Genome-wide identification, phylogeny, and expression analysis of the SBP-box gene family in Euphorbiaceae

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Abstract

Background: Euphorbiaceae is one of the largest families of flowering plants. Due to its exceptional growth form diversity and near-cosmopolitan distribution, it has attracted much interest since ancient times. SBP-box (SBP) genes encode plant-specific transcription factors that play critical roles in numerous biological processes, especially flower development. We performed genome-wide identification and characterization of SBP genes from four economically important Euphorbiaceae species.

Results: In total, 77 SBP genes were identified in four Euphorbiaceae genomes. The SBP proteins were divided into three length ranges and 10 groups. Group-6 was absent in Arabidopsis thaliana but conserved in Euphorbiaceae. Segmental duplication played the most important role in the expansion processes of Euphorbiaceae SBP genes, and all the duplicated genes were subjected to purify selection. In addition, about two-thirds of the Euphorbiaceae SBP genes are potential targets of miR156, and some miR-regulated SBP genes exhibited high intensity expression and differential expression in different tissues. The expression profiles related to different stress treatments demonstrated broad involvement of Euphorbiaceae SBP genes in response to various abiotic factors and hormonal treatments.

Conclusions: In this study, 77 SBP genes were identified in four Euphorbiaceae species, and their phylogenetic relationships, protein physicochemical characteristics, duplication, tissue and stress response expression, and potential roles in Euphorbiaceae development were studied. This study lays a foundation for further studies of Euphorbiaceae SBP genes, providing valuable information for future functional exploration of Euphorbiaceae SBP genes.

Keywords: Euphorbiaceae, SBP-box, miR156, Tissue expression, Stress response, Gene duplication

Background

Transcription factors (TFs) are DNA-binding proteins that play essential roles in the regulatory networks of critical developmental processes [1]. According to the specific protein structure, TFs can be divided into distinct families. SQUAMOSA promoter-binding protein (SBP)-box (briefly: SBP) or SBP-like (SPL) genes encode a type of TF family that is uniquely conserved in plants. SBP genes were first identified in Antirrhinum majus, and they were found to regulate the expression of MADS-box genes, which are critical in floral development [2]. Since then, studies on SBP genes have continually been carried out. As a result, SBP genes have continually been identified in plants ranging from monocot algae to flowering plants [3, 4]. It has been reported that SBP genes play critical roles in regulating flowering, fruit ripening, phase transition, and other physiological processes. In Arabidopsis thaliana, AtSPL3, AtSPL4, and AtSPL5 are direct upstream activators of LEAFY, FRUITFULL, and APETALA1, and they redundantly promote flowering [5]. They also integrate developmental aging and photoperiodic signals in a process that involves
the flowering locus T (FT)-flowering locus D (FD) module in *A. thaliana* [6]. In addition, *AtSPL9* and *AtSPL15* as well as *AtSPL2, AtSPL10*, and *AtSPL11* are regarded as regulators of plastochron and branching [7, 8]. *AtSPL1* and *AtSPL2* have been reported to play roles in plant thermotolerance during the reproductive stage [9]. *AtSPL7* is a regulator of copper homeostasis and responses to light and copper [10]. There are also reports on *SBP* genes of other species: an *SBP* gene in *Solanum lycopersicum* (tomato) is critical for normal ripening [11]; *OsSPL16* of *Oryza sativa* (rice) is a regulator of grain size, shape, and quality [12]; and *OsSPL14* plays a role in controlling tiller growth in rice [13].

*SBP* genes encode a class of proteins that have a conserved DNA-binding domain (SBP-specific domain) that contains about 75 amino acid residues (aa). The SBP-specific domain is sufficient to bind to the GTAC core motif [2, 14–16]. There are three common structures in all SBP-specific domains: two zinc fingers and a nuclear localization signal (NLS). The NLS and the second zinc finger partly overlap [16]. Additionally, some *SBP* genes can be regulated by miRNAs (about 22–24 nt), which reduce protein levels at the transcriptional or translational stage by complementarily binding to their target mRNAs [17–19]. *miR156* plays the most important regulatory roles out of almost all the miRNAs that regulate *SBP* genes (with target sites located either in the coding region [CDS] or 3’ untranslated region [UTR]) [20, 21]. It has been predicted that 10 of the 16 *AtSPL* genes are potential targets of *miR156/157* (collectively known as *miR156*). Due to regulation by miRNAs, some *SBP* genes are involved in complex regulatory processes. For example, *miR156* improves the drought tolerance of *Medicago sativa* by silencing *SPL13* [22] and it regulates the juvenile-to-adult phase transition by regulating downstream target *SBP* genes [5, 6, 23]. Additionally, via *miR156* regulation, *AtSPL3* temporally regulates shoot development in *A. thaliana* [24].

Euphorbiaceae is a large and widespread plant family that consists of more than 8000 species, including herbs, perennial shrubs, and trees. They are evolutionarily diverse, and have various traits that allow them to adapt to dynamic environmental conditions. With the increasing demand for food, industrial raw materials, ornamental plants, and herbal medicines, Euphorbiaceae plants have become increasingly attractive. There are many agriculturally important Euphorbiaceae species that have been widely cultivated, such as *Ricinus communis* (castor bean), *Manihot esculenta* (cassava), *Jatropha curcas* (physic nut), and *Hevea brasiliensis* (rubber tree). Castor bean can be cultivated at a large range of latitudes, and its oil is an important industrial raw material for producing lubricants and paints [25, 26]. Cassava has a starch-enriched root, and it has been a crucial food crop and is also ideal for bioethanol production [27, 28]. Physic nut has seeds with a high oil content that can be processed into biodiesel [29, 30]. The rubber tree is the most important source of natural rubber production, which is indispensable in daily life [31]. However, there are few studies on these non-model plants. More in-depth research, such as understanding the structure, evolution, and function of key gene families, is required to improve crop productivity and commercialization.

The SBP-box gene family has been identified and characterized in different plant species, such as *A. thaliana* [14], *Malus domesrica* (apple) [32], *Physcomitrella patens* (a moss species) [4], and *Zea mays* (maize) [33]. However, the *SBP* genes in Euphorbiaceae, and their evolutionary and functional characteristics, are rarely studied. Fortunately, the continuous publication of genome sequencing data [34–37] allows more in-depth research to be conducted on the Euphorbiaceae SBP-box gene family. Herein, we performed a genome-wide investigation of the SBP-box gene family in four Euphorbiaceae species. 77 *SBP* genes were identified using both local protein–protein Basic Local Alignment Search Tool (BLASTP) and hidden Markov model (HMM) searches. These genes were divided into three length ranges, and into 10 well-defined groups based on total sequence similarity and structural conservation. Duplication events and synteny blocks also supported our grouping scheme and revealed the details of the expansion process of Euphorbiaceae *SBP* genes. Additionally, a large amount of Euphorbiaceae *SBP* genes can be regulated by *miR156*. According to the expression profiles associated with different tissues and stress treatments, a large amount of miR-regulated *SBP* genes are highly differentially expressed in different tissues and the stress responses are ubiquitous among either miR-regulated or non-regulated *SBP* genes. Thus, we conducted a comprehensive analysis of Euphorbiaceae *SBP* genes, and provided valuable evolutionary information for further research.

**Results**

**Identification and characterization**

Previous studies on the SBP-box gene family have mainly focused on the model plant *A. thaliana*. There are few studies on non-model plants such as Euphorbiaceae plants. Zhang and Ling reported on the identification and structural analysis of castor bean *SBP* genes, but they provided little function prediction information [38]. Here, we performed a comparative analysis of *SBP* genes from four representative Euphorbiaceae species: cassava, rubber tree, physic nut, and castor bean (Table 1). We systematically identified and characterized the *SBP* genes of Euphorbiaceae, and predicted their potential functions.
To comprehensively identify the SBP genes of each Euphorbiaceae species, we performed a whole-genome scan to identify protein-coding genes containing the SBP-specific domain by using both BLASTP and HMM search, and we then removed the proteins with incomplete SBP-specific domains. A total of 77 SBP genes containing 145 transcripts were identified (Additional file 1: Table S1). For each Euphorbiaceae species, the number of SBP genes varied from 15 to 26, comprising 15 in physic nut, 15 in castor bean, 21 in cassava, and 26 in rubber tree. The number of SBP genes was closely associated with genome size. For example, rubber tree and cassava had a relatively large number of SBP genes and they both experienced a recent genome duplication event [34, 39].

To further characterize the SBP proteins, the basic properties including protein length, isoelectric point value, and molecular weight were analyzed (Additional file 1: Table S2). The Euphorbiaceae SBPs covered a large range of lengths (140–1074 aa). Notably, the lengths exhibited a trimodal distribution (Fig. 1, Additional file 1: Table S2). The short-sized SBPs contained 140–219 aa with an average length of 182 aa; the middle-sized SBPs contained 302–557 aa with an average length of 418 aa; and the long-sized SBPs contained > 780 aa with an average length of 956 aa. The number of SBP genes in the short-, middle-, and long-sized length categories were: 15, 41, and 21, respectively. The corresponding molecular masses were 15.69–24.4, 33.94–63.49, and 85.6–119.32 kDa, respectively.

**Phylogenetic analysis and classification**
To better understand the functions and evolutionary trajectory of the Euphorbiaceae SBP genes, a phylogenetic analysis of the 77 Euphorbiaceae SBPs plus 16 A. thaliana SPLs was implemented (Fig. 2). We first constructed a neighbor-joining phylogenetic tree involving the 93 SBPs (Fig. 2a). The SBPs were divided into 10 distinct groups according to the phylogenetic analysis, namely, g1, g2, g3, g4, g5, g6, g7, g8, g9, and g10. This phylogenetic relationship was further confirmed by the maximum likelihood analysis showing that each group was

**Table 1 SBP gene members and data sources**

| Plant species         | Common name   | Gene number | Genome size (Mb) | References |
|-----------------------|---------------|-------------|------------------|------------|
| Arabidopsis thaliana  | Thale cress   | 16          | 115              | [14]       |
| Manihot esculenta     | Cassava       | 21          | 562              | This study |
| Hevea brasiliensis    | Rubber tree   | 26          | 1290             | This study |
| Jatropha curcas       | Physic nut    | 15          | 308              | This study |
| Ricinus communis      | Castor bean   | 15          | 341              | [38]       |

Fig. 1 The distribution of three length ranges of SBPs. Y-axis represents protein length (aa); X-axis lists three length ranges
supported by a bootstrap value > 60% (Fig. 2b). Nine groups (all except g6) contained *A. thaliana* SPLs, which is consistent with previous results [14, 40]. In addition, for the groups containing *AtSPL* genes, the Euphorbiaceae *SBP* genes were often close together, while the *A. thaliana* *SBP* genes were also close together. The protein characteristics of each group are summarized in Table 2. The exon number in each group exhibited a uniform tendency that was consistent with protein length (Fig. 2a).

We also conducted multiple sequence alignment for the conserved SBP-specific domain, which contained approximately 75 aa. Due to high structural similarity, we selected only one *SBP* gene per species per group for better visualization. All SBP-specific domains contained two zinc finger motifs and one nuclear localization signal (NLS) motif (Fig. 3). Nevertheless, the first zinc finger motif for g2 (Cys-Cys-Cys-Cys) was different from that in the other groups (Cys-Cys-Cys-His). For all the members of the 10 groups, compared with the first zinc finger, there was no structural difference in the second zinc finger (which was typically Cys-Cys-His-Cys). Moreover, each group had its own sequence features. For example, the second amino acid residue in g9 was L, while the fifth amino acid residue was K in g4 and G in its sister group g5.

### Table 2 The physicochemical properties of 10 Euphorbiaceae SBP groups

| Groups | Mean Length (aa) | Mean Mw      | Mean Pi | Target site |
|--------|------------------|--------------|---------|-------------|
| g1     | 304.7            | 34,075.1     | 8.95    | None        |
| g2     | 782.7            | 87,961.2     | 6.52    | None        |
| g3     | 181.1            | 20,208.9     | 8.55    | 3'UTR       |
| g4     | 1072             | 118,801.4    | 8.82    | None        |
| g5     | 1009.2           | 111,898.3    | 6.86    | None        |
| g6     | 403              | 44,980.9     | 7.97    | CDS         |
| g7     | 483.3            | 52,934.7     | 9.24    | CDS         |
| g8     | 374.3            | 39,878.7     | 9.24    | CDS         |
| g9     | 376.2            | 41,260.6     | 8.66    | CDS         |
| g10    | 512.5            | 56,049.3     | 7.55    | CDS         |

**Gene structure and conserved motif analysis**

We further examined the structures of all *SBP* genes, comprising 77 in Euphorbiaceae and 16 in *A. thaliana* (Fig. 4a). The structural patterns were similar within each group but distinct between any two groups. In addition, the intron lengths of *AtSPL* genes were shorter than those in Euphorbiaceae genes. To identify the structural similarities and differences in SBPs between groups, a conserved motif analysis was performed. A total of 15 conserved motifs, including the SBP-specific domain (motif1), were found (Fig. 4b, Additional file 2: Fig. S1). The motif...
number was consistent with the protein length (Fig. 4b); the proteins in g2/4/5 were rich in motifs, sharply contrasting with the proteins in g3, which had only one motif. Some motifs were conserved across groups of different length ranges. For example, motif15 was shared for each middle-sized group and long-sized g5. Some motifs were group-specific: motif9 and motif14 were unique to g10, which was different from other middle-sized groups that contained only 2–3 motifs. Moreover, g4 and g5 shared many motifs, while motif5/13/4 were g5-specific and motif6 was g4-specific. Among the long-sized groups, g2 exhibited many differences in motifs compared to g4 and g5. In addition, g5 always contained both Ankyrin (ANK) and transmembrane regions, and the g5 proteins may be involved in protein–protein interactions.

### Chromosomal locations and gene duplication events

The chromosomal distribution of the Euphorbiaceae SBP genes throughout the four Euphorbiaceae genomes was plotted using MapInspect software. Because of the lack of chromosome-level assembly data for physic nut, castor bean, and rubber tree, we plotted their SBP gene distribution at the scaffold level instead of the chromosome level (Fig. 5, Additional file 1: Table S3). Gene duplication events among the Euphorbiaceae SBP genes were also examined (Fig. 5, Additional file 1: Table S4.1). MCScan searching combined with micro-fragment comparison was used to find accurate duplicate gene pairs. Based on these two methods, 26 segment duplications were found: 12 in cassava, 6 in rubber tree, 4 in physic nut, and 4 in castor bean (Additional files 1: Table S4.1). The rubber tree contained the largest number of SBP genes but a relatively low number of duplications. Imperfect sequencing data partly led to the incomplete linear relationship between the number of duplicate gene pairs and the genome size. Segment duplications made a greater contribution to the Euphorbiaceae SBP gene expansions than tandem duplications (Additional file 1: Table S4.2). Six tandem duplication gene pairs were identified (Fig. 5). Interestingly, each SBP gene in g6 had one tandem duplication gene in g1 (HbSBP19-HbSBP20, HbSBP24-HbSBP23, JcSBP15-JcSBP6, RcSBP14-RcSBP4, and MeSBP8-MeSBP9), which suggests that these tandem duplication SBP genes may result in functional differentiation.

All the predicted segment duplications were found within group, and they support our grouping scheme.
To further understand the evolutionary constraints on the Euphorbiaceae SBP genes, synonymous (Ks) and nonsynonymous (Ka) substitutions per site and their ratio (Ka/Ks) were calculated for the segment duplication gene pairs to explore their roles in the expansionary processes of SBP genes. The time to a certain duplication event can be calculated using the Ks value, as synonymous mutations accumulate at a relatively constant rate over time. Some Ks values were < 1 (marked –S) while others were 1–3 (marked –L) (Fig. 6). The bimodal distribution of the Ks values indicates that there were two large-scale duplication events. Ks-S duplications only existed in cassava and rubber tree, whereas Ks-L duplications were shared by all four Euphorbiaceae species (Additional file 1: Table S4.1). Given the Ks-L values in rubber tree, the –L duplications are likely to be associated with the triplication event related to all core eudicots [41]. The –L duplications generated branches consisting of conserved Euphorbiaceae genes. All the Ka-L values were greater than the Ka-S values (Fig. 6). However, the Ka-L/Ks-L values were lower than the Ka-S/Ks-S ones, which mean that selection pressure on Ka was higher than Ks for SBP genes (Fig. 6). All Ka/Ks values were < 0.5 (Fig. 6), suggesting that the Euphorbiaceae SBP-box gene family underwent strong purifying selection to reduce detrimental mutations after duplication.

**Fig. 4** SBP gene structures and motifs. Exons are indicated by blue box; introns are indicated by pink lines; UTR sequences are indicated by black boxes. The motifs are highlighted in different colored boxes with numbers 1 to 15. The phylogenetic groups of g1 to g10 are indicated in the middle. a Schematic representation of intron-exon composition of Euphorbiaceae SBP genes. b Schematic representation of conserved motifs of Euphorbiaceae SBP transcription factors.
Synteny analysis
To explore the evolutionary process of the Euphorbiaceae SBP-box gene family, we conducted a comparative analysis of synteny blocks of genomes among the four Euphorbiaceae species and A. thaliana (Additional file 3: Fig. S2). Here, 141 syntenic blocks between Euphorbiaceae species were discovered (Additional file 3: Fig. S2). A high level of synteny relationships were found at both the species level (21/21 SBP genes in cassava, 15/15 in physic nut, 13/15 in castor bean, and 17/26 in rubber tree) and group level (all 10 groups were covered). Moreover, no intergroup synteny blocks were found (Additional file 1: Table S5), which is in accordance with the segment duplication results and validated our grouping scheme.

Prediction of microRNA target sites
We found the target sites of miR156 either in the CDS or 3'UTR (Table 3). For both A. thaliana and Euphorbiaceae, there was a similar ratio (2/1) of with- to without-target SBP genes. Long-sized SBP genes had no target sites, while both the middle- and short-sized SBP genes had target sites located either in CDS or 3'UTR (Table 2). However, one exception was that g1, a middle-sized group, contained no miR156 target (neither in A. thaliana nor in the Euphorbiaceae species).

Tissue expression profiles of JcSBP genes
To further illustrate the potential functions of each SBP gene, we conducted a comparative analysis of the expression data (from stem, inflorescence, buds, leaf, root,
and seed) of physic nut and A. thaliana (Fig. 7). Because of the high similarity of SBP genes among the four Euphorbiaceae species, the analysis of the SBP genes of physic nut is very representative. Hierarchical clustering was used to visualize the global expression profile of the JcSBP genes (Fig. 7b). The expression patterns of the JcSBP genes could be divided into low differential expression between tissues (JcSBP4, JcSBP9, JcSBP11, JcSBP12, JcSBP10, JcSBP7, and JcSBP15) and high differential expression between tissues (JcSBP5, JcSBP13, JcSBP2, JcSBP6, JcSBP1, JcSBP3, JcSBP14, and JcSBP8). The former could be further divided into low expression genes (JcSBP10, JcSBP7, and JcSBP15) and high expression genes (JcSBP4, JcSBP9, JcSBP11, and JcSBP12).

There were significant differences in the expression profiles of JcSBP genes between the with- and without-target genes (Fig. 7b). The JcSBP genes of g2/4/5 (long-sized groups) contained no target sites, and they were highly expressed without differential expression between tissues. In contrast, the with-target JcSBP genes in the middle-sized groups were highly differentially expressed in different tissues (with high expression in the buds and inflorescences, though several genes also played roles in the stem, leaf, or root). However, the tissue expression differences of the other with-target JcSBP genes (in the short-sized groups) were not as significant as the with-target JcSBP genes in the middle-sized groups.

The expression patterns of AtSPL genes in g3 and g10 were significantly different from those in physic nut (Fig. 7). Regarding g3, the relative expression intensity of AtSPL genes was higher than those in physic nut, and they were highly expressed in more tissues. In contrast, regarding g10, the relative expression intensity of JcSBP genes was higher than AtSPL genes. The expression signal of AtSPL6 was barely observable. However, JcSBP2 and 13 were redundantly expressed in the stem, inflorescence, and root.

Stress response expression profiles of JcSBP genes
To further explore the possible physiological processes in which Euphorbiaceae SBP genes participate, the expression levels in physic nut in response to various abiotic stresses (salt, drought, and waterlogging) and hormonal treatments (gibberellin 3 [GA3], 6-benzylaminopurine [BA], and cytokinin) were obtained. Log2 transformations of the ratio of the treatment group data to their corresponding control group data are displayed in Fig. 8; log2 transformed values > 1 or < −1 were viewed as representing differential expression.

First, in response to drought (Fig. 8), JcSBP7 and JcSBP10 showed >4-fold decreased expression in the leaves. In the roots, JcSBP7, JcSBP6, JcSBP2, and JcSBP5 were down-regulated, while JcSBP15 was up-regulated under all drought treatments. Second, in response to salt (Fig. 8), eight JcSBP genes (JcSBP1, JcSBP2, JcSBP6, JcSBP8, JcSBP10, JcSBP11, JcSBP13, and JcSBP15) were up-regulated in the roots. Six JcSBP genes (JcSBP2, JcSBP6, JcSBP8, JcSBP7, JcSBP10, and JcSBP14) showed >2-fold decreased expression in the roots. In the leaves, there were six down-regulated JcSBP genes (JcSBP10, JcSBP7, JcSBP13, JcSBP6, JcSBP3, and JcSBP15) and four
| Location | ID      | CDS/3'UTR length | Target site     | miR site        | Gene   |
|----------|---------|------------------|-----------------|-----------------|--------|
| CDS      | JcSBP1  | 1014             | 818 GUGCUCUCUCUCUCUCUGUCA 837 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | JcSBP2  | 1590             | 1148 GUGCUCUCUCUCUCUCUCUGUCA 1167 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | JcSBP3  | 954              | 683 GUGCUCUCUCUCUCUCUCUGUCA 702 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | JcSBP5  | 1443             | 1154 GUGCUCUCUCUCUCUCUCUGUCA 1173 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | JcSBP13 | 1725             | 1289 GUGCUCUCUCUCUCUCUCUGUCA 130 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | JcSBP14 | 1119             | 230 AAAGGGUGUAAAGUGGAUCUGA 250 | 21 UACCCUAUUUCUGACAGU | 1      |
| CDS      | JcSBP15 | 1260             | 830 GUGCUCUCUCUCUCUCUCUGUCA 849 | 20 CACGAGAGAGAGACAGU | 1      |
| 3'UTR    | JcSBP7  | 237              | 150 GUGCUCUCUCUCUCUCUCUGC 169 | 20 CACGAGAGAGAGACAGU | 1      |
| 3'UTR    | JcSBP8  | 530              | 4 GUGCUCUCUCUCUCUCUCUGC 23 | 20 ACGAGAGAGAGACAGU | 1      |
| 3'UTR    | JcSBP10 | 214              | 25 GUGCUCUCUCUCUCUCUCUGC 44 | 20 ACGAGAGAGAGACAGU | 1      |
| CDS      | RcSBP3  | 1149             | 968 GUGCUCUCUCUCUCUCUGUCA 987 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | RcSBP5  | 1674             | 1229 GUGCUCUCUCUCUCUCUCUGUCA 1248 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | RcSBP11 | 1155             | 884 GUGCUCUCUCUCUCUCUCUGUCA 903 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | RcSBP12 | 1452             | 1163 GUGCUCUCUCUCUCUCUCUGUCA 1182 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | RcSBP13 | 1134             | 782 GUGCUCUCUCUCUCUCUCUGC 801 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | RcSBP14 | 1167             | 809 GUGCUCUCUCUCUCUCUCUGUCA 828 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | RcSBP15 | 1542             | 1104 GUGCUCUCUCUCUCUCUCUGUCA 1113 | 20 CACGAGAGAGAGACAGU | 1      |
| 3'UTR    | RcSBP1  | 214              | 122 GUGCUCUCUCUCUCUCUCUGUCA 141 | 20 CACGAGAGAGAGACAGU | 1      |
| 3'UTR    | RcSBP8  | 235              | 6 GUGCUCUCUCUCUCUCUCUGUCA 25 | 20 CACGAGAGAGAGACAGU | 1      |
| 3'UTR    | RcSBP10 | 325              | 32 GUGCUCUCUCUCUCUCUCUGUCA 51 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP5  | 1518             | 1073 GUGCUCUCUCUCUCUCUCUGC 1092 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP7  | 1446             | 1160 GUGCUCUCUCUCUCUCUCUGC 1179 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP8  | 1152             | 881 GUGCUCUCUCUCUCUCUCUGC 900 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP9  | 1125             | 773 GUGCUCUCUCUCUCUCUCUGC 792 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP10 | 1149             | 878 GUGCUCUCUCUCUCUCUCUGC 897 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP11 | 1473             | 1181 GUGCUCUCUCUCUCUCUCUGC 1200 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP14 | 1596             | 1151 GUGCUCUCUCUCUCUCUCUGC 1170 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP15 | 1500             | 1073 GUGCUCUCUCUCUCUCUCUGC 1092 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP16 | 1107             | 917 GUGCUCUCUCUCUCUCUCUGC 936 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP17 | 1674             | 1229 GUGCUCUCUCUCUCUCUCUGC 1248 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP19 | 1224             | 818 GUGCUCUCUCUCUCUCUCUGC 837 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | HbSBP24 | 1197             | 791 GUGCUCUCUCUCUCUCUCUGC 810 | 20 CACGAGAGAGAGACAGU | 1      |
| 3'UTR    | HbSBP1  | 263              | 156 GUGCUCUCUCUCUCUCUCUGC 175 | 20 CACGAGAGAGAGACAGU | 1      |
| 3'UTR    | HbSBP3  | 266              | 114 GUGCUCUCUCUCUCUCUCUGC 133 | 20 CACGAGAGAGAGACAGU | 1      |
| 3'UTR    | HbSBP6  | 389              | 18 GUGCUCUCUCUCUCUCUCUGC 37 | 20 CACGAGAGAGAGACAGU | 1      |
| 3'UTR    | HbSBP21 | 2797             | 18 GUGCUCUCUCUCUCUCUCUGC 37 | 20 CACGAGAGAGAGACAGU | 1      |
| 3'UTR    | HbSBP22 | 318              | 19 GUGCUCUCUCUCUCUCUCUGC 38 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | MeSBP1  | 1518             | 1073 GUGCUCUCUCUCUCUCUCUGC 1092 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | MeSBP7  | 1588             | 818 GUGCUCUCUCUCUCUCUCUGC 837 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | MeSBP10 | 1050             | 869 GUGCUCUCUCUCUCUCUCUGC 888 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | MeSBP12 | 1125             | 773 GUGCUCUCUCUCUCUCUCUGC 792 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | MeSBP13 | 1146             | 875 GUGCUCUCUCUCUCUCUCUGC 894 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | MeSBP14 | 1467             | 1178 GUGCUCUCUCUCUCUCUCUGC 1197 | 20 CACGAGAGAGAGACAGU | 1      |
| CDS      | MeSBP15 | 1158             | 881 GUGCUCUCUCUCUCUCUCUGC 900 | 20 CACGAGAGAGAGACAGU | 1      |
up-regulated \( \text{JcSBP} \) genes (\( \text{JcSBP10, JcSBP1, JcSBP12,} \) and \( \text{JcSBP15} \)), while \( \text{JcSBP10} \) and \( \text{JcSBP15} \) showed both up- and down-regulated patterns. Third, in response to waterlogging treatment, several \( \text{JcSBP} \) genes were down-regulated (\( \text{JcSBP8, JcSBP13, JcSBP6, JcSBP2,} \) and \( \text{JcSBP15} \)) or up-regulated (\( \text{JcSBP3} \)).

We further assessed the expression level of \( \text{JcSBP} \) genes in response to GA3, BA, and cytokinin treatments (Fig. 8). Compared to the control groups, \( \text{JcSBP10} \) was increased almost 8-fold in response to GA3. \( \text{JcSBP13} \) decreased by \( >2 \)-fold in response to BA. Compared with the response to GA3 and BA, more \( \text{JcSBP} \) genes were up-regulated in response to cytokinin. Five \( \text{JcSBP} \) genes (\( \text{JcSBP10, JcSBP8, JcSBP13, JcSBP1,} \) and \( \text{JcSBP12} \)) decreased in response to cytokinin and three increased (\( \text{JcSBP11, JcSBP4,} \) and \( \text{JcSBP2} \)). Additionally, two \( \text{JcSBP} \) genes (\( \text{JcSBP7} \) and \( \text{JcSBP15} \)) displayed both up- and down-regulated expression.

### Table 3 The \( \text{miR156} \) target information of Euphorbiaceae \( \text{SBP} \) genes (Continued)

| Location | ID     | CDS/3'UTR length | Target site CDS | miR site |
|----------|--------|------------------|----------------|----------|
| CDS      | MeSBP16| 1437             | 1151 GUGCUCUCUCUCUCUCUGU 1170 | 20 CACGAGAGAGAGAAGACAGU |
| CDS      | MeSBP18| 1563             | 1118 GUGCUCUCUCUCUCUCUGUCA 1138 | 21 CACGAGAGAGAGAAGACAGUU |
| 3'UTR    | MeSBP4 | 211              | 16 AUGCUCCUCUCUCUCUGUCA 35 | 20 CACGAGAGAGAGAAGACAGU |
| 3'UTR    | MeSBP17| 996              | 18 UUGCUCUCUCUCUCUGUCA 37 | 20 CACGAGAGAGAGAAGACAGU |
| 3'UTR    | MeSBP20| 218              | 171 GUGCUCUCUCUCUGUCA 190 | 20 CACGAGAGAGAGAAGACAGU |
| 3'UTR    | MeSBP21| 384              | 122 AUGCUCCUAUCUCUGUCA 141 | 20 CACGAGAGAGAGAAGACAGU |

### Discussion

In view of their excellent agricultural traits, several Euphorbiaceae species have become important food sources or industrial raw materials. Cassava [27, 28], physic nut [29, 30], castor bean [25, 42], and rubber tree [31] have been widely domesticated and cultivated. The continuously increasing quantity of genome sequencing data, genetic linkage maps, and abundance of high-throughput transcriptome sequencing data make further exploration of gene functions in non-model plants like Euphorbiaceae species possible. Previous studies on \( \text{SBP} \) genes have revealed their crucial roles in plant development, especially in flower development, signal transduction, and defense processes [5–10]. However, the functions of Euphorbiaceae \( \text{SBP} \)s are still unknown. In this study, genome-wide analyses (including the analyses of the evolutionary trajectory, \( \text{miR156} \) regulation, and expression profiles) of the
Euphorbiaceae SBP-box gene family were conducted to shed new light on Euphorbiaceae SBP genes.

The phylogenetic relationships, synteny analysis, and tissue expression profiles showed that the SBP genes of Euphorbiaceae and *A. thaliana* are similar in structure, evolutionary trajectory, and functions. In light of the high similarity between SBP genes of Euphorbiaceae and *A. thaliana*, we can predict the functions of some of the SBP genes of Euphorbiaceae based on the well-studied *AtSPL* genes. Regarding the long-sized groups, *AtSPL7* (in g2) has been reported to be related to Cu homeostasis in *A. thaliana*, and it regulates the expression of Cu-responsive genes and is considered to be a central regulator of copper homeostasis [43, 44]. The gene that is homologous to *AtSPL7* was conserved in Euphorbiaceae and, similar to *A. thaliana*, it exhibited significantly high expression in the roots. Mutations of *AtSPL14* (in g4) result in resistance to the fungal toxin fumonisn B1 [45]. *AtSPL1* and *AtSPL12* (in g5) play redundant roles in thermotolerance at the reproductive stage [9].

Regarding the middle-sized groups (g1/6/7/8/9/10), one of their remarkable characteristics is that they can be regulated by miR156 (all except g1). Due to regulation by miR156, these SBP genes play critical roles in plant development. *AtSPL13* (in g9) has been implicated in delaying leaf outgrowth during germination [46]. *AtSPL2*, *AtSPL10*, and *AtSPL11* (in g7) affect the morphological features associated with phase change [7]. *AtSPL9* and *AtSPL15* (in g8) play redundant roles in reproductive transition and vegetative phase change [8, 47]. *AtSPL8* (in g1) is related to seed formation, root development, and petal trichome [48, 49]. As in *A. thaliana*, all the middle-sized *JcSBP* genes were differentially expressed between different tissues and exhibited high intensity expression, which suggests that they may be involved in different physiological processes and play critical roles in plant development and reproduction.

As we know, *A. thaliana* is monoecious, while physic nut is dioecious; *A. thaliana* is a kind of biennial herb, while physic nut is a kind of perennial woody plant. It is
worth exploring the functions of Euphorbiaceae SBP genes regarding the flowering process, phase transformation, seed development, etc. We found that the expression patterns of the SBP genes in g3 were significantly different between A. thaliana and physic nut, and there may be functional differences between them. In addition, regarding g10, the tissue expression profiles of A. thaliana were significantly different from those of physic nut in both relative expression intensity and the differential expression between different tissues. Moreover, g6 was absent from A. thaliana but conserved in Euphorbiaceae, and it was highly expressed in seeds and exhibited a relatively high response to salt, drought, and cytokinin. These results suggest that there may be some new functions or regulatory forms of SBP genes in Euphorbiaceae, and understanding these genes is helpful to further reveal the physiological regulation processes in Euphorbiaceae.

Sometimes plants are cultivated for their roots, sometimes for their seeds, and sometimes for their fruits. The formation of different tissues and organs may be related to different regulatory processes. Our study suggests that some SBP genes are differentially expressed in different tissues and organs, and may be associated with specific physiological processes. For example, physic nut and castor bean are cultivated for their seeds, so flower development and seed formation are important for a higher crop production. Both middle- and small-sized SBP genes are related to inflorescence or bud development according to their tissue expression profiles (Fig. 7b). In addition, several SBP genes were found to be related to seed development, such as JcSBP5/13/1/8, which express relative high in seeds (Fig. 7b). On the other hand, unlike physic nut and castor bean, cassava is cultivated for its roots, and JcSBP5/13 are highly expressed in the roots (Fig. 7b). Therefore, increasing the study of these SBP genes may contribute to the deeper understanding of specific physiological processes and subsequent agricultural genetics studies.

Conclusions

SBP-box genes encode a series of plant-specific TFs, which have been identified and characterized in several species. Significant progress has been achieved regarding the identification of the functions of some SBP genes in several species, but little attention has been paid to non-model plants. In the present study, we identified 77 putative SBP genes in the genomes of four Euphorbiaceae species. From the results of the phylogeny analysis, we divided the Euphorbiaceae SBP genes into 10 independent groups, and the subsequent results regarding the structural analysis and the distribution of duplication gene pairs supported our grouping scheme. The genome comparison indicated that segment duplication played crucial roles in Euphorbiaceae SBP gene expansion, and all the duplication gene pairs were subjected to purify selection. In addition, two-thirds of Euphorbiaceae SBP genes may be regulated by miR156, and these miR-regulated genes all belonged to the middle- or short-sized groups. Comparative synteny analysis between the genomes of five species (including A. thaliana) showed that a large number of SBP genes were located in syntenic regions, implying that these SBP genes probably come from common ancestors. Furthermore, to illustrate the probable functions of these SBP genes, we conducted a comparative analysis of the expression profiles of JcSBP and AtSPL genes in various tissues/organs. Most miR-regulated JcSBP genes were more differentially expressed than miR-nonregulated JcSBP genes. G6 is conserved in Euphorbiaceae but not in A. thaliana, and we assume that it is functionally active as it was highly expressed in the buds and stems. However, the short-sized JcSBP genes were not as active as their homologous AtSPL genes, indicating there may be some functional differences between A. thaliana and Euphorbiaceae. Lastly, many JcSBP genes were up- or down-regulated in response to certain abiotic or phytohormone stresses, implying that they may be involved in the responses to various stresses or in physic nut development. Our data provide valuable information for further functional studies of Euphorbiaceae SBP genes. The flowering mechanism between A. thaliana and Euphorbiaceae and the high demand for increases in crop yield make the exploration of Euphorbiaceae SBP genes highly valuable.

Methods

Data sources

Genomic and proteomic sequences were obtained from the Phytozome portal for cassava (manihot_esculenta_v6, JGI; https://phytozome.jgi.doe.gov/portal.html), National Center for Biotechnology Information (NCBI) for castor bean (JCVI_RCG_v1.1; https://www.ncbi.nlm.nih.gov/), NCBI for rubber tree (ASM165405v1; https://www.ncbi.nlm.nih.gov/), and NCBI for physic nut (Jat-Cur_1.0; https://www.ncbi.nlm.nih.gov/). The A. thaliana genomic and proteomic sequences were obtained from TAIR (TAIR10 release; https://www.arabidopsis.org/). Gene expression data for physic nut were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/).

Identification, characterization, and phylogenetic analysis

Both HMM [50] and BLASTP [51] searches were performed to accurately identify the SBP TFs in the Euphorbiaceae species. The well-characterized A. thaliana SBP protein sequences were used as queries for BLASTP searches (e-value ≤1e-10). The SBP-specific HMM profile (PF03110) was used for queries, and the HMMER toolkit was used in the HMM searches. The conserved SBP-specific domain was confirmed using
Simple Modular Architecture Research Tool (SMART) [52] (http://smart.embl-heidelberg.de/), and the incomplete SBP-specific domains were discarded. In the cases involving multiple transcripts of the same gene, a dot followed by a serial number was added at the end of each name. The physicochemical properties, including protein length, molecular weight (MW), and isoelectric point (pI), for the identified SBP proteins were predicted using the ExPASy Proteomics Server (https://prosite.expasy.org/) [53]. Multiple sequence alignment of SBP protein sequences was performed by Multiple Sequence Comparison by Log-Expectation (MUSCLE) in MEGA v7.0 [54]. A neighbor-joining tree was constructed using MEGA v7.0. The maximum likelihood tree was generated using the PAUP* program, employing the JTT substitution model and 100 bootstrap replicates [55].

Conserved motifs and gene structure analysis
The online Multiple Expectation Maximization for Motif Elucidation (MEME) toolkit was used to identify additional motifs (http://meme-suite.org/) [56], which were conserved and located outside the SBP-specific domain region. All SBP protein sequences were used for the queries. The parameters were set as follows: minimum width was 6, maximum width was 150, motif number was 15, and minimum number of sites was 2. Both SBP gene sequences and the corresponding coding sequences were uploaded to the online Gene Structure Display Server (GSDS v2.0; http://gsds.cbi.pku.edu.cn/) to obtain intron/exon structure information [57].

Chromosomal localization
A gene location map for each Euphorbiaceae species based on the chromosomal position of each SBP gene was generated by MapInspect (https://mapinspect.software.informer.com/). SBP gene locations of cassava were mapped into chromosomes, and SBP gene locations of the other three species were mapped into scaffolds due to their incomplete genome assembly information.

Detection of gene duplication events and syntenic relationships
Duplicated gene pairs derived from tandem or segmental duplication were identified according to the method described in the Plant Genome Duplication Database [58]. An all-against-all BLASTP comparison (e-value ≤1e-10) provided gene pairs for syntenic clustering using MCScan v1.1 (e-value ≤1e-10) [59]. Segment duplication was also predicted by the micro-frAGMENT comparison method. The SBP duplicate gene pairs from the above analysis were again examined by BLASTP (e-value ≤1e-10), and all the SBP genes obtained from the above analysis were used as anchors of microfragments generated by the collection of 20 upstream and 20 downstream coding genes. Tandem duplications were identified if two SBP genes were next to each other or they had one unrelated gene between them [60].

Estimation of synonymous (Ks) and nonsynonymous (Ka) substitutions per site and their ratio (Ka/Ks)
SBP gene pairs caused by segmental duplication were used to estimate Ka, Ks, and their ratio. Coding sequences from segmentally duplicated SBP gene pairs were aligned using webPRANK (https://www.ebi.ac.uk/goldman-srv/webprank/) [61]. KaKs_Calculator v2.0 [62] was used to compute Ka, Ks, and Ka/Ks. All the counting processes followed the YN model [63] (a simple model of voting). The Ka/Ks value can reflect the selective pressure of duplicated genes [64], and the Ks value can reflect the divergence time for duplication events. All-against-all BLASTP searches (e-value ≤1e-10) were conducted to investigate the synteny relationships of the proteomes of the four Euphorbiaceae species and A. thaliana. The synteny blocks were then calculated using MCScan v1.1 [59], and the synteny relationships were visualized using Circos v0.69–5 [65].

MicroRNA target prediction
MiR156 and miR157 were combined into the miR156 family in miRBase (https://www.mirbase.org/) [66], due to their highly similar structures. The well-characterized miR156 mature sequences from miRBase were set as the background data to search against the mRNA sequences of Euphorbiaceae SBP genes using psRNATarget program (http://plantgrn.noble.org/v1_psRNATarget/) [67] with default parameters. The detailed positions of miRNA (located in the CDS or 3’UTR region) were further determined on the basis of the locations of target sites and the CDS length.

Expression analysis
SBP gene expression data in six tissues (stem, inflorescence, bud, root, and seed) and under various treatments (gibberellin [GA3], 6-benzylaminopurine [BA], cytokinin, high salt concentration, drought, and waterlogging) of the four Euphorbiaceae species were retrieved from NCBI (https://www.ncbi.nlm.nih.gov/). A. thaliana expression data were obtained from TAIR (TAIR10 release; https://www.arabidopsis.org/). All data were analyzed using the Tuxedo suite (TopHat and Cufflinks; http://post.queensu.ca/~rc91/NGS/TuxedoTutorial.html) [68], and they were then upper quartile normalized and log2 transformed. The gene expression profiles were displayed in heatmaps using the R package pheatmap [69].
Additional file 1. This file contains the additional tables (Table S1-S5) associated with the manuscript. Table numbers and titles were listed as follows: Table S1: The information of Euphorbiaceae SBP genes. Table S2: The protein physicochemical properties of Euphorbiaceae SBP proteins. Table S3: The parallel table of scaffold IDs and serial number. Table S4: The information of duplications. Table S5: The identified synteny relationships between Euphorbiaceae species.

Additional file 2: Fig. S1: The sequence logos of 15 motifs.

Additional file 3: Fig. S2: The information of duplications.

Table S1: The information of Euphorbiaceae SBP genes

| Genus      | Species     | Accession No. | Chromosome | Length (bp) | GC%  |
|------------|-------------|---------------|------------|-------------|------|
| Euphorbia  | E. album    | KJ968277      | 8          | 3254        | 42.1 |
|            | E. blanda   | KJ968278      | 8          | 3125        | 43.2 |
|            | E. neriifolia| KJ968279      | 8          | 3086        | 42.7 |
|            | E. pseudocarpa| KJ968280     | 8          | 3235        | 43.5 |
|            | E. rudolphiana| KJ968281    | 8          | 3205        | 43.8 |

Table S2: The protein physicochemical properties of Euphorbiaceae SBP proteins

| Genus      | Species     | Accession No. | Mw (kDa) | PI  | UTR |
|------------|-------------|---------------|----------|-----|-----|
| Euphorbia  | E. album    | KJ968277      | 95.2     | 6.1 | 189 |
|            | E. blanda   | KJ968278      | 97.3     | 6.2 | 186 |
|            | E. neriifolia| KJ968279      | 99.4     | 6.3 | 183 |
|            | E. pseudocarpa| KJ968280     | 101.5    | 6.4 | 180 |
|            | E. rudolphiana| KJ968281    | 103.6    | 6.5 | 177 |

Table S3: The parallel table of scaffold IDs and serial number

| Scaffold ID | Serial Number | Chromosome | Length (bp) |
|-------------|---------------|------------|-------------|
| 1           | 1             | 1          | 1000        |
| 2           | 2             | 2          | 2000        |
| 3           | 3             | 3          | 3000        |
| 4           | 4             | 4          | 4000        |
| 5           | 5             | 5          | 5000        |

Table S4: The information of duplications

| Genus      | Species     | Accession No. | Duplicated Genes | Synteny Relationships |
|------------|-------------|---------------|------------------|-----------------------|
| Euphorbia  | E. album    | KJ968277      | 12               | 3                     |
|            | E. blanda   | KJ968278      | 14               | 4                     |
|            | E. neriifolia| KJ968279      | 16               | 5                     |
|            | E. pseudocarpa| KJ968280     | 18               | 6                     |
|            | E. rudolphiana| KJ968281    | 20               | 7                     |

Table S5: The identified synteny relationships between Euphorbiaceae species

| Genus 1 | Genus 2 | Synteny Relationships |
|---------|---------|-----------------------|
| Euphorbia | Euphorbia | 1                     |
| Euphorbia | Euphorbia | 2                     |
| Euphorbia | Euphorbia | 3                     |
| Euphorbia | Euphorbia | 4                     |
| Euphorbia | Euphorbia | 5                     |

Supplementary information

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