Identification of the basic leucine zipper transcription factor and analysis of expression responses to biotic and abiotic stresses in kiwifruit (*Actinidia* Lindl.)

Zhaobin Jing^{1,2} and Hongqi Fu²

¹ College of Horticulture, Northwest A&F University, Yangling, Shaanxi, China

² Weinan Vocational and Technical College, Weinan Fruit Industry Institute of Shaanxi, Weinan, Shaanxi, China

Summary

The basic leucine zipper (bZIP) transcription factor family is involved in regulating organ differentiation, embryogenesis, flower and vascular development, seed maturation, and plays important roles in responding to biotic and abiotic stress. To date, bZIP gene family in kiwifruit (*Actinidia* spp.) remains poorly understood. In this study our objective was to identify the bZIP transcription factor of kiwifruit; in this study 80 *AcbZIP* genes were identified in the kiwifruit genome. Based on domain architecture and phylogenetic analyses, kiwifruit bZIP genes can be classified into ten superfamilies. Synteny analysis indicated that segmental duplication events contributed to the expansion of the kiwifruit *AcbZIP* family. In addition, the synteny analysis between kiwifruit and *Arabidopsis* suggested that some of the *AcbZIP* genes were derived from common ancestors before the divergence of these two genera. Conserved motifs outside the *AcbZIP* domain may reflect their functional conservation. Furthermore, the analysis of 16 Group A *AcbZIP* genes showed a variety of expression patterns in five different organs and suggested some potential genes during biotic and abiotic stresses. The genome-wide identification and characterization of kiwifruit bZIP transcription factors could facilitate functional analysis of these genes and provide important information of the molecular basis of development and stress tolerance in kiwifruit.

Keywords

genome-wide, kiwifruit, bZIP transcript factor, expression analysis

Introduction

Kiwifruit is one of the most important fruits in the world, and it is widely used in food production due to its high nutritional value. The native distribution of this genus is in China and southeastern Asia, and includes a genus of 55 species and 75 subspecific taxa (Huang and Ferguson, 2007). Recently, the quality and production of kiwifruit have been affected by some abiotic and biotic stresses. For example, bacterial canker of kiwifruit has influenced kiwifruit orchards in almost all areas of its production worldwide since 2008. Plants need to adapt their growth process to abiotic and biotic stresses by internal gene expression and regulation. Transcriptomes play a large role in the regulatory networks and signal transduction of plant abiotic and biotic biological pro-

Significance of this study

What is already known on this subject?

- The basic leucine zipper (bZIP) family is one of the largest and most diverse transcription factor families in plants. bZIP family were identified in some plants. The availability of complete kiwifruit genome sequences allows the genome-wide identification of bZIP TFs.

What are the new findings?

- In this study, 80 *AcbZIP* genes have been identified in the kiwifruit genome. Synteny analysis indicated that segmental duplication events contributed to the expansion of the kiwifruit *AcbZIP* family. The analysis of *AcbZIP* genes showed a variety of expression patterns in five different organs and suggested some potential genes during biotic and abiotic stresses.

What is the expected impact on horticulture?

- We expected to provide reference and resources for studying functional analysis of *AcbZIP* genes and to provide important information of the molecular basis of development and stress tolerance in horticultural plants.

cesses (Rabara et al., 2013). The basic leucine zipper (bZIP) family is one of the largest and most diverse transcription factor families in plants (Zhao et al., 2016). The bZIP TFs are named according to the domain that contains a conserved 60–80 amino acids, which has two structural features: a basic region that binds DNA and a leucine zipper dimerization motif (Hurst, 1994; Talanian et al., 1990). The basic region contains a N-x7-R/K-x9 motif with about 18 amino acid residues, which are responsible for DNA binding and nuclear localization. The leucine zipper is composed of a heptad repeat of leucine residues or other bulky hydrophobic amino acids that involved recognition and dimerization of bZIP.

Numerous studies showed that bZIP TFs are involved in regulating organ differentiation (Silveira et al., 2007), embryogenesis, flower and vascular development (Guan et al., 2009; Zou et al., 2008), seed maturation (Jakovy et al., 2002). bZIP TFs also play important roles in responding to biotic and abiotic stress, including salt and drought (Huang et al., 2016; Zhao et al., 2016; Ying et al., 2012), low temperatures (Liu et al., 2012), osmotic stress (Weltmeier et al., 2006), and pathogens (Thurrow et al., 2005). Increasing studies have also indicated that bZIP TFs take part in hormone and sugar sig-

naling (Nieva et al., 2005), light treatment (Ulm et al., 2004), nitrogen/carbon metabolism (Wang et al., 2011). To date, a number of bZIP factors have been identified in some plants, such as 75 bZIP *Arabidopsis thaliana* (Jakoby et al., 2002), 89 in rice (Nijhawan et al., 2008), 88 in sorghum (Wang et al., 2011), 125 in maize (Wei et al., 2012), 100 in castor bean (Jin et al., 2014), 64 in cucumber (Baloglu et al., 2014), 55 in grape (Li et al., 2017; Gao et al., 2014), 112 or 144 in apple (Zhao et al., 2016), 136 in Chinese cabbage (Bai et al., 2016), and 77 in cassava (Hu et al., 2016).

Despite studies of the plant bZIP TFs from different plant species, only a few have been identified in tree or vine species. Based on the available genome sequences, the bZIP TFs had not yet been studied in the kiwifruit. The availability of complete kiwifruit genome sequences allows the genome-wide identification of bZIP TFs. In this study, our objective was to identify kiwifruit bZIP TFs based on the complete genome sequence of the diploid kiwifruit variety Hongyang. In addition, the phylogenetic relationships, exon-intron structures, synteny and expression profiles of selected bZIP genes in different tissues and under abiotic and biotic stresses were evaluated.

Materials and methods

Plant material and treatments

'Hongyang' (*A. chinensis* var. *chinensis* f. *rufopulpa* M.Z. Li) kiwifruit variety were used this study and cultivated in an experimental field at the College of Horticulture, Northwest A&F University, Yangling, China (34°20'N; 108°24'E). Different tissues, including apical buds, young leaves, stems, flower buds, and fruits were collected from 'Hongyang' trees (aged 6 years) during the 2016 growing season. The 2-year-old 'Hongyang' kiwifruit seedlings were used for the exogenous hormone, salinity and drought stress and *Pseudomonas syringae* pv. *actinidiae* (Psa) inoculation treatments. These seedlings had been planted in 20-cm diameter pots in the greenhouse of the experimental station at the College of Horticulture, Northwest A&F University, Yangling, China.

The third to fifth fully expanded young leaves from the shoot apices were used for hormone treatments. Hormone treatments were carried out by spraying young leaves with 100 µM gibberellins (GA), 100 µM salicylic acid (SA), 50 µM methyl jasmonate (MeJA), 300 µM abscisic acid (ABA) and 0.5 g L⁻¹ ethylene (Eth) as previously described (Zhao et al., 2016; Guo et al., 2014), and leaves samples were harvested at 1, 12, 24, and 48 h after the initiation of the treatments and frozen immediately in liquid nitrogen and stored at -80°C. Leaves sprayed with sterile water and harvested at the same time were used as controls for hormone treatments. The salinity stress was performed by irrigating seedlings with 2 L 250 mM NaCl solution, and the seedlings irrigated with same value water were used as a control (Zhao et al., 2016; Guo et al., 2014). Leaves were harvested at 1, 12, 24, and 48 h post-treatment. The drought stress treatment was performed by withholding water, and young leaves of the seedlings were collected at 48, 96, and 168 h post-treatment. Subsequently, the stressed seedlings were rewatered to soil saturation and leaves were collected at 48 h after rewatering (R48). For the drought stress control, seedlings were watered to drip each day (Meng et al., 2016; Zhao et al., 2016; Guo et al., 2014). Pathogen treatment was performed using inoculating the young leaves of 'Hongyang' with Psa (*Pseudomonas syringae* pv. *actinidiae*) strain Psamx-9 (GenBank Assembly ID: KT731230) according to the method previously

SUPPLEMENTARY TABLE S1. The primer sequences used for semi-quantitative RT-PCR amplification of *AcbZIP* genes.

Gene	Product size		Primer (5' - 3')
<i>AcbZIP3</i>	231	F	TCAACTTCCAGGACTTTCTCG
		R	CAATATCAAACGGGTCAGCA
<i>AcbZIP13</i>	245	F	GACGATGTGTGGAGGGAGAT
		R	ACTCCGTCCCAAACCTAC
<i>AcbZIP15</i>	249	F	TTCAATTGCCTGGAAAAACC
		R	TTGAAGGTGGTTGTTGTTGC
<i>AcbZIP20</i>	225	F	TGGAGAGACATAGCCACTGGT
		R	TGTGTGCTGACCCATCTGTT
<i>AcbZIP21</i>	243	F	TTAATCGAAACCCAGGTCCA
		R	ACCGATCAGGACCCGAAT
<i>AcbZIP22</i>	220	F	CAACCAATGACAAGGCCAAG
		R	GATGAAGCAACTGCTGCTGA
<i>AcbZIP31</i>	219	F	GGGGTTAGATCATCACAGCAA
		R	CAGTGCACCACTTTGAACCA
<i>AcbZIP34</i>	201	F	GTGTCGTGAGATGGGATGTG
		R	TTCAACTGTGTAGGCCTGCTT
<i>AcbZIP35</i>	238	F	CCCAGTTCACAGGCTCTTC
		R	AGCCTGTTTCCTTGCTCTTG
<i>AcbZIP43</i>	237	F	ACGGGTCGTACCAGAACAAAT
		R	CGGCACTCCAACTCTCCTA
<i>AcbZIP44</i>	246	F	GACGTTGTGTGGAGGGAGAT
		R	CACCTCGTTCCCAAACCTA
<i>AcbZIP56</i>	211	F	CATCATTGGTATGGCGTTT
		R	ACCCATCATTGGTCCAGGT
<i>AcbZIP61</i>	217	F	GCCACAACATCATAGGTACGG
		R	CCCATAATAGGTCCAGGGTCA
<i>AcbZIP64</i>	222	F	CAACAGATGGGTCCGAACAT
		R	ACTGCTCGGTATAGGCATGG
<i>AcbZIP68</i>	241	F	CCCGTAATGATTAGCCCTA
		R	ATAGGCATGGGATTGGCATA
<i>AcbZIP72</i>	216	F	TGAGTCAGGAGCCAGAGATG
		R	CCATTCCTCCTCAATTTCG

described (Jing et al., 2016; Zhao et al., 2015). Leaves sprayed with sterile water were used as a negative control, and leaves were collected at 24, 48, 72, and 120 h post-inoculation. For all above treatments, at each time point of each treatment, six leaves from six different plants were mixed to form one sample, to be used later in the experiment. All experiments were repeated for three biological replicates.

RNA extraction and cDNA synthesis

The total RNA was isolated using the E.Z.N.A.® Plant RNA Kit (Omega Bio-Tek, Inc.) according to the manufacturer's protocol. The quality and quantity of RNA were assessed using electrophoresis on 1% agarose gels and a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA), respectively. 500 ng total high quality RNA were used for the first-stand cDNA synthesis. The cDNA synthesis was performed using a TransScript® cDNA sample prep kit (TransGen Biotech, China). The cDNA products were then diluted six-fold and stored at -40°C for the later semi-quantitative RT-PCR and real-time quantitative RT-PCR analysis.

Identification and analysis of the kiwifruit bZIP family

The following approach was used to search kiwifruit bZIP transcription factor families. First, we obtained the kiwifruit bZIP TFs from the iTAK-Plant Transcription Factor & Protein Kinase Identifier and Classifier Database (http://bioinfo.bti.cornell.edu/cgi-bin/itak/db_home.cgi). Then each protein sequence was examined for the presence of a BZIP domain based on the HMM of the SMART online tool (<http://smart.embl-heidelberg.de/>) (Letunic et al., 2012). All hits with an e-value less than 0.01 were collected. All non-redundant protein sequences encoding complete bZIP domains were selected as putative bZIP genes and the genes without a complete bZIP domain were discarded. Lastly, we obtained the cDNA, genes, proteins sequences, and the chromosomal location of selected kiwifruit bZIP TFs from the Kiwifruit Genome Database (<http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/search.cgi>). The information of *A. thaliana* bZIP TFs was downloaded from the TAIR database (<http://www.arabidopsis.org/>).

Multiple sequence alignment and phylogenetic analysis of the kiwifruit bZIP genes

Multiple sequence alignments of a total of 80 selected *AcbZIP* proteins were completed by DNAMAN software (Version 6.0). Then, multiple sequence alignments of the bZIP proteins of kiwifruit and *A. thaliana* were performed using ClustalW program (<http://www.ebi.ac.uk/Tools>). Phylogenetic trees were constructed using the MEGA 7.0 software with the neighbor-joining (NJ) method and a 1000 replicates of the bootstrap test. *Arabidopsis* bZIP domains of different subgroups were used as references to class the kiwifruit bZIP proteins. According to the multiple sequence alignments and the classification of *AtbZIP* (*Arabidopsis*) TFs, the *AcbZIP* TFs were assigned to corresponding groups and subgroups.

Exon-intron structure analysis, distribution of conserved motifs in kiwifruit bZIP genes

The exon-intron structures of the kiwifruit *AcbZIP* TFs were analyzed by the Gene Structure Display Server online tool (<http://gsds.cbi.pku.edu.cn/>) with all 80 CDS and the corresponding genomic sequences (Hu et al., 2015). Structure motif analysis were analyzed using the online MEME tool (<http://meme-suite.org/tools/meme>). All 80 *AcbZIP* protein sequences were put in as a file, and the parameters were set by optimum motif width ≥ 6 and ≤ 200 , and the maximum number of motifs to limit 20.

Synteny analysis of kiwifruit bZIP genes

Tandem duplicated genes are defined as adjacent homologous *AcbZIP* genes on the single chromosome, with no more than one intervening gene (Guo et al., 2014). The specific chromosome location of each *AcbZIP* gene were analyzed in order to estimate whether or not a gene resulting from a tandem duplication. For synteny analysis, synteny blocks within kiwifruit genome and between kiwifruit and *Arabidopsis* genomes were downloaded from the plant Genome Duplication Database (<http://chibba.agtec.uga.edu/duplication/>). Then, the genes of the synteny blocks were identified and analyzed. The diagram of synteny analysis were generated by the Circos program (<http://circos.ca/>). The synonymous (Ks) and non-synonymous (Ka) nucleotide substitutions between orthologous gene pairs were obtained from iTAK Database 16.03 (http://bioinfo.bti.cornell.edu/cgi-bin/itak/db_home.cgi). The chromosome localization of *AcbZIP* genes was analyzed using MapInspect based on the information of gene physical position in the kiwifruit genome.

Semi-quantitative RT-PCR and real-time PCR analysis

The kiwifruit *ACT 2* gene (GenBank Accession number EF063572) amplified with the primers F(5'-GCAGGAATCCATGAGACTACC-3') and R(5'-GCAGGAATCCATGAGACTACC-3'), *18S 2* gene (GenBank Accession number AF419792) amplified with the primers F (5'-CTGTGAAACTGCGAATGGCTC-3') and R (5'-TTCCAGAAGTCGGGGTTTGT-3') was used the reference gene for accurate normalization of real-time quantitative PCR data (Ferradás et al., 2016). Other gene-specific primers were designed using Primer 5.0 (Premier Biosoft International) based on the corresponding CDS sequences (Supplementary Table S1). Semi-quantitative RT-PCR amplification reactions were carried out according to previously established protocols by Zhao et al. (2016) in 20 μ L volume, containing 1 μ L of template cDNA, 0.8 μ L of each primer, 10 μ L 2 $\times$ TranStart[®] RTip Green qPCR Super Mix (TransGen Biotech, China), and 7.4 μ L sterile distilled water. PCR amplification was performed under the following conditions: 94°C for 3 min, 28–35 cycles of 94°C for 30 s, 55–60°C for 30 s, 72°C for 30 s and 72°C for 5 min. Semi-quantitative RT-PCR products were separated on 1.5% (w/v) agarose gels and detected by ethidium bromide and photographed under UV light. PCR was repeated three times with the purpose of testing the same trends for each gene and treatment. Then expression data were recorded, analyzed and visualized by GeneSnap and Mev (Guo et al., 2014).

Real-time PCR analysis was conducted with an ABI real-time PCR instrument (ABI Step One Plus). Each reaction was carried out in triplicate with a reaction volume of 20 μ L containing 1.0 μ L of cDNA, 0.8 μ L each primer (1.0 μ M), 10 μ L of SYBR green Passive Reference Dye 0.4 μ L (TransGen Biotech, China), and 7.4 μ L sterile distilled water. The PCR amplification parameters were 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. Melt-curve analyses were performed using a program with 95°C for 15 s and then a constant increase from 60°C to 95°C. The kiwifruit reference genes *18S 2* and *ACT 2* were used as the internal reference genes. For each assay, three independent biological replications were performed. Relative expression levels were analyzed using the ABI software and the normalized expression ΔC_t method.

Statistical analysis

Statistical analysis was performed using Tukey's *t* test. All experiments were carried out in triplicate and the results were expressed as mean $\pm$ SD.

Results

bZIP genes identification in kiwifruit

To identify bZIP gene family in the kiwifruit genome, the HMM profile of the bZIP domain (PF00170) was found from the Pfam database. We used the SMART tool to test the bZIP domain with e-value less than 0.01 for each matching sequence, resulting a total of 80 potential genes that could be annotated as kiwifruit bZIP TFs. The 80 kiwifruit bZIP genes were named *AcbZIP1* to *AcbZIP80* according to their locations on the chromosomes 1 to 29. Of them, eighteen *AcbZIP* genes (*Achn388061*, *Achn131571*, *Achn131971*, *Achn165061*, *Achn096961*, *Achn227711*, *Achn245851*, *Achn289881*, *Achn253991*, *Achn279821*, *Achn335331*, *Achn344281*, *Achn016621*, *Achn032501*, *Achn032491*, *Achn033861*, *Achn041161*, *Achn058051*) could not be mapped to any chromosome, and were renamed *AcbZIP63* to *AcbZIP80*. The information for 80 *AcbZIP* TFs was listed in Supplementary

SUPPLEMENTARY TABLE S2. The information of *AcbZIP* gene families in kiwifruit.

Group	Gene ID	Gene locus ID	Chromosome	CDS	ORF	Full length	Start site	End site
Group A	<i>AcbZIP3</i>	<i>Achn076181</i>	1	519	172	519	2,470,965	2,471,483
	<i>AcbZIP13</i>	<i>Achn305211</i>	4	864	287	958	5,205,236	5,206,193
	<i>AcbZIP15</i>	<i>Achn158851</i>	4	1191	396	3986	9,470,943	9,474,928
	<i>AcbZIP20</i>	<i>Achn315671</i>	5	1191	396	3986	4,716,539	4,720,524
	<i>AcbZIP21</i>	<i>Achn296661</i>	5	975	324	10832	18,725,958	18,736,789
	<i>AcbZIP22</i>	<i>Achn233651</i>	6	1347	448	2019	16,332,232	16,334,250
	<i>AcbZIP31</i>	<i>Achn026271</i>	11	1380	459	4307	1,539,809	1,544,115
	<i>AcbZIP34</i>	<i>Achn120161</i>	12	3372	1123	11808	5,314,924	5,326,731
	<i>AcbZIP35</i>	<i>Achn173651</i>	12	1290	429	6656	8,407,833	8,414,488
	<i>AcbZIP43</i>	<i>Achn091811</i>	20	1197	398	2055	5,853,994	5,856,048
	<i>AcbZIP44</i>	<i>Achn290761</i>	21	930	309	5152	7,204,386	7,209,537
	<i>AcbZIP56</i>	<i>Achn068551</i>	26	960	319	2615	12,000,664	12,003,278
	<i>AcbZIP61</i>	<i>Achn200201</i>	28	960	319	1919	11,291,432	11,293,350
	<i>AcbZIP64</i>	<i>Achn131571</i>	Unknown	1296	431	4897	1,987,609	1,992,505
	<i>AcbZIP68</i>	<i>Achn227711</i>	Unknown	1236	411	4046	57,279,469	57,283,514
	<i>AcbZIP72</i>	<i>Achn279821</i>	Unknown	699	232	3661	71,591,190	71,594,850
Group B	<i>AcbZIP10</i>	<i>Achn328331</i>	3	2424	807	5636	12,999,243	13,004,878
	<i>AcbZIP75</i>	<i>Achn016621</i>	Unknown	2415	804	5118	117,298,859	117,303,976
Group C	<i>AcbZIP4</i>	<i>Achn040871</i>	1	819	272	2689	11,509,065	11,511,753
	<i>AcbZIP33</i>	<i>Achn124481</i>	11	1008	335	6184	7,809,687	7,815,870
	<i>AcbZIP67</i>	<i>Achn096961</i>	Unknown	972	323	11506	31,641,397	31,652,902
	<i>AcbZIP71</i>	<i>Achn253991</i>	Unknown	888	295	6497	65,705,973	65,712,469
Group D	<i>AcbZIP1</i>	<i>Achn094351</i>	1	1878	625	17275	873,057	890,331
	<i>AcbZIP29</i>	<i>Achn136681</i>	9	1128	375	8067	7,364,137	7,372,203
	<i>AcbZIP32</i>	<i>Achn284361</i>	11	1080	359	3707	4,860,844	4,864,550
	<i>AcbZIP36</i>	<i>Achn126871</i>	12	1284	427	8159	10,258,742	10,266,900
	<i>AcbZIP52</i>	<i>Achn251821</i>	25	762	253	9986	7,251,652	7,261,637
	<i>AcbZIP58</i>	<i>Achn170521</i>	27	735	244	14501	3,334,509	3,349,009
	<i>AcbZIP62</i>	<i>Achn085011</i>	28	648	215	6426	13,981,520	13,987,945
	<i>AcbZIP63</i>	<i>Achn388061</i>	Unknown	1095	364	3477	565,935	569,411
Group E	<i>AcbZIP73</i>	<i>Achn335331</i>	Unknown	981	326	3507	90,412,479	90,415,985
Group F	<i>AcbZIP23</i>	<i>Achn340941</i>	7	810	269	810	573,431	574,240
	<i>AcbZIP26</i>	<i>Achn112471</i>	8	741	246	741	6,148,444	6,149,184
	<i>AcbZIP70</i>	<i>Achn289881</i>	Unknown	804	267	804	60,384,386	60,385,189
Group G	<i>AcbZIP18</i>	<i>Achn254431</i>	5	1884	627	26461	335,865	362,325
	<i>AcbZIP40</i>	<i>Achn278691</i>	14	1164	387	3227	6,650,726	6,653,952
	<i>AcbZIP41</i>	<i>Achn184611</i>	15	1128	375	11514	667,704	679,217
	<i>AcbZIP53</i>	<i>Achn331511</i>	25	855	284	4599	10,023,108	10,027,706
	<i>AcbZIP54</i>	<i>Achn096361</i>	25	1065	354	10294	12,246,127	12,256,420
	<i>AcbZIP55</i>	<i>Achn084391</i>	26	816	271	8699	5,091,573	5,100,271
	<i>AcbZIP59</i>	<i>Achn210631</i>	27	945	314	7818	4,588,001	4,595,818
	<i>AcbZIP69</i>	<i>Achn245851</i>	Unknown	1119	372	3195	60,031,511	60,034,705
Group H	<i>AcbZIP42</i>	<i>Achn159901</i>	20	666	221	4925	1,697,134	1,702,058
	<i>AcbZIP76</i>	<i>Achn032501</i>	Unknown	222	73	4947	118,502,354	118,507,300
	<i>AcbZIP77</i>	<i>Achn032491</i>	Unknown	387	128	5054	118,524,549	118,529,602
	<i>AcbZIP80</i>	<i>Achn058051</i>	Unknown	1038	345	7706	135,425,791	135,433,496

SUPPLEMENTARY TABLE S2. (Continued).

Group	Gene ID	Gene locus ID	Chromosome	CDS	ORF	Full length	Start site	End site
Group I	<i>AcbZIP5</i>	<i>Achn327671</i>	1	1542	513	4405	13,498,771	13,503,175
	<i>AcbZIP6</i>	<i>Achn251071</i>	2	954	317	4121	2,308,680	2,312,800
	<i>AcbZIP8</i>	<i>Achn054841</i>	3	1029	342	4409	1,540,811	1,545,219
	<i>AcbZIP9</i>	<i>Achn062651</i>	3	1092	363	4795	11,015,733	11,020,527
	<i>AcbZIP12</i>	<i>Achn121041</i>	4	1065	354	4925	4,373,887	4,378,811
	<i>AcbZIP16</i>	<i>Achn049341</i>	4	1653	550	7279	10,535,621	10,542,899
	<i>AcbZIP24</i>	<i>Achn340751</i>	7	1689	562	3519	976,206	979,724
	<i>AcbZIP27</i>	<i>Achn237931</i>	8	1227	408	15806	11,547,744	11,563,549
	<i>AcbZIP38</i>	<i>Achn214181</i>	13	1593	530	3250	7,226,747	7,229,996
	<i>AcbZIP46</i>	<i>Achn230471</i>	21	1002	333	7686	12,153,876	12,161,561
	<i>AcbZIP51</i>	<i>Achn147981</i>	24	1065	354	4393	13,104,660	13,109,052
	<i>AcbZIP78</i>	<i>Achn033861</i>	Unknown	1602	533	3586	121,934,667	121,938,252
Group S	<i>AcbZIP2</i>	<i>Achn135561</i>	1	447	148	447	1,695,035	1,695,481
	<i>AcbZIP7</i>	<i>Achn293811</i>	2	495	164	495	11,421,641	11,422,135
	<i>AcbZIP11</i>	<i>Achn057151</i>	3	408	135	5214	15,005,760	15,010,973
	<i>AcbZIP17</i>	<i>Achn049201</i>	4	474	157	474	10,931,787	10,932,260
	<i>AcbZIP14</i>	<i>Achn249321</i>	4	465	154	3137	8,351,591	8,354,727
	<i>AcbZIP19</i>	<i>Achn062291</i>	5	591	196	591	4,560,105	4,560,695
	<i>AcbZIP25</i>	<i>Achn006801</i>	7	444	147	444	1,325,736	1,326,179
	<i>AcbZIP28</i>	<i>Achn201271</i>	9	447	148	447	4,545,207	4,545,653
	<i>AcbZIP30</i>	<i>Achn025721</i>	11	363	120	363	876,192	876,554
	<i>AcbZIP39</i>	<i>Achn035031</i>	13	612	203	2494	7,666,788	7,669,281
	<i>AcbZIP37</i>	<i>Achn229321</i>	13	438	145	438	325,678	326,115
	<i>AcbZIP45</i>	<i>Achn015171</i>	21	558	185	2523	12,079,122	12,081,644
	<i>AcbZIP47</i>	<i>Achn059341</i>	22	393	130	393	11,613,437	11,613,829
	<i>AcbZIP49</i>	<i>Achn024761</i>	23	438	145	438	20,599,729	20,600,166
	<i>AcbZIP48</i>	<i>Achn355201</i>	23	705	234	2674	5,271,751	5,274,424
	<i>AcbZIP50</i>	<i>Achn103311</i>	24	399	132	399	1,097,701	1,098,099
	<i>AcbZIP57</i>	<i>Achn170171</i>	27	567	188	567	2,124,198	2,124,764
	<i>AcbZIP65</i>	<i>Achn131971</i>	Unknown	456	151	456	2,601,369	2,601,824
	<i>AcbZIP66</i>	<i>Achn165061</i>	Unknown	435	144	855	5,437,155	5,438,009
	<i>AcbZIP74</i>	<i>Achn344281</i>	Unknown	429	142	3287	94,441,515	94,444,801
	<i>AcbZIP79</i>	<i>Achn041161</i>	Unknown	567	188	567	126,599,061	126,599,627
	<i>AcbZIP60</i>	<i>Achn338531</i>	27	591	196	591	8,306,869	8,307,459

Table S2, including the group, subgroup, gene locus ID, the start sites and end sites of chromosome distribution, full-length sequences, the length coding (CDS) and ORF sequences. A multiple sequence alignment of 80 *AcbZIP* genes was performed based on the core bZIP domain using DNAMAN software, including approximated 40 to 80 amino acids for each *AcbZIP* gene (Supplementary Figure S1).

Phylogenetic analysis of kiwifruit and *Arabidopsis* bZIP genes

The phylogenetic relationships of different plants can help to perform the classification of the *AcbZIP* gene family. As a model plant, *Arabidopsis* is commonly used to predict the function of a gene in a research plant. The phylogenetic relationship of 80 *AcbZIP* genes with *AtbZIP* genes from *Arabidopsis* was analyzed using the neighbor-joining algorithm, and all *Arabidopsis AtbZIP* proteins were added as reference proteins. The protein sequences of their full-length *AcbZIP* protein sequences were used for evolutionary relationships analysis. According to the results of phylogenetic tree, the *AcbZIP* genes were clustered into 10 distinct groups (A, B, C,

D, E, F, G, H, I, S) based on the features of *AcbZIP* proteins (Figure 1).

Sequence and structure analyses of kiwifruit bZIP genes

The unrooted phylogenetic tree was constructed based on the multiple sequence alignment of 80 kiwifruit *AcbZIP* protein domains (Figure 2A), and 80 *AcbZIP* genes were clustered into ten different groups. Exon-intron structure of the 80 *AcbZIP* genes were analyzed using the genome sequences and coding sequences (Figure 2B). As shown in Figure 3B, most of the *AcbZIP* genes shared a similar structure of exon-intron in the same group with some exception. For example, *AcbZIP5*, *AcbZIP6*, *AcbZIP8*, *AcbZIP9*, *AcbZIP12*, *AcbZIP16*, *AcbZIP24*, *AcbZIP27*, *AcbZIP38*, *AcbZIP51*, *AcbZIP78* genes had three introns in group I, while other groups were similar within each group. A total of 59 *AcbZIP* genes include one to thirteen introns, which accounted for 73.75% of the total number of *AcbZIP* genes. In addition, 21 *AcbZIP* genes had been found no introns, which located in groups F and S. The conserved motifs of 80 kiwifruit *AcbZIP* genes were analyzed using the MEME program (Figure 2C). As shown in Figure 3C,

FIGURE S1. Multiple sequence alignment of the bZIP domain for kiwifruit bZIP genes. Blue indicates conserved amino acid domains. Full stop indicates gaps. The sequence conservations of each position were shown using the overall height in each stack, and each residue letter height is in proportion to the relative frequency of the corresponding residue.

FIGURE 1. Phylogenetic tree of identified *AcbZIP* kiwifruit genes (black). Selected *Arabidopsis AtbZIP* (yellow) were representatives for the different groups.

20 motifs were found (Supplementary Figure S2 and Table S3), and these genes were clustered in the same group that have a similar motif structure. All 80 *AcbZIP* genes contained a conserved basic leucine zipper amino acid domain (Motif 1), excluding *AcbZIP21*, *AcbZIP30*, *AcbZIP 76*. And almost all had more than one motif. The 80 *AcbZIP* TFs were distributed in all 29 kiwifruit chromosomes except for 10, 16, 17, 18, 19 and 29 chromosomes, with chromosome 4 including the highest percentage (6.19%) (Supplementary Figure 3).

Tandem duplication and synteny analysis of *AcbZIP* genes

In order to analyze the expansion patterns of the kiwifruit *AcbZIP* gene family, the tandem duplication events of the 80 *AcbZIP* genes on the 23 kiwifruit chromosomes (Figure 3) were identified according to their physical location of each *AcbZIP* gene on its individual chromosome. In this study, *AcbZIP* genes with tandem duplication events were defined as a chromosomal region within 0.2 Mb containing two or more genes (Guo et al., 2014). Only four *AcbZIP* genes (*AcbZIP19* and *AcbZIP20*, *AcbZIP45* and *AcbZIP46*) were

FIGURE 2. Structure characterization of kiwifruit *AcbZIP* genes. (A) Phylogenetic analysis of kiwifruit *AcbZIP* genes. (B) Intron/Exon structure of kiwifruit *AcbZIP* genes, green indicates exons; black indicates introns; red indicates upstream or downstream. (C) Distribution of conserved motifs in the 80 *AcbZIP* genes. Each motif is represented by a colored box.

FIGURE S2. The logo of each motif of 80 *AcbZIP* genes.

FIGURE 3. Synteny analysis of kiwifruit *AcbZIP* genes. *AcbZIP* genes are indicated by vertical black lines. Colored lines connect the syntenic regions between kiwifruit bZIP genes.

SUPPLEMENTARY TABLE S3. Additional conserved motifs identified from 80 *AcbZIP* proteins.

Motif No.	Length (aa)	E-value	Multi-level consensus sequences	No. of <i>AcbZIP</i> proteins in which motif is present	<i>AcbZIP</i> proteins in which motif is present
1	50	8.0E-1628	XEKVD[EK]R[K]Q[K]R[M]L[SN]RESA[RA]R[SR]E[LM]R[K]Q[K]A[Y]H[L]TVYXEL[EA]Q[K]V[X] [QK] LREENQELXX[QK]	77	<i>AcbZIP</i> 1,10,11,12,13,14,15,16,17,18,19,20,22,23,24,25,26,27,28,29,3,31,32,33,34,35,36,37,38,39,4,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56,57,58,59,60,61,62,63,64,65,66,67,68,69,7,70,71,72,73,74,75,77,78,79,80,9
2	50	2.10E-261	LT[L]FQ[RD]T[ST]G[L]S[T]NSV[JE]Q[N]N[T]E[L]K[L]FRLQAMEQQA[Q]H[L]RDALNEAL[K] [H]Q[E]V[I] [Q]E[R]L[K]IAT	13	<i>AcbZIP</i> 9,6,51,8,24,12,78,16,27,38,5,46,73
3	41	3.60E-183	[L]I[NS]I[S]T[V]I[S]T[Q]E[I]HOM[S]Y[H]L[D]I[N]V[M]E[V]A[Q]E[N]S[VOR]L[R]K[A]E[I]Q[E]L[AGS]E[L] [SR]T[Q]R[M]L[T]SD[L]N[Q]I[DE]I[V]L[S]I[DP]S[Y]M[P]I[DN]S[I]L[A]	20	<i>AcbZIP</i> 17,30,14,19,2,25,28,37,39,45,47,48,49,50,57,60,65,66,74,79
4	29	1.70E-136	[R]Q[Q]Q[P]T[L]F[E]G[K]E[M]I[T]L[E]D[E]F[L]V[A]R[K]A[G]V[A]VRE[DE]I[S]Q[S]L[V]P[G]M[J]K	14	<i>AcbZIP</i> 13,15,20,22,31,34,35,43,44,56,61,64,68,72
5	29	4.70E-132	SG[K]D[N]F[G]SMNDEL[K]N[S]I[V]W[S]TAEEN[T]QTMG[S]T[S]	13	<i>AcbZIP</i> 15,20,22,31,34,35,43,56,61,64,68,72,80
6	29	3.50E-136	[G]Q[G]Y[N]LQ[RG]S[L]T[P]R[T]P[L]S[C]Q[K]T[V]D[E]E[V]W[R]D[L] [A]H	11	<i>AcbZIP</i> 20,15,68,64,31,22,43,34,61,56,35
7	41	4.30E-105	HYD[EN]LFRMKA[ET]AAKADV[Y]H[L]M[S]G[M]T[W]K[R]TSAER[F]C[F]L[S]W[L]GGFRP	6	<i>AcbZIP</i> 29,32,1,63,62,36
8	50	6.30E-90	KLD[AT]LENFVNQADHLRQQT[L]Q[H]Q[L]M[SH]R[L]T[I]I[R]H[Q]IAT[AR]GLL[AN]LGEY[F]C[Q]R[L]R [A]T[L]S	6	<i>AcbZIP</i> 63,32,1,52,36,29
9	49	2.00E-83	[NPV]I[Q]A[I]L[Q]E[L]I[P]D[L]I[TA]DQ[Q]L[V]M[G]A[V]I[CN]L[Q]R[Q]S[SC]QQAEDAL[SQ]G[M]I[DE] [A]K [L]Q[S]I[L]A[S]V[ED]Q[T]I[L]V[A]S[I]S[A]I[DG]N[I]P[Q]I[L]T[G]	7	<i>AcbZIP</i> 52,63,58,1,32,29,36
10	29	4.20E-79	[GPV]G[R]K[P]L[N]I[P]Q[IG]S[P]D[F]I[P]P[S]L[AG]R[Q]S[T]S[V]Y[S]L[T]F[L]D[F]Q[S]H[N]T[L]V[I] [G]C	9	<i>AcbZIP</i> 20,15,64,31,68,22,43,34,35
11	50	2.20E-73	SD[AS]Y[C]NFGM[Q]P[I]H[Y]L[P]L[Y]TQSSFY[QQ]PQ[N]Q[G]T[R]I[S]N[T]D[P]A[S]I[H]Q[L]H[Q] [T]I[M] Q[M]V[P]Q[F]H[P]LQSNM[L]N[H]G[Q]I[H]Q	4	<i>AcbZIP</i> 8,6,51,9
12	32	6.00E-60	[P]A[I]QSANL[P]V[L]N[V]N[V]R[S]T[Q]Q[Q]L[M]I[Y]F[I]M[P]Q[P]Q[Q]Q[Q]Q	6	<i>AcbZIP</i> 20,15,68,64,31,22
13	29	9.60E-58	[A]I[AT]T[F]E[D]I[M]E[Y]A[E]I[H]R[W]V[E]K[E]Q[H]Q[R]H[Q]I[N]C[L]R[K]T[I]A[V]L[V]N[Q]C[H]	6	<i>AcbZIP</i> 29,63,32,1,36,62
14	29	3.70E-56	[GR]I[H]N[RS]I[H]N[JS]K[T]F[N]S[L]V[DE]I[F]G[S]I[N]V[I]G[F]I[E]L[I]F[S]I[S]E[I]GAS[I]I[A]L[K]K [A]I [M]A[P]N[I]D[E]K[L]	9	<i>AcbZIP</i> 9,51,8,6,78,24,16,46,27
15	29	2.00E-55	SDG[R]L[AG]I[K]R[S]N[G]D[N]I[L]M[T]S[S]V[S]P[V]Y[V]A[F]N[G]G[L]RGRK	6	<i>AcbZIP</i> 20,15,64,31,68,22
16	32	9.50E-43	[GE]I[S]R[I]F[K]I[D]P[E]I[G]I[E]G[S]V[E]V[D]D[L]F[S]C[I]A[Y]M[D]N[L]M[V]E[K]N[I]L[F]I[GD]I[S]T[I]G[L] [N]S [S]A[I]D[S]G[I]G[I]D[G]I[D]C	8	<i>AcbZIP</i> 51,9,6,8,78,24,16,27
17	15	1.80E-36	[M]F[I]N[L]N[S]P[W]N[S]W[M]Y[L]F[N]Q[P]M	7	<i>AcbZIP</i> 17,45,65,30,2,28,25
18	41	4.20E-59	[H]Q[I]A[V]P[H]P[H]Y[L]M[W]G[S]I[P]T[P]Q[P]T[L]M[M]P[S]Y[G]V[I]A[L]V[P]Y[AS]I[AT]I[Y]A[S]I[HP]G [G]V [Y]A[H]P[AG]V[P]F[L]P[V]I[AD]I[D]T	4	<i>AcbZIP</i> 69,40,41,53
19	29	3.10E-40	[CP]I[L]T[I]H[Y]I[H]T[L]H[I]T[Q]I[C]I[T]N[P]P[Q]G[P]Q[T]E[D]I[F]S[I]S[T]I[P]H[I]Q[T]I[P]H[T]C[F]Y[I]H[S]I[N]S[V] [H]N[Y]S[I]T[T]K[I]S[I]D[E]S	7	<i>AcbZIP</i> 23,70,26,19,60,7,11
20	29	2.90E-38	[SH]Q[L]N[L]G[K]N[S]I[P]Q[I]G[V]I[P]R[G]S[I]G[E]I[V]N[G]I[N]G[I]N[G]I[T]M[S]D[S]K[Q]S[P]V[M]N[V]G[N]I[L]T [V]G [Q]A[I]G[S]T[I]G[K]I[AG]I[L]M	8	<i>AcbZIP</i> 68,20,15,64,31,69,40,22

SUPPLEMENTARY TABLE S4. Tandem duplication events in the 80 kiwifruit *AcbZIP* genes.

Cluster number	Gene ID	Chromosome	Start site	End site
1	<i>AcbZIP19</i>	5	4,560,105	4,560,695
	<i>AcbZIP20</i>	5	4,716,539	4,720,524
2	<i>AcbZIP45</i>	21	12,079,122	12,081,644
	<i>AcbZIP46</i>	21	12,153,876	12,161,561

SUPPLEMENTARY TABLE S5. Synteny blocks of *bZIP* genes within kiwifruit genome.

ID	Region1 (kiwifruit)			Region2 (kiwifruit)			Gene in the synteny region		Gene name	
	Chr	Start	Stop	Chr	Start	Stop	Gene 1	Gene 2	Gene 1	Gene 2
1	Chr1	2371829	2874852	Chr5	17827057	18823165	<i>Achn076181</i>	<i>Achn296661</i>	<i>AcbZIP3</i>	<i>AcbZIP21</i>
2	Chr1	834573	1014187	Chr9	7339414	7517854	<i>Achn094351</i>	<i>Achn136681</i>	<i>AcbZIP1</i>	<i>AcbZIP29</i>
3	Chr1	1403633	1919298	Chr9	4376982	4958615	<i>Achn135561</i>	<i>Achn201271</i>	<i>AcbZIP2</i>	<i>AcbZIP28</i>
4	Chr11	762760	1035549	Chr1	1483731	2023713	<i>Achn025721</i>	<i>Achn135561</i>	<i>AcbZIP30</i>	<i>AcbZIP2</i>
5	Chr11	623477	1086143	Chr4	10548353	11146162	<i>Achn025721</i>	<i>Achn049201</i>	<i>AcbZIP30</i>	<i>AcbZIP17</i>
6	Chr11	1275804	2385657	Chr4	8481738	9993279	<i>Achn026271</i>	<i>Achn158851</i>	<i>AcbZIP31</i>	<i>AcbZIP15</i>
7	Chr11	403340	2555158	Chr6	15484537	17821077	<i>Achn026271</i>	<i>Achn233651</i>	<i>AcbZIP31</i>	<i>AcbZIP22</i>
8	Chr11	623477	1313843	Chr9	4304002	5189407	<i>Achn025721</i>	<i>Achn201271</i>	<i>AcbZIP30</i>	<i>AcbZIP28</i>
9	Chr12	8294314	8672132	Chr20	12545699	12722021	<i>Achn173651</i>	<i>Achn270881</i>	<i>AcbZIP35</i>	-
10	Chr15	578066	1197478	Chr25	9930983	10438361	<i>Achn184611</i>	<i>Achn331511</i>	<i>AcbZIP41</i>	<i>AcbZIP53</i>
11	Chr2	10582131	11760758	Chr27	7396311	8589526	<i>Achn293811</i>	<i>Achn338531</i>	<i>AcbZIP7</i>	<i>AcbZIP60</i>
12	Chr21	11809820	12570028	Chr1	1348842	1980234	<i>Achn015171</i>	<i>Achn135561</i>	<i>AcbZIP45</i>	<i>AcbZIP2</i>
13	Chr21	11210647	12081644	Chr11	876192	1370339	<i>Achn015171</i>	<i>Achn025721</i>	<i>AcbZIP45</i>	<i>AcbZIP30</i>
14	Chr21	7052064	7524912	Chr4	5049812	5247304	<i>Achn290761</i>	<i>Achn305211</i>	<i>AcbZIP44</i>	<i>AcbZIP13</i>
15	Chr22	11094441	11880883	Chr24	747029	1302588	<i>Achn059341</i>	<i>Achn103311</i>	<i>AcbZIP47</i>	<i>AcbZIP50</i>
16	Chr23	19721715	20666117	Chr13	221213	967308	<i>Achn024761</i>	<i>Achn229321</i>	<i>AcbZIP49</i>	<i>AcbZIP37</i>
17	Chr23	16583963	16786550	Chr13	7057100	7314165	<i>Achn191271</i>	<i>Achn214181</i>	-	<i>AcbZIP38</i>
18	Chr24	13028939	13330770	Chr2	2127381	2353519	<i>Achn147981</i>	<i>Achn251071</i>	<i>AcbZIP51</i>	<i>AcbZIP6</i>
19	Chr26	4834952	5604687	Chr25	11995337	12634770	<i>Achn084391</i>	<i>Achn096361</i>	<i>AcbZIP55</i>	<i>AcbZIP54</i>
20	Chr26	11450709	12729232	Chr28	10716428	12066768	<i>Achn068551</i>	<i>Achn200201</i>	<i>AcbZIP56</i>	<i>AcbZIP61</i>
21	Chr27	3096850	3470623	Chr25	7098369	7359352	<i>Achn170511</i>	<i>Achn251821</i>	-	<i>AcbZIP52</i>
22	Chr27	4437680	4858600	Chr5	48486	565179	<i>Achn210621</i>	<i>Achn254431</i>	-	<i>AcbZIP18</i>
23	Chr3	14611063	16114725	Chr2	11123156	12530279	<i>Achn057151</i>	<i>Achn293811</i>	<i>AcbZIP11</i>	<i>AcbZIP7</i>
24	Chr3	10451976	11695808	Chr2	1965465	2988546	<i>Achn062651</i>	<i>Achn251071</i>	<i>AcbZIP9</i>	<i>AcbZIP6</i>
25	Chr3	11015733	11785438	Chr23	16180360	16640371	<i>Achn062651</i>	<i>Achn191271</i>	<i>AcbZIP9</i>	-
26	Chr3	10155487	11206639	Chr24	12943189	13691997	<i>Achn062651</i>	<i>Achn147981</i>	<i>AcbZIP9</i>	<i>AcbZIP51</i>
27	Chr3	1518906	2233352	Chr24	11679371	13127713	<i>Achn054841</i>	<i>Achn147981</i>	<i>AcbZIP8</i>	<i>AcbZIP51</i>
28	Chr3	14364874	15332373	Chr27	7396311	8589526	<i>Achn057151</i>	<i>Achn338531</i>	<i>AcbZIP11</i>	<i>AcbZIP60</i>
29	Chr3	14611063	15646650	Chr5	4482927	5847398	<i>Achn057151</i>	<i>Achn062291</i>	<i>AcbZIP11</i>	<i>AcbZIP19</i>
30	Chr4	10556611	10932260	Chr1	1062750	1695481	<i>Achn049201</i>	<i>Achn135561</i>	<i>AcbZIP17</i>	<i>AcbZIP2</i>
31	Chr4	10501611	10902278	Chr21	12122999	12521802	<i>Achn049341</i>	<i>Achn230471</i>	<i>AcbZIP16</i>	<i>AcbZIP46</i>
32	Chr4	3205541	4900887	Chr21	6467739	7424965	<i>Achn121041</i>	<i>Achn290821</i>	<i>AcbZIP12</i>	-
33	Chr4	10548353	10932260	Chr9	4545207	5189407	<i>Achn049201</i>	<i>Achn201271</i>	<i>AcbZIP17</i>	<i>AcbZIP28</i>
34	Chr5	3571004	4602360	Chr2	10582131	11461478	<i>Achn062291</i>	<i>Achn293811</i>	<i>AcbZIP19</i>	<i>AcbZIP7</i>
35	Chr5	2945099	4975044	Chr27	6954897	8589526	<i>Achn062291</i>	<i>Achn338531</i>	<i>AcbZIP19</i>	<i>AcbZIP60</i>
36	Chr7	1303601	2216331	Chr11	863151	1857439	<i>Achn006801</i>	<i>Achn025721</i>	<i>AcbZIP25</i>	<i>AcbZIP30</i>
37	Chr8	11204864	13156651	Chr1	13163591	14648527	<i>Achn237931</i>	<i>Achn327661</i>	<i>AcbZIP27</i>	-

found that belong to two tandem duplication event regions on chromosome 5 (one cluster), 21 (one cluster) (Supplementary Table S4). In addition, 37 segregation duplication events were identified on kiwifruit chromosomes 1, 2, 4, 5, 6, 9, 11, 13, 20, 21, 23, 24, 25, 27, 28 (Figure 3; Supplementary Table S5).

In order to clarify the origin of kiwifruit bZIP TFs and the evolutionary relationship between the kiwifruit and *Arabidopsis*, we also performed a comparative bZIP gene families synteny map of the kiwifruit and *Arabidopsis* genomes. The corresponding orthologs of 29 kiwifruit *AcbZIP* genes and 29 *AcbZIP Arabidopsis* were identified (Figure 4; Supplementary Table S6).

FIGURE S3. The distribution of *AcbZIP* genes in kiwifruit chromosomes. The number of bZIP genes is shown at the top in each chromosome. Eighteen of the 80 *AcbZIP* could not be mapped to any chromosome and are not shown.

SUPPLEMENTARY TABLE S6. Synteny blocks of *bZIP* genes between kiwifruit and *Arabidopsis* genomes.

ID	Region 1 (Kiwifruit)			Region 2 (<i>Arabidopsis</i>)			Gene in the synteny region			Gene name		Ka	KS	Ka/KS
	Chr	Start	Stop	Chr	Start	Stop	Gene 1	Gene 2	Gene 1	Gene 2				
1	Chr1	13466555	14047186	Chr2	13378944	13450095	Achn327661	AT2G31370	-	AtbZIP59	0.2499	1.3374	0.186855	
8	Chr1	2110462	2484488	Chr4	16796256	17021060	Achn076181	AT4G35900	AcbZIP3	AtbZIP14	0.6647	1.7438	0.381179	
30	Chr11	823968	1262833	Chr1	28157530	28315122	Achn025721	AT1G75390	AcbZIP30	AtbZIP44	0.3846	0	-	
47	Chr11	607089	2174695	Chr5	16866040	17335234	Achn026271	AT5G42910	AcbZIP31	AtbZIP15	0.6047	3.2349	0.18693	
56	Chr12	7782870	10369225	Chr3	20941502	21448690	Achn173651	AT3G56850	AcbZIP35	AtbZIP66	0.3638	0	-	
82	Chr13	6686753	7229996	Chr1	2105048	2250529	Achn214181	AT1G06850	AcbZIP38	AtbZIP52	0.6546	1.9146	0.341899	
94	Chr13	45261	1665062	Chr3	22983138	23422399	Achn229321	AT3G62420	AcbZIP37	AtbZIP53	0.3135	2.2145	0.141567	
121	Chr14	6086324	7492153	Chr2	18935301	19093315	Achn278691	AT2G46270	AcbZIP40	AtbZIP55	0.3214	1.7635	0.182251	
135	Chr14	6214463	7492153	Chr4	362169	566310	Achn278691	AT4G01120	AcbZIP40	AtbZIP54	0.385	1.507	0.255474	
302	Chr2	2122681	3236492	Chr2	16588696	17036208	Achn251071	AT2G40620	AcbZIP6	AtbZIP18	0.272	2.3961	0.113518	
332	Chr20	5853994	6238330	Chr2	15204764	15300990	Achn091811	AT2G36270	AcbZIP43	AtbZIP39	0.3753	0	-	
338	Chr20	12659948	14015433	Chr3	20984752	21177119	Achn270881	AT3G56850	-	AtbZIP66	0.3901	0	-	
347	Chr20	395162	3566708	Chr5	3377663	3832944	Achn159901	AT5G11260	AcbZIP42	AtbZIP56	0.3002	2.0202	0.148599	
352	Chr21	11471476	14174099	Chr1	28171018	28747845	Achn015171	AT1G75390	AcbZIP45	AtbZIP44	0.3551	0	-	
359	Chr21	6159914	7524912	Chr1	16263813	16709713	Achn290821	AT1G43700	-	AtbZIP51	1.4319	2.431	0.589017	
415	Chr23	19868338	20666117	Chr3	23072786	23269324	Achn024761	AT3G62420	AcbZIP49	AtbZIP53	0.3225	1.7629	0.182937	
447	Chr24	9803769	13660633	Chr2	16260853	17071657	Achn147981	AT2G40620	AcbZIP51	AtbZIP18	0.2758	1.967	0.140214	
479	Chr25	11251612	12634770	Chr1	11436719	11800988	Achn096361	AT1G32150	AcbZIP54	AtbZIP68	0.2883	2.1468	0.134293	
491	Chr25	12045043	12639470	Chr2	14839091	14945357	Achn096361	AT2G35530	AcbZIP54	AtbZIP16	0.2406	1.4704	0.163629	
506	Chr26	4847568	5370993	Chr1	11552926	11654794	Achn084391	AT1G32150	AcbZIP55	AtbZIP68	0.3665	2.7445	0.13354	
520	Chr26	11546572	12013046	Chr2	15082855	15210845	Achn068551	AT2G36270	AcbZIP56	AtbZIP39	0.5941	0	-	
523	Chr26	11366490	12207317	Chr3	15866039	16195833	Achn068551	AT3G44460	AcbZIP56	AtbZIP67	0.57	0	-	
560	Chr27	4220165	5187294	Chr4	17225912	17492010	Achn210631	AT4G36730	AcbZIP59	AtbZIP41	0.2752	2.4196	0.113738	
587	Chr28	10472888	11306249	Chr2	15106940	15210845	Achn200201	AT2G36270	AcbZIP61	AtbZIP39	0.592	2.4838	0.238344	
589	Chr28	10725668	11487576	Chr3	15888902	16195833	Achn200201	AT3G44460	AcbZIP61	AtbZIP67	0.6085	0	-	
635	Chr3	10789897	11732521	Chr2	16658656	17034600	Achn062651	AT2G40620	AcbZIP9	AtbZIP18	0.2816	2.7538	0.102259	
638	Chr3	1518906	1650042	Chr2	16877223	16961144	Achn054841	AT2G40620	AcbZIP8	AtbZIP18	0.2868	3.0051	0.095438	
688	Chr5	18269065	18823165	Chr2	7703856	7802230	Achn296661	AT2G17770	AcbZIP21	AtbZIP27	0.5007	0	-	
708	Chr5	4713831	4860099	Chr5	17142820	17209545	Achn315671	AT5G42910	AcbZIP20	AtbZIP15	0.4794	0	-	
716	Chr6	15571693	17030730	Chr1	18246305	18543948	Achn233651	AT1G49720	AcbZIP22	ABF1	0.4925	1.7755	0.277387	
734	Chr6	15571693	16940187	Chr3	6501668	6842320	Achn233651	AT3G19290	AcbZIP22	AtbZIP38	0.4719	2.0242	0.233129	
741	Chr6	15923018	16334250	Chr4	16295430	16406240	Achn233651	AT4G34000	AcbZIP22	AtbZIP37	0.4663	1.5565	0.299582	
752	Chr7	380271	1313141	Chr2	8998686	9210231	Achn340751	AT2G21230	AcbZIP24	AtbZIP30	0.332	1.84	0.180435	
755	Chr7	131078	2265847	Chr2	6637878	7490176	Achn340941	AT2G16770	AcbZIP23	AtbZIP23	0.2	2.2391	0.089322	

SUPPLEMENTARY TABLE S6. (Continued).

ID	Region 1 (Kiwifruit)			Region 2 (Arabidopsis)			Gene in the syntenic region			Gene name		Ka	KS	Ka/KS
	Chr	Start	Stop	Chr	Start	Stop	Gene 1	Gene 2		Gene 1	Gene 2			
765	Chr7	25965	1481549	Chr4	17904558	18284930	Achn340751	AT4G38900		AcbZIP24	AtbZIP29	0.2471	0	-
769	Chr7	131078	1936782	Chr4	16412773	16898876	Achn006801	AT4G34590		AcbZIP25	AtbZIP11	0.4353	0	-
769	Chr7	131078	1936782	Chr4	16412773	16898876	Achn340941	AT4G35040		AcbZIP23	AtbZIP19	0.2362	1.5623	0.151187
780	Chr8	11547744	13060564	Chr1	1749682	1837648	Achn237931	AT1G06070		AcbZIP27	AtbZIP69	0.2083	1.9046	0.109367
787	Chr8	11547744	12988671	Chr2	13378944	13462181	Achn237931	AT2G31370		AcbZIP27	AtbZIP59	0.2333	1.9517	0.119537
801	Chr8	5689059	6174740	Chr3	19277778	19343466	Achn112471	AT3G51960		AcbZIP26	AtbZIP24	0.5794	0	-
828	Chr9	4545207	5277950	Chr2	7784288	7899242	Achn201271	AT2G18160		AcbZIP28	AtbZIP2	0.4704	0	-

Expression patterns of kiwifruit group A *AcbZIP* genes in different organs

To identify potential functions of the *AcbZIP* genes, semi-quantitative RT-PCR was carried out to assess their expression pattern in five different tissue organs including apical buds, young leaves, stems, flower buds, and fruits. Some studies have indicated that Group A bZIP genes involved in abiotic stress regulation and ABA signal transduction in seeds (Jakoby et al., 2002). Therefore, 16 Group A bZIP kiwifruit genes were selected to analyze the expression patterns by semi-quantitative RT-PCR. The results indicated that the 16 selected *AcbZIP* genes were expressed in all organs, and each *AcbZIP* gene showed a different expression level among the different organs (Supplementary Figure S4). Most of the genes showed a relatively high expression in apical buds, flower buds, and fruits compared to young leaves and stems.

Expression profiles of kiwifruit group A *AcbZIP* genes in response to Psa, salt, drought and hormone treatments

To further identify potential roles for kiwifruit *AcbZIP* genes in response to Psa, salt, drought and hormone treatments, semi-quantitative RT-PCR was used to analyze the expression profiles of 16 Group A bZIP genes in response to Psa, salt, drought and hormone treatments at different times (Supplementary Figures S5 and S6). The results showed that diverse expression patterns were found and some of the genes were up-regulated, while others were down-regulated. As shown in Figure S2, several genes (such as *AcbZIP13*, *AcbZIP15*, *AcbZIP20*, *AcbZIP22*) showed decreased 72 h post Psa treatment, but then increased at 120 h after Psa treatment. *AcbZIP13*, *AcbZIP15*, *AcbZIP20*, *AcbZIP22* and *AcbZIP64* showed an obvious increase in expression from 96 h post-treatment with drought, while the expression decreased after R48 h treatment. As shown in Figure S3,

FIGURE S4. Expression analysis of Group A *AcbZIP* genes in five organs. Organs are indicated as follows: 1 - young leaves; 2 - stems; 3 - apical buds; 4 - flower buds; 5 - fruits.

FIGURE 4. Synteny analysis of bZIP genes between kiwifruit and *Arabidopsis*. Kiwifruit and *Arabidopsis* bZIP genes are indicated by vertical black lines. Colored lines connect the syntenic regions between kiwifruit and *Arabidopsis* bZIP genes.

FIGURE S5. Expression levels of Group A *AcbZIP* genes following Psa, salt, and drought treatments by semi-quantitative RT-PCR.

AcbZIP13, *AcbZIP15*, *AcbZIP22* and *AcbZIP31* showed similar expression levels and no obvious increased expression at different time points following the hormone treatments. To verify the efficacy of the semi-quantitative RT-PCR analyses, the expression of seven selected *AcbZIP* genes (*AcbZIP13*, *AcbZIP15*, *AcbZIP20*, *AcbZIP22*, *AcbZIP31*, *AcbZIP64* and *AcbZIP68*) following Psa, salt, drought and hormone treatments were analyzed using quantitative real-time PCR (Figure 5).

Discussion

In this study, we performed a genome-wide identification of kiwifruit bZIP genes and analyze their evolutionary histories and expression pattern in different kiwifruit organs and biotic and abiotic stresses. These results may provide some research resource to further analyze the function of *AcbZIP* genes in kiwifruit.

In the present research, we identified 80 *AcbZIP* genes according to the core bZIP domain. Multiple sequence alignment showed that 80 of the *AcbZIP* genes contain a highly conserved sequence N-x7-R/K-x9 motif and a leucine zipper motif. These results have also been found in the grape, apple bZIP TFs (Zhao et al., 2016; Gao et al., 2014). Genomic comparison could provide an efficient method for transferring genomic information within one taxon (Peterson et al., 2012). *Arabidopsis* is considered to be the best model plant species for the phylogenetic relationship of bZIP TFs (Zhao et al., 2016; Gao et al., 2014). In this study, an unrooted neighbor-joining (NJ) tree was constructed using a multiple alignment of full-length protein sequence. The kiwifruit *AcbZIP* TFs is divided into ten groups (A, B, C, D, E, F, G, H, I, and S) depending on the characteristics of bZIP sequence that including highly conserved sequence N-x7-R/K-x9 motif and

FIGURE 5. Expression levels of Group A *AcbZIP* genes following Psa, salt, and drought, and different hormone treatments (salicylic acid-SA; Amethyl jasmonic acid-MeJA; Ethylene-Eth; gibberellins-GA; abscisic acid-ABA) treatments by quantitative real-time RT-PCR. The error bars were calculated by three biological and technical replicates.

FIGURE S6. Expression levels of Group A *AcbZIP* genes with different hormone treatments (SA – salicylic acid; MeJA – methyl jasmonic acid; Eth – ethylene; GA – gibberellins; ABA – abscisic acid) by semi-quantitative RT-PCR.

a leucine zipper motif, which was in accordance with *Arabidopsis*, and sorghum (Wang et al., 2011; Jakoby et al., 2002). In addition, 13 groups also were present in some plants, such as *Arabidopsis*, rice, black cottonwood, apple and grape (Zhao et al., 2016; Gao et al., 2014; Corrêa et al., 2008). In this study, we found that kiwifruit has a larger number of bZIP genes than other plants, such as cassava (77) (Hu et al., 2016), *Arabidopsis* (75) (Jakoby et al., 2002), cucumber (64) (Baloul et al., 2014), and grape (55) (Gao et al., 2014), while compared with rice (89) (Nijhawan et al., 2008), sorghum (88) (Wang et al., 2011), maize (125) (Wei et al., 2012), castor bean (100) (Jin et al., 2014), apple (112 or 144) (Li et al., 2016; Zhao et al., 2016), Chinese cabbage (136) (Bai et al., 2016), the kiwifruit *AcbZIP* genes are few. The reason may be related to the size of genome. For example, the genomic size of several plants in this order: kiwifruit (616.1 Mb) (Huang et al., 2013), grape (490 Mb) (Gao et al., 2014), cucumber (367 Mb) (Huang et al., 2009), *Arabidopsis* (119 Mb) (International Rice Genome Sequencing Project, 2005). However, the number of TFs and the genome size are not necessarily correlated (Song et al., 2016). For example, the genome size of rice (389 Mb) (International Rice Genome Sequencing Project) is smaller than kiwifruit, but the number of bZIP are higher than kiwifruit.

Previous studies indicated that exon/intron structure can also provide crucial information to analyze the phylogenetic relationships for a gene family (Hu et al., 2015). And exon/intron also play important roles in the evolution of multiple gene families (Zhang et al., 2012). In this study, we analyzed the exon/intron of the 80 *AcbZIP* genes to help us further understand the evolution. Among 80 *AcbZIP* genes, the number of introns ranged from 0 to 12. However, most studies showed that some bZIP TF families had no introns, such as bZIP TFs in apple, grape, rice and sorghum (Zhao et al., 2016; Gao et al., 2014; Wang et al., 2011; Nijhawan et al., 2008). In general, introns of the same group showed similar conserved positions and phases. This is similar with *VvbZIP* in grape and *MdbZIP* in apple (Zhao et al., 2016; Gao et al., 2014). Additionally, the intron/exon loss/gain were found within the same *AcbZIP* gene. For example, *AcbZIP5*, *AcbZIP38*, *AcbZIP16*, *AcbZIP24*, *AcbZIP78*, *AcbZIP12*, *AcbZIP27*, *AcbZIP6*,

AcbZIP8, *AcbZIP9*, *AcbZIP51* have three or four introns, while *AcbZIP46* genes in the same group I have only one intron. This intron/exon loss or gain may be caused by the rearrangement and fusions of different chromosome fragments (Guo et al., 2014). Conserved motifs could also provide further confirmation of the evolutionary relationship among the kiwifruit *AcbZIP* genes. Phylogenetic relationship results indicated that the same group generally contains the similar number of amino acids and motif composition, which may show that the molecular structures were highly conserved during evolution in the same group (Figure 3C). Similar patterns have also been reported in grape and apple (Zhao et al., 2016; Gao et al., 2014).

This has been made possible to reconstruct the evolutionary history of a gene by comparing the sequences of all genes among genomes from different taxa or within each genome (Koonin, 2005). Previous researches indicated that *Arabidopsis* is considered to be the best model plant species of bZIP TFs studies (Zhao et al., 2016; Gao et al., 2014). Thus, the origin and evolutionary history of the *AcbZIP* genes were analyzed by comparative synteny map between the kiwifruit and *Arabidopsis* genomes (Figure 5). According to this syntenic map, 29 *AcbZIP* genes have 29 *Arabidopsis* corresponding orthologs, suggesting that the expansion might have occurred before the divergence of kiwifruit and *Arabidopsis*. Of these, eight pairs revealed to be single kiwifruit-to-*Arabidopsis* pairs (Supplementary Table S3), indicating that these *AcbZIP* genes and their *Arabidopsis* counterparts share a common ancestor (Gao et al., 2014). From these homologs results, we may be obtaining the function information of some kiwifruit *AcbZIP* genes. Other gene combinations showed a different syntenic relationship, with eight gene-pairs that one single kiwifruit gene corresponded to multiple *Arabidopsis* genes, seven gene-pairs that kiwifruit segmental duplications corresponded to single *Arabidopsis* gene (Supplementary Table S3). In addition, we found that more *AcbZIP* genes could not be mapped into any synteny blocks, but it cannot be concluded that these genes did not share a common ancestor with *Arabidopsis* bZIP genes. The reasons may be explained by the fact that after the speciation of kiwifruit and *Arabidopsis*, their genomes may have undergone multiple rounds of sig-

nificant chromosomal rearrangement and fusions, followed by selective gene loss, which can severely obscure chromosomal synteny (Zhao et al., 2016; Gao et al., 2014; Paterson et al., 2012). Moreover, the Ka, Ks, and Ka/Ks of the syntenic orthologous gene-pairs showed Ka/Ks ration < 1 that these genes may underwent a strong Darwinian purifying positive selection; based on these results we also inferred that some of the kiwifruit genes shared a common ancestor with their *Arabidopsis* bZIP counterparts (Zhao et al., 2016).

The *AcbZIP* genes were located to the 23 kiwifruit chromosomes based on the chromosome annotation of the kiwifruit genome, indicating that not all 29 chromosomes contain an *AcbZIP* gene, and chromosomes 10, 16, 17, 18, 19, and 29 have no *AcbZIP* genes. The largest numbers of *AcbZIP* genes are chromosomes 4, eighteen *AcbZIP* genes were not assigned on any of the chromosomes; these results were also found in grape *MdbZIP* genes (Li et al., 2016; Zhao et al., 2016). Tandem and segmental duplicated events of genome duplication play an important role in generating new duplicated genes and evolution of organisms (Song et al., 2016; Zhao et al., 2016; Gao et al., 2014). Whole-genome duplication (γ , β , α) followed by gene loss is regarded as the major evolutionary force (Huang et al., 2013), and often lead to gene family expansion (Cannon et al., 2004). Kiwifruit has undergone an ancient hexaploidization event (γ) shared by core eudicots and two more recent whole-genome duplication events (Huang et al., 2013). Furthermore, kiwifruit also has undergone large gene duplication in its evolution process and 10,581 duplicated genes have been found in the kiwifruit genome (Huang et al., 2013). In this study, we only identified two pairs of tandem duplications (*AcbZIP19/AcbZIP20*; *AcbZIP45/AcbZIP46*) on kiwifruit chromosomes 5 and 21, respectively, indicating a few contribution of tandem duplication to *AcbZIP* gene family expansion. Same results were found in grape and sorghum, including two and three tandem duplication event regions, respectively (Gao et al., 2014; Wang et al., 2011). However, 37 *AcbZIP* genes were associated with segmental duplication events, indicating that segmental duplication events could be regarded as a major mechanism to drive the expansion of kiwifruit *AcbZIP* gene families. As a whole, tandem and segmental duplications may play important roles in the expansion and evolution of the *AcbZIP* gene family in kiwifruit.

From most previous researches we know that bZIP genes play essential roles in response to various abiotic and biotic stress for many plants. Compared with other plant species, the responses of kiwifruit bZIP genes to abiotic and biotic stress, such as salt, drought, pathology resistance, and hormone, have not been studied. In addition, the expression patterns of bZIP genes also have not been studied in kiwifruit different tissues. The function of bZIP genes had been studied in lots of plant species, especially in *Arabidopsis*, rice and tomato. For example, overexpression of *AtAREB1* plays a role in response to abiotic stress in tomato (Yáñez et al., 2009). *AREB1* could enhance drought stress tolerance in *A. thaliana* (Fujita et al., 2005). *ABF3* was reported to reduce transpiration and enhance drought tolerance (Kang et al., 2002). Drought and salt are major abiotic stresses affecting growth of plants and result in severe yield losses for economic crops (Li et al., 2013). bZIP TF gene could be induced by drought and salt in some plants, such as *Arabidopsis*, grape, tomato. For example, *ABP9* showed enhanced drought stresses (Zhang et al., 2008), *VvbZIP23* was induced by drought and cold stress (Tak and Mhatre, 2013), and *StABF1* was reported to enhance drought tolerance (García et al., 2012). In the

present study, we analyzed the *AcbZIP* genes expression patterns in response to drought and salt abiotic stresses. *AcbZIP13*, *AcbZIP15*, *AcbZIP20*, *AcbZIP22*, *AcbZIP31*, *AcbZIP64* and *AcbZIP68* showed a peak of expression at 168 h after drought treatment, indicating that a certain response time is needed for bZIP genes to response to drought stress, similar results were found in WRKY genes (Guo et al., 2014). The reasons may be that the temporal and spatial diversification of bZIP gene expression is widespread. Similar results were also found in *Psa* biotic stress, including *AcbZIP13*, *AcbZIP15*, *AcbZIP20*, *AcbZIP22*, *AcbZIP31*, *AcbZIP64* showed down-regulation during early infection, and up-regulated during a later time. Some plant hormones, including Eth, SA, MeJA, ABA and GA have been reported to play important roles in the growth and development of plants, and involved in the different biotic and abiotic stress responses in plants. For example, Eth, SA and GA have key roles in biotic stress signaling upon pathogen infection; ABA is involved in response to pathogen infection, water-deficit, cold and osmotic stress (Zhao et al., 2016; Gao et al., 2014; Bari et al., 2009). Our studies show that the *AcbZIP* genes have a variety of response patterns after various hormone treatments, indicating that they may be induced by the hormones.

Conclusions

In conclusion, we performed the first genome-wide analysis of 80 kiwifruit *AcbZIP* TF family. Synteny analyses showed that tandem and segmental duplications mainly have contributed to the expansion of the kiwifruit *AcbZIP* gene family. Phylogenetic and gene structure analyses revealed the classification and evolution relationship of 80 *AcbZIP* genes. Some *AcbZIP* genes expression indicated that they play an important role in response to various biotic and abiotic stresses. These results may provide some information for further gene clone and functional research in kiwifruit.

Acknowledgments

This work was supported by the earmarked fund for China Postdoctoral Science Foundation (2015M582712), Postdoctoral Science Foundation of Shaanxi Province (2016BSHYDZZ07), Science and Technology Research and Development Program of Shaanxi Province (2015KTZD-NY02-03-01, 2016KJXX-58). We thank AJE (<https://www.aje.com/>) for editing this manuscript.

References

- Bai, Y., Zhu, W., Hu, X., Sun, C., Li, Y., Wang, D., Wang, Q., Pei, G., Zhang, Y., Guo, A., Zhao, H., Lu, H., Mu, X., Hu, J., Zhou, X., and Xie, C.G. (2016). Genome-wide analysis of the bZIP gene family identifies two ABI5-like bZIP transcription factors, BrABI5a and BrABI5b, as positive modulators of ABA signalling in Chinese cabbage. *PLoS ONE* 11(7), e0158966. <https://doi.org/10.1371/journal.pone.0158966>.
- Baloglu, M.C., Eldem, V., Hajyzadeh, M., and Unver, T. (2014). Genome-wide analysis of the bZIP transcription factors in cucumber. *PLoS ONE* 9, e96014. <https://doi.org/10.1371/journal.pone.0096014>.
- Bari, R., and Jones, J.D. (2009). Role of plant hormones in plant defense responses. *Plant Mol. Biol.* 69(4), 473–488. <https://doi.org/10.1007/s11103-008-9435-0>.
- Cannon, S.B., Mitra, A., Baumgarten, A., Young, N.D., and 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, 10. <https://doi.org/10.1186/1471-2229-4-10>.
- Corrêa, L.G., Riaño-Pachón, D.M., Schrago, C.G., dos Santos, R.V., Mueller-Roeber, B., and Vincentz, M. (2008). The role of bZIP

- transcription factors in green plant evolution: adaptive features emerging from four founder genes. *PLoS ONE* 3, e2944. <https://doi.org/10.1371/journal.pone.0002944>.
- Ferradás, Y., Rey, L., Martínez, O., Rey, M., and González, M.V. (2016). Identification and validation of reference genes for accurate normalization of real-time quantitative PCR data in kiwifruit. *Plant Physiol. Biochem.* 102, 27–36. <https://doi.org/10.1016/j.plaphy.2016.02.011>.
- Fujita, Y., Fujita, M., Satoh, R., Maruyama, K., Parvez, M.M., Seki, M., et al. (2005). AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in *Arabidopsis*. *Plant Cell* 17, 3470–3488. <https://doi.org/10.1105/tpc.105.035659>.
- Gao, M., Zhang, H.J., Guo, C.L., Cheng, C.X., Guo, R.R., Mao, L.Y., Fei, Z.J., and Wang, X.P. (2014). Evolutionary and expression analyses of basic zipper transcription factors in the highly homozygous model grape PN40024 (*Vitis vinifera* L.). *Plant Mol. Biol. Rep.* 32, 1085–1102. <https://doi.org/10.1007/s11105-014-0723-3>.
- García, M.N.M., Giammaria, V., Grandellis, C., Téllez-Iñón, M.T., Ulloa, R.M., and Capiati, D.A. (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. <https://doi.org/10.1007/s00425-011-1540-7>.
- Guan, Y., Ren, H., Xie, H., Ma, Z., and Chen, F. (2009). Identification and characterization of bZIP-type transcription factors involved in carrot (*Daucus carota* L.) somatic embryo genesis. *Plant J.* 60, 207–217. <https://doi.org/10.1111/j.1365-3113X.2009.03948.x>.
- Guo, C., Guo, R., Xu, X., Gao, M., Li, X., Song, J., Zheng, Y., and Wang, X. (2014). Evolution and expression analysis of the grape (*Vitis vinifera* L.) WRKY gene family. *J. Exp. Bot.* 65(6), 1513–1528. <https://doi.org/10.1093/jxb/eru007>.
- Hu, B., Jin, J., Guo, A.Y., Zhang, H., Luo, J., and Gao, G. (2015). GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics* 31(8), 1296–1297. <https://doi.org/10.1093/bioinformatics/btu817>.
- Hu, W., Yang, H., Yan, Y., Wei, Y., Tie, W., Ding, Z., Zuo, J., Peng, M., and Li, K. (2016). Genome-wide characterization and analysis of bZIP transcription factor gene family related to abiotic stress in cassava. *Sci. Rep.* 6, 22783. <https://doi.org/10.1038/srep22783>.
- Huang, C.J., Zhou, J.H., Jie, Y.C., Xing, H.C., Zhong, Y.L., She, W., Wei, G., Yu, W.L., and Ma, Y.S. (2016). A ramie (*Boehmeria nivea*) bZIP transcription factor *BnbZIP3* positively regulates drought, salinity and heavy metal tolerance. *Mol. Breeding* 36, 120. <https://doi.org/10.1007/s11032-016-0470-2>.
- Huang, H.W., and Ferguson, A.R. (2007). *Actinidia* in China: natural diversity, phylogeographical evolution, interspecific gene flow and kiwifruit cultivar improvement. *Acta Hort.* 753, 31–40. <https://doi.org/10.17660/ActaHortic.2007.753.1>.
- Huang, S., Ding, J., Deng, D., Tang, W., Sun, H., Liu, D., Zhang, L., Niu, X., Zhang, X., Meng, M., Yu, J., Liu, J., Han, Y., Shi, W., Zhang, D., Cao, S., Wei, Z., Cui, Y., Xia, Y., Zeng, H., Bao, K., Lin, L., Min, Y., Zhang, H., Miao, M., Tang, X., Zhu, Y., Sui, Y., Li, G., Sun, H., Yue, J., Sun, J., Liu, F., Zhou, L., Lei, L., Zheng, X., Liu, M., Huang, L., Song, J., Xu, C., Li, J., Ye, K., Zhong, S., Lu, B.R., He, G., Xiao, F., Wang, H.L., Zheng, H., Fei, Z., and Liu, Y. (2013). Draft genome of the kiwifruit *Actinidia chinensis*. *Nat. Commun.* 4, 2640. <https://doi.org/10.1038/ncomms3640>.
- Huang, S., Li, R., Zhang, Z., Li, L., Gu, X., Fan, W., Lucas, W.J., Wang, X., Xie, B., Ni, P., Ren, Y., Zhu, H., Li, J., Lin, K., Jin, W., Fei, Z., Li, G., Staub, J., Kilian, A., van der Vossen, E.A., Wu, Y., Guo, J., He, J., Jia, Z., Ren, Y., Tian, G., Lu, Y., Ruan, J., Qian, W., Wang, M., Huang, Q., Li, B., Xuan, Z., Cao, J., Asan, W., Zhang, J., Cai, Q., Bai, Y., Zhao, B., Han, Y., Li, Y., Li, X., Wang, S., Shi, Q., Liu, S., Cho, W.K., Kim, J.Y., Xu, Y., Heller-Uszynska, K., Miao, H., Cheng, Z., Zhang, S., Wu, J., Yang, Y., Kang, H., Li, M., Liang, H., Ren, X., Shi, Z., Wen, M., Jian, M., Yang, H., Zhang, G., Yang, Z., Chen, R., Liu, S., Li, J., Ma, L., Liu, H., Zhou, Y., Zhao, J., Fang, X., Li, G., Fang, L., Li, Y., Liu, D., Zheng, H., Zhang, Y., Qin, N., Li, Z., Yang, G., Yang, S., Bolund, L., Kristiansen, K., Zheng, H., Li, S., Zhang, X., Yang, H., Wang, J., Sun, R., Zhang, B., Jiang, S., Wang, J., Du, Y., and Li, S. (2009). The genome of the cucumber, *Cucumis sativus* L. *Nat. Genet.* 41(12), 1275–1281. <https://doi.org/10.1038/ng.475>.
- Hurst, H.C. (1994). Transcription factors.1: bZIP proteins. *Protein Profile* 1, 123–168.
- International Rice Genome Sequencing Project (2005). The map-based sequence of the rice genome. *Nature* 436, 793–800. <https://doi.org/10.1038/nature03895>.
- Jakoby, M., Weisshaar, B., Dröge-Laser, W., Vicente-Carbajosa, J., Tiedemann, J., Kroj, T., and Parcy, F. (2002). bZIP transcription factors in *Arabidopsis*. *Trends Plant Sci.* 7, 106–111. [https://doi.org/10.1016/S1360-1385\(01\)02223-3](https://doi.org/10.1016/S1360-1385(01)02223-3).
- Jin, Z.W., Xu, W., and Liu, A.Z. (2014). Genomic surveys and expression analysis of bZIP gene family in castor bean (*Ricinus communis* L.). *Planta* 239, 299–312. <https://doi.org/10.1007/s00425-013-1979-9>.
- Jing, Z.B., Yang, H.B., Yang, Y.W., Shen, J., Zhou, S.M., Yan, R.L., and Xu, M. (2016). Isolation and identification of *Pseudomonas syringae* pv. *Actinidiae* in Northern Area of Qinling Mountains. *J. Northwest Forestry University* 31(5), 188–193 (in Chinese).
- Kang, J.Y., Choi, H.I., Im, M.Y., and Kim, S.Y. (2002). *Arabidopsis* basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. *Plant Cell* 14, 343–357. <https://doi.org/10.1105/tpc.010362>.
- Koonin, E.V. (2005). Orthologs, paralogs, and evolutionary genomics. *Annu. Rev. Genet.* 39, 309–338. <https://doi.org/10.1146/annurev.genet.39.073003.114725>.
- Letunic, I., Doerks, T., and Bork, P. (2012). SMART 7: recent updates to the protein domain annotation resource. *Nucleic Acid Res.* 40(D1), D302–D305. <https://doi.org/10.1093/nar/gkr931>.
- Li, H., Gao, Y., Xu, H., Dai, Y., Deng, D.Q., and Chen, J.M. (2013). ZmWRKY33, a WRKY maize transcription factor conferring enhanced salt stress tolerances in *Arabidopsis*. *Plant Growth Regul.* 70, 207–216. <https://doi.org/10.1007/s10725-013-9792-9>.
- Li, Y., Zhu, Y., Yao, J., Zhang, S., Wang, L., Guo, C., van Nocker, S., and Wang, X. (2017). Genome-wide identification and expression analyses of the homeobox transcription factor family during ovule development in seedless and seeded grapes. *Sci. Rep.* 7, 12638. <https://doi.org/10.1038/s41598-017-12988-y>.
- Li, Y.Y., Meng, D., Li, M.J., and Cheng, L.L. (2016). Genome-wide identification and expression analysis of the bZIP gene family in apple (*Malus domestica*). *Tree Genet. Genomes* 12, 82. <https://doi.org/10.1007/s11295-016-1043-6>.
- Liu, C., Wu, Y., and Wang, X. (2012). bZIP transcription factor OsbZIP52/RISBZ5: a potential negative regulator of cold and drought stress response in rice. *Planta* 235, 1157–1169. <https://doi.org/10.1007/s00425-011-1564-z>.
- Meng, D., Li, Y., Bai, Y., Li, M., and Cheng, L. (2016). Genome-wide identification and characterization of WRKY transcriptional factor family in apple and analysis of their responses to waterlogging and drought stress. *Plant Physiol. Biochem.* 103, 71–83. <https://doi.org/10.1016/j.plaphy.2016.02.006>.
- Nieva, C., Busk, P.K., Domínguez-Puigjaner, E., Lumberras, V., Testillano, P.S., Risueño, M.C., and Pagès, M. (2005). Isolation and functional characterisation of two new bZIP maize regulators of the ABA responsive gene *rab28*. *Plant Mol. Biol.* 58, 899–914. <https://doi.org/10.1007/s11103-005-8407-x>.
- Nijhawan, A., Jain, M., Tyagi, A.K., and Khurana, J.P. (2008). Genomic survey and gene expression analysis of the basic leucine zipper

- transcription factor family in rice. *Plant Physiol.* **146**, 333–350. <https://doi.org/10.1104/pp.107.112821>.
- Paterson, A.H., Wang, X., Tang, H., and Lee, T.H. (2012). Synteny and genomic rearrangements. *Plant. Gen. Diversity* **1**, 195–207. https://doi.org/10.1007/978-3-7091-1130-7_13.
- Rabara, R.C., Tripathi, P., Lin, J., and Rushton, R.J. (2013). Dehydration-induced WRKY genes from tobacco and soybean respond to jasmonic acid treatments in BY-2 cell culture. *Biochem. Biophys. Res. Commun.* **431**, 409–414. <https://doi.org/10.1016/j.bbrc.2012.12.156>.
- Silveira, A.B., Gauer, L., Tomaz, J.P., Cardoso, P.R., Carmello-Guerreiro, S., and Vincentz, M. (2007). The *Arabidopsis* AtbZIP9 protein fused to the VP16 transcriptional activation domain alters leaf and vascular development. *Plant Sci.* **172**, 1148–1156. <https://doi.org/10.1016/j.plantsci.2007.03.003>.
- Song, H., Wang, P., Hou, L., Zhao, S., Xia, H., Li, P., Zhang, Y., Bian, X., and Wang, X. (2016). Global analysis of WRKY genes and their response to dehydration and salt stress in soybean. *Front. Plant Sci.* **7**, 9. <https://doi.org/10.3389/fpls.2016.00009>.
- Tak, H., and Mhatre, M. (2013). Cloning and molecular characterization of a putative bZIP transcription factor VvbZIP23 from *Vitis vinifera*. *Protoplasma* **250**(1), 333–345. <https://doi.org/10.1007/s00709-012-0417-3>.
- Talanian, R.V., McKnight, C.J., and Kim, P.S. (1990). Sequence-specific DNA binding by a short peptide dimer. *Science* **249**, 769–771. <https://doi.org/10.1126/science.2389142>.
- Thurrow, C., Schiermeyer, A., Krawczyk, S., Butterbrodt, T., Nickolov, K., and 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**, 100–113. <https://doi.org/10.1111/j.1365-3113X.2005.02513.x>.
- Ulm, R., Baumann, A., Oravec, A., Máté, Z., Ad, E., Oakeley, E.J., Schäfer, E., and Nagy, F. (2004). Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **101**, 1397–1402. <https://doi.org/10.1073/pnas.0308044100>.
- Wang, J., Zhou, J., Zhang, B., Vanitha, J., Ramachandran, S., and Jiang, S.Y. (2011). 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**, 212–231. <https://doi.org/10.1111/j.1744-7909.2010.01017.x>.
- Wei, K., Chen, J., Wang, Y., Chen, Y., Chen, S., Lin, Y., Pan, S., Zhong, X., and Xie, D. (2012). Genome-wide analysis of bZIP-encoding genes in maize. *DNA Res.* **19**, 463–476. <https://doi.org/10.1093/dnares/dss026>.
- Weltmeier, F., Ehlert, A., Mayer, C.S., Dietrich, K., Wang, X., Schütze, K., Alonso, R., Harter, K., Vicente-Carbajosa, J., and Dröge-Laser, W. (2006). Combinatorial control of *Arabidopsis* proline dehydrogenase transcription by specific hetero dimerisation of bZIP transcription factors. *EMBO J.* **25**, 3133–3143. <https://doi.org/10.1038/sj.emboj.7601206>.
- Yáñez, M., Cáceres, S., Orellana, S., Bastías, A., Verdugo, I., Ruiz-Lara, S., and Casaretto, J.A. (2009). An abiotic stress-responsive bZIP transcription factor from wild and cultivated tomatoes regulates stress-related genes. *Plant Cell Rep.* **28**, 1497–1507. <https://doi.org/10.1007/s00299-009-0749-4>.
- Ying, S., Zhang, D.F., Fu, J., Shi, Y.S., Song, Y.C., Wang, T.Y., and Li, Y. (2012). Cloning and characterization of a maize bZIP transcription factor, ZmbZIP72, confers drought and salt tolerance in transgenic *Arabidopsis*. *Planta* **235**, 253–266. <https://doi.org/10.1007/s00425-011-1496-7>.
- Zhang, X., Wollenweber, B., Jiang, D., Liu, F., and 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. <https://doi.org/10.1093/jxb/erm364>.
- Zhang, Y.C., Miao L., Wang, H., Brocker, C., Yin, X., Vasiliou, V., Fei, Z., and Wang, X. (2012). Genome-wide identification and analysis of grape aldehyde dehydrogenase (ALDH) gene superfamily. *PLoS ONE* **7**, e32153. <https://doi.org/10.1371/journal.pone.0032153>.
- Zhao, J., Guo, R., Guo, C., Hou, H., Wang, X., and Gao, H. (2016). Evolutionary and expression analyses of the apple basic leucine zipper transcription factor family. *Front. Plant Sci.* **7**, 376. <https://doi.org/10.3389/fpls.2016.00376>.
- Zhao, Z.B., Gao, X.N., Yang, D.H., Huang, L.L., Qin, H.Q., Kang, Z.S., and Wang, N.N. (2015). Field detection of canker-causing bacteria on kiwifruit trees: *Pseudomonas syringae* pv. *actinidiae* is the major causal agent. *Crop. Prot.* **75**, 55–62. <https://doi.org/10.1016/j.cropro.2015.05.008>.
- Zou, M., Guan, Y., Ren, H., Zhang, F., and Chen, F. (2008). A bZIP transcription factor, OsABI5, is involved in rice fertility and stress tolerance. *Plant. Mol. Biol.* **66**, 675–683. <https://doi.org/10.1007/s11103-008-9298-4>.

Received: Apr. 26, 2018

Accepted: Jun. 6, 2018

Addresses of authors:

Zhaobin Jing^{1,2,*} and Hongqi Fu²¹College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China²Weinan Vocational and Technical College, Weinan Fruit Industry Institute of Shaanxi, Weinan 714026, Shaanxi, China*Corresponding author; E-mail: jingzhaobin@nwsuaf.edu.cn
Tel.: +86-(0)913-303-3264