Original article

Identification, evolution, and expression analysis of SBP-box gene family in kiwifruit (*Actinidia chinensis* Planch.)

Zhaobin Jing^{1,2}, Xiangning Qi² and Linsen Zhang²¹ Weinan Vocational and Technical College, Weinan Fruit Industry Institute of Shaanxi, Weinan, Shaanxi, China² College of Horticulture, Northwest A&F University, Yangling, Shaanxi, China

Summary

The Squamosa promoter binding protein (SBP)-box genes encode a kind of plant-specific transcription factors, and play important roles in plant growth, development, biotic and abiotic stress. Although the genome-wide analysis of SBP-box gene family has been performed in some species, SBP-box gene family in kiwifruit (*Actinidia chinensis* Planch.) remains poorly understood. In this study, 25 SBP-box genes of kiwifruit were identified in the kiwifruit genome. Based on phylogenetic and comprehensive bioinformatics analysis, 25 kiwifruit SBP-box genes can be classified into eight groups. Additionally, detailed gene structure, conserved domain, motif compositions, and phylogenetic relationship of the *AcSBP* genes were systematically characterized. Synteny analysis indicated that segmental duplication has likely contributed to the expansion and evolution of the *AcSBP* genes. Expression analysis revealed that all the *AcSBP* genes showed distinct expression levels in five different tissues, and suggested *AcSBP* genes are significantly involved in response to different plant hormone, drought, salt, and Psa treatments to mimic biotic and abiotic stresses. The genome-wide identification and characterization of SBP-box gene family could facilitate the functional analysis of *AcSBP* genes and provide information of the molecular basis of development and stress tolerance in kiwifruit.

Keywords

Actinidia chinensis Planch., genome-wide analysis, SBP-box genes, phylogenetic analysis, abiotic stress response, expression analysis

Significance of this study

What is already known on this subject?

- The Squamosa promoter binding protein (SBP)-box genes encode a kind of plant-specific transcription factors. SBP-box family was identified in some plants. The availability of complete kiwifruit genome sequences allows the genome-wide identification of SBP-box TFs.

What are the new findings?

- In this study, 25 SBP-box genes have been identified in the kiwifruit genome. Synteny analysis indicated that segmental duplication contributed to the expansion and evolution of the *AcSBP* genes. The analysis of *AcSBP* 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 *AcSBP* genes and provide important information of the molecular basis of development and stress tolerance in horticultural plants.

Introduction

Transcription factors (TFs) are DNA-binding proteins that exist in almost all living organisms. In plants, TFs play a critical role in the regulation networks of many significant developmental processes. Squamosa promoter binding protein genes (SBP) encode a family of transcription factors that are unique to plants (Li et al., 2013; Klein et al., 1996; Xie et al., 2006). The common feature of SBP genes is that their protein products contain a highly conserved SBP-box DNA-binding domain, which consists of approximately 76 amino acid residues that include ten conserved cysteine and histidine residues (Cardon et al., 1999). The SBP domain comprises two zinc-binding sites and individual DNA-binding and nuclear localization domains (Birkenbihl et al., 2005; Yamasaki

et al., 2004). Since the first SBP-box gene was identified in *Antirrhinum majus*, and many genes have been characterized from different plant species. At present, the SBP-box genes family has been isolated, identified, and characterized in a number of plant species, including rice (Xie et al., 2006), *Arabidopsis* (Cardon et al., 1999), maize (Chuck et al., 2010; Mao et al., 2016; Zhang et al., 2016), tomato (Salinas et al., 2011), grape (Hou et al., 2013), apple (Li et al., 2013), Moso Bamboo (Pan et al., 2017), pepper (Zhang et al., 2015), castor bean (Zhang and Li, 2014), *Salvia miltiorrhiza* (Zhang et al., 2014), *Gossypium hirsutum* (Zhang et al., 2015), melons (Ma et al., 2014), *Prunus mume* (Xu et al., 2015), petunia (Preston et al., 2015), peanut (Li et al., 2015), citrus (Shalom et al., 2015), *Chrysanthemum* (Song et al., 2016), potato (Kavas et al., 2017), and so on.

Previous studies have indicated that the SBP-box genes family has positive regulatory roles in plant development process. For example, in *Arabidopsis*, the 16 of SBP TFs have been shown to play important roles in different developmental processes, including leaf development (Schwarz et al., 2008), shoot development and shoot maturation (Shikata et al., 2009; Wu et al., 2006), flowering (Gandikota et al., 2007), fertility (Xing et al., 2010), plant hormone signaling and cop-

per homeostasis (Zhang et al., 2007; Yamasaki et al., 2009), floral transition (Yamaguchi et al., 2009), GA-mediated anther development (Zhang et al., 2007; Unte et al., 2003), gynoecium patterning through interference with auxin homeostasis (Xing et al., 2013). In tomato, the SBP-box gene Colorless Non-Ripening (CNR) is critical for fruit ripening, and an epigenetic mutation in the promoter of this gene was shown to inhibit fruit ripening (Manning et al., 2006). In addition, SBP-box genes also play essential roles in fruit ripening and in response to abiotic and biotic stresses in other plants. Several *ZmSPL* genes were involved in response to abiotic stresses in maize (Mao et al., 2016). Many *MdSBP* genes may be involved in responses to hormone signaling during stress in apple (Li et al., 2013).

Kiwifruit is one of the most important fruit worldwide. However, kiwifruit quality and production have been affected by some abiotic and biotic stresses, such as the majority of currently growing kiwifruit cultivars, like 'Hongyang', are susceptible to Psa stress. The pathogen can affect the plant at any stage of development causing damping-off, seedling blight, and wilting, followed by plant death (Zhao et al., 2015). Analysis of *AcSBP* genes in response to Psa and hormones is important for identification of candidate genes in kiwifruit, and further used to breeding resistance cultivar. In addition, a thorough knowledge of kiwifruit resistance mechanisms will help to limit crop loss due to stress, and to decrease the economic losses. However, genome-wide identification of *AcSBP* genes has not been reported in kiwifruit. Recently, a complete genome sequence has been released for kiwifruit (Huang et al., 2013). This genome sequence database provides unique opportunities for the genome-wide investigation of *AcSBP* genes in kiwifruit. In this study, we performed a genome-wide search for *AcSBP* genes in the kiwifruit genome and the gene structures, gene characterization, sequence alignment, synteny, and phylogenetic analysis of the SBP genes were analyzed. Moreover, we investigated the expression patterns of all *AcSBP* genes in various tissues and response to abiotic and biotic stress. To our knowledge, this is the first study that aimed to identify and expression analysis of the SBP-box gene family in kiwifruit. Our study will provide a foundation for further researches of *AcSBP* genes function in kiwifruit.

Materials and methods

Identification and annotation of kiwifruit SBP-box genes

The following approach was used to search for kiwifruit SBP TFs. First, the database of kiwifruit was downloaded from the Kiwifruit Genome Database (http://itak.feilab.net/cgi-bin/itak/db_family.cgi?plant=3625). Then, all of the obtained protein sequences were submitted to SMART (<http://smart.embl-heidelberg.de/>) (Letunic et al., 2012) to detect the presence of the SBP domain. Lastly, the cDNA, genes, protein sequences, and chromosomal location of SBP TFs were obtained from the iTAK-Plant Transcription factor and Protein Kinase Identifier and Classifier database (http://bioinfo.bti.cornell.edu/cgi-bin/itak/db_home.cgi). *Arabidopsis* SBP-box genes were downloaded from TAIR using the accession numbers reported by Yang (Yang et al., 2008). Potential chemical characteristics of all SBP-box were predicted using the Prosite ExPASy server (<https://www.expasy.org/>) (Artimo et al., 2012).

Multiple sequence alignment and phylogenetic analysis of *AcSBP* genes

First, multiple sequence alignment of SBP-box protein sequences was carried out using DNAMAN software (v. 6.0). Then, phylogenetic tree was constructed using MEGA 7.0 software using the neighbor-joining (NJ) method with the following parameters: Poisson substitution model, pairwise deletion, and 1,000 bootstrap replications (Kumar et al., 2016). Sequence logos were created using the Weblogo online application (<http://weblogo.threeplusone.com/create.cgi>).

Gene structure, conserved motif and exon-intron structure analysis of *AcSBP* genes

Information on *AcSBP* gene structures was downloaded from the Kiwifruit Genome Database (<http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/search.cgi>). Online version 4.9.1 of the Multiple Expectation for MEME server (<http://meme-suite.org/>) was used to analyze conserved motifs for all full-length *AcSBP* protein sequences (Bailey et al., 2006). The identified motifs were annotated by SMART (<http://smart.embl-heidelberg.de/>) (Letunic et al., 2012). The exon-intron structure of the *AcSBP* genes was analyzed by the Gene Structure Display Server (GSDS) online tool (<http://gsds.cbi.pku.edu.cn/>) by comparing the CDS and the corresponding genomic sequences (Hu et al., 2015). The genomic DNA sequence upstream of the start codon of each *AcSBP* gene was obtained from the kiwifruit genome.

Tandem duplication, synteny analysis and chromosome localization of *AcSBP* genes

Tandem duplications genes are defined as adjacent homologous *AcSBP* genes on the same chromosome, with no more than one intervening gene (Guo et al., 2014). The specific chromosomal location of each *AcSBP* gene was analyzed to estimate whether a gene resulted from a tandem duplication based on the Kiwifruit Genome Database. For synteny analysis, Synteny blocks within the kiwifruit genome, as well as between the kiwifruit and *Arabidopsis thaliana* genomes, were downloaded from the Plant Genome Duplication Database (<http://chibba.agtec.uga.edu/duplication>) (Lee et al., 2012; Tang et al., 2008a, b), and those including kiwifruit and *A. thaliana* SBP-box genes were identified and analyzed. The diagrams of synteny analysis were carried out using Circos v. 0.63 (<http://circos.ca/>). The chromosome localization of *AcSBP* genes were analyzed using MapInspect based on the information of gene physical position in the kiwifruit genome.

Plant material

'Hongyang' was used 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 (the fourth to fifth young leaves from the shoot apices when the length of newly growing shoots was approximately 50 cm), stems (at the apices of newly growing shoots and approximately 2 mm diameter), flower buds, and fruits (green young fruit, approximately 4 cm diameter) 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, drought stresses and Psa inoculation treatments. These 2-year-old seedlings had been planted in 20-cm diameter pots in the greenhouse of the experimental station.

Abiotic, biotic and hormone treatments

Hormone treatments were conducted by spraying young leaves of 2-year-old seedlings with abscisic acid (ABA: 300 μ M), methyl jasmonate (MeJA: 50 μ M), ethylene (Eth: 0.5 g L⁻¹), salicylic acid (SA: 100 μ M), gibberellic acid (GA3: 100 μ M), and followed by leaf sampling at 1, 12, 24, and 48 h post-treatment. And leaves sprayed with sterile distilled water were collected as the control (Guo et al., 2014; Jing et al., 2018a, b; Zhao et al., 2016). Drought stress was applied by withholding water from the 2-year-old seedlings, and harvesting leaves at 48, 96, and 168 h post-treatment. Drought-stressed plants were then re-watered to soil saturation, and leaves were collected 48 h after re-watering (R48) (Guo et al., 2014; Jing et al., 2018a, b; Meng et al., 2016; Zhao et al., 2016). The salinity stress was performed by irrigating seedlings with 2 L of 250 mM NaCl solution, and seedlings that were irrigated with the same volume of water were used as a control (Guo et al., 2014; Jing et al., 2018a, b; Zhao et al., 2016). Leaves were harvested at 1, 12, 24, and 48 h post-treatment. Pathogen treatment was performed by inoculating the young leaves of 'Hongyang' with *Psa* (*Pseudomonas syringae* pv. *actinidiae*) strain Psamx-9 (GenBank Assembly ID: KT731230) according to the method previously 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 12, 48, 72, and 120 h post-inoculation.

For all the above treatments, at each time point of each treatment, six leaves from six different plants were mixed to form one sample then used later in the experiment. All experiments were repeated for three biological replicates.

Expression analysis of *AcSBP* genes

The total RNA of samples was isolated using an E.Z.N.A.R. Plant RNA Kit (Omega Bio-Tek, Inc.). 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. The first-strand cDNA was synthesized from 1 μ g of total RNA using a TransCripT[®] cDNA sample prep kit (TransGen Biotech, China).

All gene-specific primers were designed using in the internal database platform from The New Zealand Institute for Plant & Food Research Limited (Supplemental Information – Table S1). Semi-quantitative RT-PCR was carried out according to previously established protocols by Jing et al. (2018a, b) in a reaction volume of 20 μ L containing 1 μ g of cDNA template, 0.8 μ L of each primer, 10 μ L 2 × TranStart[®] Tip Green qPCR Super Mix (TransGen Biotech, China), and 7.4 μ L sterile distilled water. The PCR parameters were as follows: 94°C for 3 min, 30–35 cycles at 94°C for 30 s, 55–60°C for 30 s, 72°C for 30 s, with a final extension of 72°C for 5 min. PCR products were separated on a 1.5% (w/v) agarose gel with ethidium bromide staining and photographed under UV light. Each reaction was repeated three times and the three independent analyses showed the same trends for each gene and treatment. Real-time PCR was conducted in optical 96-well plates using an IQ5 real-time PCR system (Bio-Rad, Hercules, CA, USA). Reactions were carried out according to previously established protocols by Jing et al. (2018a, b) in a 20- μ L volume containing 1.0 μ L of cDNA, 0.8 μ L of each primer (1.0 μ M), 10 μ L of SYBR green Passive Reference Dye (TransGen Biotech, China), and 7.4 μ L sterile distilled water with the following conditions: 30 s at 94°C, 45 cycles of 5 s at 94°C, and 30 s at 60°C. The specificity of the amplification for each primer pair was verified by melting curve analysis. All real-time RT-PCR experiments were performed with

three biological replicates, and each replicate was analyzed in triplicate. The relative expression levels were calculated using the 2^{- $\Delta\Delta C_t$} method, with the kiwifruit actin gene 18S 2 (GenBank Accession number AF419792: F: 5'-CTG TGA AAC TGC GAA TGG CTC-3', R 5'-TTC CAG AAG TCG GGG TTT GT-3') and ACT 2 (GenBank Accession number EF063572: F: 5'-GCA GGA ATC CAT GAG ACT ACC-3', R: 5'-GCA GGA ATC CAT GAG ACT ACC-3') used as an internal control for gene expression normalization (Ferradás et al., 2016). Relative expression levels were analyzed using the IQ5 software and the normalized expression method.

Results

Genome-wide identification of the kiwifruit SBP-box genes

A total of 30 potential SBP-box genes were identified in the Kiwifruit Genome Database. Then we used the SMART tool to test the SBP domains with *e*-value less than 0.01 for each matching sequence, and five of them (*Achn016511*, *Achn225111*, *Achn234991*, *Achn235351*, *Achn279201*) were excluded due to the lack of a complete SBP domain. The other 25 candidate SBP-box genes were defined as *AcSBP1*–*AcSBP25* based on their location on chromosomes 1 to 29. The 25 *AcSBP* genes were mapped into 18 kiwifruit chromosomes. Each chromosome (Chr 2, Chr 3, Chr 4, Chr 6, Chr 8, Chr 9, Chr 12, Chr 15, Chr 16, Chr 17, Chr 19, Chr 20, Chr 23, Chr 26, Chr 27, and Chr 28) contained only one *AcSBP* gene (*AcSBP1*, *AcSBP2*, *AcSBP3*, *AcSBP4*, *AcSBP5*, *AcSBP6*, *AcSBP7*, *AcSBP10*, *AcSBP11*, *AcSBP12*, *AcSBP13*, *AcSBP14*, *AcSBP15*, *AcSBP18*, *AcSBP19*, and *AcSBP20*, respectively). Information of 25 genes were given in Table 1, including gene accession numbers, genomic locations, amino acid numbers, molecular weights, instability indexes, aliphatic indexes, grand average of hydropathicity (GRAVY) values, and coding sequence (CDS) lengths. From Table 1, we found that the lengths of CDS were between 429 and 3,213 bp, molecular weights ranged from 15.89 to 119.16 kDa, the aliphatic indexes of the 25 *AcSBP* proteins ranged from 35.7 to 85.63, isoelectric points ranged from 5.38 to 9.48, with the majority above 7. Most *AcSBP* proteins within the same group had similar theoretical isoelectric points. The proteins were all hydrophilic, as reflected by grand average of hydropathicity (GRAVY) values less than 0. The in-stability index, a measure of protein stability (Guruprasad et al., 1990), was >40 for each protein, and they all belonged to the instable proteins. With respect to major amino acid contents of the 25 *AcSBP* genes, Ser was the most abundant amino acid followed by Leu.

Sequence alignment and phylogenetic analysis of the *AcSBP* genes

DNAMAN software was used to perform multiple sequence alignment of the *AcSBP* protein sequences. According to the sequence alignment results, we found that most *AcSBP* proteins contained the complete SBP domain except for *AcSBP13*, *AcSBP15*, and *AcSBP18* (Figure 1a). The SBP domain of *AcSBP* genes was found to be highly conserved and shared a high or absolute conservation at certain positions (Figure 1b). All the *AcSBP* genes include two zinc-binding sites (Zn-1 and Zn-2) and a bipartite nuclear localization signal (NLS).

To further reveal the phylogenetic relationships of *AcSBP* genes in kiwifruit and other plant species, an unrooted phylogenetic tree was constructed with SBP domain protein se-

TABLE 1. The information of *AcSBP* genes for the kiwifruit genome.

Gene name	Gene ID	CDS size (bp)	Length of ORF(bp)	Chr.	Start site	End site	Full length	Amino acids	PI	Molecular mass (Da)	Aliphatic index	GRAVY	Instability index
AcSBP1	Achn039041	1,128	375	2	14 272 648	14 276 689	4,042	375	6.89	41,460.16	69.87	-0.663	61.59
AcSBP2	Achn185971	3,186	1,061	3	19 739 355	19 759 322	19,938	1,061	5.71	119,160.38	85.63	-0.208	48.63
AcSBP3	Achn343391	3,213	1,070	4	12 913 680	12 918 349	4,670	1,070	8.31	118,320.60	77.9	-0.437	56.84
AcSBP4	Achn252971	1,020	339	6	640 804	647 306	6,503	339	8.42	37,166.29	61.5	-0.645	57.46
AcSBP5	Achn181301	429	142	8	2 298 167	2 299 668	1,502	142	7.05	15,887.44	35.7	-1.27	78.99
AcSBP6	Achn064091	1,047	348	9	760 560	765 466	4,907	348	8.64	37,218.90	55.26	-0.673	50.85
AcSBP7	Achn126821	1,080	359	12	10 348 980	10 356 268	7,289	359	9.1	38,637.82	47.05	-0.705	54.20
AcSBP8	Achn144801	1,299	432	13	16 041 792	16 044 686	2,895	432	9.16	47,782.82	71.27	-0.504	55.72
AcSBP9	Achn350751	1,221	406	13	14 040 019	14 044 173	4,155	406	8.45	44,649.77	58.42	-0.668	59.42
AcSBP10	Achn219131	633	210	15	16 311 516	16 314 630	3,115	210	8.88	23,484.27	53.43	-0.918	84.07
AcSBP11	Achn017541	957	318	16	6 967 788	6 970 583	2,796	318	6.03	34,529.64	64.65	-0.333	46.77
AcSBP12	Achn251461	3,072	1,023	17	133 632	139 968	6,337	1,023	5.38	113,639.26	81.4	-0.444	54.68
AcSBP13	Achn138051	1,344	447	19	6 500 181	6 504 418	4,238	447	8.41	49,007.46	65.84	-0.605	49.66
AcSBP14	Achn235741	2,568	855	20	9 985 514	9 998 587	13,074	855	6.34	94,851.31	84.95	-0.214	44.31
AcSBP15	Achn121261	1,425	474	23	5 129 888	5 134 375	4,488	474	8.01	51,991.00	66.22	-0.564	50.90
AcSBP16	Achn102591	3,150	1,049	24	269 781	277 213	7,433	1,049	6.91	115,948.37	80.59	-0.368	49.79
AcSBP17	Achn082431	2,547	848	24	2 842 040	2 855 094	13,055	848	5.86	95,142.26	79.93	-0.347	57.88
AcSBP18	Achn298021	1,092	363	26	8 435 511	8 438 106	2,596	363	8.03	39,765.46	64.71	-0.579	56.88
AcSBP19	Achn353841	594	197	27	9 789 881	9 792 207	2,327	197	9.18	22,254.07	65.28	-0.808	47.76
AcSBP20	Achn195011	3,072	1,023	28	666 212	673 174	6,963	1,023	5.86	113,839.75	82.62	-0.457	53.58
AcSBP21	Achn196331	1,335	444	29	3 228 099	3 230 419	2,321	444	9.24	48,574.03	63.24	-0.502	51.22
AcSBP22	Achn355701	1,263	420	29	7 035 422	7 038 312	2,891	420	9.48	46,626.56	66.33	-0.551	56.78
AcSBP23	Achn225601	894	297	Unknown	7 556 979	7 559 319	2,341	297	9.3	32,938.52	46.43	-0.755	59.89
AcSBP24	Achn129111	1,074	357	Unknown	39 362 568	39 371 390	8,823.00	357	8.53	38,611.62	47.82	-0.718	57.68
AcSBP25	Achn324461	1,143	380	Unknown	86 222 981	86 228 737	5,757	380	8.47	41,919.77	69.24	-0.675	60.71

FIGURE 1. Multiple sequence alignment of the SBP domain for kiwifruit SBP genes. (a) Multiple alignment of the kiwifruit SBP-box domains proteins. The two conserved zinc finger structures (Zn-1 and Zn-2) and NLS are indicated. (b) Sequence logo of the SBP domain of *AcSBPs*. The overall height of each stack represents the degree of conservation at this position, while the height of the letters within each stack indicates the relative frequency of the corresponding amino acids.

quences from apple (*MdSBP*), rice (*OsSPL*), grape (*VvSBP*), snapdragon (*AmSBP* or *AmQ9SNV*), *Chlamydomonas reinhardtii* (*CRR1* or *CrSPL*), *Physcomitrella patens* (*PpSBP*), tomato (*CNR* or *SlySBP*) and *Arabidopsis* (*AtSPL*) using the neighbor-joining algorithm methods (Figure 2). According to the results of cluster analysis, the *AcSBP* genes were clustered into eight groups (G1 to G8). The group 8 only included seven genes from hydrophytic green alga, while other plants that from land plants were clustered into the other groups (G1 to G7). Almost all the *AcSBP* genes of kiwifruit generally exhibited closer relationships to SBP genes from eudicots (such as tomato, grape, and *Arabidopsis*) than to those from monocots (such as rice).

Gene structure and conserved motif of the *AcSBP* genes

An unrooted phylogenetic tree was constructed based on the multiple sequence alignment of 25 kiwifruit *AcSBP* protein domains (Figure 3a). An exon-intron diagram of the 25 *AcSBP* genes was analyzed based on their genome sequences and coding sequences (Figure 3b). As shown in Figure 3b, the *AcSBP* genes of the same group have a similar exon-intron structure, with some exceptions. For example, fifteen *AcSBP* genes in G1, G2, G5, and G6 shared one intron except for *AcSBP8* and *AcSBP14*, while five *AcSBP* genes shared two to three introns except for *AcSBP19*. However, no exon-intron structures of some genes (*AcSBP18*, *AcSBP2*, and *AcSBP17*) have been found. The MEME tool was used to further analyze the evolutionary relationships among the *AcSBP* genes (Figure 4b). In total, 25 motifs were identified (Supple-

mental Information – Table S2). Likewise, MEME analysis indicated extensive conservation in motif architecture within the SBP genes of each subgroup, which further supported the group definitions. All 25 *AcSBP* proteins contained a basic SBP domain: GCNADLSSAKDYHRRHKVCEVHSKTPKVVVG-GIEQRFCQQC. For instance, these motifs occupied the same relative positions in some sequences (Figure 4c).

Tandem duplication and synteny analysis of the *AcSBP* genes

To understand the expansion patterns of the *AcSBP* genes in kiwifruit genome, we performed the synteny analysis of the *AcSBP* genes. Tandem duplication events of *AcSBP* gene were defined as adjacent homologous genes on a single chromosome, while segmental duplications were characterized for its gene duplication events occurred between different chromosomes (Guo et al., 2014). No tandem duplication event regions were found in *AcSBP* genes. A total of thirteen segregation duplication events were obtained on kiwifruit different chromosomes (Figure 5; Supplemental Information – Table S3). In order to further analyze the evolutionary history and putative function of kiwifruit *AcSBP* genes, a comparative syntenic map between kiwifruit and *Arabidopsis* SBP-box genes were completed. The corresponding orthologs of 12 kiwifruit *AcSBP* genes and 11 *AtSPL* were identified (Figure 6; Supplemental Information – Table S4).

Expression profile of *AcSBP* genes in different tissues

To elucidate the potential roles of *AcSBP* genes in organ

FIGURE 2. Phylogenetic tree of identified *AcSBP* kiwifruit genes (Red). Phylogenetic tree was constructed with selected *Arabidopsis* (*AtSPL*), apple (*MdSBP*), grape (*VvSBP*), rice (*OsSPL*), tomato (*CNR* or *SlySBP*), *A. majus* (*AmSBP* or *AmQ9SNV*), *C. reinhardtii* (*CRR1* or *CrSPL*), and *P. patens* (*PpSBP*).

FIGURE 3. Phylogenetic analysis and exon-intron structures of kiwifruit *AcSBP* genes. (a) Phylogenetic analysis of kiwifruit *AcSBP* genes. (b) Intron/Exon structure of kiwifruit *AcSBP* genes; green indicates exons, black indicates introns, red indicates upstream or downstream.

FIGURE 4. Conserved motifs and logo analysis of kiwifruit *AcSBP* genes. (a) Phylogenetic analysis of kiwifruit *AcSBP* genes. (b) Distribution of conserved motifs of kiwifruit *AcSBP* genes; each motif is represented by a colored box. (c) The logo of each motif.

FIGURE 5. Chromosomal distribution and synteny analysis of kiwifruit *AcSBP* genes. The chromosomes positions of the *AcSBP* genes are shown in the kiwifruit chromosomes (chr1–29). The different colored lines connect the syntenic regions between kiwifruit *AcSBP* genes.

FIGURE 6. Synteny analysis of SBP genes between kiwifruit and *Arabidopsis*. The positions of many related *AcSBP* genes and *AtSPL* genes are shown in the kiwifruit chromosomes (chr3, 4, 9, 12, 13, 15, 17, 24, and 29) and *Arabidopsis* chromosomes (chr1, 2, 3, and 5), respectively. The different colored lines connect the syntenic regions between kiwifruit and *Arabidopsis* SBP genes.

development, the expression of 25 *AcSBP* genes in five different organs (including young leaves, stems, apical buds, flower buds, fruits) were analyzed. Most *AcSBP* genes showed continuous expression in these five tissues. Most of the genes were expressed in all the organs but at different varying levels. About 25 predicted genes were expressed in at least three of the five organs (Figure 7).

Differential expression profiling of *AcSBP* genes in response to abiotic and biotic stresses

SBP genes are widely involved in biotic and abiotic stress responses under normal and stressed growth conditions (Li et al., 2013; Pan et al., 2017; Zhang et al., 2007). To identify *AcSBP* gene with a potential role in biotic and abiotic stresses response of plants, qRT-PCR was performed for 25 *AcSBP* genes expression after drought, salt, GA, SA, ABA, MeJA, Eth, and Psa treatments, respectively. Overall, the analysis indicated that these genes are differentially expressed in response to one or more stresses.

In the hormone treatment (Figure 8), 25 *AcSBP* genes were found to be up-regulated at some time points. After GA treatment, 22 *AcSBP* genes (*AcSBP1*, *AcSBP3*, *AcSBP4*, *AcSBP5*, *AcSBP6*, *AcSBP8*, *AcSBP9*, *AcSBP10*, *AcSBP11*, *AcSBP12*, *AcSBP13*, *AcSBP15*, *AcSBP16*, *AcSBP17*, *AcSBP18*,

AcSBP19, *AcSBP20*, *AcSBP21*, *AcSBP22*, *AcSBP23*, *AcSBP24*, and *AcSBP25*) were highly expressed at least at 1, 12, 24, and 48 h post-treatment. *AcSBP12* and *AcSBP25* displayed a significant increase in transcript levels from 1 h to 48 h, and *AcSBP5*, *AcSBP15*, and *AcSBP23* increased in expression from 12 h to 48 h. After SA treatment, 19 *AcSBP* genes (*AcSBP1*, *AcSBP4*, *AcSBP5*, *AcSBP6*, *AcSBP7*, *AcSBP8*, *AcSBP9*, *AcSBP10*, *AcSBP11*, *AcSBP12*, *AcSBP13*, *AcSBP14*, *AcSBP15*, *AcSBP19*, *AcSBP20*, *AcSBP21*, *AcSBP23*, *AcSBP24*, and *AcSBP25*) were highly expressed at least at 1, 12, 24, and 48 h post-treatment. *AcSBP24* and *AcSBP25* displayed a significant increase in transcript levels from 12 h to 48 h. After Eth treatment, 18 *AcSBP* genes (*AcSBP1*, *AcSBP2*, *AcSBP4*, *AcSBP7*, *AcSBP8*, *AcSBP10*, *AcSBP11*, *AcSBP12*, *AcSBP13*, *AcSBP14*, *AcSBP15*, *AcSBP16*, *AcSBP17*, *AcSBP18*, *AcSBP19*, *AcSBP23*, *AcSBP24*, and *AcSBP25*) showed the up-regulation at least at 1, 12, 24, and 48 h post-treatment. *AcSBP13* and *AcSBP19* showed a significant increase in transcript levels from 1 h to 24 h. After MeJA treatment, 19 *AcSBP* genes (*AcSBP1*, *AcSBP2*, *AcSBP3*, *AcSBP5*, *AcSBP7*, *AcSBP8*, *AcSBP9*, *AcSBP10*, *AcSBP11*, *AcSBP12*, *AcSBP13*, *AcSBP15*, *AcSBP18*, *AcSBP19*, *AcSBP20*, *AcSBP21*, *AcSBP23*, *AcSBP24*, and *AcSBP25*) showed the up-regulation at least at 1, 12, 24, and 48 h post-treatment. *AcSBP2*, *AcSBP11*, *AcSBP13*, and *AcSBP23* showed an increase

FIGURE 7. Expression analysis of the 25 *AcSBP* genes in five organs. Organs are indicated as follows: 1: young leaves, 2: stems, 3: apical buds, 4: flower buds, 5: fruits.

in transcript levels from 12 h to 48 h. After ABA treatment, 19 *AcSBP* genes (*AcSBP1*, *AcSBP2*, *AcSBP3*, *AcSBP4*, *AcSBP5*, *AcSBP6*, *AcSBP7*, *AcSBP8*, *AcSBP9*, *AcSBP12*, *AcSBP13*, *AcSBP14*, *AcSBP18*, *AcSBP19*, *AcSBP20*, *AcSBP21*, *AcSBP23*, *AcSBP24*, and *AcSBP25*) were highly expressed at least at 1, 12, 24, and 48 h post-treatment. *AcSBP1* and *AcSBP8* showed increase in transcript levels from 12 h to 48 h.

In the NaCl, drought and Psa treatments (Figure 9), 25 *AcSBP* genes were also found to be up-regulated at some time points. After NaCl treatment, 16 *AcSBP* genes (*AcSBP1*, *AcSBP2*, *AcSBP3*, *AcSBP4*, *AcSBP8*, *AcSBP11*, *AcSBP12*, *AcSBP13*, *AcSBP14*, *AcSBP15*, *AcSBP16*, *AcSBP17*, *AcSBP18*, *AcSBP21*, *AcSBP23*, and *AcSBP24*) were highly expressed at least at 1, 12, 24, h and 48 h post-treatment. *AcSBP1* and *AcSBP3* showed increase in transcript levels from 1 h to 24 h. After drought treatment, 21 *AcSBP* genes (*AcSBP1*, *AcSBP2*, *AcSBP3*, *AcSBP5*, *AcSBP6*, *AcSBP7*, *AcSBP9*, *AcSBP10*, *AcSBP11*, *AcSBP13*, *AcSBP14*, *AcSBP15*, *AcSBP16*, *AcSBP17*, *AcSBP18*, *AcSBP19*, *AcSBP20*, *AcSBP22*, *AcSBP23*, *AcSBP24*, and *AcSBP25*) were highly expressed at least at 48, 96, 168, and R48 h post-treatment. *AcSBP7*, *AcSBP11*, and *AcSBP25* increased in expression from 48 h to R48 h. After Psa treatment, 19 *AcSBP* genes (*AcSBP1*, *AcSBP2*, *AcSBP3*, *AcSBP5*, *AcSBP6*, *AcSBP8*, *AcSBP10*, *AcSBP11*, *AcSBP12*, *AcSBP13*, *AcSBP15*, *AcSBP16*, *AcSBP17*, *AcSBP18*, *AcSBP19*, *AcSBP20*, *AcSBP22*, *AcSBP23*, and *AcSBP24*) showed high expression at least at 24, 48, 72, and 120 h post-treatment. *AcSBP1*, *AcSBP8*, *AcSBP13*, and *AcSBP20* showed increase in transcript levels from 24 h to 120 h, and *AcSBP2* and *AcSBP10* showed increase in transcript levels from 48 h to 120 h and 24 h to 72 h, respectively. The results suggested that these *AcSBP* genes may be involved in regulating biotic and abiotic stresses in kiwifruit.

Discussion

In this study, we performed genome-wide identification of kiwifruit SBP-box genes and analyzed their evolutionary history and expression patterns in different organs, and biotic and abiotic stresses.

Multiple sequence alignment showed that 25 *AcSBP* genes contain two zinc-binding sites and individual DNA-binding, and nuclear localization domains, and the highly conserved sequence GCNADLSSAKDYHRRHKVCEVHSTPKVVGGL-EQRFCCQC. This result has also been found in the other fruit trees, such as grape (Hou et al., 2013), apple (Li et al., 2013), and melon (Ma et al., 2014). In this study, the kiwifruit *AcSBP*

FIGURE 8. Expression levels of the kiwifruit *AcSBP* genes with different hormone treatments (Eth: ethylene; MeJA: methyl jasmonic acid, ABA: abscisic acid, GA: gibberellins, SA: salicylic acid) by qRT-PCR. The error bars were calculated by three biological and technical replicates.

FIGURE 8 (continued). Expression levels of the kiwifruit *AcSBP* genes with different hormone treatments (Eth: ethylene; MeJA: methyl jasmonic acid, ABA: abscisic acid, GA: gibberellins, SA: salicylic acid) by qRT-PCR. The error bars were calculated by three biological and technical replicates.

FIGURE 9. Expression levels of the kiwifruit *AcSBP* genes following drought, salt, and Psa treatments by qRT-PCR. The error bars were calculated by three biological and technical replicates.

genes were clustered into eight groups (G1 to G8), which is in accordance with apple and other previous findings (Li et al., 2013; Hou et al., 2013; Pan et al., 2017; Zhang et al., 2016; Salinas et al., 2011). In addition, we found that kiwifruit has a smaller number of *SBP* genes than apple (Han et al., 2007), while compared with grape (Jaillon et al., 2007), the number of kiwifruit *AcSBP* genes is larger. The reason may be related to the size of the genome, and the number of *SBP-box* genes has increased with the genome during the evolution of plants (Meng et al., 2016). For example, the genomic size of several plants is in the following order: apple (927 Mb and 742.3 Mb) (Han et al., 2007), kiwifruit (616.1 Mb) (Huang et al., 2013), grape (490 Mb) (Jaillon et al., 2007), and the order of *SBP-box* genes in these plants thus corresponds to the genome size order.

Exon/intron structures can also provide crucial information for analyzing the phylogenetic relationships of a gene family (Hu et al., 2015). Exon/intron structures play important roles in the evolution of multiple gene families (Zhang et al., 2012). In this study, the number of introns ranged from 1 to 7 among the 25 *AcSBP* genes. However, most studies showed that some TFs families have no introns, such as bZIP TFs in apple and grape (Gao et al., 2014; Zhao et al., 2016). In this study, we also found that *AcSBP18*, *AcSBP17*, and *AcSBP2* have no introns. In general, introns of the same group showed similar conserved positions and phases, such as G1, G2, G6 containing two exons. Additionally, intron/exon loss or gain was found within the same *AcSBP* genes. For example, *AcSBP1* and *AcSBP25* have two introns, while *AcSBP19* genes in the same group have only one intron. This intron/exon loss or gain may be due to the rearrangement and fusion of different chromosome fragments (Guo et al., 2014). Phylogenetic relationship results indicated that genes in the same group typically contain a similar number of amino acids and motif composition, which may show that these molecular structures in the same group were highly conserved during evolution (Table 1, Figure 4). Similar patterns have also been reported in grape and apple (Hou et al., 2013; Li et al., 2013).

It is 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 et al., 2005). Thus, the origin and evolutionary history of the *AcSBP* genes were analyzed by comparative synteny mapping between the kiwifruit and *Arabidopsis* genomes (Figure 6). According to this syntenic map, 12 *AcSBP* genes have 11 corresponding *Arabidopsis* orthologs, suggesting that the expansion may have occurred before the divergence of kiwifruit and *Arabidopsis*. Of these, four pairs were revealed to be single kiwifruit-to-*Arabidopsis* pairs, including *AcSBP7* – *AtSPL15*, *AcSBP10* – *AtSPL5*, and *AcSBP11* – *AtSPL13*, *AcSBP12* – *AtSPL8* (Supplemental Information – Table S3), indicating that these *AcSBP* genes and their *Arabidopsis* counterparts share a common ancestor (Zhang et al., 2012). From these homology results, we may be obtaining functional information for some kiwifruit *AcSBP* genes, such as *AcSBP6* and *AcSBP7* may be related with shoot development and shoot maturation (Shikata et al., 2009; Wu et al., 2006), *AcSBP9*, *AcSBP21*, and *AcSBP22* may be related with leaf development (Shikata et al., 2009). However, further function studies need to complete. Other gene combinations showed a different syntenic relationship, with two gene-pairs in which one single kiwifruit gene corresponds to multiple *Arabidopsis* genes, including *AcSBP3* – *AtSPL14/AtSPL16*; *AcSBP6* – *AtSPL9/AtSPL15*; *AcSBP22* – *AtSPL6/AtSPL10/AtSPL16*. And seven gene-pairs in which kiwifruit segmental duplications

correspond to a single *Arabidopsis* gene, including *AtSPL8/AtSPL22* – *AcSBP6*; *AtSPL2/AtSPL17* – *AcSBP7*; *AtSPL9/AtSPL21* – *AcSBP11*; *AtSPL6/AtSPL7* – *AcSBP15*; *AtSPL3/AtSPL22* – *AcSBP16* (Supplemental Information – Table S3). In addition, we found some *AcSBP* genes that could not be mapped into any synteny blocks, but it is unclear whether these genes share a common ancestor with *Arabidopsis* *SBP* genes. This finding may be explained by the fact that after the speciation of kiwifruit and *Arabidopsis*, their genomes may have undergone multiple rounds of significant chromosomal rearrangement and fusions, followed by selective gene loss, which can severely obscure chromosomal synteny (Paterson et al., 2012; Zhang et al., 2012). Based on these results, we also inferred that some of the kiwifruit genes share a common ancestor with their *Arabidopsis* *SBP* counterparts. The *AcSBP* genes were localized to the 19 kiwifruit chromosomes, indicating that not all 29 chromosomes contain an *AcSBP* gene. Three *AcSBP* genes were not assigned to any of the chromosomes, and this result was also found in grape and apple *SBP-box* genes (Li et al., 2013; Hou et al., 2013).

Genome duplication, including tandem and segmental duplication events, play an important role in generating new duplicated genes and the evolution of organisms (Song et al., 2016; Zhao et al., 2016; Zhang et al., 2012). 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 abundant gene duplication in its evolutionary process, and 10,581 duplicated genes have been found in the kiwifruit genome (Huang et al., 2013). In this study, no tandem duplication event regions were found in *AcSBP* genes, indicating no contribution of tandem duplication to *AcSBP* genes expansion. However, thirteen segregation duplication events were found in kiwifruit genome, indicating that segmental duplication events could be regarded as a major mechanism driving the expansion of kiwifruit *AcSBP* genes. In summary, segmental duplications may have played an important role in the expansion and evolution of the *AcSBP* genes in kiwifruit.

The function of *AcSBP* genes has been studied in many plant species. However, the responses of kiwifruit *AcSBP* genes to biotic and abiotic stresses have not been studied. In addition, the expression pattern of kiwifruit different tissues for *AcSBP* is also not known. From previous studies, we know that *AcSBP* genes play essential roles in response to various biotic and abiotic stresses in many plants, such as *Arabidopsis* (Zhang et al., 2007), apple (Li et al., 2013), and Moso Bamboo (Pan et al., 2017). The *PeSPL* genes are highly regulated by environmental signals and play positive roles in response to biotic and abiotic stresses (Pan et al., 2017), and some *MdSBP* genes were up-regulated or down-regulated by plant hormone signals (Li et al., 2013). Similarly, we found that the *AcSBP* genes showed significant differential expression patterns in response to drought, salt, GA, SA, ABA, MeJA, Eth, and PsA treatments. Eth, SA, MeJA, and GA have key roles in biotic stress signaling upon pathogen infection (Li et al., 2013). ABA is involved in response to pathogen infection, water deficit, and cold and osmotic stress (Zhao et al., 2016; Zhang et al., 2012). Therefore, we investigated the responses of *AcSBP* genes to some plant hormone stresses. Our studies showed that the *AcSBP* genes have a variety of response patterns after various hormone treatments, indicating that they may be induced by hormones and responses to plant hormone stresses in the development process. Drought and salt are major abiotic stresses that affect the

growth of plants, and they can result in severe yield losses for economic crops (Li et al., 2013). In the present study, some *AcSBP* genes (*AcSBP7*, *AcSBP11*, and *AcSBP25*) showed peak expression from 48 h to R48 h after drought treatment, indicating that a certain response time is needed for *SBP* genes to respond to drought stress. The reason may be that the temporal and spatial diversification of *SBP* gene expression is widespread (Guo et al., 2014). Similar results were also in *Psa* biotic stress: *AcSBP1*, *AcSBP8*, *AcSBP13*, and *AcSBP20* showed up-regulation during 24 h to 120 h. *AcSBP* genes also showed a complex response pattern for the regulatory networks of plant biotic stress. For instance, some *AcSBP* genes may have a positive effect on kiwifruit *Psa* resistance, while others may have a negative effect. Therefore, it is important to further study the potential function of *AcSBP* genes in biotic and abiotic stresses.

Conclusions

In this study, we performed the first genome-wide analysis of the 25 kiwifruit *AcSBP* genes, and the sequence alignment, gene structure, conserved motifs, phylogenetic analysis, promoter regions, and gene duplication were analyzed. We also analyzed the expression profiles of *AcSBP* genes in different tissues and under biotic and abiotic stresses. Overall, the analysis indicates that these genes are differentially expressed in response to one or more stresses. These results provide additional information of kiwifruit *AcSBP* genes, which should elucidate to revealing the potential important function of *AcSBP* genes in kiwifruit according to present results.

Acknowledgments

This work was supported by the earmarked fund for Science and Technology Research and Development Program of Shaanxi Province, China (2020NY-074), Scientific Research Projects of Shaanxi Education Department (18JK0982), Weinan Science and Technology Research and Development Program of Shaanxi Province (2018-ZDTY-NYCX-7), and China Agriculture Research System (CARS-28). Special thanks for the anonymous reviewers for their comments on this paper.

References

- Artimo, P., Jonnalagedda, M., Arnold, K., Baratin, D., Csardi, G., de Castro, E., Duvaud, S., Flegel, V., Fortier, A., Gasteiger, E., Grosdidier, A., Hernandez, C., Ioannidis, V., Kuznetsov, D., Liechti, R., Moretti, S., Mostaguir, K., Redaschi, N., Rossier, G., Xenarios, L., and Stockinger, H. (2012). ExPASy: SIB bioinformatics resource portal. *Nucleic Acids Res.* 40(W1), W597–W603. <https://doi.org/10.1093/nar/gks400>.
- Bailey, T.L., Williams, N., Mislé, C., and Li, W.W. (2006). MEME: Discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Res.* 34, 369–373. <https://doi.org/10.1093/nar/gkl198>.
- Birkenbihl, R.P., Jach, G., Saedler, H., and Huijser, P. (2005). Functional dissection of the plant-specific SBP-domain: Overlap of the DNA-binding and nuclear localization domains. *J. Mol. Biol.* 352, 585–596. <https://doi.org/10.1016/j.jmb.2005.07.013>.
- Cardon, G., Höhmann, S., Klein, J., Nettesheim, K., Saedler, H., and Huijser, P. (1999). Molecular characterization of the Arabidopsis SBP-box genes. *Gene* 237, 91–104. [https://doi.org/10.1016/s0378-1119\(99\)00308-x](https://doi.org/10.1016/s0378-1119(99)00308-x).
- Chuck, G., Whipple, C., Jackson, D., and Hake, S. (2010). The maize SBP-box transcription factor encoded by *tasselsheath4* regulates bract development and the establishment of meristem boundaries. *Development* 137, 1243–1250. <https://doi.org/10.1242/dev.048348>.
- Ferradás, Y., Rey, L., Martínez, Ó., 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>.
- Gandikota, M., Birkenbihl, R.P., Höhmann, S., Cardon, G.H., Saedler, H., and Huijser, P. (2007). The miRNA156/157 recognition element in the 3'UTR of the Arabidopsis SBP box gene *SPL3* prevents early flowering by translational inhibition in seedlings. *Plant J.* 49, 683–693. <https://doi.org/10.1111/j.1365-3113.2006.02983.x>.
- 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>.
- 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>.
- Guruprasad, K., Reddy, B.V.B., and Pandit, M.W. (1999). Correlation between stability of a protein and its dipeptide composition: A novel approach for predicting *in vivo* stability of a protein from its primary sequence. *Protein Engin.* 4(2), 155–161. <https://doi.org/10.1093/protein/4.2.155>.
- Han, Y., Gasic, K., Marron, B., Beever, J.E., and Korban, S.S. (2007). A BAC-based physical map of the apple genome. *Genomics* 89(5), 630–637. <https://doi.org/10.1016/j.ygeno.2006.12.010>.
- Hou, H.M., Li, J., Gao, M., Singer, S.D., Wang, H., Fei, Z.J., and Wang, X.P. (2013). Genomic organization, phylogenetic comparison and differential expression of the SBP-box gene family in grape. *PLoS ONE* 8(3), e59358. <https://doi.org/10.1371/journal.pone.0059358>.
- Hu, B., Jin, J.P., Guo, A.Y., Zhang, H., Luo, J.C., 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>.
- 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>.
- Jaillon, O., Aury, J.M., Noel, B., Policriti, A., Clepet, C., and Casagrande, A. (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 449(7161), 463–467. <https://doi.org/10.1038/nature06148>.
- Jing, Z.B., and Liu, Z.D. (2018a). Genome-wide identification of WRKY transcription factors in kiwifruit (*Actinidia* spp.) and analysis of WRKY expression in responses to biotic and abiotic stresses. *Genes Genomics* 40(4), 429–446. <https://doi.org/10.1007/s13258-017-0645-1>.
- Jing, Z.B., and Fu, H.Q. (2018b). Identification of the basic leucine zipper transcription factor and analysis of expression responses to biotic and abiotic stresses in kiwifruit (*Actinidia* Lindl.). *Eur. J. Hort. Sci.* 83(4), 212–230. <https://doi.org/10.17660/eJHS.2018/83.4.2>.
- Jing, Z.B., Yang, H.B., Yang, Y.W., Shen, J., Zhou, S.M., and Xu, M. (2016). Isolation and identification of *Pseudomonas syringae* pv. *actinidiae* in Northern area of Qinling Mountains. *J. Northwest Forestry Univ.* 31(5), 188–193 (in Chinese).
- Kavas, M., Kizildogan, A.K., and Abanoz, B. (2017). Comparative genome-wide phylogenetic and expression analysis of SBP genes from potato (*Solanum tuberosum*). *Comput. Biol. Chem.* 67, 131–140. <https://doi.org/10.1016/j.compbiolchem.2017.01.001>.

- Klein, J., Saedler, H., and Huijser, P. (1996). A new family of DNA binding proteins includes putative transcriptional regulators of the *Antirrhinum majus* floral meristem identity gene SQUAMOSA. *Mol. Gen. Genet.* 250, 7–16. <https://doi.org/10.1007/bf02191820>.
- 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>.
- Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis v. 7.0 for bigger datasets. *Mol. Biol. Evol.* 33(7), 1870–1874. <https://doi.org/10.1093/molbev/msw054>.
- Lee, T.H., Tang, H., Wang, X., and Paterson, A.H. (2012). PGDD: A database of gene and genome duplication in plants. *Nucleic Acids Res.* 41, D1152–1158. <https://doi.org/10.1093/nar/gks1104>.
- 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, J., Hou, H.M., Li, X.Q., Xiang, J., Yin, X.J., Gao, H., and Wang, X.P. (2013). Genome-wide identification and analysis of the SBP-box family genes in apple (*Malus × domestica* Borkh.). *Plant Physiol. Biochem.* 70, 100–114. <https://doi.org/10.1016/j.plaphy.2013.05.021>.
- Li, M., Zhao, S.Z., Zhao, C.Z., Zhang, Y., Xia, H., Lopez-Baltazar, J., Wan, S.B., and Wang, X.J. (2015). Cloning and characterization of SPL family genes in the peanut (*Arachis hypogaea* L.). *Genet. Mol. Res.* 15(1). <https://doi.org/10.4238/gmr.15017344>.
- Ma, Y., Guo, J.W., Bade, R., Men, Z.H., and Hasi, A. (2014). Genome-wide identification and phylogenetic analysis of the SBP-box gene family in melons. *Genet. Mol. Res.* 13, 8794–8806. <https://doi.org/10.1007/s00344-014-9443-z>.
- Manning, K., Tör, M., Poole, M., Hong, Y.G., Thompson, A.J., King, G.J., Giovannoni, J.J., and Seymour, G.B. (2006). A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat. Genet.* 38, 948–952. <https://doi.org/10.1038/ng1841>.
- Mao, H.D., Yu, L.J., Li, Z.J., Yan, Y., Han, R., and Liu, H. (2016). Genome-wide analysis of the SPL family transcription factors and their responses to abiotic stresses in maize. *Plant Gene* 6, 1–12. <https://doi.org/10.1016/j.plgene.2016.03.003>.
- Meng, D., Li, Y.Y., Bai, Y., Li, M.J., and Cheng, L.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>.
- Pan, F., Wang, Y., Liu, H.L., Wu, M., Chu, W.Y., Chen, D.M., and Xiang, Y. (2017). Genome-wide identification and expression analysis of SBP-like transcription factor genes in Moso Bamboo (*Phyllostachys edulis*). *BMC Genomics* 18, 486. <https://doi.org/10.1186/s12864-017-3882-4>.
- Paterson, A.H., Wang, X., Tang, H., and Lee, T.H. (2012). Synteny and genomic rearrangements. *Plant. Gen. Divers.* 1, 195–207. https://doi.org/10.1007/978-3-7091-1130-7_13.
- Preston, J.C., Jorgensen, S.A., Orozco, R., and Hileman, L.C. (2015). Paralogous SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes differentially regulate leaf initiation and reproductive phase change in petunia. *Planta* 243, 429–440. <https://doi.org/10.1007/s00425-015-2413-2>.
- Salinas, M., Xing, S., Höhmann, S., Berndtgen, R., and Huijser, P. (2011). Genomic organization, phylogenetic comparison and differential expression of the SBP-box family of transcription factors in tomato. *Planta* 235, 1171–1184. <https://doi.org/10.1007/s00425-011-1565-y>.
- Schwarz, S., Grande, A.V., Bujdoso, N., Saedler, H., and Huijser, P. (2008). The microRNA regulated SBP-box genes *SPL9* and *SPL15* control shoot maturation in Arabidopsis. *Plant. Mol. Biol.* 67, 183–195. <https://doi.org/10.1007/s11103-008-9310-z>.
- Shalom, L., Shlizerman, L., Zur, N., Doronfaigenboim, A., Blumwald, E., and Sadka, A. (2015). Molecular characterization of SQUAMOSA PROMOTER BINDING PROTEINLIKE (SPL) gene family from *Citrus* and the effect of fruit load on their expression. *Front. Plant Sci.* 6, 389. <https://doi.org/10.3389/fpls.2015.00389>.
- Shikata, M., Koyama, T., Mitsuda, N., and Ohme-Takagi, M. (2009). Arabidopsis SBP-box genes *SPL10*, *SPL11* and *SPL2* control morphological change in association with shoot maturation in the reproductive phase. *Plant Cell Physiol.* 50, 2133–2151. <https://doi.org/10.1093/pcp/pcp148>.
- Song, A., Gao, T., Wu, D., Xin, J., Chen, S., Guan, Z., Wang, H., Jin, L., and Chen, F. (2016). Transcriptome-wide identification and expression analysis of chrysanthemum SBP-like transcription factors. *Plant Physiol. Biochem.* 102, 10–16. <https://doi.org/10.3390/ijms17020198>.
- Tang, H., Bowers, J.E., Wang, X., Ming, R., Alam, M., and Paterson, A.H. (2008a). Synteny and collinearity in plant genomes. *Science* 320, 486–488. <https://doi.org/10.1126/science.1153917>.
- Tang, H., Wang, X., Bowers, J.E., Ming, R., Alam, M., and Paterson, A.H. (2008b). Unraveling ancient hexaploidy through multiply-aligned angiosperm gene maps. *Genome Res.* 18, 1944–1954. <https://doi.org/10.1101/gr.080978.108>.
- Unte, U.S., Sorensen, A.M., Pesaresi, P., Gandikota, M., Leister, D., Saedler, H., and Huijser, P. (2003). *SPL8*, a SBP-box gene that affects pollen sac development in Arabidopsis. *Plant Cell* 15, 1009–1019. <https://doi.org/10.1105/tpc.010678>.
- Wu, G., and Poethig, R.S. (2006). Temporal regulation of shoot development in *Arabidopsis thaliana* by *miR156* and its target *SPL3*. *Development* 133, 3539–3547. <https://doi.org/10.1242/dev.02521>.
- Xie, K., Wu, C., and Xiong, L. (2006). Genomic organization, differential expression, and interaction of SQUAMOSA promoter-binding-like transcription factors and microRNA156 in rice. *Plant Physiol.* 142, 280–293. <https://doi.org/10.2307/20205922>.
- Xing, S., Salinas, M., Höhmann, S., Berndtgen, R., and Huijser, P. (2010). *miR156*-targeted and nontargeted SBP-box transcription factors act in concert to secure male fertility in *Arabidopsis*. *Plant Cell* 22, 3935–3950. <https://doi.org/10.1105/tpc.110.079343>.
- Xing, S.P., Salinas, M., Garcia-Molina, A., Höhmann, S., Berndtgen, R., and Huijser, P. (2013). *SPL8* and *miR156*-targeted *SPL* genes redundantly regulate *Arabidopsis gynoecium* differential patterning. *Plant J.* 75(4), 566–577. <https://doi.org/10.1111/tpj.12221>.
- Xu, Z., Sun, L., Zhou, Y., Yang, W., Cheng, T., Wang, J., and Zhang, Q.X. (2015). Identification and expression analysis of the SQUAMOSA promoter-binding protein (SBP)-box gene family in *Prunus mume*. *Mol. Gen. Genet.* 290, 1701–1715. <https://doi.org/10.1007/s00438-015-1029-3>.
- Yamaguchi, A., Wu, M.F., Yang, L., Wu, G., Poethig, R.S., and Wagner, D. (2009). The microRNA-regulated SBP-box transcription factor *SPL3* is a direct upstream activator of *LEAFY*, *FRUITFULL*, and *APETALA1*. *Developm. Cell* 17, 268–278. <https://doi.org/10.1016/j.devcel.2009.06.007>.
- Yamasaki, H., Hayashi, M., Fukazawa, M., Kobayashi, Y., and Shikanai, T. (2009). *SQUAMOSA* promoter binding protein-like7 is a central regulator for copper homeostasis in *Arabidopsis*. *Plant Cell* 21, 347–361. <https://doi.org/10.1105/tpc.108.060137>.

Yamasaki, K., Kigawa, T., Inoue, M., Tateno, M., Yamasaki, T., Yabuki, T., Aoki, M., Seki, E., Matsuda, T., Nunokawa, E., Ishizuka, Y., Terada, T., Shirouzu, M., Osanai, T., Tanaka, A., Seki, M., Shinozaki, K., and Yokoyama, S. (2004). A novel zinc-binding motif revealed by solution structures of DNA-binding domains of *Arabidopsis* SBP-family transcription factors. *J. Mol. Biol.* 337, 49–63. <https://doi.org/10.1016/j.jmb.2004.01.015>.

Yang, Z., Wang, X., Gu, S., Hu, Z., Xu, H., and Xu, C. (2008). Comparative study of SBP-box gene family in *Arabidopsis* and rice. *Gene* 407, 1–11. <https://doi.org/10.1016/j.jmb.2004.01.015>.

Zhang, H.X., Jin, J.H., He, Y.M., Lu, B.Y., Li, D.W., Chai, W.G., Khan, A., and Gong, Z.H. (2015). Genome-wide identification and analysis of the SBP-box family genes under *Phytophthora capsici* stress in pepper (*Capsicum annuum* L.). *Front. Plant Sci.* 7, 504. <https://doi.org/10.3389/fpls.2016.00504>.

Zhang, L.S., Wu, B., Zhao, D.G., Li, C.L., Shao, F.G., and Lu, S.F. (2014). Genome-wide analysis and molecular dissection of the *SPL* gene family in *Salvia miltiorrhiza*. *J. Integr. Plant Biol.* 56, 38–50. <https://doi.org/10.1111/jipb.12111>.

Zhang, S.D., and Ling, L.Z. (2014). Genome-wide identification and evolutionary analysis of the SBP-box gene family in Castor Bean. *PLoS ONE* 9(1), e86688. <https://doi.org/10.1371/journal.pone.0086688>.

Zhang, W., Li, B., and Yu, B. (2016). Genome-wide identification, phylogeny and expression analysis of the SBP-box gene family in maize (*Zea mays*). *J. Integr. Agr.* 15(1), 29–41. [https://doi.org/10.1016/S2095-3119\(14\)60955-2](https://doi.org/10.1016/S2095-3119(14)60955-2).

Zhang, X.H., Dou, L.L., Pang, C.Y., Song, M.Z., Wei, H.L., Fan, S.L., Wang, C., and Yu, S. (2015). Genomic organization, differential expression, and functional analysis of the *SPL* gene family in *Gossypium hirsutum*. *Mol. Genet. Genomics* 290, 115–126. <https://doi.org/10.1007/s00438-014-0901-x>.

Zhang, Y., Schwarz, S., Saedler, H., and Huijser, P. (2007). SPL8, a local regulator in a subset of gibberellin-mediated developmental processes in *Arabidopsis*. *Plant Mol. Biol.* 63, 429–439. <https://doi.org/10.1007/s11103-006-9099-6>.

Zhang, Y.C., Mao, L.Y., Wang, H., Brocker, C., Yin, X.J., Vasiliou, V., Fei, Z.J., and Wang, X.P. (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.R., Guo, C.L., Hou, H.M., Wang, X.P., 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>.

Received: Oct. 14, 2019

Accepted: Apr. 6, 2020

Addresses of authors:

Zhaobin Jing^{1,2,*a}, Xiangning Qi^{2,a} and Linsen Zhang²

¹ Weinan Vocational and Technical College, Weinan Fruit Industry Institute of Shaanxi, Weinan 714026, Shaanxi, China

² College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China

* Corresponding author;

E-mail: jingzhaobin@nwsuaf.edu.cn;

Tel.: +86-(0)913-303-3264

^a These authors contributed equally to this work.

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL INFORMATION – TABLE S1. The sequence used in this study.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
<i>AcSBP1</i>	AACTCCAAGCGTGCTCTCTC	GTTTCTCCTCCAGCCCTTGT
<i>AcSBP2</i>	GTTSP LTCAGAGSPLSPLGAGAGTG	TCTTCTTCCTSPLTTGTCCGCTAAT
<i>AcSBP3</i>	AAGCTTGCTSPLCGGAAAAA	GGTGAAGGAACCCTTGTGAA
<i>AcSBP4</i>	AGAATSPLGGTGGTGTGGCTCA	GTTTTGGGACGTGGGTAATATTTTT
<i>AcSBP5</i>	CTAGTGGAGGAGGTCAACG	CTGACAGCTCATGGAACCTG
<i>AcSBP6</i>	GGCTCAAGAGACCSPLCCTTG	CATGTGAACCACCACCAGCTTC
<i>AcSBP7</i>	CACCCGTCAACCACTTCTCT	AGTGTGCTGAGCCAATTCAA
<i>AcSBP8</i>	CAAGAATCATCTGGGGTCTCA	TCCCACTTGCTTCAACTTCA
<i>AcSBP9</i>	TTTCAACTGSPLGCAGCAC	SPLGAAGCTAGCGGCAGACT
<i>AcSBP10</i>	GAGGGTCAACACAGCCTTCT	TTGACAGCTCATGGAACCTG
<i>AcSBP11</i>	CGGTACAGTGGTTAATGCAGG	AGGCACSPLGCTATCTGAACTCCAT
<i>AcSBP12</i>	CAGCTGCCAGTTTTCASPL	AAAGAAAAGGGCCACACAAA
<i>AcSBP13</i>	GCAGCCATCCTCGTSPLAGT	CCTGGCATCCATGTATTGTG
<i>AcSBP14</i>	TTGCTCSPLATCCCTTGTC	CCACTSPLGTTCTGGTGGTG
<i>AcSBP15</i>	CCTGGCTGTGCTCTCTCTCT	AACTTGGGTGACTGGCTTG
<i>AcSBP16</i>	AAGCATGGAAGGGTGCCCGT	CCCGTCCATCAGTTTCTGCT
<i>AcSBP17</i>	TTGSPLGCTGTAGCAGGACA	TCCAAAACAAACAGCAGCAG
<i>AcSBP18</i>	GTGAGAAGCACTCTAAGACTGCAGA	ATCAAGACGCTTCCTGCAACTTCTC
<i>AcSBP19</i>	TACAATGGGCGAGACAAACAGTTAC	CTGACGSPLAATGCTTTCTGTGAAC
<i>AcSBP20</i>	ACTGGACATGTGGTGGTTGA	TGGCATTTCCATAAGCCAAC
<i>AcSBP21</i>	AAGTACTCCATGGGAATGCACAAG	AGTCTATTGGAATTCTGACCAGGCA
<i>AcSBP22</i>	TCCAGCAGTGCTCTCTCTCTT	GCCCAAGTTTAGCCTTCACA
<i>AcSBP23</i>	CGGAGTTTGACAATGGGAAG	ACCGTGACAGAGSPLGAGGA
<i>AcSBP24</i>	AATCAGCCCTGGGTCTCAC	CCCASPLCAGAAGGCATCTC
<i>AcSBP25</i>	TCTACATGAGTTCTGGGACCTATTT	TTGGTTSPLCAGTGGCATGCAAG
<i>ACT2</i>	GCAGGAATCCATGAGACTACC	GTCTGCSPLACCAGGGAACAT
<i>18S2</i>	CTGTGAAACTGCGAATGGCTC	TTCCAGAAGTCGGGGTTTGT

SUPPLEMENTAL INFORMATION – TABLE S2. Additional conserved motifs identified from 25 *AcSBP* proteins.

Motif No.	Length (aa)	E-value	Multi-level consensus sequences	No. of <i>AcWRKY</i> proteins in which motif is present	<i>AcWRKY</i> proteins in which motif is present
1	41	5.8e-637	GCNADLSSAKDYHRRHKVCEVHSTPKVVGGIEQRCQQC	25	AcSBP1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25
2	29	3.8e-390	EFDEGRSCRKRLAGNRRRKQPESLS	23	AcSBP1,2,3,4,5,6,7,8,9,10,12,13,14,15,16,17,18,20,21,22,23,24,25
3	50	7.1e-159	RTDRIVFKLFGKNPSDFPLVLRQILDLWSHSPDIESYIRPGCVILTY	7	AcSBP16,20,12,14,3,17,2
4	49	1.2e-085	HDZLQFRNFSQIPDLTGRGFIEVEDNSLSSFFPFVAEKDVCSEIKT	6	AcSBP20,12,16,14,2,17
5	49	1.3e-080	KYPKIQSIKPIAVSASEPAQFLVKGFNLRKPTTRLLCALEGKYLVEAC	6	AcSBP12,14,20,16,17,2
6	40	2.5e-064	FPLKRFKWLMEFSVEHDWCVAVKLLGILLDGTGVAGEHP	5	AcSBP12,16,20,14,3
7	50	2.3e-070	GLTPLHIAAGRDGEDVLDALTDPGKVGIEAWKSARDSTGLTPEDYARL	5	AcSBP12,20,16,14,3
8	37	2.0e-063	ATSYLLYRPAMLSMVAIAVCVALLFKSSPELVV	5	AcSBP20,12,14,16,3
9	28	2.8e-055	GVNSGCALSLLSSQPQQTSEHSSIPV	12	AcSBP11,15,13,18,24,8,22,1,4,7,25,6
10	11	7.1e-048	GSSQPPSCQVE	25	AcSBP1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25
11	41	4.4e-045	DDIQPESEQIEARNQALDFIHEMGWLLHRNQLKSLRGLDLP	4	AcSBP12,20,16,14
12	50	8.2e-042	EFEGGYMDRRDYLKSEDGPTIDLLQLSSYLQRVEQQRNSMQVKQENDFY	4	AcSBP13,8,22,15
13	34	2.4e-035	RGHYSYHLVQKKINRKAGTGHVLDIPAYLPZC	4	AcSBP12,20,16,14
14	29	9.0e-035	ALSEMGLLHRAVRRNCRSMVECLLKYIE	5	AcSBP20,12,14,16,3
15	50	6.5e-034	PYLHSQGLEEKPSALVPNASDTNVHCQGMFHMGRDDLLENEASZVLPFSW	4	AcSBP18,11,25,1
16	29	4.9e-029	DTFWTTGWLYTRMQHQIAFYNGQVVAET	3	AcSBP20,12,16
17	20	1.6e-027	GSSNSSDLSIELKLGLAD	17	AcSBP24,7,18,15,11,19,8,13,1,22,21,6,25,20,12,23,3
18	49	1.3e-031	AVGKRSEWDPNDRWDGDLFIASPLNSQSNYSQRQFFPIGGPIT	3	AcSBP12,20,16
19	50	2.8e-037	MDFTFPRFNGRDPWPPTTKAPDQISGNQPAATGKFLHPWQNESENPPPD	3	AcSBP7,24,6
20	49	4.4e-032	ALDQQNLLPYSFARSYNGGDKQFPFLPTDPERSNQAVPEVSTSQLHK	5	AcSBP1,25,18,11,19
21	6	3.5e-027	SRFHSL	21	AcSBP24,7,6,5,10,20,12,16,14,4,23,8,22,21,9,3,11,25,1,17,2
22	50	1.5e-024	MDWNAKWDWENLVFNSTKIESPKKQPTDWGIEEGEIDPGSFNLYWGGG	2	AcSBP9,21
23	50	1.1e-021	IHNKSTCILERYIHVMENMDNLLNSLANGIVDTDMKLLQSNLDNAREI	2	AcSBP2,17
24	50	2.3e-019	IAMPKSMWFKLLEDPLVLYIHJVAAPVKMLSGRGTLLVYLDNVIFRAMRV	2	AcSBP17,2
25	21	1.9e-018	KRELEKRRRVVVEDDNLDE	4	AcSBP20,12,16,14

SUPPLEMENTAL INFORMATION – TABLE S3. Synteny blocks of SBP genes within kiwifruit genome.

ID	Region 1 (kiwifruit)			Region 2 (kiwifruit)			Gene in the synteny region		Gene name	
	Chr	Start	Stop	Chr	Start	Stop	Gene 1	Gene 2	Gene 1	Gene 2
108	Chr13	15565224	16851276	Chr29	5446223	8272374	Achn144801	Achn355701	AcSBP8	AcSBP
191	Chr16	6890950	7469086	Chr6	184584	812266	Achn017541	Achn252971	AcSBP11	AcSBP4
289	Chr2	13073211	14276689	Chr26	8435511	9143922	Achn039041	Achn298021	AcSBP1	AcSBP18
291	Chr2	14042900	14430482	Chr27	9627699	9906611	Achn039041	Achn353841	AcSBP1	AcSBP19
346	Chr21	14056075	14285644	Chr4	12847365	12986663	Achn016511	Achn343391	–	AcSBP3
389	Chr23	2760441	5148107	Chr19	6487728	8505054	Achn121261	Achn138051	AcSBP15	AcSBP13
420	Chr24	133486	2812331	Chr20	8593487	10048449	Achn102591	Achn235741	AcSBP16	AcSBP14
432	Chr24	175279	2870942	Chr3	18796808	19776136	Achn082431	Achn185971	AcSBP17	AcSBP2
435	Chr24	2333735	2917182	Chr3	19554784	20178333	Achn082431	Achn185971	AcSBP17	AcSBP2
531	Chr28	54076	695396	Chr17	6439	157645	Achn195011	Achn251461	AcSBP20	AcSBP12
558	Chr29	2706547	4613975	Chr13	13655185	15232241	Achn196331	Achn350751	AcSBP21	AcSBP9
666	Chr5	13034702	13222288	Chr27	9743423	9837695	Achn235351	Achn353841	–	AcSBP19
699	Chr6	640804	1674399	Chr26	8013664	8438106	Achn252971	Achn298021	AcSBP4	AcSBP18
718	Chr6	640804	1194464	Chr7	16917157	17997492	Achn252971	Achn261591	AcSBP4	–
771	Chr8	2224027	2875621	Chr15	15695233	16384918	Achn181301	Achn219131	AcSBP5	AcSBP10
796	Chr9	760560	966268	Chr12	10065402	10356268	Achn064091	Achn126821	AcSBP6	AcSBP7

SUPPLEMENTAL INFORMATION – TABLE S4. Synteny blocks of SBP genes between kiwifruit and *Arabidopsis* genomes.

Id	Region 1 (kiwifruit)			Region 2 (<i>Arabidopsis</i>)			Gene in the synteny region			Gene name	
	Chr	Start	Stop	Chr	Start	Stop	Gene 1	Gene 2		Gene 1	Gene 2
56	Chr12	7782870	10369225	Chr3	20941502	21448690	Achn126821	AT3G57920		AcSBP7	AtSPL15
76	Chr13	15906876	16194882	Chr1	25883616	26099388	Achn144801	AT1G69170		AcSBP8	AtSPL6
77	Chr13	14000614	14153729	Chr1	9483157	9516940	Achn350751	AT1G27360		AcSBP9	AtSPL11
156	Chr15	15978966	16781235	Chr3	5066879	5236088	Achn219131	AT3G15270		AcSBP10	AtSPL5
204	Chr15	6100215	7668849	Chr5	20201101	20755714	Achn017541	AT5G50570		AcSBP11	AtSPL13
210	Chr17	133632	1733167	Chr1	365209	687315	Achn251461	AT1G02065		AcSBP12	AtSPL8
465	Chr24	2356699	3450254	Chr5	6171661	6516205	Achn082431	AT5G18830		AcSBP17	AtSPL7
613	Chr29	7035422	8197302	Chr1	26005068	26240708	Achn355701	AT1G69170		AcSBP22	AtSPL6
614	Chr29	3077622	3404200	Chr1	9373926	9516940	Achn196331	AT1G27360		AcSBP21	AtSPL11
617	Chr29	6796323	7130266	Chr1	25941675	26086481	Achn355701	AT1G69170		AcSBP22	AtSPL16
619	Chr29	7035422	8197302	Chr1	9329512	9508481	Achn355701	AT1G27370		AcSBP22	AtSPL10
674	Chr3	19540741	20010378	Chr5	620260	6338100	Achn185971	AT5G18830		AcSBP2	AtSPL7
681	Chr4	12519764	12986663	Chr1	28651154	28771409	Achn343391	AT1G76580		AcSBP3	AtSPL16
682	Chr4	12685978	12937815	Chr1	7242920	7346973	Achn343391	AT1G20980		AcSBP3	AtSPL14
822	Chr9	556848	953881	Chr2	17507509	17624841	Achn064091	AT2G42200		AcSBP6	AtSPL9
833	Chr9	701552	1321468	Chr3	21225540	21477569	Achn064091	AT3G57920		AcSBP6	AtSPL15