Genome-wide analysis of structures and characteristics of the SPL gene family in wheat (Triticum aestivum L.)

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Abstract

Background: The SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) genes encode a family of plant-specific transcription factors that contain a conservative SBP domain. SPL proteins play important roles in plant growth and development, such as plant architecture, flowering regulation, and grain yield. However, the systematic analysis of TaSPL gene family in wheat is lacking.

Results: In this study, 56 TaSPL genes were identified from wheat genome and divided into eight groups (G1-G8), according to the phylogenetic analysis of TaSPL proteins among numbers of plant species. Bioinformatics method were applied to analyse the gene structure, motif, chromosome localization, segmental duplication and synteny of total TaSPL genes and the results showed that their characteristics were different among group in the exon-intron constitution, conserved and specific motif. The expansion and evolution of the TaSPL genes occurred within the wheat genome. Total 28 of 56 TaSPL genes were predicted to be putative targets for miR156, which revealed the importance of miR156-mediated regulation in wheat. Moreover, transcript level analysis of TaSPL genes in wheat tissues by qRT-PCR discovered the diversified spatiotemporal expression patterns, based on the comparison with reference RNA-seq data. Some TaSPL genes were subject to various stress treatments including drought and hormones, etc. suggesting that these part genes probably involved in responding to hormone signals during different wheat development stages.

Conclusions: Our findings show that TaSPL genes may regulate the development of spike and grain, resistance to abiotic stresses, and involve in responding to hormone signals. These results could provide a fundamentally information to further study of the functions of TaSPL genes in wheat growth and development.

Background

Transcriptional regulations of genes are the one of most important mechanisms for regulating gene expression [1]. Transcriptional control relies largely on transcription factors (TFs), which are commonly defined as proteins with sequence-specific DNA binding domain that activate or inhibit transcription of target genes [2]. Typical structures of TFs include DNA-binding, transcription regulatory and oligomerization domains, and nuclear localization signal (NLS) [3]. SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) proteins are a plant-specific transcription factor family and have a highly conserved SBP domain, with approximately 78 amino acid residues [4, 5]. This domain contains a novel zinc finger motif with two Zn$^{2+}$ binding sites: Cys-Cys-His-Cys (CCHC) and Cys-Cys-Cys-His (CCCH) and a nuclear localization signal [4]. This domain can specifically bind to the cis-element TNCGTACAA for the target gene promoter region [6, 7].

According to the binding activity of SBP1 and SBP2 with cis-element upstream of the gene SQUAMOSA, AmSBP1 and AmSBP2 were first found to be involved in early flowering in Antirrhinum majus L. in vitro. [8]. Since then, SPL genes have been extensively identified and functionally studied in different plant
species, such as 17 AtSPL genes in Arabidopsis thaliana (L.) Heynh., 19 OsSPL genes in rice (Oryza sativa L.), 31 ZmSPL genes in maize (Zea mays L.), 15 SISPL genes in tomato (Solanum lycopersicum L.), 27 MdSPL genes in apple (Malus × domestica Borkh.), 18 VvSPL genes in grape (Vitis vinifera L.) and 15 SmSPL genes in Salvia miltiorrhiza were predicted [4, 9–14].

However, these SPL genes showed significant difference in gene size and structure, so that there are significant differences in their functions. Previous studies have shown that SPL genes exhibit functional diversity in different plant species, including shoot architecture [15], axillary bud formation [16], plant architecture [17–20], male fertility [21], flowering regulation [7, 22–25], inflorescence branching [24, 26], organ size [27–30] and grain yield [17, 27]. In Arabidopsis, AtSPL3 has been first identified gene and involved in the floral transition [7]. AtSPL14 was involved in regulating plant development and sensitivity to fumonisin B1 [31]. AtSPL15 and SOC1 form a complex and cooperate to activate target gene transcription and control flowering signal at the Arabidopsis shoot meristem [32]. In rice, OsSPL13 determined plant architecture by controlling the number of panicle branches and tillers [19]. In maize, liguleless1 (lg1) gene regulated ligule and auricle formation [34]. Besides, miR156-targeted SPL genes with distinct expression in developing flowers and ovaries has been shown to regulate the development of ovary and fruit in tomato [35]. Such as, miR156a-targeted SISPL13 with the suppressed expression level could increase inflorescence and lateral branches and decrease fruit number, size and yield [36].

The SPL genes also exerted considerable influence in biotic and abiotic stresses. Overexpression of OsSPL9 induced Cu accumulation in rice grains [37]. FBR6/SPL14, as a transcriptional regulator, was considered to contribute not only to the development of plant architecture but also to FB1 sensitivity [31]. Recent studies showed that IPA1 was involved in the regulation of resistance to blast in rice [38]. Ectopic expression of BpSPL9 increased the ROS (Reactive Oxygen Species) scavenging ability under salt and drought stresses in Betula platyphylla Suk. [39]. MiRNA156-targeted SPL13 increased drought stress tolerance in alfalfa (Medicago sativa L.) [40]. Interestingly, the functions of partial SPL genes were regulated by miRNA156 [16, 23, 35, 36].

MiRNAs are the classes of 20 to 22 nucleotide RNAs as vital regulators of gene expression [41]. As negative regulators, miRNAs could repress target gene expression through post-transcriptional degradation and translational repression and play indispensable roles in diverse stress responses [42, 43]. MicroRNA156, as previously reported, was involved in a variety signalling pathways by inhibiting SPL gene family members [16, 19, 23, 36, 44]. In Arabidopsis, 11 of 17 SPL genes were predicted to be miR156/miR157 targets [23]. There were target sites of miR156 on AtSPL3/AtSPL4/AtSPL5 genes, which could promote shoot development and prevent early flowering [23, 45, 46]. The 11 of 19 OsSPL genes in rice were regulated by miR156 [12]. OsSPL14 were regulated by OsmiR156 in vivo, while OsSPL14 mutant inhibited the OsmiR156-directed regulation, generating an ideal plant architecture in rice plants [19]. OsSPL16 also was the one of targets of miRNA156 involved in the regulation of grain shape, size and quality [30]. Similarly, 7 out of the 17 SPL genes had targets for miR156 in tomato [35, 47]. Subsequently,
miR156-targeted GmSPL9d controlled axillary bud formation and branching development in soybean [Glycine max (L.) Merr.][15]. In switchgrass (Panicum virgatum L.), a dedicated bioenergy crop, miR156 could down-regulated SPL4 gene expression and regulated axillary bud formation, and miR156-targeted SPL7 and SPL8 regulated flowering [16, 25]. Importantly, TaSPL17, a target gene of miR156, was also found in wheat, but the location and function of miR156 are not clear [48].

The allohexaploid common bread wheat genome was very large approximately 17 G in size and consisted of three sub-genomes (A, B, and D) originating from the fusion of genomes from diploid ancestral species [49, 50]. The establishment of the International Wheat Genome Sequencing Consortium (IWGSC) provided reference sequence for the acquisition of the TaSPL genes sequence, and provided the possibility for the identification and functional research of TaSPL gene family in wheat [51]. Until now, there are only few reports about the role that TaSPL genes may play in wheat. For example, TaSPL16 controlled precocious flowering and yield-related traits in Arabidopsis [52]. Expression of TaSPL20 and TaSPL21 regulated panicle branch and seed yields in rice [53]. Expression TaSPL3 and TaSPL6 regulated flowering and biomass accumulation in Arabidopsis [54]. Besides above-mentioned, TaSPL8 modulated the leaf cushion development of wheat by regulating the signalling pathways of auxin and brassinosteroid [55].

In our present studies, 56 TaSPL genes were identified in wheat. Subsequently, further analysis including phylogenetic construction, chromosomal location, duplication event, synteny analysis, gene structure, conserved motif, prediction of miR156 recognition site and expression pattern under various stresses were applied by using various databases. Moreover, quantitative real-time PCR (qRT-PCR) method was used to examine the expression patterns of the eight TaSPL genes in various tissues and organs, and to suggest the expression patterns of these genes in wheat. These genes treated with various treatments, including drought, polyethylene glycol (PEG), NaCl, cold, and six hormones such as Abscisic acid (ABA), Auxin (IAA), Gibberellin (GA), Strigolactones (GR24), Methyl jasmonate (MeJA), and Brassinolide (BR).

### Results

#### Identification of 56 TaSPL genes in wheat

Programs of HMMER, SMART and Interpro were adopted to identify TaSPL genes in wheat genome [56-58]. After removing the redundant sequences, total 56 presumed SBP-box genes with E-values less than 0.01 found in the wheat genome. Meanwhile, 18 AetSPL, 10 TuSPL, and 16 BdSPL genes in Aegilops tauschii Coss., Triticum urartu, and Brachypodium distachyon were respectively identified and named with reference to the OsSPL genes. The relevant information including gene name, gene accession number, amino acid sequence and amino acid length was provided in Additional file 1: Table S1. Program of ExPASy server was used to calculate the physical parameters of each TaSPL protein. As shown in Additional file 2: Table S2, Additional file 3: Table S3 and Additional file 4: Table S4, the cDNA length of TaSPL genes ranged from 1,062 (TaSPL-7-B) to 4,510 bp (TaSPL-1-B), the full-length coding sequences (CDS) ranged from 579 (TaSPL-13-A, -B, -D) to 3,390 bp (TaSPL-15-D), and the protein sequence included
192-1,129 amino acid residues. The isoelectric point ranged from 5.54 to 9.90, with the range of molecular weight, 20.11-123.69. **Phylogenetic analysis of TaSPL genes** To examine the phylogenetic relationship among SPL genes of plant species, and 136 putative SPL proteins from six species were selected, including 56 from the wheat, 18 from *Ae. tauschii*, 10 from *T. urartu*, 16 from *B. distachyon*, 19 from rice, and remaining 17 from *Arabidopsis*. The full length protein sequences were used for phylogenetic analysis using the neighbour-joining (N-J) method by MEGA X [59]. The 136 SPL proteins were aggregated into eight distinct groups (G1-G8) (Fig. 1). TaSPL proteins were distributed in all eight groups, containing 3, 9, 3, 17, 6, 3, 6 and 9 proteins, respectively. Groups 3-8 included short TaSPL proteins with no more than 473 (TaSPL-3-B, -3-D) amino acid residues, whereas the members of group 1 and 2 were longer and vary from 822 (TaSPL-1-D) to 1,129 (TaSPL-15-B, -15-D) amino acid residues (Additional file 2: Table S2). According to phylogenetic tree analysis, some TaSPL proteins clustered closer to *Ae. tauschii*, *T. urartu*, *B. distachyon* and rice than *Arabidopsis*, indicating the majority of wheat TaSPL genes were more closely related to monocots. Thus, although the plant SBP-box genes may have originated from a common ancestor, it appears to be a unique pattern of differentiation among many family members after the separation of each lineage. Taken together, our results suggest that the TaSPL genes may have profound evolutionary origin and diversity of biological functions. **Sequence alignments and SBP domain of TaSPLs in wheat** The full length protein sequences of 19 OsSPL and 56 TaSPL proteins were used for multiple sequence alignment, and the ClustalW2.0 software was selected to determine the domain structures of the TaSPL proteins in detail [60]. The results showed that there was only one very conservative SBP domain, containing approximately 78 amino acid residues, found to be shared by all TaSPL and OsSPL proteins (Additional file 5: Figure S1). Sequence alignments of the SBP domain showed that zinc-binding sites, zinc finger 1 and zinc finger 2, located in SBP domain of all TaSPL proteins. A conservative NLS appeared at the C-terminus of the SBP domain. The zinc finger 2 partially overlapped with the NLS (Additional file 5: Figure S1a), as previously reported [5]. For 56 TaSPL proteins, the type of zinc finger 1 in the SBP domain was Cys-Cys-Cys-His (CCCH), except for TaSPL-9-A, -9-B and -9-D with Cys-Cys-Cys-Cys (CCCC), and the zinc finger 2 of all the proteins is Cys-Cys-His-Cys (CCHC) (Additional file 5: Figure S1b). **Analysis of gene structure and conservative motif** For gene structure, number and distribution of introns and exons were performed in 56 TaSPL genes (Fig. 2a). The results suggested that introns were existed in 56 TaSPL genes and the number of exons varies from 2 (TaSPL-13-A, -B, -D) to 12 (TaSPL-6-D). Most TaSPL genes harboured a similar gene structure in the same group. Such as, genes for each group (1, 3, 4, 5, 6, 7 and 8) were made up of equal number of introns or exons but with different intron or exon lengths, whereas those within most of group 2 consisted of 11 exons, except that TaSPL-15 contained only 10 exons, and TaSPL-6-D had 12 exons.

The conservative motifs among the TaSPL proteins were examined using MEME motif program. Ten motifs were identified, and the distributions and sequences of these motifs in TaSPL proteins were showed in Fig. 2b-c. Motif 1 and motif 2, which together constituted the SBP-domain, were found in all TaSPL proteins. Protein sequence alignment (Additional file 5: Figure S1b) supported the result, which
showed that the SBP domain was highly conserved in SPL proteins, consistent with previous reports [12]. In addition to the conservative SBP domain, other motifs could also play important role in the functions of *TaSPL* genes [12]. For example, motif 6 was encoded by a conservative sequence that was complementary to the mature sequence of miR156 (Fig. 2c). Some motifs (5, 6, 7, 8, 9 and 10) were present in most TaSPL proteins, while motifs (3 and 4) only were present in fewer TaSPL proteins, and these motifs in gene functions need further study.

**Chromosome localization, segmentally duplication and syntenic analysis**

Corresponding maps were constructed for the chromosomal localization of 56 *TaSPL* genes (Fig. 3). A total of 56 *TaSPL* genes were distributed on 19 wheat chromosomes except for 4B and 4D, which 19, 18 and 19 *TaSPL* genes were located in the A, B and D sub-genomes, respectively. The wheat chromosome 7 was the most abundant, containing 23 of the 56 *TaSPL* genes, followed by chromosome 6. By contrast, only one *TaSPL* gene was found on chromosome 4A, which contained the minimum number of *TaSPL* genes (Fig. 3a). The relative positions of the 56 *TaSPL* genes on the chromosomes were shown in Fig. 3b. Notably, some genes were tightly distributed across wheat chromosomes, for example, *TaSPL*22-A and 21-A, *TaSPL*20-A and 10-A genes. Within this type of genes, the gene structures and motifs were quite similar and conservative (Fig. 2). Therefore, we also examined gene segmental duplication events to explore the expansion patterns of the *TaSPL* genes in wheat. In this study, there are 63 gene pairs identified cross on 21 chromosomes (Additional file 6: Table S5 and Fig. 4). In addition, by calculating the ratio of nonsynonymous substitutions (Ka) to synonymous substitutions (Ks) of each gene pair, the selection constraints of duplicated *TaSPL* genes were investigated. In general, duplicated genes with a high Ka/Ks (>1) are indicated to be evolving under positive selection, Ka/Ks =1 manifested neutral selection, while Ka/Ks (<1) indicated negative or purifying selection [61]. The values of Ka/Ks for the 63 *TaSPL* gene pairs were shown in Additional file 6: Table S5. The results showed that all of the calculated genes pair values were Ka/Ks (<1), and the ratio of segmental duplications ranged from 0.05 (*TaSPL*-8-B/ *TaSPL*-8-D) to 0.62 (*TaSPL*-22-A/ *TaSPL*-22-D), which implied that *TaSPL* genes have mainly undergone purifying selection or negative selection after the segmental duplication events with limited functional divergence. Subsequently, to understand the original, evolutionary history, and supposed function of the *TaSPL* genes, a syntenic map were made between wheat and rice. The 55 gene pairs syntenic relationships between 19 *OsSPL* and 56 *TaSPL* genes were analysed (Additional file 7: Table S6 and Fig 5). The results indicated that some *TaSPL* genes and their *OsSPL* counterparts appeared to be derived from a common ancestor, suggesting that the function of some *TaSPL* genes could be inferred from their *OsSPL* homolog, which could facilitate research on the roles of the *TaSPL* genes in wheat.

**The miR156 may regulate the expression of *TaSPL* genes**

According to the available annotation information, the 11 *OsSPL* genes and 10 *AtSPL* genes are targets by miR156 respectively, and the complementary sites of miR156 are located in the coding regions or 3’ UTRs of *SPL* genes in rice and *Arabidopsis* [12, 62]. To understand the post-transcriptional regulation of miR156-mediated *TaSPL* genes in wheat, miRBase database was used to predict putative members of the
miR156 in wheat. Tae-miR156 (MI0016450) precursor in the public database and the psRNATarget server can be used to search for targets of miR156 according to cDNA region. The results showed that 28 TaSPL genes had targets for miR156, which divided into six groups (I-VI), according to the different complementary sequences existing on the target genes (Fig. 6). Group I contained 15 TaSPL genes, the mature sequence of tae-miR156 was completely complementary to target sites in the TaSPL genes, while other groups showed different degree of nucleotide mismatch. Interestingly, there were 11 target sites that were conserved across 28 TaSPL genes, showing that a functional studies of these genes could be performed by mutating miR156 target sites.

The miR156-target sites of 24 TaSPL genes were located in the coding region, and the other four TaSPL genes target sites were existed in the 3' UTR, near the termination codon (Additional file 8: Table S7 and Additional file 9: Figure S2), almost the same to miR156 targets of SPL genes in Arabidopsis and rice. The results indicated that miR156-mediated regulation of the SPL genes were conserved in diverse plant species. Further analysis of SPL genes in wheat showed the miR156-targeted SPL genes were distributed in six subgroups (G3, G4, G5, G6, G7 and G8) but except other two subgroups (G1 and G2).

The multiple alignments of the miR156 mature sequences from wheat, Ae. tauschii, B. distachyon, rice and Arabidopsis, revealed few polymorphism of nucleotides existed in the complementary region of the mature miR156 sequence, indicating the precursor sequences of miR156 family is relatively conserved in various plant species (Additional file 10: Table S8 and Fig. 7).

**Tissue-specific expression analysis TaSPL genes**

Although the TaSPL genes have different gene size, structure, location and segmental duplication, their functions in wheat remain unclear. The expression patterns of TaSPL genes were analysed by RNA-seq data (Fig. 8). The results showed that 56 TaSPL genes exhibited an extensive range of expression in different tissues, while homologous genes across the A, B, and D subgenomes revealed similar expression patterns. Remarkably, TaSPL13 and TaSPL15 were highly expressed in spikes, suggesting that they might be regulate spike development, consistent with our previous study. In addition to the expression patterns of the six genes previously analysed, we randomly selected another eight genes for tissue expression analysis by qRT-PCR method in this study [63].

The tissues-specific expression profiles of eight TaSPL genes were examined by qRT-PCR in different vegetative and reproductive tissues and organs. Our results showed that the transcript levels of eight TaSPL genes were varied greatly (Fig. 9). At the vegetative growth stage (Z00-Z49), the eight TaSPL genes were constitutively expressed in all detected tissues, while TaSPL-2 exhibited a high level of expression in root, which was associated with RNA-seq data showing that TaSPL-2 was involved in drought stress (Additional file 11: Figure S3a). The expression level of TaSPL-3 was highly detected in tiller base and axillary bud (Z20-Z29), implying their specific roles in tiller base and axillary bud development. TaSPL-4 was expressed at high levels in coleoptile and tiller base (Z00-Z09, Z20-Z29), indicating the may be involvement in coleoptile and tiller base development. TaSPL-6 was highly expressed in root and axillary bud (Z10-Z19, Z20-Z29), which might be involve in root and axillary bud development. TaSPL-8 exhibit
relatively high expression levels in radicle, tiller base and ligule (Z00-Z09, Z20-Z29 and Z40-Z49). TaSPL-10 was highly expressed in coleoptile and axillary bud (Z00-Z09, Z20-Z29), TaSPL-14 at high levels in coleoptile and flag leaf (Z00-Z09, Z30-Z39) and TaSPL-18 at high levels at coleoptile and node (Z00-Z09, Z30-Z39). At the reproductive stage, TaSPL-2, -3, -4, -6, -10, -14 and -18 genes had high expression level during spike developmental stage (Z50-Z69), while the transcript of TaSPL-8 exhibit low expression level. Besides, TaSPL-2, -8 and -18 genes showed relatively higher expression levels in the grains (Z70-Z79), except for TaSPL-3, -4, -6, -10 and -14. These results indicated that some TaSPL genes might be involved in the development of axillary bud, spike and grain, and provide a theoretical basis for our future research on the functional mechanism of genes.

Expression of TaSPL genes of wheat under abiotic stresses and plant hormones

To better understand the responses of TaSPL genes to various stresses, RNA-seq data of three abiotic stresses (drought, heat, and cold) and two biotic stresses (powdery mildew pathogen and stripe rust pathogen infection) treatments were acquired and the results were performed by row clustering (Additional file 11: Figure S3). The expression of TaSPL-2-B, -2-D and -6-D genes were significantly up-regulated after 1 h drought treatment (Additional file 10: Figure S3a and Additional file 12: Table S9a). Meanwhile, TaSPL-2-A, -2-B and -2-D genes were also up-regulated after 6 h of combined drought and heat stresses (Additional file 11: Figure S3a and Additional file 12: Table S9c). There are seven TaSPL genes may be respond to cold stress (Additional file 11: Figure S3b and Additional file 12: Table S9d). Besides, TaSPL-6-A, -6-D and -15-B genes were distinctly up-regulated with prolonged injection of powdery mildew pathogen and stripe rust pathogen from 24 h to 72 h (Additional file 11: Figure S3c and Additional file 12: Table S9e).

Previous studies have shown that SPL genes could response to drought [39], heat [64], auxin (IAA) and brassinolide signal pathways [55], biotic and abiotic tresses [37, 39]. To examine the effects of different abiotic stresses and plant hormone on TaSPL gene expression, wheat cultivar Chinese Spring seedlings were treated with drought, PEG6000, NaCl, Cold, ABA, IAA, GA, GR24, MeJA and BR, and the qRT-PCR method also used to investigate the expression levels of the eight TaSPL genes (Fig. 10).

Under drought treatment, the expression of these eight genes was up-regulated or down-regulated at different time points. TaSPL-6 was down-regulated during the whole treatment period, and TaSPL-10 was up-regulated by more than seven-fold after 3 h treatment. After PEG treatment, the expression of TaSPL-2, -4, -6, -8 and -18 genes peaked at 1 h, TaSPL-10 peaked at 12 h, while TaSPL-3 was down-regulated at all treatment time points. Under NaCl stress treatment, the expression level of TaSPL-2 was up-regulated, TaSPL-3, -4 and -14 genes were significantly down-regulated throughout the treatment time, and the expression levels of TaSPL-6 and -10 genes peaked at 1 h. After cold stress treatment, the expression level TaSPL-2 was up-regulated distinctly at different time points, the expression of TaSPL-4, -6, -10, -14 and -18 genes peaked at 1h, while TaSPL-8 peaked at 24 h.

Under ABA treatment, the expression levels of these eight genes showed a trend of up-regulation or down-regulation at the whole time point of treatment, the expression of TaSPL-2, -3, -8 and -18 genes peaked at
3 h, TaSPL-4, -6 and -14 genes peaked at 6 h, and TaSPL-10 peaked at 1 h. After IAA treatment, the expression levels of the TaSPL-2, -3, -4, -6, -14 and -18 genes were distinctly up-regulated at the whole treatment process, more remarkably for TaSPL-2 and TaSPL-18 and TaSPL-10 peaked at 12 h. Under GA treatment, the expression levels of the TaSPL-3 and -6 genes were down-regulated, TaSPL-10 was obviously up-regulated at each point in time of treatments, and TaSPL-2 and -4 genes peaked at 24 h, TaSPL-8, -14 and -18 genes peaked at 12 h. Under GR24 treatment, the expression levels of the TaSPL-2, -3, -6 and -10 genes were up-regulated at each treatment time points, and TaSPL-4 and -8 genes peaked at 12 h, TaSPL-14 and -18 genes peaked at 24 h. Under MeJA treatment, TaSPL-3, -8 and -18 were down-regulated during the whole treatment time points, TaSPL-2 and -10 peaked at 3 h, TaSPL-4 peaked at 1h, and TaSPL-6 and -14 genes peaked at 6 h. Under BR treatment, the expression levels of the TaSPL-3 and -4 genes were up-regulated at each time point of treatment, and TaSPL-2 and -14 peaked at 12 h, TaSPL-6 peaked at 3 h and TaSPL-8, -10 and -18 genes peaked at 24 h. These results showed that some TaSPL genes could respond to at least one hormone and participate in the abiotic stress process. For example, the TaSPL-3 was up-regulated under IAA treatment and down-regulated under GA treatment. TaSPL-10 was up-regulated under GA and GR24 treatments.

**Discussion**

**Evolution relationship analysis of TaSPL genes**

In plants, SPL proteins, containing the SBP domain, are plant-specific transcription factors and is not found in human-being, animals, or bacteria [4, 10-12, 14, 39]. There are many SPL genes have been exclusively identified among plant species [4, 9-14, 65, 66]. In our present study, 56 putative TaSPL genes were identified in wheat genome (Additional file 2: Table S2, Additional file 3: S3, Additional file 4: Table S4 and Fig.1), and a phylogenetic analysis of the SPL genes from different plant species was performed including monocots (Ae. tauschii, T. urartu, rice, and B. distachyon) and dicotyledon (Arabidopsis). A total of 136 SPL genes were divided into eight groups (G1-G8) (Fig. 1). Within the same group, gene structure and motif composition were uniformly distributed. In Arabidopsis, AtSPL2/AtSPL10/ AtSPL 11 controlled proper development of lateral organs, which belong to G7 [67]. Moreover, due to high sequence homology, TaSPL genes probably play similar biological functions in the same group.

**Motif analysis of TaSPL proteins**

The results of motifs distribution showed that most of the TaSPL proteins in the same group had similar motif distribution, while several motifs varied greatly among different groups. For example, the SBP-domain, including motif 1 and motif 2, existed in 56 TaSPL proteins, and motif 6 contained the miR156 complementary site and presented in some TaSPL proteins (Figs. 2 and 7). Motif 3 was more specific to G4. The functions of other motifs are still mysterious. These results were consistent with those for OsSPL proteins, which motif 1 and motif 2 were presented in all OsSPL proteins, while motif 6 was only presented in some proteins [12, 62]. Several TaSPL proteins (TaSPL-1-D, -6-A, -6-B, -15-A and -15-B) were searched by using the CD-search tool, and they had ankyrin repeats, which are domains for protein-
protein interactions, while ankyrin repeats were also found in OsSPL1, OsSPL6 and OsSPL15 proteins in rice [12]. The functions of ankyrin repeats are still unknown.

**Duplication and synteny of TaSPL genes**

In the process of gene evolution, gene duplication events could influence the gene numbers, structures and functions. More SPL genes identified in wheat (56) than in rice (19), Arabidopsis (17), maize (31), tomato (15), apple (27) and S. miltiorrhiza (15) [4, 9-13]. Phylogenetic analysis indicated that 37 SPL genes were obtained after the division of wheat and rice, indicating the expansion events of TaSPL genes in wheat. In our study, it was found that 63 gene pairs were found to be involved in segmental duplication events in 56 TaSPL genes (Additional file 7: Table S6 and Fig. 4). Similarly, SPL gene pairs have been found in rice (OsSPL16/18, OsSPL5/10, OsSPL3/12, OsSPL4/11 and OsSPL2/19) and Arabidopsis (AtSPL4/5, AtSPL1/12 and AtSPL10/11) were located within respective segmental duplication regions [68]. The number of TaSPL gene pairs found in wheat was significantly higher than that of Arabidopsis and rice, suggesting that more segment duplication events occurred within wheat genome. Additionally, the ratio of Ka/Ks was less than 1.00, suggesting that TaSPL genes underwent extensive purifying selection. Allopolyploid and high-level of gene replication provide a large genomes with many functional genes for wheat adaptation in complex environments.

**TaSPL genes may be targets by miR156**

Most of the target genes of microRNAs found encode transcription factors in plants. In Arabidopsis, AtmiR156 acted on AtSPL genes and regulated many biological processes, such as, leaf development [69], shoot development [45], male fertility [21], restrain early flowering [23], organ size [28] and anthocyanin biosynthesis [70]. Previous reports indicated that OsmiRNA156 negative regulated the expression of OsSPL genes and determined the ideal plant architecture [19] and grain yield [26]. In switchgrass, SPL genes targeted by miRNA156 regulated axillary bud formation and promoted flowering [16, 25]. In tomato, miRNA156-targeted SlSPL genes regulated inflorescence morphogenesis, ovary and fruit development and dramatic alterations (small leaves and fruit, reduced height) [36, 44, 47]. In addition, miRNA156-targeted SPL13 increased drought stress tolerance in alfalfa [40].

Our studies showed that 28 TaSPLs were predicted to be putative targets of miR156 because of these genes containing complementary miRNA sequences in the 3'UTR or coding region. Meanwhile, some TaSPL genes were targeted by other miRNAs, such as TaSPL16 and TaSPL13 targeted by miR9780, TaSPL2-B and TaSPL14-D by miR9677b and TaSPL3 by miR319. As reported, miR9780 may be involved in low-nitrogen-promoted root growth [71], miR9677b in pollen development [72] and miR319 in the development of embryo patterning and leaf morphogenesis [73]. These results revealed that complex regulatory network between TaSPL genes and miRNAs affected the potential function of TaSPL genes, which should be confirmed by genetic modification in the expression levels of specific miRNAs or target TaSPL genes of wheat in future.

**TaSPL genes are responsive to various stresses treatment and plant hormones**
Although diversified functions of SPL genes have been explained during different plant growth and developmental processes [16, 19, 25-27, 33]. There are only a few studies on SPL genes involved in stresses, such as, ectopic expression of BpSPL9 could enhance scavenging of ROS under salt and drought stresses in B. platyphylla Suk. [39]. These results suggest that some SPL genes were involved in response to various stresses [31, 37, 39]. In our study, the expression analysis of TaSPL genes under various stresses by RNA-seq data indicated that some TaSPL genes responded to drought, heat, cold, stripe rust or powdery mildew stresses (Additional file 11: Figure S3 and Additional file 12: Table S9).

Our results revealed that most TaSPL genes not only involved in drought stress but also to fungal pathogens, which has not been reported previously by RNA-seq data. Some TaSPL genes were obviously up-regulated in heat treatment, but not in drought treatment, which may contribute to improving the cold tolerance of wheat. And some plant hormonal signals such as IAA, GA and GR24 had been reported to be involved in plant response to various stresses by activating transcription of many defence-related genes [19, 26, 74]. We also investigated the responses of some TaSPL genes to different plant hormone signals by examining their transcript levels in wheat root upon various hormone treatments. The results showed that many TaSPL genes were up-regulated or down-regulated by different plant hormones, indicating TaSPL genes may be involved in the responses to various plant hormones signalling (Fig.10).

Conclusions

In our study, the 56 putative TaSPL genes were identified in the wheat genome and divided into eight groups (G1-G8), according to phylogenetic tree construction among the six plant species. The 28 of 56 TaSPL genes were predicted to be putative targets of miRNA156, revealing the important regulatory mechanism between miRNA and SPL genes. Gene structure, conserved motif, chromosome location, duplication event and syntenic relationship between wheat and rice were analysed using different databases. Expression patterns analysis in various tissues showed that some TaSPL genes could regulate the development of spike and grain. TaSPL genes were subject to various stress treatments, suggesting part genes probably involved in responding to hormone signals. These results could provide a fundamentally information to further study of the functions of TaSPL genes in wheat growth and development.

Methods

Identification of TaSPL genes

To identify the TaSPL proteins, the HMM (hidden Markov model) was downloaded from the Pfam containing the SBP domain (Accession no. PF03110), and URGI (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies) was used to download the whole genome sequence of wheat cv. Chinese Spring [75, 76]. Programs of SMART (http://smart.embl-heidelberg.de) and Interpro (http://www.ebi.ac.uk/interpro/search/sequence-search) were used to check the TaSPL proteins containing the SBP domain [56, 57]. Information on the TaSPL genes, including the numbers and lengths of
complementary deoxyribonucleic acid (cDNA), coding sequences (CDS), amino acids and location coordinates was analysed by Ensemble Plants (http://plants.ensembl.org/index.html). Physicochemical properties of TaSPL proteins were obtained by ExPASy program (https://web.expasy.org/protparam/) [77]. Additionally, the protein sequences of 17 AtSPL and 19 OsSPL proteins were downloaded from UniProt (http://www.uniprot.org/). Besides, 18 AetSPL genes in Ae. tauschii, 10 TuSPL genes in T. urartu and 16 BdSPL genes in B. distachyon were identified using the above-described method.

**Phylogenetic tree construction and sequence alignment**

The SPL protein sequences of wheat, Ae. tauschii, T. urartu and B. distachyon, rice and Arabidopsis, were imported together to Clustal X (http://www.clustal.org/clustal2/) to align default parameters. An un-rooted phylogenetic tree with 1,000 replicates of bootstrap was constructed by using the neighbour-joining (N-J) method with MEGA X software [59]. All Protein sequences were listed in Additional file 1: Table S1 and Additional file 2: Table S2. Multiple sequence alignments of SBP domain-containing sequences were applied with ClustalW2.0 software [60].

**Conserved protein motif and exon-intron structure determination** The MEME motif search tool was used to determine the conserved motifs of 56 TaSPL proteins (http://meme-suite.org/) [78]. After that, the results were rearranged by TBtools [79]. The gene structure of TaSPL genes was analysed by Gene Structure Display Server 2.0 (http://gsds.cbi.pku.edu.cn/index.php) [80].

**Chromosome localization and gene duplication** Information of chromosome localization was sorted out according to genome annotation and analysed using MG2c (http://mg2c.iask.in/mg2c_v2.0/). Considering that wheat is allohexaploid, the segmental duplications in three sub-genomes were separately identified. Multiple possible gene duplication events of TaSPL genes between wheat and rice were examined using the Multiple Collinearity Scan toolkit (MCScanX) and mapped with Circos v0.69 [81-83]. Non-synonymous (Ka) and synonymous (Ks) values were figured out using TBtools software, which determine whether selective pressure applied to repeated events [79].

**Prediction of target sites of miR156**

The stem-loop and mature sequences of miR156 were downloaded from the miRBase (http://www.mirbase.org/) [84]. TaSPL genes targeted by miR156 were forecasted by searching the cDNA regions of TaSPL genes with the psRNATarget server (http://plantgrn.noble.org/psRNATarget/) [85]. The sequences of stem regions (mature miRNA sequences and the complementary sequences) were aligned by Clustal X software. The map was optimized by using IBS1.0 software (http://ibs.biocuckoo.org/) [86].

**Expression pattern analysis of TaSPL genes by RNA-seq**

To analyse the tissue specific expression of TaSPL genes and its expression under biotic and abiotic stresses, RNA-seq data of the project SRP041017, SRP043554 and SRP045409 were analysed from expVIP platform (http://www.wheat-expression.com) [87-90]. For spatiotemporal expression patterns analysis, there are five tissues at three stages of wheat growth and development, including roots (at
seedling stage) (RS), roots (at three leaf stage) (RTL), roots (at flag leaf stage) (RFL), stems (at 1 cm spike) (SS), stems (at two-node stage) (STN), stems (at anthesis stage) (SA), leaves (at seedling stage) (LS), leaves (flag leaf at tillering stage) (LFLT), leaves (at 2 DAP stage) (L2D), grains (at 2DAP stage) (G2D), grains (at 15 DAP stage) (G15D), grains (at 30 DAP stage) (G30D), spikes (at two-node stage) (SPTN), spikes (at flag leaf stage) (SPFL) and spikes (at anthesis stage) (SPA). The OriginPro 8.5.1 was used to draw the diagram based on transcripts per million (TPM) value. For various stresses analysis, TBtools was used to generate heat maps via log\textsubscript{2} based on TPM value.

**TaSPL gene expression pattern analysis by qRT-PCR**

Allohexaploid wheat (*T. aestivum* L. cv. Chinese Spring) seeds were planted in a climate room with a cycle of 12 h of light/12 h of dark at 20-22 °C. The various tissues and organs were collected following the scales of Zadoks and Tottman. They showed the decimal code for the growth stage of the cereals [91, 92]. The various vegetative and reproductive tissues of wheat were harvested with three replicates at each tissue, including coleoptile and radicle (germination stage, Z00-Z09), root, basal stem, leaf blade and leaf sheath (seedling growth stage, Z10-Z19), tiller base, leaf sheath, axillary bud and pulvinus (tillering stage, Z20-Z29), internode, node and flag leaf (stem elongation stage, Z30-Z39), ligule and auricle (booting stage, Z40-Z49), stamen, pistil and the different length of young spike (YS) (inflorescence emergence and anthesis stage, Z50-Z69), and the developing seed (milk development stage, Z70-Z79).

The root of two-week-old seedlings were treated with drought, 20% (w/w) polyethylene glycol (PEG) 6000, 200 mM NaCl, cold, 100 μM abscisic acid (ABA), 50μM auxin (IAA), 50 μM gibberellin (GA), 6μM strigolactones (GR24), 100 μM methyl jasmonate (MeJA) and 50μM brassinolide (BR), followed by sampling at 1, 3, 6, 12 and 24 h post treatment. The seedling roots treated with distilled water were used as the control. The roots of seedlings treated with distilled water were used as the control. All root samples, including treated and controlled samples, were collected with three biological replicates. Then the samples were collected and stored at –80 °C for later RNA extraction.

**Expression analysis of representative TaSPL genes in wheat**

Plant Total RNA Extraction Kit (Zomanbio, Beijing, China) was used to extract total RNA of the mentioned-above samples. The gDNase treated RNA was used for synthesis of first-strand cDNA with FastKing RT Kit (Tiangen, Beijing, China) and SYBR Green Master Mix (Vazyme, Nanjing, China) was used to amplify reaction. Reactions were performed using the CFX96 real-time system (Bio-Rad, Hercules, CA, USA), and each sample was replicated three times, with three technical replicates per condition. Wheat *TaActin* (TraesCS1B02G283900) gene was used as internal control. All primers used in this research are listed in Additional file 13: Table S10. Relative transcript levels were analysed using the relative quantitative method [93], and the Sigma Plot 10.0 software was used to drawn graphs. Value are the mean ±SE (n=3,* P<0.05; ** P<0.01 by Tukey's test).

**Abbreviations**
Declarations

Authors' contributions

GH and YW conceived, designed, revised and finalized the manuscript. LL and FS participated in all experiments, performed the bioinformatics analysis, data processing, writing and review draft. GW, YG and YZ participated qRT-PCR experiment. JC, MC, and GY put forward valuable suggestions. All authors read and approved the final manuscript. LL and FS contributed equally to this work.

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Availability of data and materials

The sequencing data are available in the URGI (https://wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies), the miR156 sequences information are available from miRBase (http://www.mirbase.org/) and the public RNA-seq data are available on expVIP website (http://www.wheat-expression.com/).

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

SPL: SQUAMOSA-promoter binding protein-like; TFs: Transcription factors; NLS: Nuclear Localization Signal; ROS: Reactive Oxygen Species; IWGSC: International Wheat Genome Sequencing Consortium; PEG: Polyethylene glycol; ABA: Abscisic acid; IAA: Auxin; GA: Gibberellin; GR24: Strigolactones; MeJA: Methyl jasmonate; BR: brassinolide; HMM: The hidden Markov model; CDS: coding sequences; cDNA: complementary deoxyribonucleic acid; PI: Isoelectric point; MW: Molecular weight; N-J: Neighbour-joining; GSDS: Gene Structure Display Server; Ka: non-synonymous substitutions; Ks: synonymous substitutions; MCScanX: Multiple Collinearity Scan toolkit; TPM: Transcripts per million; qRT-PCR: Quantitative real time polymerase chain reaction.
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