GENOME-WIDE CHARACTERIZATION AND ANALYSIS OF SBP TRANSCRIPTION FACTOR FAMILY IN COMMON BEAN (*PHASEOLUS VULGARIS* L.)

ILHAN, E.

Department of Molecular Biology and Genetics, Erzurum Technical University
Erzurum, Turkey
(e-mail: emre.ilhan@erzurum.edu.tr; phone:+90-444-5388-2387)

(Received 6th Jul 2018; accepted 14th Aug 2018)

Abstract. SQUAMOSA promoter binding proteins (SBPs) are considered as being a major family of plant-specific transcription factors which plays an important role in flower improvement process. To discover SBP genes in common bean [*Phaseolus vulgaris* (L.)] genome, in silico analysis methods were carried out in this study. Twenty-three members of Pvul-SBP gene family were identified from common bean genome. A phylogenetic tree drawn using SPB proteins of *Phaseolus vulgaris*, *Arabidopsis thaliana* and *Glycine max* was separated into three main groups (A, B, and C). The C group was clustered into nine subclades. The chromosome locations of *Pvul-SBP* genes showed that *Pvul-SBP-1*/*Pvul-SBP-6*, *Pvul-SBP-2*/*Pvul-SBP-16*, *Pvul-SBP-5*/*Pvul-SBP-14* and *Pvul-SBP-9*/*Pvul-SBP-15* gene couples were segmentally-duplicated. Also, orthologue genes were defined using SBP proteins of *Arabidopsis thaliana* and *Glycine max*. The expression levels of *Pvul-SBP* genes in different plant tissues such as roots, leaf and floral organs showed some of *Pvul-SBP* genes were up or down regulated in certain tissue types. The results of this research would provide the estimation of the evolutionary relationships between *SBP* genes in various plants such as *P. vulgaris*, *G. max* and *A. thaliana*.
Keywords: *gene expression, orthologue relationship, phylogeny, synteny*

Introduction

Identification and characterization of transcription factor (TF) families in plants has been turned into one of the most important topics for plant scientists in recent years due to their importance in regulation networks of plant growing processes (Buyuk et al., 2016; Inal et al., 2017; Ilhan et al., 2018). There have been many well-conducted studies for characterization of several plant-specific TFs in many plant species. However, there is no information about some of them including SBP (SQUAMOSA promoter binding protein family) in certain plant species (Wang et al., 2015).

SBPs are considered as being a major family of plant-specific transcription factors which plays an important role in flower improvement processes (Pan et al., 2017). SBP domain, a highly-conserved DNA-binding domain, has specific properties for each member of this TF family. Even though this domain is known to be a zinc-binding domain, structures within this domain are not similar to those ones in other zinc-binding domains and thus has been declared as representing a novel type of zinc binding motif (Wang et al., 2015).

Recent studies which were aimed at defining the roles of SBP family of transcription factors showed divergent roles of them in plant developmental processes (Zhang et al., 2016).

Among sixteen identified *SBP* genes in *Arabidopsis* genome, many of them have been revealed to play role in embryogenesis (Unte et al., 2003), development of shoots and leaves (Schwarz et al., 2008), flowering process (Gandikota et al., 2007), transition

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

between vegetative and generative phases (Jung et al., 2012) and plant hormone signaling (Zhang et al., 2007)

Genome-wide characterization and expression studies of SBP genes have been conducted in a number of plant species including *Betula pendula*, *Chlamydomonas*, *Oryza sativa*, *Zea mays*, *Populus trichocarpa*, *L. esculentum*, *Malus* × *domestica* Borkh., *Salvia miltiorrhiza*, *Gossypium hirsutum*, *Arachis hypogaea* L., petunia, *Capsicum annuum* L., *Chrysanthemum morifolium*, *Phyllostachys edulis*, *Solanum tuberosum*, *Ziziphus jujuba* and (Lannenpaa et al., 2004; Kropat et al., 2005; Xie et al., 2006; Chuck et al., 2010; Lu et al., 2011; Salinas et al., 2012; Li et al., 2013; Zhang et al., 2014, 2015, 2016; Li et al., 2016; Preston et al., 2016; Song et al., 2016; Pan et al., 2017; Kavas et al., 2017; Shao et al., 2017).

The common bean [Phaseolus vulgaris (L.)] is a self-pollinated legume species. It has a small genome size of 588 Mbp and a diploid genome ($2n = 2x = 22$) (Arumuganthan and Earle, 1991). It is one of the earliest plant species cultivated. As a result of its nitrogen fixation ability, it enriches the nitrogen content of soil. It has an important role in human nutrition, especially in developing countries, due to its high protein content (Broughton et al., 2003). It has been possible to analyze and characterize the SBP gene family with the publication of the whole genome sequence of common bean by Schmutz in 2014 (Schmutz et al., 2014). However, the SPB proteins in *Phaseolus vulgaris* L. have not been identified and characterized yet. To fill this gap in the literature and due to importance of this gene family in plant developmental processes, this study aimed at describing and characterizing SBP proteins in *Phaseolus vulgaris* L. Accordingly, 23 Phvul-SBP members were identified and comprehensive analysis of the sequence phylogeny, genomic organization, exon-intron architecture variation, conserved protein motifs, gene duplication events, and expression analysis were performed.

Materials and methods

Identification of SBP genes in P. vulgaris genome

Phaseolus vulgaris of SBP protein sequences were retrieved from Phytozome database v12.1 (www.phytozome.net) using keyword searching with Pfam Accession Number (PF03110) obtained from Pfam Database (http://pfam.xfam.org). BLASTP and BLASTX searches (National Center for Biotechnology Information [NCBI]: http://www.ncbi.nlm.nih.gov) was used to confirm Pvul-SBP proteins. Non-redundant sequences were obtained using decrease redundancy tool (http://web.expasy.org/decrease_redundancy/). SBP domains in non-redundant sequences were checked by HMMER (http://www.ebi.ac.uk). The solid and chemical traits of SBP proteins in *Phaseolus vulgaris*, such as the theoretical isoelectric point (pI), the number of amino acids, and the molecular weight (Da), were identified using the ProtParam tool (http://web.expasy.org/protparam/).

Phylogenetic analysis, physical location, conserved motifs of phvul-SBP genes, gene structure and gene duplication events

Chromosomal locations and CDS sizes (bp) were identified by using Phytozome database v12.1. The *Pvul-SBP* genes were mapped with MapChart (Voorrips, 2002).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

Multiple sequence alignment of Pvul-SBP proteins was conducted with ClustalW. Phylogenetic analysis was performed using MEGA v7 (Tamura et al., 2013) and Neighbor-joining (NJ) algorithm with 1000 replicated-bootstrap value.

The conserved motifs in Pvul-SBP protein sequences were identified using the MEME (Multiple Expectation Maximization for Motif Elucidation) online tool (Bailey et al., 2006). The limits for maximum number of motifs and minimum/maximum width were adjusted as 20 and 2, 50, respectively. Motif sites were among 2 and 300. Site distribution was set as any number of repetitions. The described conserved motifs were examined in InterProscan with default adjusting (Quevillon et al., 2005).

Online Gene Structure Display Server program tool (GSDS) was used (Guo et al., 2007) to predict the exon/intron organization of the *Pvul-SBP* genes. Genomic DNA sequences and coding sequences of Pvul-SBP genes were utilized.

Segmental duplicate gene pairs were examined on the Plant Genome Duplication Database server (PGDD) (Lee et al., 2013), with a display range of 100 kb. The nonsynonymous rates (Ka), synonymous rates (Ks) and developmental constraints (Ka/Ks) with the duplicated pairs of Pvul-SPLs were evaluated using the CODEML program in PAML (Yang, 2007). The roughly date of the duplication events was determined using $T = Ks / 2\lambda \times 10^{-6}$ Mya (million years ago), in terms of the synonymous substitution rates (λ) in common bean of 8.46×10^{-9} (Schmutz et al., 2014).

Synteny analysis

P. vulgaris and *A. thaliana*, *P. vulgaris* and *G. max* of orthologue *SBP* genes were identified with PGDD (Lee et al., 2013). Then, the protein sequences of orthologue genes were retrieved from Phytozome v12.1. The retreived synteny map was created using iTAK - Plant Transcription factor & Protein Kinase Identifier and Classifier (Zheng et al., 2016).

Gene expression analysis in silico

The expression levels of Pvul-SBP genes examined in special tissue libraries of plants at different stages of growth, including root_10 (10 days after planting), nodules, root_19 (19 days after planting), young pods, stem_10 (10 days after planting), stem_19 (19 days after planting), green mature buds, leaves, young triloliates, flower buds and flowers were retrieved from Phytozome Database v12.1 (http://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Pvulgaris). FPKM (expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced) units were used for the expression levels *in silico*. FPKM values were log2 transformed and the heatmap was produced with the algorithm CIMMiner (http://discover.nci.nih.gov/cimminer).

Results and discussion

Identification of SBP gene family in common bean

Sequences of SBP proteins in the *P. vulgaris* genome were downloaded from Phytozome database v12.1 (www.phytozome.net) using keyword searching with Pfam Accession Number (PF03110) retrieved from Pfam Database. Subsequently, SBP domains were analyzed by searching HMMER and Pfam databases in candidate Phvul-

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

SBP proteins and the redundant sequences were discarded after the confirmation. A total of 23 candidate *Phvul-SBP* genes in *Phaseolus vulgaris* genome was discovered and some descriptive information was given in *Table 1* which involves the information about chromosomal location, the coding sequences (CDS, lengths), amino acid (length), molecular weight and number of isoelectric point (pI). As shown in *Table 1* and *Figure 1*, all of the non-redundant *Phvul-SBP* genes were distributed on 10 chromosomes of P. vulgaris. While the lowest number of *Phvul-SBP* genes was obtained on chromosome 11 (one *Phvul-SBP* gene), the highest number of Phvul-SBPs was found on chromosome 3 (4 *Phvul-SBP* genes) (*Fig. 1*). The CDS of the *Phvul-SBP* genes extended from 378 (*Phvul-SBP-4*) to 3210 (*Phvul-SBP-9*). The length of Phvul-SBP proteins extended from 125 (Phvul-SBP-4) to 1039 (Phvul-SBP-9) amino acids (aa). pIs of Phvul-SBP proteins were among 5.8 (Phvul-SBP-15) and 9.46 (Phvul-SBP-12) ranging from acidic to alkaline, and the molecular weight of Phvul-SBPs were between 14756.09 Da (Phvul-SBP-14) and 114838.01 Da (Phvul-SBP-9) (*Table 1*). *SBP* gene family which is essential to plants was detected and classified in various species such as *Arabidopsis* (Rhoades et al., 2002), rice (Xie et al., 2006), tomato (Salinas et al., 2012), *Citrus* (Shalom et al., 2015), *Gossypium raimondii* (Ali et al., 2017), wheat (Wang et al., 2015), maize (Mao et al., 2016) and grape (Hou et al., 2013).

Table 1. *The information of 23 Phvul-SBP proteins*

Gene ID	Phytozome ID	Chromosomal location	CDS length	aa length	Molecular weight (kDa)	pI
Phvul-SBP-1	Phvul.001G091400	Chr01:15552500..15556152	1095	364	40.66	7.16
Phvul-SBP-2	Phvul.001G141000	Chr01:38299114..38303396	1074	357	38.20	8.97
Phvul-SBP-3	Phvul.001G262600	Chr01:50971750..50975439	1362	453	49.63	7.58
Phvul-SBP-4	Phvul.002G114500	Chr02:24504136..24505511	378	125	13.99	9.19
Phvul-SBP-5	Phvul.002G230300	Chr02:40279842..40282041	510	169	19.49	5.47
Phvul-SBP-6	Phvul.002G286000	Chr02:45529658..45533339	1152	383	41.91	8.82
Phvul-SBP-7	Phvul.003G008800	Chr03:989197..995182	3030	1009	111.88	8.45
Phvul-SBP-8	Phvul.003G039600	Chr03:4337189..4341787	1443	480	53.96	6.4
Phvul-SBP-9	Phvul.003G120700	Chr03:30548630..30555522	3120	1039	114.84	7.35
Phvul-SBP-10	Phvul.003G182900	Chr03:40612076..40617217	1650	549	60.30	6.49
Phvul-SBP-11	Phvul.005G074000	Chr05:13162582..13164100	576	191	21.61	8.86
Phvul-SBP-12	Phvul.005G101900	Chr05:31789454..31791276	510	169	19.30	9.46
Phvul-SBP-13	Phvul.006G159700	Chr06:26373187..26387876	2289	762	85.64	6.85
Phvul-SBP-14	Phvul.006G183900	Chr06:28501741..28503445	384	127	14.76	6.45
Phvul-SBP-15	Phvul.007G210600	Chr07:33265157..33272512	3045	1014	112.51	5.8
Phvul-SBP-16	Phvul.007G258400	Chr07:37986117..37990987	1107	368	39.58	8.76
Phvul-SBP-17	Phvul.008G157100	Chr08:28607666..28613632	1035	344	37.55	9.15
Phvul-SBP-18	Phvul.008G157200	Chr08:28427029..28429343	951	316	34.55	8.82
Phvul-SBP-19	Phvul.009G165100	Chr09:24615068..24618431	1194	397	43.71	7.67
Phvul-SBP-20	Phvul.009G219200	Chr09:33103356..33114413	3096	1031	113.97	6.59
Phvul-SBP-21	Phvul.010G056000	Chr10:10002440..10005618	993	330	36.35	8.85
Phvul-SBP-22	Phvul.010G056200	Chr10:10088279..10091188	1119	372	41.02	6.9
Phvul-SBP-23	Phvul.011G164800	Chr11:46749842..46751202	561	186	20.80	8.97

pI: Theoretical isoelectric point, kDa: kiloDalton

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

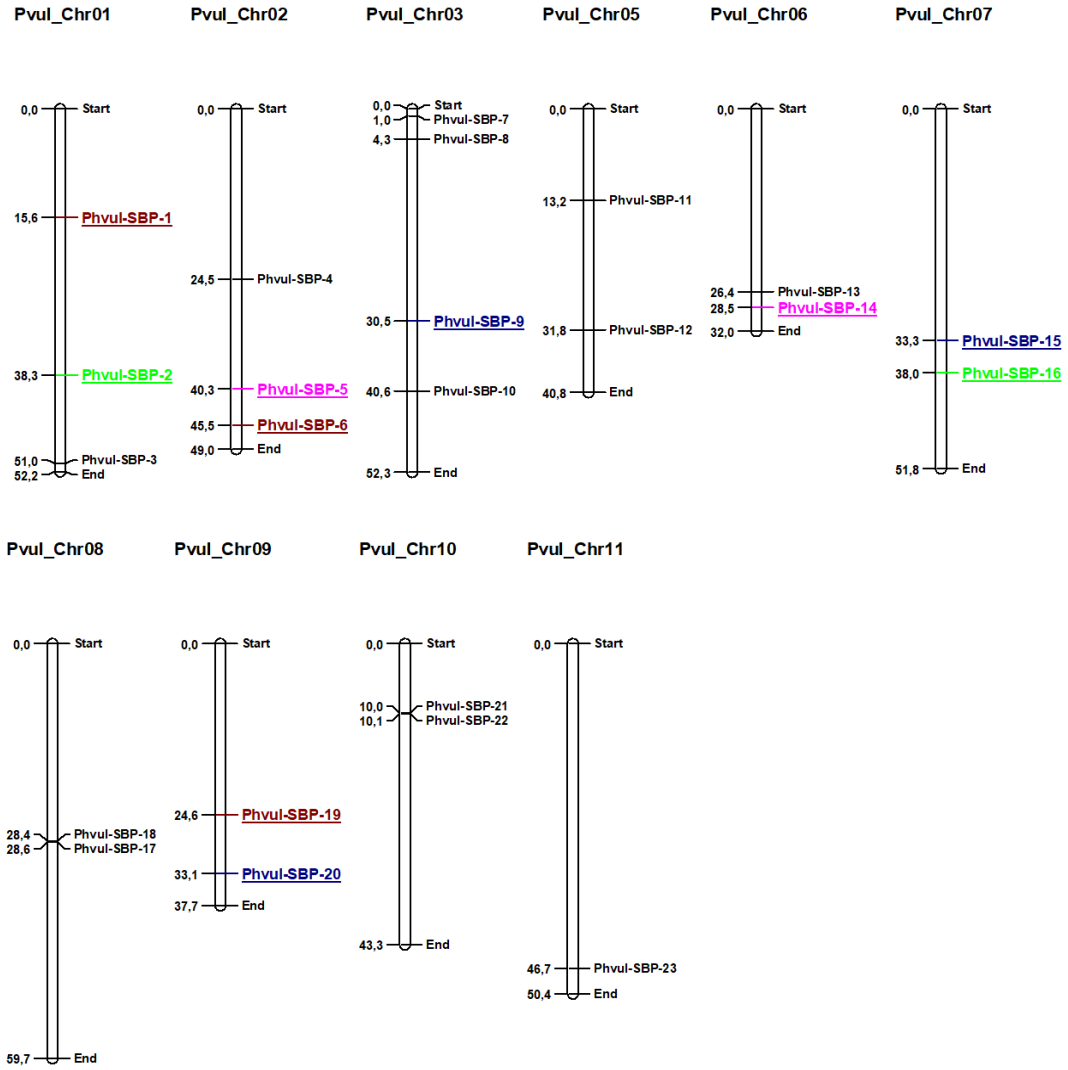

Figure 1. *Distribution of Phvul-SBP genes on common bean chromosomes. Similar colors present segmentally – duplicated genes*

Several paralogous gene pairs are originated from gene duplication events (Cannon et al., 2004). Furthermore, organisms adapt to different environments via gene duplication events throughout processes of development and growth (Bowers et al., 2003; Gu et al., 2003). Plant genomic structure, which can occur by independent events involving in segmental or tandem duplications, is one of most important characteristics of gene duplication (Flagel and Wendel, 2009). Based on significance of gene duplications on evolution of gene families in plants, the gene duplication events of putative *Phvul-SBP* genes in common bean genome have been examined. After the duplication analysis, six segmentally-duplicated gene couples (*Phvul-SBP-1*/*Phvul-SBP-19*, *Phvul-SBP-2*/*Phvul-SBP-16*, *Phvul-SBP-5*/*Phvul-SBP-14*, *Phvul-SBP-6*/*Phvul-SBP-19*, *Phvul-SBP-9*/*Phvul-SBP-15* and *Phvul-SBP-9*/*Phvul-SBP-20*) among the identified 23 *Phvul-SBP* genes in common bean (*Table 2*) were detected. According to the gene duplication analysis, none of the gene couples have been found to include tandem repeats.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

The Ka/Ks ratio has been become a popular parameter for genome-wide identification researches of gene families in recent years. This ratio gives a worthwhile information about the selective evolutionary pressures that are acting on that gene (Yang and Swanson, 2002; Kryazhimskiy and Plotkin, 2008). According to Juretic et al. (2005), a Ka/Ks larger than 1 reflects the positive selection during the evolution of the gene sequence. Additionally, a Ka/Ks lower than 1 indicates duplications have undergone purifying selection and a Ka/Ks = 1 means neutral selection in duplication events. As displayed in *Table 2*, the divergence time of segmentally duplicated Phvul-SBP gene pairs extended from 32.77 to 101.61 million years (MYA). Except for one, all of the duplicated Phvul-SBP gene pairs showed Ka/Ks ratio lower than 1 which reflects the occurrence of purifying selection after the duplication events. The Ka/Ks ratio (=1) of Phvul-SBP-1/Phvul-SBP-19 indicated the predominance of neutral selection during duplication events of this gene pair (*Table 2*).

Table 2. *The Ka/Ks ratios and identified divergence times for the segmentally duplicated Phvul-SBP proteins*

Paralogous gene pairs		Ka	Ks	Ka/Ks	Duplication date (MYA)
Phvul-SBP-1	Phvul-SBP-19	0	0	1	-
Phvul-SBP-2	Phvul-SBP-16	0.1922	0.8346	0.2075	49.33
Phvul-SBP-5	Phvul-SBP-14	0.3358	1.0685	0.0917	63.15
Phvul-SBP-6	Phvul-SBP-19	0.2174	0.7511	0.2834	44.39
Phvul-SBP-9	Phvul-SBP-15	0.3989	1.7193	0.1484	101.61
	Phvul-SBP-20	0.1836	0.5544	0.2868	32.77

Phylogenetic analysis, gene structure and conserved motifs of Phvul-SBPs

To discover the relationships between Phvul-SBP proteins, a phylogenetic tree of SBP domain proteins in *Phaseolus vulgaris*, *Arabidopsis* and *Glycine max* was structured using amino acid sequences with contributed bootstrap values (1000 replicates; *Fig. 2*) via neighbour joining method. Phvul-SBP proteins were clustered into twelve groups, A - C9 (*Fig. 2*). Similar coding and exon-intron sequences were observed in most of the Phvul-SBP genes which were found in the same subgroup in phylogenetic tree.

To investigate conserved motifs in Phvul-SBP proteins, MEME (v5.0.1) was used. MEME software finds the most statistically significant (low E-value) motifs based on its log likelihood ratio, its width and number of occurrences, the background letter frequencies and the size of the training set. Motifs with E-values larger than 0.01 (1e-2) are possibly just statistical artifacts, and not real motifs (Bailey et al., 2009).

A total of 20 conserved motifs were described (*Fig. 3* and *Table 3*). The lengths of identified motifs were between 8 and 50 amino acids. A total of Phvul-SBP proteins consist of Motif 1 which is one of the two motifs which have SBP domain among all motifs. A similar result was found in Ali et al. (2017).

Twenty-three common bean SBP gene structures were investigated and the estimated number of exons among the 23 *Phvul-SBP* genes extended from two to 10 (*Fig. 4*). As seen in *Figure 4*, these genes were also clustered into twelve groups in terms of the gene structure analysis in line with the phylogenetic tree of Phvul-SBP proteins shown in *Figure 2*. Deep examinations of the segmentally duplicated gene pairs, which have

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

same exon–intron structures and similar motifs, would contribute to a comprehension of the different roles they play in development and growth. In a recent research, it has been reported that the gene pairs, which have similar motif sequences and orders could show similar functions in moso bamboo (Pan et al., 2017). These gene pairs could be originated from an ancestor in evolutionary process.

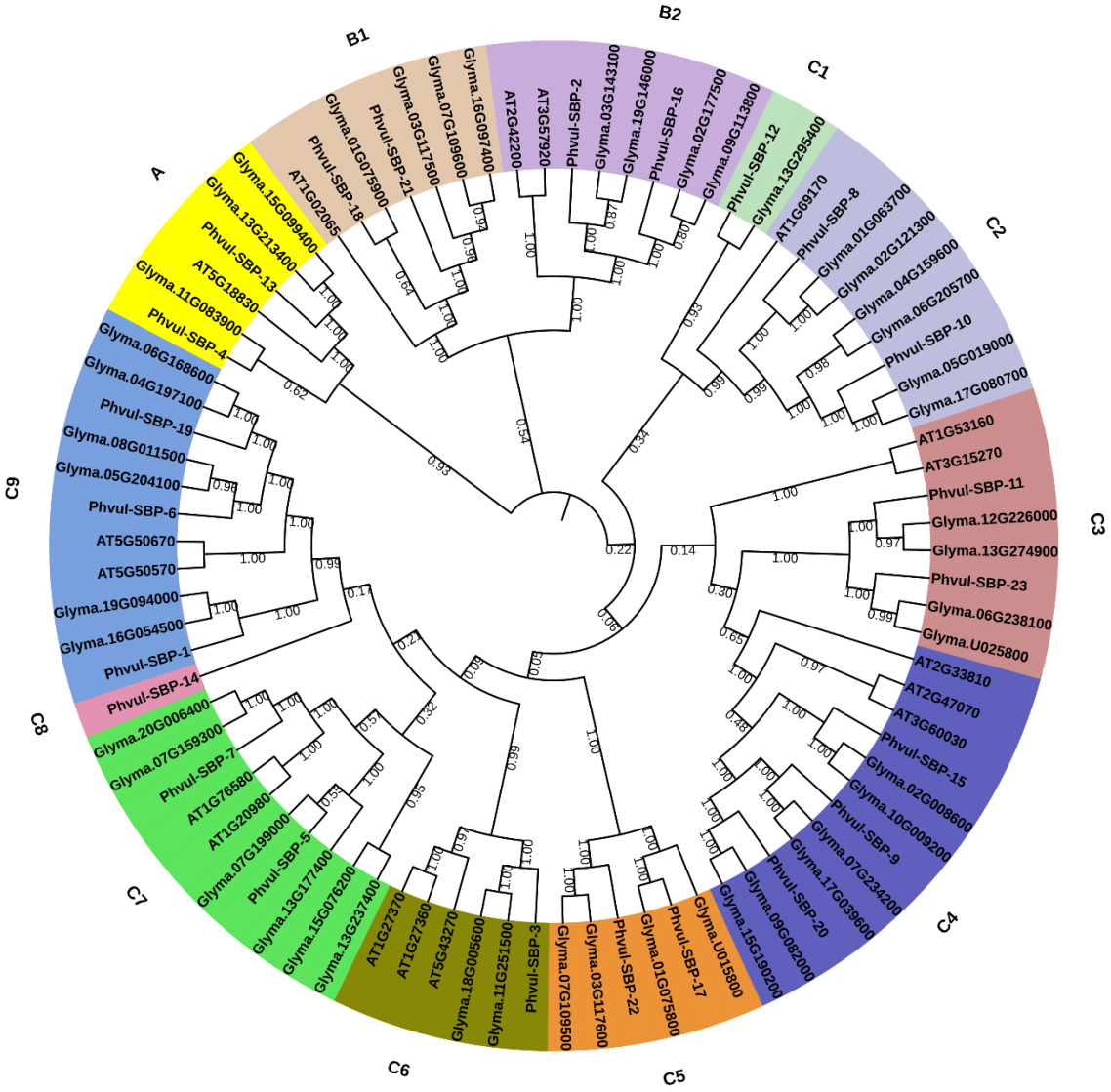

Figure 2. *A phylogenetic tree of SBP proteins of common bean, soybean and Arabidopsis thaliana. The tree was generated with MEGA v7 and Neighbor-joining (NJ) algorithm with 1000 replicated-bootstrap values*

Table 3. *The MEME motif sequences and length of Pvul-SBP proteins*

Motif ID	Conserved sequence	E-value*	Sites	Width	Domain
1	RCQVEGCNADLSNAKDYHRRHKVCEMHSKAP KVJVAGLEQRFCQQCSRFH	2.3E-730	23	50	SBP
2	VLSEFDEGKRSCRRRLAGHNERRRKPQPE	5.5E-351	22	29	SBP
3	RTDRIVFKLFGKDPNDFPLVLRAQILNWLSHSP TEIESYIRPGCIILTIY	5E-92	5	50	Unknown

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

4	PAGLTPLHVAASISGSDNVLDALTDDPGMVGIE AWKSARDSTGLTPHDYA	8E-48	4	50	Unknown
5	TAMVYRPAMLSMVAIAAVCVCVALLFKSSPKV YYVFRPF	7E-41	4	39	Unknown
6	FIQEMGWLLHRSRLKVRLGPVAPIQDJFQFNRF KWLVDFSMDHDWCAVMK	3E-34	3	50	Unknown
7	GKRSLEWDLNDWKWDGDLFTASRLNSVPSDC	1E-24	3	31	Unknown
8	PKILCVKPLAVPASKRAQFIVKGVNLLQPATRL LCALEGKYLVCE	1E-24	5	45	Unknown
9	FWRTGWVYVRVQHQLAFLYNGQVVIDVPL	1E-21	4	29	unknown
10	DSSCALSLLSSQSW	2E-16	10	14	Unknown
11	SLIGLKLGKRIYFED	5E-15	9	15	Unknown
12	ALLEMGLLHKAVKRNSRPMVELLLRYVP	1E-13	4	28	Unknown
13	PVIVAEEEICSEICTLENVJE	2E-11	5	21	Unknown
14	YLLMSLLRILSNMHSNGSDHTTBQDILSHLLRN LASLAGPNNG	2E-13	3	43	Unknown
15	HJKFSCTIPNVVGRGFIEVED	2E-10	5	21	Unknown
16	WEELDYGT	6E-09	10	8	n/a
17	LRGHYSYIQLVQKKINKKGGAAHVVDIP	1E-08	3	28	Unknown
18	WVQCDSLKSSPPQTSRNSDSTSTQ	9E-09	3	24	Unknown
19	MDWNLKAPSWDLVEVDKANJPNIESMEEHNRF GMFRMEGEFSVDLKLGQV	2E-07	2	50	Unknown
20	CRQADERGRIQMNJPRNSGYKSFHIR	5E-06	3	26	Unknown

*The expected value of each motif prediction was given in the MEME program

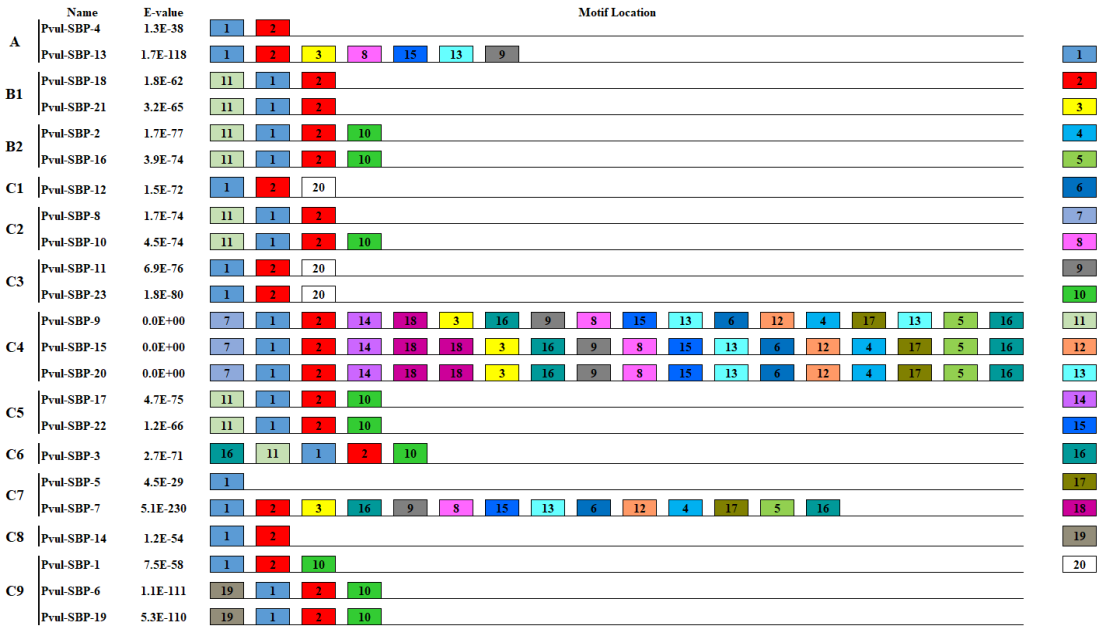

Figure 3. *Shematic representation of the 20 conserved motifs in Pvul-SBP proteins. The 20 conserved motifs were classified using MEME Suite v5.0.1. Each motifs were shown by different colored boxes. The numbers in the boxes represent motif 1 – motif 20, respectively*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

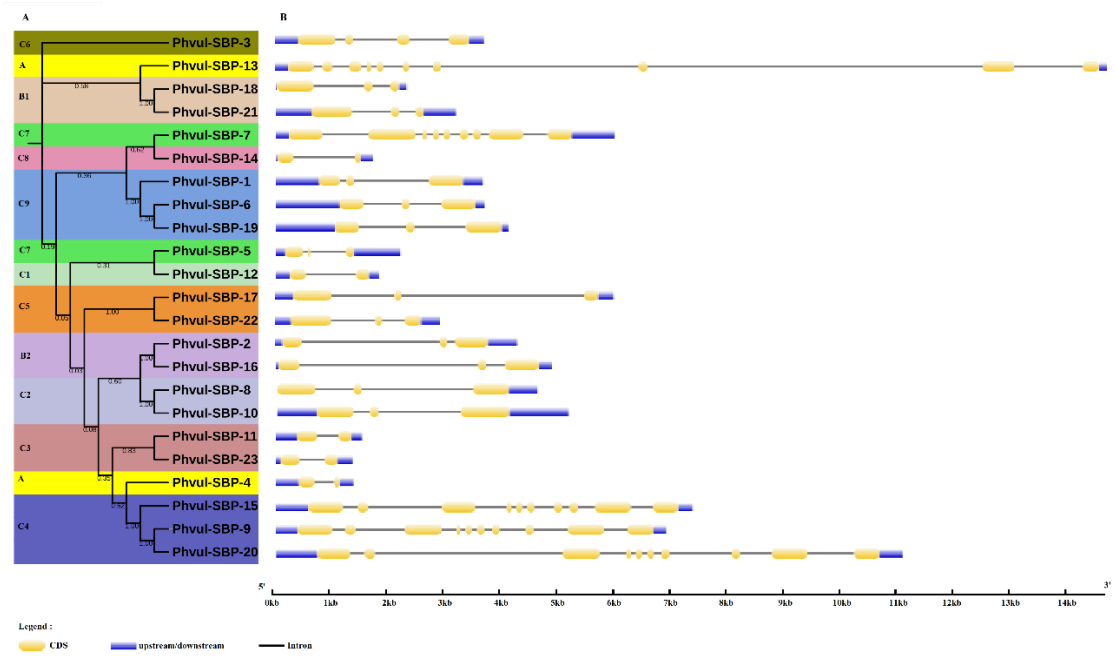

Figure 4. *Gene structure of Phvul-SBP genes.* ***A.*** *The number of below branches of the phylogenetic tree display bootstrap values. The tree was structured with MEGA v7 by the Neighbor-Joining (NJ) method with 1000 bootstrap replicates. The A – C9 numerals on the tree correspond to 12 Pvul-SBP groups shown in Figure 2.* ***B.*** *Yellow colors represent exons, grey lines represent introns and blue colors represent untranslated regions (UTRs)*

Comparative and synteny events among SBP genes of P. vulgaris, Arabidopsis thaliana and Glycine max

To calculate evolutionary relationship of the *SBP* gene family in *P. vulgaris*, *Arabidopsis* and *Glycine max*, the synteny events among SBP protein sequences of these plants are shown in *Figure 5*.

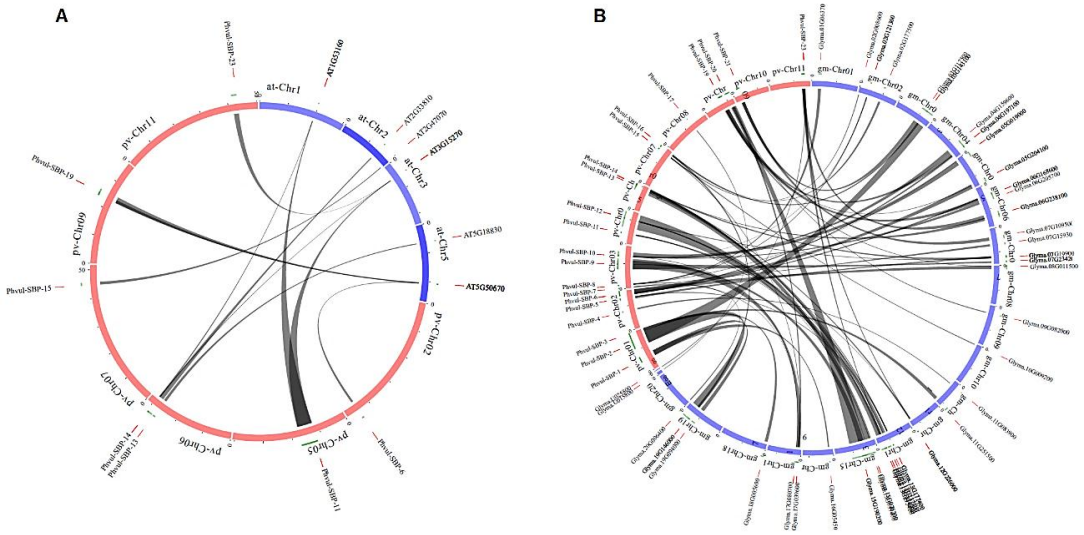

Figure 5. *Genome wide synteny analysis of SBP genes.* ***A.*** *Comparative map between P. vulgaris and A. thaliana.* ***B.*** *Comparative map between P. vulgaris and G. max*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

This synteny analysis was established on SBP proteins of 6 *Arabidopsis* and 40 *Glycine max*. Since *P. vulgaris* and *G. max* were closely related genomes (Schmutz et al., 2014), *SPB* genes of these plants showed high similarity. However, 22 *Pvul-SBP* genes were matched to 40 *G. max SBP* genes distributed on all chromosomes of *G. max*. In a recent study, it was reported that some *Arabidopsis SBP* genes were syntenic to those of diploid cotton genes and these genes showed an evolutionary relationship (Ali et al., 2017). In another study, the synteny analysis between apple and *Arabidopsis* demonstrated that 11 apple and *Arabidopsis* genes were located in the duplicated genomic regions. In the same study, it was notified that duplications such as segmental, tandem and genomic duplications have important roles in evolutionary processes (Li et al., 2013).

Expression profiles of Phvul-SBPs in different tissues

In this research, a common mRNA analysis of *Phvul-SBP* genes were performed via publicly available expression data in Phytozome v12.1 online plant genomics resource (https://phytozome.jgi.doe.gov). The heatmap shows the expression variance between identified 21 *Phvul-SBP* genes in different plant tissues (*Fig. 6*).

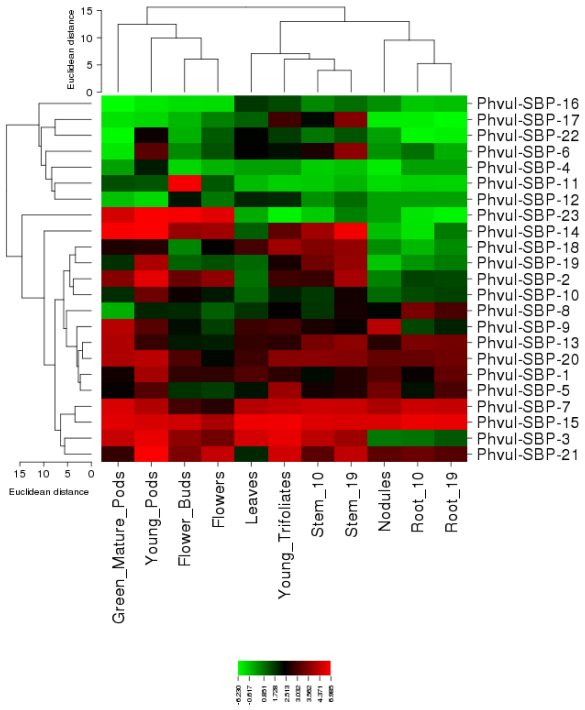

Figure 6. *Hierarchical clustering of expression profiles of Phvul-SBP genes in 11 different tissues of common bean. Heat map was drawn with CIMminer online tool*

As seen in the *Figure 6*, the *Phvul-SBP-16, -17, -22, -6, -4, -11* and *-12* genes revealed relatively low expression levels in almost all tissues including green mature pods, young pods, flower buds, flowers, leaves, young trifoliates, stem_10, stem_19, nodules, root_10 and root_19. The rest of the *Phvul-SBP* genes exhibited relatively high mRNA levels in most of the developmental stages of common bean. *Phvul-SBP-14, -15* and *-23* were the genes which exhibited the highest expression levels at least in one of the

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

certain tissue types among all genes. Additionally, some of the genes were only up- or down-regulated in certain tissue types. For instance, *Phvul-SBP-1*, *-3*, *-5*, *-7*, *-8*, *-9*, *-13*, *-15*, *-20* and *-21* were highly expressed in root tissues. *Phvul-SBP-1*, *-2*, *-3*, *-5*, *-7*, *-8*, *-9*, *-10*, *-13*, *-14*, *-15*, *-18*, *-19*, *-20* and *-21* were highly expressed in stem tissues. *Phvul-SBP-1*, *-2*, *-3*, *-5*, *-7*, *-8*, *-9*, *-10*, *-13*, *-14*, *-15*, *-18*, *-19*, *-20*, *-21* and *-23* were highly expressed in floral organs. The other *Pvul-SBP* genes were low expressed in these tissues of *P. vulgaris*. Similar results were obtained in grapevine genome (Wang et al., 2010). *SBP* genes in this genome were up or down regulated in various tissues such as root, leaf, flower and fruit. *SBP* genes in *P. vulgaris* were suggested to express in different tissues.

Conclusion

In this study, genome sequences, identification, conserved motifs of 23 *Pvul-SBP* genes were identified in *P. vulgaris* genome. The Pvul-SBPs were divided into twelve classes and 23 *Pvul-SBP* genes were distributed across 10 chromosomes with different densities. Also, expression levels of 23 *Pvul-SBP* genes in different plant tissues such as roots, leaf and floral organs were determined using *in silico* analysis. To the best of our knowledge, this is the first report of a genome wide analysis of the common bean SBP gene family. This study would provide beneficial information for understanding the classification and functions of the *SBP* genes in common bean.

REFERENCES

[1] Ali, M. A., Alia, K. B., Atif, R. M., Rasul, I., Nadeem, H. U., Shahid, A., Azeem, F. (2017): Genome-wide identification and comparative analysis of squamosa-promoter binding proteins (Sbp) transcription factor family in *Gossypium raimondii* and *Arabidopsis thaliana*. – Pakistan Journal of Botany 49: 1113-1126.

[2] Arumuganthan, K., Earle, E. (1991): Nuclear DNA content of some important plant species. – Plant Molecular Biology Reports 9: 208-218.

[3] Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., Ren, J., Li, W. W., Noble, W. S. (2009): MEME SUITE: tools for motif discovery and searching. – Nucleic Acids Research 37(suppl_2): W202-W208.

[4] Bailey, T. L., Williams, N., Misleh, C., Li, W. W. (2006): MEME: discovering and analyzing DNA and protein sequence motifs. – Nucleic Acids Research 34: W369-W373.

[5] Bowers, J. E., Chapman, B. A., Rong, J., Paterson, A. H. (2003): Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events – Nature 422(6930): 433-8.

[6] Broughton, W. J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., Vanderleyden, J. (2003): Beans (*Phaseolus* spp.) – model food legumes. – Plant and Soil 252(1): 55-128.

[7] Buyuk, I., Inal, B., Ilhan, E., Tanriseven, M., Aras, S., Erayman, M. (2016): Genome-wide identification of salinity responsive HSP70s in common bean. – Molecular Biology Reports 43: 1251-1266.

[8] Cannon, S. B., Mitra, A., Baumgarten, A., Young, N. D., May, G. (2004): The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. – BMC Plant Boilogy 4(1): 10.

[9] Chuck, G., Whipple, C., Jackson, D., Hake, S. (2010): The maize SBP-box transcription factor encoded by tasselsheath4 regulates bract development and the establishment of meristem boundaries. – Development 137: 1585-1585.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

[10] Flagel, L. E., Wendel, J. F. (2009): Gene duplication and evolutionary novelty in plants. – New Phytologist 183: 557-564.
[11] Gandikota, M., Birkenbihl, R. P., Hohmann, S., Cardon, G. H., Saedler, H., 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 Journal 49: 683-693.
[12] Gu, Z. L., Steinmetz, L. M., Gu, X., Li, W. H. (2003): Role of duplicate genes in genetic robustness against null mutations. – Nature 421: 63-6.
[13] Guo, A., Zhu, Q., Chen, X., Luo, J. (2007): GSDS: a gene structure display server. Yi chuan. – Hereditas 29: 1023-1026.
[14] Hou, H. M., Li, J., Gao, M., Singer, S. D., Wang, H., Mao, L. Y., Fei, Z. J., Wang, X. P. (2013): Genomic organization, phylogenetic comparison and differential expression of the SBP-box family genes in grape. – Plos One 8.
[15] Ilhan, E., Buyuk, I., Inal, B. (2018): Transcriptome - Scale characterization of salt responsive bean TCP transcription factors. – Gene 642: 64-73.
[16] Inal, B., Buyuk, I., Ilhan, E. and Aras, S. (2017): Genome-wide analysis of *Phaseolus vulgaris* C2C2-YABBY transcription factors under salt stress conditions. – 3Biotech 7(5): 302.
[17] Jung, J. H., Ju, Y., Seo, P. J., Lee, J. H., Park, C. M. (2012): The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in *Arabidopsis.* – Plant Journal 69: 577-588.
[18] Juretic, N., Hoen, D. R., Huynh, M. L., Harrison, P. M., Bureau, T. E. (2005): The evolutionary fate of MULE-mediated duplications of host gene fragments in rice. – Genome Research 15: 1292-1297.
[19] Kavas, M., Kızıldoğan, A. K., Abanoz, B. (2017): Comparative genome-wide phylogenetic and expression analysis of SBP genes from potato (*Solanum tuberosum*). – Computational Biology and Chemistry 67: 131-140.
[20] Kropat, J., Tottey, S., Birkenbihl, R. P., Depege, N., Huijser, P., Merchant, S. (2005): A regulator of nutritional copper signaling in Chlamydomonas is an SBP domain protein that recognizes the GTAC core of copper response element. – Proceedings of the National Academy of Sciences of the United States of America 102: 18730-18735.
[21] Kryazhimskiy, S., Plotkin, J. B. (2008): The population genetics of dN/dS. – Plos Genetics 4.
[22] Lannenpaa, M., Janonen, I., Holtta-Vuori, M., Gardemeister, M., Porali, I. and Sopanen, T. (2004): A new SBP-box gene BpSPL1 in silver birch (*Betula pendula*). – Physiologia Plantarum 120: 491-500.
[23] Lee, T. H., Tang, H. B., Wang, X. Y., Paterson, A. H. (2013): PGDD: a database of gene and genome duplication in plants. – Nucleic Acids Research 41: D1152-D1158.
[24] Li, J., Hou, H. M., Li, X. Q., Xiang, J., Yin, X. J., Gao, H., Zheng, Y., Bassett, C. L., Wang, X. P. (2013): Genome-wide identification and analysis of the SBP-box family genes in apple (*Malus* × *domestica* Borkh.). – Plant Physiology and Biochemistry 70: 100-114.
[25] Li, M., Zhao, S. Z., Zhao, C. Z., Zhang, Y., Xia, H., Lopez-Baltazar, J., Wan, S. B., Wang, X. J. (2016): Cloning and characterization of SPL-family genes in the peanut (*Arachis hypogaea* L.). – Genetics and Molecular Research 15.
[26] Lu, S. F., Yang, C. M., Chiang, V. L. (2011): Conservation and diversity of MicroRNA-associated copper-regulatory networks in *Populus trichocarpa.* – Journal of Integrative Plant Biology 53: 879-891.
[27] Mao, H. D., Yu, L. J., Li, Z. J., Yan, Y., Han, R., Liu, H., Ma, M. (2016): Genome-wide analysis of the SPL family transcription factors and their responses to abiotic stresses in maize. – Plant Gene 6: 1-12.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

[28] Pan, F., Wang, Y., Liu, H. L., Wu, M., Chu, W. Y., Chen, D. M., Xiang, Y. (2017): Genome-wide identification and expression analysis of SBP-like transcription factor genes in Moso Bamboo (*Phyllostachys edulis*). – BMC Genomics 18: 486.
[29] Preston, J. C., Jorgensen, S. A., Orozco, R., Hileman, L. C. (2016): Paralogous SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes differentially regulate leaf initiation and reproductive phase change in petunia. – Planta 243: 429-440.
[30] Quevillon, E., Silventoinen, V., Pillai, S., Harte, N., Mulder, N., Apweiler, R., Lopez, R. (2005): InterProScan: protein domains identifier. – Nucleic Acids Research 33: W116-W120.
[31] Rhoades, M. W., Reinhart, B. J., Lim, L. P., Burge, C. B., Bartel, B., Bartel, D. P. (2002): Prediction of plant microRNA targets. – Cell 110: 513-520.
[32] Salinas, M., Xing, S. P., Hohmann, S., Berndtgen, R., Huijser, P. (2012): Genomic organization, phylogenetic comparison and differential expression of the SBP-box family of transcription factors in tomato. – Planta 235: 1171-1184.
[33] Schmutz, J., McClean, P. E., Mamidi, S., Wu, G. A., Cannon, S. B., Grimwood, J., Jenkins, J., Shu, S. Q., Song, Q. J., Chavarro, C., Torres-Torres, M., Geffroy, V., Moghaddam, S. M., Gao, D. Y., Abernathy, B., Barry, K., Blair, M., Brick, M. A., Chovatia, M., Gepts, P., Goodstein, D. M., Gonzales, M., Hellsten, U., Hyten, D. L., Jia, G. F., Kelly, J. D., Kudrna, D., Lee, R., Richard, M. M. S., Miklas, P. N., Osorno, J. M., Rodrigues, J., Thareau, V., Urrea, C. A., Wang, M., Yu, Y., Zhang, M., Wing, R. A., Cregan, P. B., Rokhsar, D. S., Jackson, S. A. (2014): A reference genome for common bean and genome-wide analysis of dual domestications. – Nature Genetics 46: 707-713.
[34] Schwarz, S., Grande, A. V., Bujdoso, N., Saedler, H., Huijser, P. (2008): The microRNA regulated SBP-box genes *SPL*9 and *SPL*15 control shoot maturation in *Arabidopsis*. – Plant Molecular Biology 67: 183-195.
[35] Shalom, L., Shlizerman, L., Zur, N., Doron-Faigenboim, A., Blumwald, E., Sadka, A. (2015): Molecular characterization of SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) gene family from *Citrus* and the effect of fruit load on their expression. – Frontiers in Plant Science 6: 389.
[36] Song, A., Gao, T., Wu, D., Xin, J., Chen, S., Guan, Z., Wang, H., Jin, L., Chen, F. (2016): Transcriptome-wide identification and expression analysis of chrysanthemum SBP-like transcription factors. – Plant Physiology and Biochemistry 102: 10-16.
[37] Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. – Molecular Biology and Evolution 30: 2725-2729.
[38] Unte, U. S., Sorensen, A. M., Pesaresi, P., Gandikota, M., Leister, D., Saedler, H., Huijser, P. (2003): SPL8, an SBP-Box gene that affects pollen sac development in *Arabidopsis*. – Plant Cell 15: 1009-1019.
[39] Voorrips, R. E. (2002): MapChart: Software for the graphical presentation of linkage maps and QTLs. – Journal of Heredity 93: 77-78.
[40] Wang, B. N., Geng, S. F., Wang, D., Feng, N., Zhang, D. D., Wu, L., Hao, C. Y., Zhang, X. Y., Li, A. L., Mao, L. (2015): Characterization of SQUAMOSA promoter binding protein-like genes in wheat. – Journal of Plant Biology 58: 220-229.
[41] Wang, Y., Hu, Z., Yang, Y., Chen, X., Chen, G. (2010): Genome-wide identification, phylogeny, and expression analysis of the SBP-box gene family in grapevine. – Russian Journal of Plant Physiology 57: 273-282.
[42] Xie, K. B., Wu, C. Q., Xiong, L. Z. (2006): Genomic organization, differential expression, and interaction of SQUAMOSA promoter-binding-like transcription factors and microRNA156 in rice. – Plant Physiology 142: 280-293.
[43] Yang, Z. H. (2007): PAML 4: Phylogenetic analysis by maximum likelihood. – Molecular Biology and Evolution 24: 1586-1591.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480

[44] Yang, Z. H., Swanson, W. J. (2002): Codon-substitution models to detect adaptive evolution that account for heterogeneous selective pressures among site classes. – Molecular Biology and Evolution 19: 49-57.
[45] Zhang, H. X., Jin, J. H., He, Y. M., Lu, B. Y., Li, D. W., Chai, W. G., Khan, A., Gong, Z. H. (2016): Genome-wide identification and analysis of the SBP-box family genes under Phytophthora capsici stress in pepper (*Capsicum annuum* L.). – Frontiers in Plant Science 7: 504.
[46] Zhang, L. S., Wu, B., Zhao, D. G., Li, C. L., Shao, F. J., Lu, S. F. (2014): Genome-wide analysis and molecular dissection of the SPL gene family in *Salvia miltiorrhiza.* – Journal of Integrative Plant Biology 56: 38-50.
[47] Zhang, X. H., Dou, L. L., Pang, C. Y., Song, M. Z., Wei, H. L., Fan, S. L., Wang, C. S., Yu, S. X. (2015): Genomic organization, differential expression, and functional analysis of the SPL gene family in *Gossypium hirsutum.* – Molecular Genetics and Genomics 290: 115-126.
[48] Zhang, Y., Schwarz, S., Saedler, H., Huijser, P. (2007): SPL8, a local regulator in a subset of gibberellin-mediated developmental processes in *Arabidopsis*. – Plant Molecular Biology 63: 429-439.
[49] Zheng, Y., Jiao, C., Sun, H. H., Rosli, H. G., Pombo, M. A., Zhang, P. F., Banf, M., Dai, X. B., Martin, G. B., Giovannoni, J. J., Zhao, P. X., Rhee, S. Y., Fei, Z. J. (2016): iTAK: A program for genome-wide prediction and classification of plant transcription factors, transcriptional regulators, and protein kinases. – Molecular Plant 9: 1667-1670.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1605_54675480