentiation and development of the source or sink organs in apple. Meanwhile, the higher transcript abundance of many *MdbZIP* genes in apple leaves suggests that these genes may perform their function primarily in apple leaves. These results provide us useful clues to further study the functions of *MdbZIP*s in apple organs.

Expression of *MdbZIP* genes during salt and drought stresses

Previous studies showed that some *bZIP*s were involved in responses to multiple stresses (Hossain et al. 2010). Many bZIP family genes that participated in salt and drought stresses are reported in many plants. For example, OsbZIP71 participate in ABA-mediated salt and drought tolerance in rice (Liu et al. 2014a, b). Although, regulation expression level of stress-response genes, *PtrABF* (a bZIP gene isolate from *Poncirus trifoliate*), can enhance dehydration and drought tolerance in transgenic tobacco (Huang et al. 2010). All those reports reveal that bZIP family plays important roles during environmental stress, such as salt and drought. However, little information on bZIP TFs responding to stress in apple has been reported. To investigate the salt and drought responses of *MdbZIP* genes in apple, qRT-PCR was performed to analyze the expression patterns of some of the *MdbZIP* genes. Based on the phylogenetic tree and the RNA-seq results, 16 *MdbZIP* genes (*MdbZIP* 2, 8, 10, 39, 46, 60, 69, 72, 76, 78, 79, 94, 96, 98, 104, 109) from different groups were selected to determine the transcript levels in both leaves and roots under salt (NaCl) and drought stress treatments. According to the RNA-seq results, the 16 *bZIP* genes were expressed

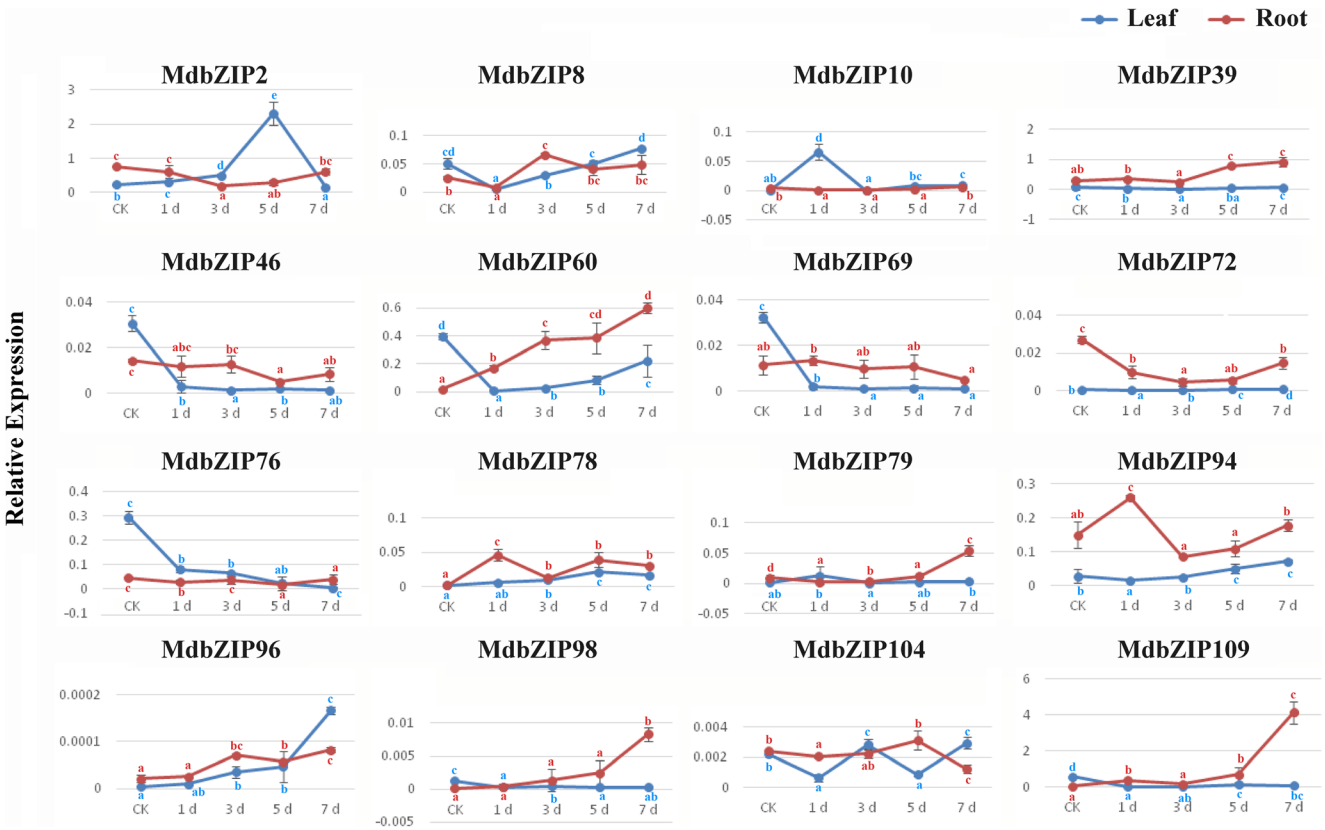


Fig. 7 Relative expression levels determined by quantitative real-time PCR of 16 *MdbZIP* genes in response to NaCl stress treatment. The expression levels of 16 *MdbZIP*s in apple leaves and roots were examined after 100 mM NaCl treatment for 0, 1, 3, 5, and 7 days. Means were separated at the 5 % significance level

highly during apple fruit development (Fig. 5a) and in apple fruits and leaves (Fig. 6a). Meanwhile, there is obvious expression difference between fruits and leaves of the 16 genes. All the genes analyzed showed responses to both NaCl and drought stresses (Figs. 7 and 8). It was obvious that in apple leaves, *MdbZIP* 46, 60, 69, and 76 were negatively regulated by NaCl stress, in contrast to *MdbZIP* 8, 78, 94, and 96, which were positively regulated. In the roots, expression of 9 *MdbZIP* genes (*MdbZIP* 8, 39, 60, 78, 79, 94, 96, 98, and 109) was elevated under NaCl stress, while *MdbZIP* 46, 69, 72, and 104 were down-regulated. We also found that, in both leaves and roots, transcript levels of *MdbZIP* 8, 46, 69, 78, 94, and 96 showed the same expression trend under NaCl treatment in both organs; however, the patterns among them were different (Fig. 7). For instance, the accumulation of *MdbZIP46* and *MdbZIP69* transcripts in the leaves declined dramatically the first day after treatment and subsequently decreased gradually in the following 6 days of NaCl treatment. However, both genes were down-regulated to a much lesser degree in the roots during the entire NaCl stress treatment. These findings suggest that *MdbZIP* genes might play an important role in mediating salt signaling transduction in apple.

We found that expression of the majority of the *MdbZIP* genes selected was up-regulated in roots in response to drought stress (Fig. 8). Of the 16 *MdbZIP* genes followed, only *MdbZIP72* declined gradually upon drought treatment, but its expression increased and then plateaued after 2 days of re-watering. In leaves, however, transcript abundance of most of the *MdbZIP*s showed very limited changes. The only exception was *MdbZIP76*, which was expressed at high levels in the control group and on the first day after the initiation of drought treatment; then, the expression was significantly decreased. This supports the notion that the signal for water deficit is primarily generated in the roots (Neill and Burnett 1999). Interestingly, we found that several salt-associated *MdbZIP* genes were also regulated by drought stress. Some of the up-regulated genes in salt treatment were down-regulated by drought stress, indicating opposite directions in their involvement in salt and drought responses; other *MdbZIP*s were co-expressed in both salt and drought stresses, suggesting a common pathway shared by salt and drought stresses. When cellular effects of salinity or drought stress are not only imbalances of ionic or osmotic homeostasis

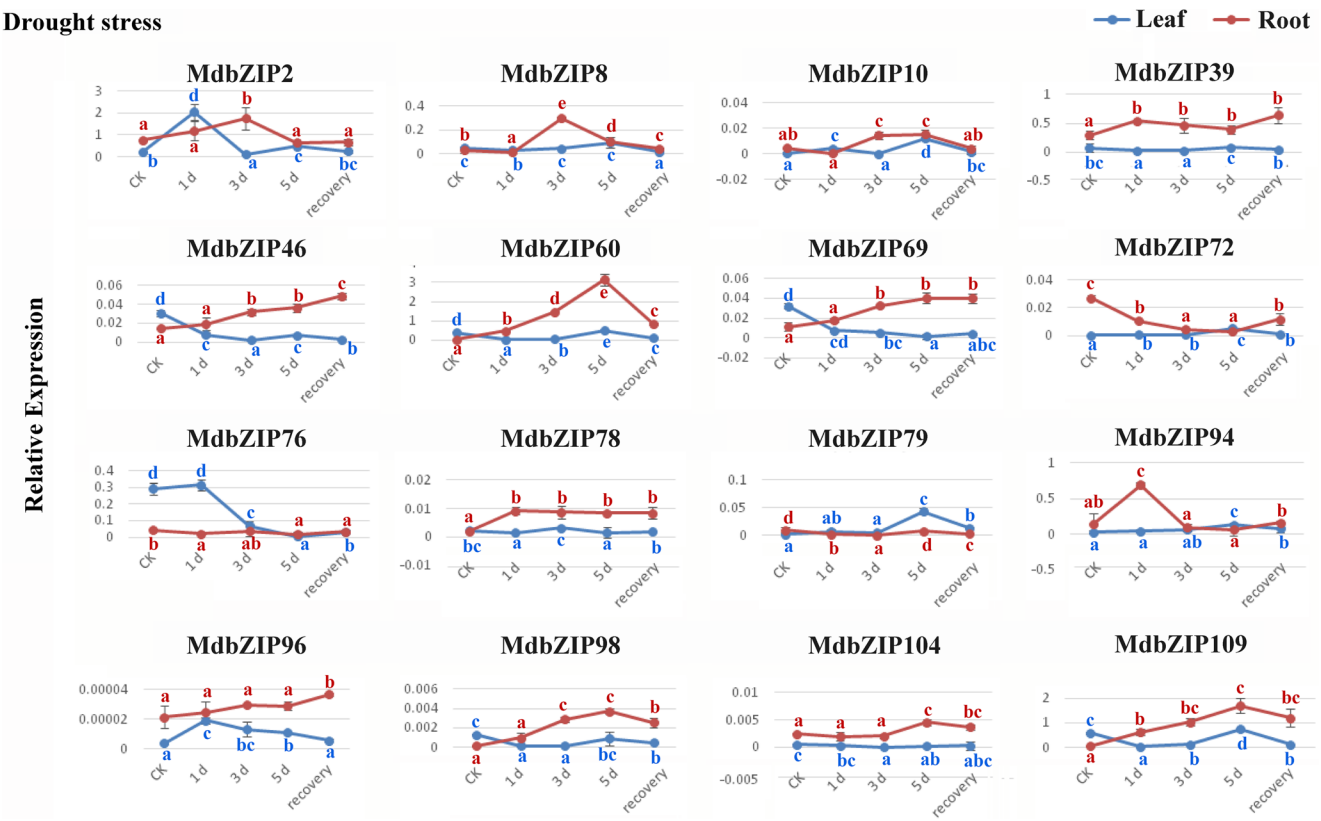


Fig. 8 Relative expression levels determined by real-time PCR of 16 *MdbZIP* genes in response to drought stress. The expression levels of 16 *MdbZIP*s in apple leaves and roots were examined after water was withheld for 0, 1, 3, and 5 days and then after 2 days of re-watering. Means were separated at the 5 % significance level

but also impaired others, such as water deficit and redox imbalances, it is possible that some of the *MdbZIP*s that are responsive to both salt and drought stresses may be involved in regulating the perception of the water deficit or ROS signal, whereas those that are only regulated by salt stress may play crucial functions when the cells encounter osmotic stress or ion toxicity. Furthermore, our data demonstrates that some *MdbZIP* genes were involved in the drought response signaling pathway through distinct gene regulation patterns, and their expression was dramatically influenced by drought conditions in apple.

Finally, STRING software (version 10.0; http://string-db.org/cgi/) was used to draw an interaction network map of MdbZIP proteins in apple and related genes in *Arabidopsis* to identify their functional and physical interactions. For example, MdbZIP2 exhibited strong interaction with six bZIP proteins in *Arabidopsis*, bZIP53, bZIP9, bZIP1, bZIP25, bZO2H3 (bZIP63), and bZO2H1 (bZIP10) (Fig. 9). Of the seven bZIP proteins, only bZIP2, bZIP53, and bZIP1 are involved in response to low energy and sugar signaling through interaction with the KIN10 protein.

Taken together, our transcript analysis of *MdbZIP*s at different fruit development stages and in various tissues, as well as under different abiotic stress conditions, suggests that bZIP proteins are involved in the development of both source and sink organs of apple and in the response to abiotic stresses. Although the functions of most MdbZIPs have not been discovered, this phylogenetic and expression profile analysis provides an important foundation for further functional analysis of the apple *bZIP* TF gene family.

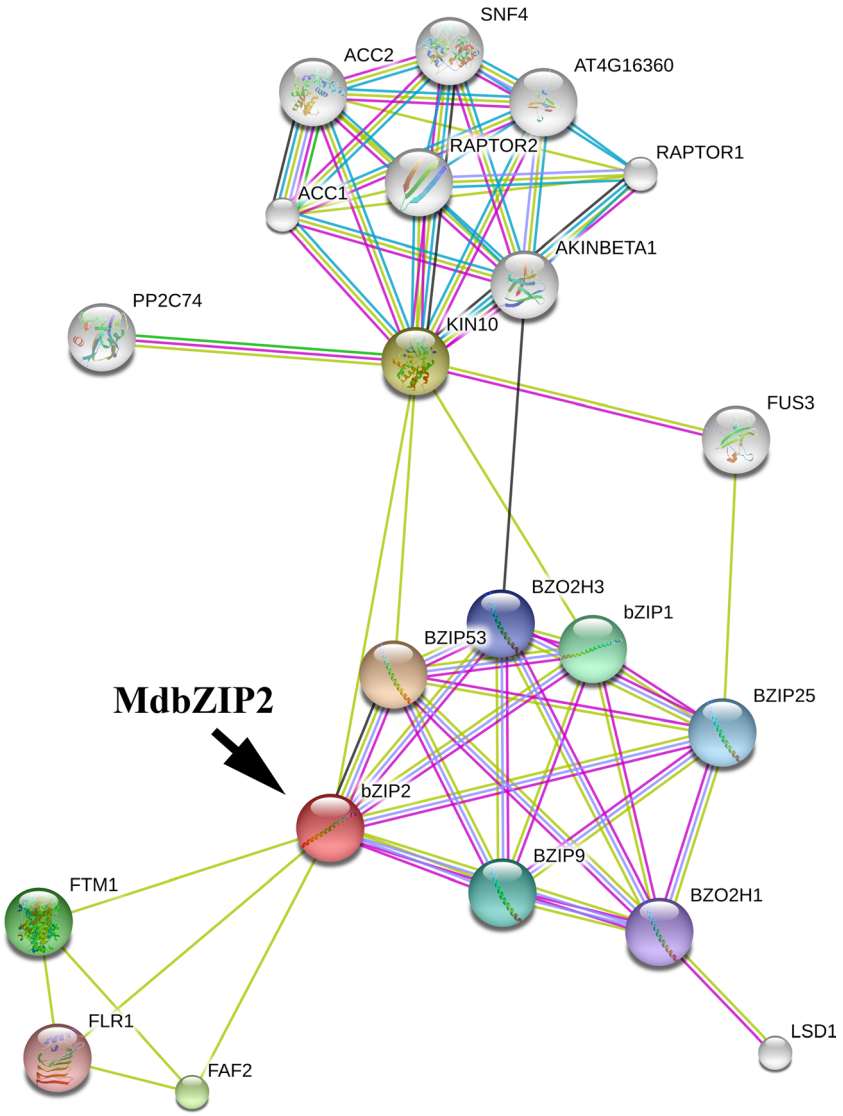


Fig. 9 Interaction network of *MdbZIP2* in apple and related genes in *Arabidopsis*. The chart of interaction network used MdbZIP2 amino acid sequence to search the STRING database, and the *Arabidopsis* bZIP2 was chosen to establish the protein interaction network. The *small and big nodes* indicate the proteins with unknown 3D structure and known or predicted 3D structures, respectively. The *colored nodes* indicate the query proteins and first shell of interactors, and the *white nodes* indicate the second shell of interactors. The *colored lines* between the nodes indicate the different kinds of interactions. The known interactions used *light blue lines* (from curate databases) and *purple lines* (from experiments). The predicated interactions used *green* (gene neighborhood), *red* (gene fusions), and *blue lines* (gene co-occurrence). The *yellow, black, and cyan lines* represent the text mining, co-expression, and protein homology, respectively. All the detailed information is in the STRING website (http://string-db.org)

Compliance with ethical standards

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

Data archiving statement The apple bZIP gene family sequences have been submitted to GDR (Genome Database for *Rosaceae*), and the accession number been shown in the Table 1.

References

Alba R, Payton P, Fei Z, McQuinn R, Debbie P, Martin GB, Tanksley SD, Giovannonia JJ (2005) Transcriptome and selected metabolite analyses reveal multiple points of ethylene control during tomato fruit development. Plant Cell 17: 2954–2965. doi: 10.1105/tpc.105.036053

Baloglu MC, Eldem V, Hajyzadeh M, Unver T (2014) Genome-wide analysis of the bZIP transcription factors in cucumber. PLoS One 9(4): e96014. doi:10.1371/journal.pone.0096014

Chen H, Chen W, Zhou J, He H, Chen L, Deng XW (2012) Basic leucine zipper transcription factor OsbZIP16 positively regulates drought resistance in rice. Plant Sci 193-194: 8–17. doi:10.1016/j.plantsci.2012.05.003

Chung MY, Vrebalov J, Alba R, Lee JM, McQuinn R, Chung JD, Klein P, Giovannoni JJ (2010) A tomato (*Solanum lycopersicum*) APETALA2/ERF gene, SlAP2a, is a negative regulator of fruit ripening. Plant J 64:936–947. doi:10.1111/j.1365-313X.2010.04384.x

Du H, Yang S, Liang Z, Feng B, Liu L, Huang Y, Tang Y (2012) Genome-wide analysis of the MYB transcription factor superfamily in soybean. BMC Plant Biol 12:106. doi:10.1186/1471-2229-12-106

Ellenberger TE, Brandl CJ, Struhl K, Harrison SC (1992) The GCN4 basic region leucine zipper binds DNA as a dimer of uninterrupted a helices: crystal structure of the protein-DNA complex. Cell 71(7): 1223–1237. doi:10.1016/S0092-8674(05)80070-4

Gasic K, Hernandez A, Korban SS (2004) RNA Extraction from Different Apple Tissues Rich in Polyphenols and Polysaccharides for cDNA Library Construction. Plant Mol Biol Reporter 22:437–437. doi:10.1007/BF02772687

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. doi:10.1111/j.1365-313X.2009.03948.x

Huang XS, Liu JH, Chen XJ (2010) Overexpression of *PtrABF* gene, a bZIP transcription factor isolated from *Poncirus trifoliata*, enhances dehydration and drought tolerance in tobacco via scavenging ROS and modulating expression of stress-responsive genes. BMC Plant Biol 10:230. doi:10.1186/1471-2229-10-230

Hossain MA, Lee Y, Cho J, Ahn C, Lee S, Jeon J, Kang H, Lee C, An G, Park PB (2010) The bZIP transcription factor OsABF1 is an ABA responsive element binding factor that enhances abiotic stress signaling in rice. Plant Mol Biol 72:557–566. doi:10.1007/s11103-009-9592-9

Hsieh T, Li C, Su R, Cheng C, Sanjaya Tsai Y, Chan M (2012) A tomato bZIP transcription factor, SlAREB, is involved in water deficit and salt stress response. Planta 231(6):1459–1473. doi:10.1007/s00425-010-1147-4

Izawa T, Foster R, Nakajima M, Shimamoto K, Chua NH (1994) The rice bZIP transcriptional activator RITA-1 is highly expressed during seed development. Plant Cell 6(9):1277–1287. doi:10.1105/tpc.6.9.1277

Jakoby M, Weisshhaar B, Droge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in *Arabidopsis*. Trends in Plant Sci 7(3):106–111. doi:10.1016/S1360-1385(01)02223-3

Jin Z, Xu W, Liu A (2014) Genomic surveys and expression analysis of bZIP gene family in castor bean (*Ricinus communis* L.). Planta 239: 299–312. doi:10.1007/s00425-013-1979-9

Landschulz WH, Johnson PF, McKnight SL (1988) The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science 240(4860): 1759–1764. doi:10.1126/science.3289117

Li D, Fu F, Zhang H, Song F (2015) Genome-wide systematic characterization of the bZIP transcriptional factor family in tomato (*Solanum lycopersicum* L.). BMC Genomic 16:771. doi:10.1186/s12864-015-1990-6

Liao Y, Zou HF, Hao YJ, Tian AG, Huang J, Liu YF, Zhang JS, Chen SY (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. doi:10.1007/s00425-008-0731-3

Liu C, Mao B, Ou S, Wang W, Liu L, Wu Y, Chu C, Wang X (2014b) OsbZIP71, a bZIP transcription factor, confers salinity and drought tolerance in rice. Plant Mol Biol 84(1–2):19–36. doi:10.1007/s11103-013-0115-3

Liu GT, Wang JF, Cramer G, Dai ZW, Duan W, HG X, BH W, Fan PG, Wang LJ, Li SH (2012) Transcriptomic analysis of grape (*Vitis vinifera L.*) leaves during and after recovery from heat stress. BMC Plant Biol 12:174. doi:10.1186/1471-2229-12-174

Liu X, Chu Z (2015) Genome-wide evolutionary characterization and analysis of bZIP transcription factors and their expression profiles in response to multiple abiotic stresses in *Brachypodium distachyon*. BMC Genomics 16:227. doi:10.1186/s12864-015-1457-9

Liu J, Chen N, Chen F, Cai B, Santo SD, Tornielli GB, Pezzotti M, Cheng Z (2014a) Genome-wide analysis and expression profile of the bZIP transcription factor gene family in grapevine (*Vitis vinifera*). BMC Genomics 15:281. doi:10.1186/1471-2164-15-281

Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT, Chua NH (2002) ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. Plant J 32(3):317–328. doi:10.1046/j.1365-313X.2002.01430.x

Lovisetto A, Guzzo F, Tadiello A, Confortin E, Pavanello A, Botton A, Casadoro G (2013) Characterization of a bZIP gene highly expressed during ripening of the peach fruit. Plant Physiol Biochem 70:462–470. doi:10.1016/j.plaphy.2013.06.014

Neill SJ, Burnett EC (1999) Regulation of gene expression during water deficit stress. Plant Growth Regul 29:23–33. doi:10.1023/A:1006251631570

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. doi:10.1104/pp.107.112821

Perez-Rodriguez P, Riano-Pachon DM, Correa LG, Rensing SA, Kersten B, Mueller-Roeber B (2010) PlnTFDB: updated content and new features of the plant transcription factor database. Nucleic Acids Res 38:822–827. doi:10.1093/nar/gkp805

Rook F, Gerrits N, Kortstee A, van Kampen M, Borrias M, Weisbeek P, Smeekens S (1998) Sucrose-specific signalling represses translation of the *Arabidopsis ATB2* bZIP transcription factor gene. Plant J 15: 253–263. doi:10.1046/j.1365-313X.1998.00205.x

Shimizu H, Sato K, Berberich T, Miyazaki A, Ozaki R, Imai R, Kusano T (2005) LIP19, a basic region leucine zipper protein, is a Fos-like molecular switch in the cold signaling of rice plants. Plant Cell Physiol 46(10):1623–1634. doi:10.1093/pcp/pci178

Soding J (2005) Protein homology detection by HMM-HMM comparison. Bioinformatics 21:951–960. doi:10.1093/bioinformatics/bti125

Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerte-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M,

Bork P, Jensen LJ, Mering C (2014) STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucl Acids Res 1–6. doi:10.1093/nar/gku1003

Talanian RV, Mcknight CJ, Kim PS (1990) Sequence-specific DNA-binding by a short peptide dimer. Science 249:769–771. doi:10.1126/science.2389142

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. doi:10.1093/molbev/mst19

Thurow C, Schiermeyer A, Krawczyk S, Butterbrodt T, Nickolov K, Gatz C (2005) Tobacco bZIP transcription factor TGA2.2 and related factor TGA2.1 have distinct roles in plant defense responses and plant development. Plant J 44(1):100–113. doi:10.1111/j.1365-313X.2005.02513.x

Toh S, McCourt P, Tsuchiya Y (2012) HY5 is involved in strigolactone-dependent seed germination in *Arabidopsis*. Plant Signal Behav 7(5):556–558. doi:10.4161/psb.19839

Udrardi MK, Czechowski T, Scheible WR (2008) Eleven golden rules of quantitative RT-PCR. Plant Cell 20:1736–1737. doi:10.1105/tpc.108.061143

Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, Kalyanaraman A, Fontana1 P, Bhatnagar SK, Troggio M, Pruss D, Salvi S, Pindo M, Baldi P, Castelletti S, Cavaiuolo M, Coppola G, Costa F, Cova V, Dal Ri A, Goremykin V, Komjanc M, Longhi S, Magnago P, Malacarne G, Malnoy M, Micheletti D, Moretto M, Perazzolli M, Si-Ammour A, Vezzulli S, Zini E, Eldredge G, Fitzgerald LM, Gutin N, Lanchbury J, Macalma T, Mitchell JT, Reid J, Wardell B, Kodira C, Chen Z, Desany B, Niazi F, Palmer M, Koepke T, Jiwan D, Schaeffer S, Krishnan V, Wu C, Chu VT, King ST, Vick J, Tao Q, Mraz A, Stormo A, Stormo K, Bogden R, Ederle D, Stella A, Vecchietti A, Kater MM, Masiero S, Lasserre P, Lespinasse V, Allan AC, Bus V, Chagné D, Crowhurst RN, Gleave AP, Lavezzo E, Fawcett JA, Proost S, Rouzé P, Sterck L, Toppo S, Lazzari B, Hellens RP, Durel C, Gutin A, Bumgarner RE, Gardiner SE, Skolnick M, Egholm M, Van de Peer Y, Salamini F, Viola R (2010) The genome of the domesticated apple (*Malus* × *domestica Borkh.*). Nat Genet 42(10): 833–841. doi:10.1038/ng.654

Walsh J, Freeling M (1999) The liguleless2 gene of maize functions during the transition from the vegetative to the reproductive shoot apex. Plant J 19(4):489–495. doi:10.1046/j.1365-313X.1999.00541.x

Wang J, Zhou J, Zhang B, Vanitha J, Ramachandran S, Jiang S (2011) Genome-wide expansion and expression divergence of the basic leucine zipper transcription factors in higher plants with an emphasis on sorghum. J of Integrative Plant Biol 53(3):212–231. doi:10.1111/j.1744-7909.2010.01017.x

Wang JC, Xu H, Zhu Y, Liu QQ, Cai XL (2013) OsbZIP58, a basic leucine zipper transcription factor, regulates starch biosynthesis in rice endosperm. J Exp Bot 64(11):3453–3466. doi:10.1093/jxb/ert187

Wang XL, Zhong Y, Cheng ZM, Xiong JS (2015) Divergence of the bZIP gene family in strawberry, peach, and apple suggests multiple modes of gene evolution after duplication. International Journal of Genomics 536943:11. doi:10.1155/2015/536943

Wei K, Chen J, Wang Y, Chen Y, Chen S, Lin Y, Pan S, Zhong X, Xie D (2012) Genome-wide analysis of bZIP-encoding genes in maize. DNA Res 19:463–476. doi:10.1093/dnares/dss026

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. Science 309(5737):1056–1059. doi:10.1126/science.1114358

Yin Y, Zhu Q, Dai S, Lamb C, Beachy RN (1997) RF2a, a bZIP transcriptional activator of the phloem-specific rice tungro bacilliform virus promoter, functions in vascular development. EMBO J 16(17): 5247–5259. doi:10.1093/emboj/16.17.5247