Comparative analysis of the *SPL* genes among four *Ipomoea* species and the evidence for a role of some *lbSPLs* in storage root development in sweet potato (*Ipomoea batatas*)

Haoyun Sun

Jiangsu Normal University

Jingzhao Mei

Tianjin Medical University

Wenqian Hou

Jiangsu Normal University

Yang Zhang

Jiangsu Normal University

Tao Xu

Jiangsu Normal University

Shaoyuan Wu

Jiangsu Normal University

Lei Zhang (✉ leizhang@jsnu.edu.cn)

Jiangsu Normal University

Research Article

Keywords: Ipomoea, SPL transcription factor, evolutionary patterns, root development, expression profiles

Posted Date: August 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-654042/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background

As a major family of plant-specific transcription factors, SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes play crucial regulatory roles in plant growth, development, and stress tolerance. SPL transcription factor family has been widely studied in various plant species, however, there are no systematic studies on *SPL* genes in genus *Ipomoea*.

Results

In this study, a total of 29, 27, 26, 23 *SPL* genes were identified in *Ipomoea batatas*, *Ipomoea trifida*, *Ipomoea triloba*, and *Ipomoea nil*, respectively. Phylogenetic analysis indicated that *Ipomoea SPL* genes could be clustered into eight clades. *SPL* members within the same clade showed similar gene structures, domain organizations, and cis-acting element compositions, suggesting similarity of biological function potentially. Evolutionary analysis revealed that segmental duplication events played a major role in the *Ipomoea* genus-specific expansion of *SPL* genes. Of these *Ipomoea SPL* genes, 69 were predicted as the target genes of miR156, and 7 *IbSPL* genes were further confirmed by degradome data. Additionally, *IbSPL* genes showed diverse expression patterns in various tissues, implying their functional conservation and divergence. Finally, by combining the information from expression patterns and regulatory sub-networks, we found that four *IbSPL* genes (*IbSPL16/IbSPL17/IbSPL21/IbSPL28*) may be involved in the formation and development of storage roots.

Conclusions

This study not only provides novel insights into the evolutionary and functional divergence of the *SPL* genes in all available sequenced species in genus *Ipomoea*, but also lays a foundation for further elucidation of the potential functional roles of *IbSPL* genes during storage root development.

1. Background

The SQUAMOSA promoter-binding protein-like (*SPL*) gene family is one of the plant-specific transcription factor families, which play fundamental roles in plant growth, development, and stress tolerance [1–5]. The most common feature of *SPL* gene members is that they all contain SQUAMOSA promoter-binding (SBP) domain, which was composed of three distinct motifs: two non-interleaved zinc-binding sites (Cys-Cys-Cys-His and Cys-Cys-His-Cys), and one nuclear localization signal (NLS) at the C-terminus [1]. *SPL* genes (*AmSBP1* and *AmSBP2*) were first discovered in *Antirrhinum majus* based on their functions on flower development [6]. Since then, numerous homologs have been identified and characterized in model species, such as *Arabidopsis thaliana* [7], *Oryza sativa* [8], and *Solanum lycopersicum* [9]. With the

increasing number of sequenced genomes, more and more *SPL* members have been annotated and reported in non-model plants, such as apple [10], pepper [11], soybean [12], and so on.

The functions of *SPL* genes in model plants have been well studied, and showed functional divergence. In *A. thaliana*, AtSPL3, AtSPL9 and AtSPL10 have been demonstrated to regulate root development [13, 14]. Meanwhile, similar roles have been reported in rice [15] and apple [16]. Other studies have found that *SPL* proteins participate in vegetative and reproductive phase transitions. For instance, AtSPL3/4/5 promote flowering by directly activating the expression of three flowering integrator genes namely, AP1, FUL and LFY [17]. In addition, *SPL* proteins have also been found to participate in fruit development and grain yield. For example, LeSPL-CNR regulates cell wall disassembly and carotenoid biosynthesis during fruit ripening in tomato [18, 19]; overexpression of *OsSPL 16* promotes cell division and grain filling, with positive consequences for grain width and yield in rice [20]. *SPL* genes are also known as the targets of miR156, thus forming a functional regulatory network of miR156-*SPL* [2]. In the miR156-*SPL* module, AtSPL9 has a negative effect on anthocyanin accumulation [21]; OsSPL7 enhances disease resistance against bacterial blight [22]. The functions of *SPL* genes have been deeply studied in *Arabidopsis* and other model plants, however, studies on *SPL* genes in genus *Ipomoea* are relatively scarce.

The genus *Ipomoea*, which includes 500–600 species, possesses the largest number of species in the family *Convolvulaceae* [23]. Species in genus *Ipomoea* are widely distributed in the world, which are of great value in the fields of industry and agriculture [23–25]. For example, Japanese morning glory, *Ipomoea nil* (L.) Roth. ($2n = 2x = 30$), is cultivated as an ornamental plant due to its various patterns of flower color [24]. Sweet potato, *Ipomoea batatas* (L.) Lam. ($2n = 6x = 90$), is ranked as the seventh most important crop globally due to its strong adaptability, stable yield and high nutritional value [25]. The storage roots of sweet potato, which are mainly harvested, are of great significance to the nutrient content and yield [26]. Up to now, the genomes of four species (*I. batatas*, *I. trifida*, *I. triloba* and *I. nil*) have been sequenced in genus *Ipomoea* [27–29]. Among these species, *I. trifida* is the most closely related diploid to *I. batatas*, followed by *I. triloba* and *I. nil* [29]. The reported haplotype-resolved genome assembly of *I. batatas* is of low-quality, making it difficult to accurately identify and characterize genes. By contrast, the genome assembly of the other three diploid relatives are of high-quality, and can be used as robust references for *I. batatas*. All in all, the availability of these genomes makes it possible to perform genome-wide comparative analysis of *SPL* genes in genus *Ipomoea*.

In this study, we first conducted the genome-wide identification and characterization of *SPL* genes in four *Ipomoea* species, including *I. batatas*, *I. trifida*, *I. triloba* and *I. nil*. We next investigated phylogenetic relationship and evolutionary patterns of *SPL* genes in these four species. We then determined the expression patterns of *IbSPL* genes using transcriptome and qRT-PCR in different tissues or under various hormones treatments. Finally, we performed the WGCNA to construct the co-expression network, and inferred the putative functions for the *IbSPL* genes in the storage root of sweet potato. Our work will not only provide insights into the evolutionary conservation and diversification of the *SPL* genes in genus *Ipomoea*, but also lays the foundation for further research on the *IbSPL* genes related to the storage root development in sweet potato.

2. Results

2.1 Identification of *SPL* genes in four *Ipomoea* species

To obtain *SPL* genes in *Ipomoea* species, two methods, BLASTP and HMM, were used for identification, and two online databases, SMART and ScanProsite, were used for confirmation. As a result, a total of 29, 27, 26, 23 *SPL* genes were identified in *I. batatas* (*Ib*), *I. trifida* (*Itf*), *I. triloba* (*Itb*), and *I. nil* (*In*), respectively. For consistency, the *Ipomoea* *SPL* genes were renamed according to their chromosomal location order in each genome (**Supplementary Table S1**). The numbers of *SPL* genes and their percentage of the total genes in each species were displayed (**Figure 1a**). We found that *I. nil* genome has the least number of *SPL* genes compared to the other three species.

Subcellular localization analysis showed that most of the *Ipomoea* *SPL* proteins (94, 89.52%) were localized in the nucleus (**Supplementary Table S1**), suggesting their critical role in regulatory functions. Besides, the physical and chemical properties of *SPL* proteins were significantly different within species, but exhibited similar patterns among the four species (**Figure 1b-d, Supplementary Table S1**). To be more specific for all of the *Ipomoea* *SPL* proteins, the number of amino acids ranged from 103 (*InSPL9*) to 1141 (*IbSPL11*), the molecular weight (MWs) varied from 12.01 (*InSPL9*) to 126.51 (*IbSPL11*) kDa, the isoelectric points (pIs) ranged from 5.40 (*ItfSPL14*) to 10.55 (*InSPL15*).

2.2 Comparative phylogenetic analysis of *Ipomoea* *SPL* genes

To explore the evolutionary relationship among the *Ipomoea* *SPL* genes, a rooted neighbor-joining phylogenetic tree was constructed using 105 *SPL* proteins from four *Ipomoea* species and 51 *SPL* proteins from other five plant species (*A. thaliana*, *S. lycopersicum*, *O. sativa* and *Chlamydomonas reinhardtii*) (**Figure 2, Supplementary Table S2**). Based on the classification of *SPLs* from *A. thaliana*, *S. lycopersicum* and *O. sativa* [7-9], *Ipomoea* *SPL* genes were classified into eight clades, which were numbered from I to VIII (**Figures 1, 2**). All clades had at least one *SPL* gene in each *Ipomoea* species, indicating the conservation of *SPLs* across *Ipomoea* genomes. However, the number of *SPLs* in certain clades were highly variable among *Ipomoea* species, suggesting the diversity of *SPLs* in genus *Ipomoea*. Clade I was the smallest subfamily, containing only one member for each *Ipomoea* species. Clade IV contained the highest number of *SPLs* (> 26%) in genus *Ipomoea*, and it could be further divided into three subclades (IV-a, IV-b, and IV-c). Members of IV-b and IV-c subclades only contain *SPL* genes from *Ipomoea* species, and no homologs of other species were found, which indicates that the *Ipomoea* *SPL* genes in these two subclades were generated in an ancient ancestor of the *Ipomoea* lineage. In addition, the phylogenetic analysis also indicated that most *IbSPL* genes were closer to *ItfSPL* genes than either *ItbSPL* or *InSPL* genes, supporting the fact that *I. trifida* is the most closely related diploid to hexaploid sweet potato [29]. When the number of *SPL* genes in *Ipomoea* species were compared with other species, we found that the number in *Ipomoea* species (the average number of *SPL* genes in 4 *Ipomoea* species was 26) was greatly increased by almost 1.6, 1.4 and 2 times than that in *A. thaliana* (16), *O. sativa* (19) and *S.*

lycopersicum (13), respectively. The results indicated that *Ipomoea* SPL genes have undergone extensive expansion after the speciation of *S. lycopersicum*.

2.3 Gene and protein structure of the *Ipomoea* SPL family

To explore the structural diversity of the *Ipomoea* SPL genes, intron/exon structure analysis were carried out (**Figure 3**). Gene structure illustrations showed the high variation in the number of exons for *Ipomoea* SPL genes, ranging from 2 to 15 (**Figure 3a, Supplementary Table S1**). For instance, *IbSPL20* possesses the largest number (15) of exons, whereas most of the *Ipomoea* SPL genes in Clade VI contained the least number (2) of exons. Moreover, most *Ipomoea* SPL genes in the same clade exhibited similar gene structures, even if they belong to different species. For example, SPL members in clade I and II contained the most exons, ranging from 10 to 15, while SPL members in the remaining clades possess 2 to 7 exons (except *IbSPL19*). Our results suggested that exon/intron gain or loss had occurred during the course of the *Ipomoea* SPL gene evolution, which may result in the functional divergence of SPL genes.

To investigate the features of *Ipomoea* SPL proteins, the conserved domains were analyzed by multiple sequence alignment. The results showed that all of the SPL members had the SBP domain, which was composed of two non-interleaved zinc finger-like structures (Zn-1/2) and one nuclear localization signal (NLS) motif (**Figure 3b, Supplementary Figure S1**). Based on the alignments, the Zn-2 motif is more conserved than the Zn-1 and NLS motif in all *Ipomoea* SPLs analyzed, which was also observed in *Rosaceae* [30] and *Oryza* species [31] (**Supplementary Figure S1**). For example, the Zn-2 motif in all *Ipomoea* SPLs was a Cys-Cys-His-Cys (C2HC) type (except *IbSPL23*); and the Zn-1 motif showed different signatures, which was Cys-Cys-Cys-Cys (C4) type in clade I and Cys-Cys-Cys-His (C3H) type in the remaining clades. Moreover, other conserved domains were identified in specific clades. For instance, SPLs in clade I and II possess an DEXDc domain (**Supplementary Figure S2**), which were involved in ATP-dependent DNA unwinding [32]; SPLs in clade II contain Ankyrin repeats (**Supplementary Figure S3**), which were considered to be significant for mediating protein-protein interactions [33].

To gain a better understanding of *Ipomoea* SPL protein characteristics, the MEME software was used to explore the motif compositions (**Figure 3d, Supplementary Figure S4**). It was found that *Ipomoea* SPL proteins within the same clade showed similar motif compositions, while *Ipomoea* SPL proteins in different clades exhibited distinct differences in motif composition. In detail, two motifs (motif 2 and 3) existed in all *Ipomoea* SPL proteins, which were actually part of the SBP domain (**Supplementary Figure S1**); four motifs (motif 4, 5, 6 and 7) existed in clade I and II, and motif 4 was actually DEXDc domain (**Supplementary Figure S2**); motif 8 existed in clade II, which were make-up of Ankyrin repeats (**Supplementary Figure S3**); motif 10 presented in clade IV with unknown function. In summary, our results revealed that some motifs only presented in the specific clade, indicating that the SPL genes may be significantly different in function between different clades.

2.4 Gene duplication, orthology relationship and selective pressure of *Ipomoea* SPLs

To investigate gene duplication modes for *Ipomoea* SPL genes, MCScanX [34] was used to perform gene collinearity analysis within each *Ipomoea* species. All of the *Ipomoea* SPL genes were estimated to be duplicated (no singleton mode was found), and segmental (51, 48.57%) is the dominant mode compared to the other duplication modes: dispersed (39, 37.14%), tandem (13, 12.38%) and proximal (2, 1.90%) (**Figure 4ab, Supplementary Table S1, Supplementary Figure S5**). Our results indicated that segmental duplication has played a predominant role in the evolution and expansion of *Ipomoea* SPL genes. In addition, we found that tandem duplication was the most frequent event in IV-b and IV-c subclades, suggesting that SPL genes in these two clades were expanded by tandem duplication.

To assign the orthology relationships of the SPL genes, OrthoMCL [35] was used to detect orthologous groups across *O. sativa*, *A. thaliana*, *S. lycopersicum* and the four *Ipomoea* species. A total of 25 (1, 2, 2, 8, 3, 5, 2 and 2) orthologous groups in the eight clades (clade I to VIII) were identified, respectively (**Supplementary Table S3**). Among these groups, nine groups had genes start from *O. sativa*, suggesting that SPL genes in these groups may have occurred prior to the split of monocots and dicots; four groups had genes from *A. thaliana* but absent in *O. sativa*, implying that they originated after the divergence between monocots and dicots; ten groups had genes only existed in genus *Ipomoea*, indicating that they may originated in the common ancestor of *Ipomoea* lineage. Furthermore, the potential functions of some *Ipomoea* SPL genes could be inferred from their orthologs in *O. sativa*, *A. thaliana* and *S. lycopersicum* species.

To understand the divergence of *Ipomoea* SPL genes, the Ka, Ks and Ka/Ks ratio for all orthologous groups were calculated using PAML software [36] (**Figure 4c, Supplementary Table S4**). As a result, the mean Ka/Ks values for all clades were lower than 1.0, suggesting that *Ipomoea* SPL genes were evolved under the pressure of purifying selection. Genes in clade VIII showed the lowest mean Ka/Ks values (0.15) than those in other clades, indicating that they may evolve under the strong positive selection. In contrast, genes in subclade IV-b and IV-c exhibited the highest Ka/Ks values, implying that these two subclades have generally diverged much more rapidly than other clades.

2.5 miR156 target site of *Ipomoea* SPL genes

Using the publicly available miRNA transcriptomes (**Supplementary Table S5**), a total of 9 lbmiR156 members were identified in *I. batatas* (**Figure 5a**). To explore the roles of miR156-mediated post-transcriptional regulation of SPLs in the genus *Ipomoea*, the transcripts of all the 105 *Ipomoea* SPL genes were searched for the target site of miR156 using psRNATarget [37]. As a result, a total of 69 SPL genes was found to be the potential targets of miR156, including 10 *InSPLs*, 19 *ItbSPLs*, 18 *ItfSPLs* and 22 *IbSPLs* (**Figure 5b, Supplementary Table S1**). Among the miR156 target SPL genes, most of which (84 %) the sites recognized by miR156 were located downstream of SBP domain in the CDS region, then followed by the 3'-UTR (**Figure 5b**).

To further validate the predicted miR156-SPL interactions in *I. batatas*, the publicly available degradomes were used (**Supplementary Table S5**). The results showed that seven miR156-SPL interactions predicted by psRNATarget were confirmed by degradome sequencing (**Figure 5c, Supplementary Table S1**). To be more specific, the miR156 target sites of *IbSPL 9*, *IbSPL 10*, *IbSPL 12*, and *IbSPL 15* were located in the CDS region, while the target sites of *IbSPL 16*, *IbSPL 17*, and *IbSPL 28* were located in the 3'-UTR region.

2.6 *Cis*-acting elements in promoters of *Ipomoea* SPL genes

To understand the regulatory mechanism and potential function of *Ipomoea* SPL genes, *cis*-acting elements were analyzed in the 2000 bp upstream sequence from the start codon for all SPL genes by using PlantCARE database [38]. A total of 4088 putative *cis*-acting elements were identified, and then were divided into four categories: light responsiveness, plant growth, phytohormone, and abiotic/biotic stress response (**Supplementary Table S6, S7**). As shown in **Figure 6a**, SPL genes in the same clades exhibited similar *cis*-acting element compositions in the promoter, indicating their conserved biological functions during the evolution in the *Ipomoea*. Among these categories, abiotic/biotic stress response category covered the largest portion (43.66%), followed by the light response (25.93%), phytohormone response (20.77%), and plant growth (9.64%) category (**Figure 6b**). In the abiotic/biotic stress response category, elements such as MYB/MYC (responds to abiotic stress signals), STRE (metal-responsive element), WUN-motif (wound-responsive element) and LTR (low-temperature-responsive element) were found. In the light responsiveness category, a series of elements were identified, such as Box 4, G-box, GT1-motif, TCT-motif, GATA-motif, and MRE, of which Box 4 motif was the most common (24%). As for the phytohormone response category, the ABRE element (responds to ABA), CGTCA-motif (responds to MeJA), ERE (responds to ethylene), TCA-element (responds to salicylic acid), and as-1 (responds to auxin) were the most common *cis*-acting elements, appeared in more than 60 *Ipomoea* SPL genes. In the plant growth category, ARE element essential for the anaerobic induction, CAT-box related to meristem expression, and O₂-site involved in zein metabolism regulation were the three major elements. In conclusion, the *cis*-acting elements analysis suggested that *Ipomoea* SPL genes participated in various biological processes.

2.7 Expression profiles of *IbSPL* genes in different tissues

Among the four *Ipomoea* species, *I. batatas* is the most important cultivated crop worldwide. To explore the putative roles of the *IbSPL* genes, the tissue-specific expression patterns of *IbSPLs* were analyzed in 8 tissues (four aboveground and four underground tissues) of two sweet potato cultivars (Xuzi3 and Yan252) using publicly available transcriptomic data (**Supplementary Table S8**) [39]. FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values were calculated and then used to evaluate gene expression levels (**Supplementary Table S9**). As shown in **Figure 7a**, the expression patterns of *IbSPLs* could be classified into three groups. The first group included 7 *IbSPL* genes (*IbSPL 6*/*IbSPL 15*/*IbSPL 22*/*IbSPL 23*/*IbSPL 24*/*IbSPL 25*/*IbSPL 26*), with lowest expression levels ($\log_2(\text{FPKM}) < 2$) in all tissues. The second group was expressed at relatively high levels in all tissues, which was composed of 5 *IbSPL* genes (*IbSPL 4*/*IbSPL 8*/*IbSPL 11*/*IbSPL 17*/*IbSPL 20*). The remaining 17

IbSPL genes belonging to the third group, which were highly expressed in some aboveground tissues, especially in shoot or young leaf. In addition, the gene expression profiles in underground tissues (fibrous and tuberous root) were investigated, and a total of 7 *IbSPL* genes were found to be expressed at a high level in underground tissues (mean FPKM > 10), such as *IbSPL 1*, *IbSPL 4*, *IbSPL 11*, *IbSPL 17*, *IbSPL 20*, *IbSPL 21* and *IbSPL 28*, implying their potential functions in root development. Furthermore, our results showed that *IbSPL* genes within the same clades exhibit distinctive expression patterns, such as *IbSPL 14*, *IbSPL 17*, *IbSPL 28*, and *IbSPL 29* in clade VI.

To further investigate the expression profiles of *IbSPL* genes in underground tissues, publicly available transcriptomes of eight different stages during root development from cultivar “Beauregard” were used (**Figure 7b, Supplementary Table S8, S9**). The results showed that overall expression patterns of *IbSPL* genes in roots of cultivar “Beauregard” were similar to that in roots of cultivar “Xuzi3” and “Yan252”. To be specific, the expression levels of *IbSPL 17*, *IbSPL 28*, and *IbSPL 29* were the highest in storage root compared with undifferentiated and fibrous root; the expression level of *IbSPL 1* was the highest in undifferentiated root; the expression level of *IbSPL 10* was the highest in fibrous root; the expression levels of *IbSPL 4*, *IbSPL 8*, *IbSPL 11*, *IbSPL 20*, and *IbSPL 21* had no distinct variation in all tissues. Our results implied that these genes may play important roles in root development.

To confirm the expression patterns of *IbSPLs* derived from transcriptomic data, qRT-PCR analysis was performed for 11 selected *IbSPL* genes (*IbSPL 1*, *IbSPL 4*, *IbSPL 5*, *IbSPL 12*, *IbSPL 16*, *IbSPL 17*, *IbSPL 20*, *IbSPL 21*, *IbSPL 27*, *IbSPL 28*, and *IbSPL 29*) in 11 tissues of cultivar “Xuyu34” (**Figure 8**). The results showed that the expression patterns of *IbSPL* genes were consistent between transcriptomic data and qRT-PCR results. We found the expression levels for *IbSPLs* were quite different in various tissues. For example, *IbSPL 27* and *IbSPL 29* were highest expressed in young leaf; *IbSPL 12*, *IbSPL 16*, and *IbSPL 21* were highest expressed in flower. Moreover, the expression levels of *IbSPL 17* and *IbSPL 28* were gradually increased during root development, indicating their differential roles in storage root development in sweet potato.

2.8 *IbSPL* genes in response to exogenous phytohormones

Phytohormones are known as regulators in plant growth and developmental processes, and the promoter analysis revealed that *IbSPL* genes might be regulated by various phytohormones. To reveal the potential roles of the *IbSPLs* in hormonal signaling pathways, qRT-PCR was used to analyze the expression of the 6 *IbSPL* genes (*IbSPL 1*, *IbSPL 4*, *IbSPL 16*, *IbSPL 17*, *IbSPL 21* and *IbSPL 28*) under exogenous phytohormone treatments, including indole-3-acetic acid (IAA), methyl-Jasmonate (MeJA), zeatin (ZT), and abscisic acid (ABA). In general, expression analysis indicated that the 6 *IbSPL* genes exhibited highly divergent response patterns under the phytohormone treatment in root (**Figure 9**). Under IAA treatment, *IbSPL 16* and *IbSPL 21* were rapidly upregulated after 6 h of IAA treatment, while the other four genes were upregulated after 12 or 24 h of IAA treatment. Under MeJA treatment, all *IbSPL* genes were significantly upregulated after 48 h of MeJA treatment, of which *IbSPL 4* and *IbSPL 21* were upregulated 45.3 and 178.1 folds. Under ZT treatment, all *IbSPL* genes were highly upregulated after 6 h of ZT treatment, then gradually declined,

and finally were expressed at least 10 folds than CK. In particular, *IbSPL21* showed extremely positive response to ZT treatment. Under ABA treatment, all examined *IbSPL* genes, particularly *IbSPL1*, *IbSPL4*, *IbSPL16*, *IbSPL17*, and *IbSPL21*, were rapidly upregulated after 6 h of ABA treatment, whereas *IbSPL28* was significantly upregulated after 24 h of ABA treatment.

2.9 Regulatory sub-networks involving *IbSPLs* and other *I. batatas* genes in the storage root of sweet potato

To identify the regulatory sub-networks involving *IbSPLs* in the storage root of sweet potato, weighted gene co-expression network analysis (WGCNA) was performed based on transcriptomic data of mature storage root from 88 sweet potato accessions (**Supplementary Figure S6, Supplementary Table S11**). A total of 19 modules were obtained, among which three modules (turquoise, blue and yellow) were found contain 8 *IbSPL* genes in total (**Figure 10a**). In the yellow module, *IbSPL20* had only one co-expressed gene. In the blue module, *IbSPL9* and *IbSPL10* were co-expressed with 17 and 107 genes, respectively. In the turquoise module, *IbSPL1*, *IbSPL16*, *IbSPL17*, *IbSPL21*, and *IbSPL28* had 12, 147, 370, 732, and 198 co-expressed genes, respectively (**Supplementary Table S12**). Interestingly, *IbSPL16*, *IbSPL17*, *IbSPL21*, and *IbSPL28* in the turquoise module share 101 co-expressed genes (**Figure 10b**), indicating they may belong to the same biological process.

To further explore putative functions of the SPLs in the storage root, GO enrichment analysis was performed for the co-expressed genes. For the *IbSPL1*, *IbSPL20* and *IbSPL9*, we can't obtain GO enrichment results due to the small number of co-expressed genes. For the *IbSPL10* in the blue module, co-expressed genes related to "response to chitin" and "response to organonitrogen compound" were enriched (**Supplementary Table S13**). For the *IbSPL16*, *IbSPL17*, *IbSPL21*, and *IbSPL28* in the turquoise module, the similar GO terms were enriched, such as "regulation of root morphogenesis", "cell division", "cytoskeleton organization", "plant-type cell wall organization or biogenesis", and "cellulose biosynthetic process" *etc.* (**Figure 10c, Supplementary Table S13**). It is known that the cytoskeleton, cell division, and cell wall organization/biogenesis are important biological processes in the storage root formation and development [40]. Our results indicated that *IbSPL16/IbSPL17/IbSPL21/IbSPL28* may play a key role in storage root development in sweet potato.

3. Discussion

The *SPL* genes are important plant-specific transcription factors with highly conserved SBP domain. Since the *SPL* genes was first discovered in *A. majus*, more and more members of *SPL* gene family have been identified in plants [6–12]. However, comprehensive molecular evolutionary and function analysis of the *SPL* genes in genus *Ipomoea* have not been reported. The genus *Ipomoea* has significant nutritional and economic value for human, including the seventh most important crop *I. batatas* and ornamental plant *I. nil*. Up to now, four species in *Ipomoea* have been sequenced, including *I. nil*, *I. triloba*, *I. trifida* and *I. batatas*. Utilizing these genomes, we systematically analyzed the *Ipomoea SPL* genes, such as molecular characteristics, evolutionary process, posttranscriptional regulation and physiological function.

3.1 Comparative analysis of *SPL* genes in the genus *Ipomoea*

Recently, some important gene families of transcription factors have been investigated in *Ipomoea* species, such as bZIPs [41], WRKYs [42], GRPs [43], MADS-[44] and DEAD-box [45]. However, these studies only focus on gene family in a single *Ipomoea* species, the comparative analysis of gene family in genus *Ipomoea* are still rare. In our study, to dissect the evolutionary dynamics of the *SPL* genes in genus *Ipomoea*, *SPL* genes in four *Ipomoea* species were identified, including 29 *IbSPLs*, 27 *ItfSPLs*, 26 *ItbSPLs* and 23 *IniSPLs* (Fig. 1). It should be noted that the number of *SPL* genes in sweet potato almost equals to that in its three diploid wild relatives (*I. nil*, *I. triloba*, and *I. trifida*) due to the haplotype-resolved genome assembly of hexaploid sweet potato [28]. In accordance with the classification in *A. thaliana*, *S. lycopersicum* and *O. sativa* [7–9], the *Ipomoea* *SPL* genes were also divided into eight clades (Fig. 2). We observed that clade IV was the largest containing 32 members, and clade I was the smallest containing only four members. By gene and protein structure analysis, it was found that most *Ipomoea* *SPLs* from the same phylogenetic clades share the similar intron/exon structures, domain organizations, and motif compositions (Fig. 3), indicating that *SPLs* within the same clade may have similar functions in *Ipomoea* species. Interestingly, except for the conserved domain and motifs presented in all *SPL* proteins, some other domains or motifs were found in clade I, II and IV, such as DEXDc domain (motif 4) and Ankyrin repeats (motif 8), which was also observed in papaya [46] and barley [47]. Our results suggesting that the *SPLs* in these clades may have undergone functional differentiation and neofunctionalization during evolution.

Ipomoea species were found possess more *SPL* genes than that in dicotyledonous model plants, such as *A. thaliana* and *S. lycopersicum* [7, 9], implying genus-specific expansion of *SPL* gene family occurred in *Ipomoea*. It is known that the expansion of gene family is mainly the result of duplication events during the evolution [48]. Our results showed that segmental duplication play a major role in the evolution and expansion of *Ipomoea* *SPL* genes (Fig. 4b), which was consistent with findings in cotton [49], *Rosacea* species [50] and *Euphorbiaceae* species [51]. Previous study revealed that a whole-genome triplication (WGT) event occurred 46.1 million years ago (Mya) in the progenitor of the genus *Ipomoea* [28], and we proposed that segmental duplication of *SPLs* may be derived from the WGT event. Furthermore, we found that tandem duplication was the most frequent event in IV-b and IV-c subclades (**Supplementary Table S1**), and no orthologs were found in dicots (**Supplementary Table S3**), implying that *SPL* members in these two subclades were tandem duplicated from a recent event. We speculated that *SPL* genes undergone replication expansion in the progenitor of the genus *Ipomoea*, and different *SPL* members were retained due to their important role in growth and development during the *Ipomoea* species differentiation. In addition, the Ka/Ks ratios were less than 1 for all *Ipomoea* *SPL* ortholog gene pairs, indicating that *Ipomoea* *SPL* genes were under strong purifying selection (**Supplementary Table S4**).

Many *SPL* genes are targeted by miR156, thus forming a functional regulatory network of miR156-*SPL*, which play an important role in plant growth and development [2, 21, 22]. It has been reported that more than half of *SPL* gene family members was targeted by miR156 in various plant species, such as rice [8],

tomato [9], and apple [10]. In this study, two-thirds of *SPL* genes in each *Ipomoea* species were predicted as the targets of miR156 (Fig. 5b). Phylogenetic analysis showed that all the members of *SPL* genes in clades III lacking miR156 binding sites, consistent with the results from *A. thaliana*, rice, and tomato [4]. In addition, two kinds of binding site of miR156 were identified in *SPL* genes, one locates in CDS and the other locates in 3'-UTR (Fig. 5b), which was also observed in other plants, such as rice [8], tomato [9], apple [10] and papaya [46]. With the degradome data in *I. batatas*, 7 *IbSPL* genes were further confirmed as the targets of miR156 (Fig. 5c, **Supplementary Table S1**). Among these *IbSPL* genes, the miR156 binding site for *IbSPL17* and *IbSPL28* was located in 3'-UTR, and the miR156 binding site of their orthologous genes (*AtSPL3* and *LeSPL-CNR*) in *A. thaliana* and tomato are also located in 3'-UTR [5, 52], suggesting the high conservation of miR156-SPL regulatory module in plants. However, most of the miR156-SPL interactions in this study were predicted by in silico analysis, more experimental verification needs to be performed for the miR156-SPL interactions in genus *Ipomoea*.

3.2 *IbSPL* genes associated with storage root development

Sweet potato, is the seventh most important crop globally, has a strong adaptability, stable yield, and high nutritional value [25]. The storage root of sweet potato is the most important economic trait in nutrient content and yield, and dissecting the mechanisms underlying the formation and development of storage roots is of great significance to improve the nutrient content and yield of sweet potato. Considering the key regulatory roles of *SPL* genes in root architecture [13, 14] and biomass enhancement [20], the expression patterns of *IbSPL* genes at different tissues or developmental stages were evaluated. Using the public transcriptome data, we found that most of the *IbSPLs* were highly expressed in aboveground tissues, especially in shoot or young leaf; however, only some *IbSPLs* were found to be expressed at a high level in underground tissues (Fig. 7). With a qRT-PCR assay, the expression levels of three *IbSPL* genes (*IbSPL17/IbSPL28*) were obviously increased with storage development (Fig. 8). The results from our study provide evidence that *SPL* genes may have important functions during the storage root development in sweet potato.

The formation and development of storage roots is a complex physiological process that includes cessation of root elongation, genesis and development of the primary and secondary vascular cambium, increase in radial growth, and accumulation of starch and storage proteins [53]. It is well known that these processes are closely related to the endogenous phytohormones, such as IAA, cytokinins (CTKs), JA, and ABA [40, 53–55]. For instance, IAA involved in the early stage of storage root formation and the primary thickening of storage roots [56], ABA had a significant role in storage root bulking by activating cell division [57], and CTKs played a key role in the initiation and expansion rate of storage roots as a prerequisite for cambial cell proliferation [40]. It is also reported that storage root yields are positively correlated with the contents of ABA and CTKs [58]. In the present study, different kinds of hormone-responsive elements were found by analyzing the *IbSPL* promoters (Fig. 6), implying *IbSPL* genes may participate in hormonal signaling pathways. qRT-PCR further confirmed that the expression of all tested *IbSPLs* (*IbSPL1*, *IbSPL4*, *IbSPL16*, *IbSPL17*, *IbSPL21* and *IbSPL28*) was strongly induced under exogenous phytohormone treatments, especially ZT and ABA, suggesting their crucial roles in root development.

The formation and development of storage roots are maintained by coordinated cellular behaviors, such as cell division, expansion, and differentiation. Previous studies revealed that cell wall biosynthesis and cytoskeleton organization are critical in these cellular behaviors [59, 60]. In this study, regulatory sub-networks analyzed by WGCNA indicated that 8 *IbSPL* genes were co-expressed with at least one other *I. batatas* genes (Fig. 10a). GO enrichment analysis of co-expressed genes revealed that these *IbSPL* genes may be involved in stress responses, root morphogenesis, and cell division (**Supplementary Table S13**). Moreover, genes co-expressed with *IbSPL 16/IbSPL 17/IbSPL 21/IbSPL 28* in the turquoise module were all significantly enriched for “regulation of root morphogenesis”, “cell division”, “cytoskeleton organization”, “plant-type cell wall organization or biogenesis”, and “cellulose biosynthetic process”. These enriched processes are essential for cell morphogenesis and cell cycles, implying their key roles in storage root development. By combining the results from expression patterns under different root developmental stages or various phytohormones treatments and regulatory sub-networks, we concluded that *IbSPL 16/IbSPL 17/IbSPL 21/IbSPL 28* may play important role in the formation and development of storage roots. In the future, functional characterization is needed to elucidate the specific roles of *IbSPLs* in storage root development.

4. Conclusions

In summary, we performed a genome-wide analysis of *SPL* gene family in four *Ipomoea* species, including *I. batatas*, *I. trifida*, *I. triloba*, and *I. nil*. A total of 105 *Ipomoea SPL* genes were identified, and could be divided into eight clades. Genes in one clade were found harbor similar gene structures, domain organizations, motif compositions and cis-acting elements, suggesting similarity of biological function potentially. Segmental duplication was the major force accounting for the expansion of *Ipomoea SPL* gene family. By combining the results from expression patterns and regulatory sub-networks, we concluded that *IbSPL 16/IbSPL 17/IbSPL 21/IbSPL 28* may play important role in the formation and development of storage roots. Taken together, our results not only provide novel insights into the evolutionary and functional divergence of the *SPL* genes in genus *Ipomoea*, but also lay a foundation for further elucidation of the potential functional roles of *IbSPL* genes during storage root development.

5. Materials And Methods

5.1 Identification of *SPL* genes in *I. nil*, *I. triloba*, *I. trifida*, and *I. batatas*

The genomes of four *Ipomoea* species (including *I. nil*, *I. triloba*, *I. trifida* and *I. batatas*) were downloaded from “*Ipomoea nil* Genome Project” (<http://viewer.shigen.info/asagao>) [27], “Sweetpotato Genomic Resource” (<http://sweetpotato.plantbiology.msu.edu>) [29] and “*Ipomoea* Genome Hub” (<http://ipomoea-genome.org>) [28], respectively. To identify the *SPL* genes in above four species, three methods described below were used. First, all of the 16 *A. thaliana* *SPL* proteins (<https://www.arabidopsis.org/>) were used as queries to find *SPL* homologs using BLASTP program with a threshold of e-value < 1e-3. Second, the Hidden Markov Model (HMM) of SBP (PF03110) domain was downloaded from Pfam database [61] and was used to identify putative *SPL* proteins by hmmsearch [61] program. Finally, all of the candidate *SPL*

proteins obtained from BLASTP and hmmsearch were submitted to SMART [62] and ScanProsite database [63] to confirm the presence of SBP domain. Those proteins lacking SBP domain were excluded, and the remaining were considered as the SPL proteins. In addition, the structures of identified *IbSPLs* were further examined manually to correct genome assembly errors.

The BUSCA online software [64] was used to predict subcellular localization of *Ipomoea* SPL proteins. An in-house Perl script was used to analysis the physical and chemical properties of *Ipomoea* SPL proteins, such as protein length, molecular weight (MW, kD) and isoelectric point (pI).

5.2 Phylogenetic analysis of SPL proteins

For the SPL proteins from *C. reinhardtii*, *A. thaliana*, *S. lycopersicum*, *O. sativa*, and the four *Ipomoea* species, phylogenetic analysis was performed using the following steps: first, the mafft software (v7.45) [65] was used to perform the multiple alignment for full-length SPL proteins; second, the Gblocks program (v0.71b) [66] was used to select conserved blocks from the multiple alignment; third, MEGA X software [67] was used to construct a neighbor-joining phylogenetic tree with 1000 bootstrap replications, CRR1 protein from *C. reinhardtii* was set as an outgroup; finally, the tree was visualized using Evolvew website [68]. For the *Ipomoea* SPL proteins, a neighbor-joining phylogenetic tree was constructed using the methods as described above.

5.3 Analysis of the gene structure, protein domain and motif

The exon/intron positions of all *Ipomoea* SPL genes were obtained based on the GFF3 files downloaded from the genomic database. The domain organizations of *Ipomoea* SPL proteins were annotated based on the results from SMART database [62]. The conserved sequences in each domain were shaded at four levels by GeneDoc. The motif compositions of *Ipomoea* SPL proteins were analyzed by MEME online database [69], with the maximum number set to 10. Finally, the gene structure, domain organization, and motif composition were drawn using Tbtools [70].

5.4 Gene duplication, orthology and selection analysis

The MCScanX software [34] was used to identify collinear blocks within or between species, and then classify the *SPL* genes into five different types: singleton, dispersed, proximal, tandem and segmental duplication. The synteny relationships of the collinearity blocks in each *Ipomoea* species were visualized using Circos [71]. The OrthoMCL software [35] was used to detect orthologous groups between diverse *SPL* genes. For each orthologue gene pairs, the Ks (synonymous substitution rate), Ka (nonsynonymous substitution rate) and Ka/Ks ratio (evolutionary constraint) were calculated by PAML [36].

5.5 Prediction of miR156-targeted genes

The publicly available datasets were used to identify miR156 sequence in *Ipomoea*. First, a total of 58 miRNA transcriptomes deposited in NCBI [72] (16 in PRJNA471495, 2 in PRJNA474012, 11 in PRJNA592001, 12 in PRJNA599544, 12 in PRJNA600587, 5 in PRJNA638516) were collected from previous studies (**Supplementary Table S5**) [73-76]. Then, Trimmomatic (version 0.39) [77] was used to filter the raw miRNA sequencing data to remove low-quality reads and sequencing adaptors. Finally, using *I. batatas* as the reference genome, miRDeep2 (version 1.1.4) pipeline [78] was employed to identify miR156 sequence with default parameters.

For the *Ipomoea* SPL genes, the miR156 target sites were predicted using the psRNATarget server [37] with default settings. To validate the predicted miR156-SPL interactions in *I. batatas*, a total of 5 degradomes (1 in PRJNA592001, 4 in PRJNA600587) [74, 76] were downloaded from the public database were used (**Supplementary Table S5**). After filtering out the low-quality reads and sequencing adaptors, CleaveLand4 pipeline [79] was used to identify miR156 cleavage sites. The identified targets with category 0-3 and *p*-value < 0.05 were considered as the reliable miR156 target genes.

5.6 Promoter analysis of *Ipomoea* SPL genes

The 2000 bp upstream sequence from the start codon for all *Ipomoea* SPL genes were retrieved from the genome using an in-house Perl script, and then were submitted to PlantCARE program [38] for predicting *cis*-acting elements as previously described [80]. The distribution of *cis*-acting elements in each promoter was drawn using TBtools [70].

5.7 Plant materials and hormone treatments

Sweet potato (*I. batatas* cv. Xuyu34) plants used in this study were provided by the Xuzhou Academy of Agricultural Sciences, Xuzhou, Jiangsu, China. According to institutional, national and international guidelines, these samples do not require specific permissions for research purposes. The plants were grown in greenhouses on the campus of Jiangsu Normal University, Xuzhou, China. For organ-specific expression analysis, the tissues of young leaves, mature leaves, flowers, roots (10DAT, 20DAT, 40DAT, 60DAT, 80DAT, 90DAT and 100DAT roots with 0.3 mm, 2mm, 7mm, 25mm, 37mm, 52mm and 60mm in diameter, respectively) were collected. For hormone treatments, adventitious roots were first induced from stem cuttings grown hydroponically for ten days, and then were treated with 100 μ M ABA, IAA, ZT, and MeJA, respectively. Adventitious roots were collected at 0, 6, 12, 24, and 48h post the treatment. Three biological replicates were collected for each sample. All samples were frozen in liquid nitrogen and finally stored at -80°C for subsequent use.

5.8 RNA extraction and qRT-PCR analysis

Total RNA for each sample was extracted using RNAPure Plant Kit (CWBio, Beijing, China), according to the manufacturers' instructions. The first strand cDNA was synthesized from 1.0 μ g total pure RNA using the

HiFiScript cDNA Synthesis Kit (CWBio, Beijing, China). Gene-specific primers for each *IbSPL* gene were designed using primer3 [81]. The qRT-PCR was performed on Bio-rad CFX Connect™ Real-Time System (Bio-Rad, CA, USA) using 2 × Q3 SYBR qPCR Master Mix (Universal) (Tolo Biotechnology, Shanghai, China) premix. The *IbARF* gene was used as a reference gene for normalizing the expression levels [82]. The relative transcript abundance for each *IbSPL* gene was calculated using the $2^{-\Delta\Delta CT}$ approach [83]. Statistical analysis was conducted by ANOVA followed by LSD test, and different letters indicate statistically significant difference at $p < 0.05$. The used primers were listed in **Supplementary Table S10**.

5.10 Analysis of expression patterns of *IbSPLs* using published transcriptomic data

To explore tissue- and developmental stage-specific expression patterns of *IbSPL* genes, publicly available transcriptome datasets from two previous studies [29, 39] (**Supplementary Table S8**) were used: one was 8 different tissues from cultivar Xuzi3 and Yan252 under BioProject accession number PRJCA000640 in NGDC [84], and the other was 8 different stages during root development from cultivar Beauregard under BioProject accession number PRJNA491292 in NCBI [72]. Transcriptome analysis was performed according to our previous study [26]. The downloaded raw fastq files were filtered using Trimmomatic (version 0.39) [77], and then were mapped to sweet potato genome Taizhong6 [28] using STAR (version 2.7.1a) software under the 2-pass mapping mode [85]. RSEM [86] was used to calculate FPKM values for each gene. Finally, heatmap was plotted based on the normalized expression values of 29 *IbSPL* genes using pheatmap package in R.

5.11 Construction of co-expression networks involving *IbSPL* and other *I. batatas* genes in the storage root of sweet potato

Transcriptomic dataset of mature storage roots of 88 sweet potato accessions were obtained from a previous study (**Supplementary Table S11**) [39] under BioProject accession number PRJCA000642 in NGDC [84]. Weighted co-expression network construction and module detection were performed using the R package WGCNA (version 1.4.9) [87] with the following parameters: the power was set as 9, the minModuleSize was set as 30, the cutHeight was set as 0.25, and the network module export weight threshold was set as 0.05. The sub-network was subsequently visualized using Cytoscape [88]. *eggNOG-mapper* (version 2) [89] was used to assign the functional annotation of all sweet potato genes, and Clusterprofiler [90] was used to perform GO enrichment analysis for genes co-expressed with *IbSPLs* with adjusted P -value < 0.05 .

List Of Abbreviations

Abbreviation	Explanation
SPL	SQUAMOSA promoter-binding protein-like
MW	Molecular Weight
pI	The isoelectric point
FPKM	Fragments Per Kilobase of transcript per Million mapped reads
IAA	Indole-3-cetic Acid
MeJA	Methyl-Jasmonate
ABA	Abscisic Acid
ZT	Zeatin
WGCNA	Weighted Gene Co-expression Network Analysis

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated or analysed during this study are included in this published article (and its supplementary information files), or are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Natural Science Foundation of Jiangsu Province (No. BK20190995), the Shanghai Municipal Afforestation & City Appearance and Environmental Sanitation Administration (No. G212402), the Natural Science Foundation of Xuzhou City (No. KC19070), Postgraduate Research & Practice Innovation Program of Jiangsu Province (No. KYCX20_2319), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). All the funders had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

L.Z., S.W., and T.X. conceived and designed the research. H.S., J.M., L.Z., W.H., and Y.Z. performed the research and analyzed the data. L.Z. and H.S. wrote the manuscript. All authors read and approved the manuscript.

Acknowledgements

We would like to thank Dr. Meng Kou (Xuzhou academy of agricultural sciences) for providing the sweet potato (*I. batatas* cv. Xuyu34) plants.

References

1. Guo AY, Zhu QH, Gu X, Ge S, Yang J, Luo J: **Genome-wide identification and evolutionary analysis of the plant specific SBP-box transcription factor family**. *Gene* 2008, **418**(1–2):1–8.
2. Wang H, Wang H: **The miR156/SPL Module, a Regulatory Hub and Versatile Toolbox, Gears up Crops for Enhanced Agronomic Traits**. *Molecular plant* 2015, **8**(5):677–688.
3. Chen X, Zhang Z, Liu D, Zhang K, Li A, Mao L: **SQUAMOSA promoter-binding protein-like transcription factors: star players for plant growth and development**. *Journal of integrative plant biology* 2010, **52**(11):946–951.
4. Preston JC, Hileman LC: **Functional Evolution in the Plant SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) Gene Family**. *Frontiers in plant science* 2013, **4**:80.
5. Chen W, Kong J, Lai T, Manning K, Wu C, Wang Y, Qin C, Li B, Yu Z, Zhang X *et al*: **Tuning LeSPL-CNR expression by SlymiR157 affects tomato fruit ripening**. *Scientific reports* 2015, **5**:7852.
6. Klein J, Saedler H, Huijser P: **A new family of DNA binding proteins includes putative transcriptional regulators of the *Antirrhinum majus* floral meristem identity gene SQUAMOSA**. *Mol Gen Genet* 1996, **250**(1):7–16.
7. Cardon G, Hohmann S, Klein J, Nettesheim K, Saedler H, Huijser P: **Molecular characterisation of the *Arabidopsis* SBP-box genes**. *Gene* 1999, **237**(1):91–104.

8. Xie K, Wu C, Xiong L: **Genomic organization, differential expression, and interaction of SQUAMOSA promoter-binding-like transcription factors and microRNA156 in rice.** *Plant physiology* 2006, **142**(1):280–293.
9. Salinas M, Xing S, Hohmann S, Berndtgen R, Huijser P: **Genomic organization, phylogenetic comparison and differential expression of the SBP-box family of transcription factors in tomato.** *Planta* 2012, **235**(6):1171–1184.
10. Li J, Hou H, Li X, Xiang J, Yin X, Gao H, Zheng Y, Bassett CL, Wang X: **Genome-wide identification and analysis of the SBP-box family genes in apple (*Malus x domestica* Borkh.).** *Plant physiology and biochemistry: PPB / Societe francaise de physiologie vegetale* 2013, **70**:100–114.
11. Zhang HX, Jin JH, He YM, Lu BY, Li DW, Chai WG, Khan A, Gong ZH: **Genome-Wide Identification and Analysis of the SBP-Box Family Genes under *Phytophthora capsici* Stress in Pepper (*Capsicum annuum* L.).** *Frontiers in plant science* 2016, **7**:504.
12. Tripathi RK, Goel R, Kumari S, Dahuja A: **Genomic organization, phylogenetic comparison, and expression profiles of the SPL family genes and their regulation in soybean.** *Dev Genes Evol* 2017, **227**(2):101–119.
13. Yu N, Niu QW, Ng KH, Chua NH: **The role of miR156/SPLs modules in Arabidopsis lateral root development.** *The Plant journal: for cell and molecular biology* 2015, **83**(4):673–685.
14. Barrera-Rojas CH, Rocha GHB, Polverari L, Pinheiro Brito DA, Batista DS, Notini MM, da Cruz ACF, Morea EGO, Sabatini S, Otoni WC *et al*: **miR156-targeted SPL10 controls Arabidopsis root meristem activity and root-derived de novo shoot regeneration via cytokinin responses.** *J Exp Bot* 2020, **71**(3):934–950.
15. Shao Y, Zhou HZ, Wu Y, Zhang H, Lin J, Jiang X, He Q, Zhu J, Li Y, Yu H *et al*: **OsSPL3, an SBP-Domain Protein, Regulates Crown Root Development in Rice.** *The Plant cell* 2019, **31**(6):1257–1275.
16. Xu X, Li X, Hu X, Wu T, Wang Y, Xu X, Zhang X, Han Z: **High miR156 Expression Is Required for Auxin-Induced Adventitious Root Formation via MxSPL26 Independent of PINs and ARFs in *Malus xiaojinensis*.** *Frontiers in plant science* 2017, **8**:1059.
17. Yamaguchi A, Wu MF, Yang L, Wu G, Poethig RS, Wagner D: **The microRNA-regulated SBP-Box transcription factor SPL3 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1.** *Developmental cell* 2009, **17**(2):268–278.
18. Manning K, Tor M, Poole M, Hong Y, Thompson AJ, King GJ, Giovannoni JJ, Seymour GB: **A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening.** *Nature genetics* 2006, **38**(8):948–952.
19. Orfila C, Huisman MM, Willats WG, van Alebeek GJ, Schols HA, Seymour GB, Knox JP: **Altered cell wall disassembly during ripening of *Cnr* tomato fruit: implications for cell adhesion and fruit softening.** *Planta* 2002, **215**(3):440–447.
20. Wang S, Li S, Liu Q, Wu K, Zhang J, Wang S, Wang Y, Chen X, Zhang Y, Gao C *et al*: **The OsSPL16-GW7 regulatory module determines grain shape and simultaneously improves rice yield and grain quality.** *Nature genetics* 2015, **47**(8):949–954.

21. Gou JY, Felippes FF, Liu CJ, Weigel D, Wang JW: **Negative regulation of anthocyanin biosynthesis in *Arabidopsis* by a miR156-targeted SPL transcription factor.** *The Plant cell* 2011, **23**(4):1512–1522.
22. Liu M, Shi Z, Zhang X, Wang M, Zhang L, Zheng K, Liu J, Hu X, Di C, Qian Q *et al*: **Inducible overexpression of Ideal Plant Architecture1 improves both yield and disease resistance in rice.** *Nature plants* 2019, **5**(4):389–400.
23. Austin DF, Huáman Z: **A synopsis of Ipomoea (Convolvulaceae) in the Americas.** *Taxon* 1996, **45**(1):3–38.
24. Morita Y, Hoshino A: **Recent advances in flower color variation and patterning of Japanese morning glory and petunia.** *Breed Sci* 2018, **68**(1):128–138.
25. Liu Q: **Improvement for agronomically important traits by gene engineering in sweetpotato.** *Breed Sci* 2017, **67**(1):15–26.
26. Zhang L, Yu Y, Shi T, Kou M, Sun J, Xu T, Li Q, Wu S, Cao Q, Hou W *et al*: **Genome-wide analysis of expression quantitative trait loci (eQTLs) reveals the regulatory architecture of gene expression variation in the storage roots of sweet potato.** *Horticulture research* 2020, **7**(1):90.
27. Hoshino A, Jayakumar V, Nitasaka E, Toyoda A, Noguchi H, Itoh T, Shin IT, Minakuchi Y, Koda Y, Nagano AJ *et al*: **Genome sequence and analysis of the Japanese morning glory *Ipomoea nil*.** *Nature communications* 2016, **7**:13295.
28. Yang J, Moeinzadeh MH, Kuhl H, Helmuth J, Xiao P, Haas S, Liu G, Zheng J, Sun Z, Fan W *et al*: **Haplotype-resolved sweet potato genome traces back its hexaploidization history.** *Nature plants* 2017, **3**(9):696–703.
29. Wu S, Lau KH, Cao Q, Hamilton JP, Sun H, Zhou C, Eserman L, Gemenet DC, Olukolu BA, Wang H *et al*: **Genome sequences of two diploid wild relatives of cultivated sweetpotato reveal targets for genetic improvement.** *Nat Commun* 2018, **9**(1):4580.
30. Jiang X, Chen P, Zhang X, Liu Q, Li H: **Comparative analysis of the SPL gene family in five Rosaceae species: *Fragaria vesca*, *Malus domestica*, *Prunus persica*, *Rubus occidentalis*, and *Pyrus pyrifolia*.** *Open Life Sci* 2021, **16**(1):160–171.
31. Zhong H, Kong W, Gong Z, Fang X, Deng X, Liu C, Li Y: **Evolutionary Analyses Reveal Diverged Patterns of SQUAMOSA Promoter Binding Protein-Like (SPL) Gene Family in *Oryza* Genus.** *Frontiers in plant science* 2019, **10**:565.
32. Caruthers JM, McKay DB: **Helicase structure and mechanism.** *Curr Opin Struct Biol* 2002, **12**(1):123–133.
33. Li J, Mahajan A, Tsai MD: **Ankyrin repeat: a unique motif mediating protein-protein interactions.** *Biochemistry* 2006, **45**(51):15168–15178.
34. Wang Y, Tang H, Debarry JD, Tan X, Li J, Wang X, Lee TH, Jin H, Marler B, Guo H *et al*: **MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity.** *Nucleic acids research* 2012, **40**(7):e49.
35. Li L, Stoeckert CJ, Jr., Roos DS: **OrthoMCL: identification of ortholog groups for eukaryotic genomes.** *Genome research* 2003, **13**(9):2178–2189.

36. Yang Z: **PAML 4: phylogenetic analysis by maximum likelihood.** *Molecular biology and evolution* 2007, **24**(8):1586–1591.
37. Dai X, Zhuang Z, Zhao PX: **psRNATarget: a plant small RNA target analysis server** (2017 release). *Nucleic acids research* 2018, **46**(W1):W49-W54.
38. Lescot M, Dehais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouze P, Rombauts S: **PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences.** *Nucleic acids research* 2002, **30**(1):325–327.
39. Ding N, Wang A, Zhang X, Wu Y, Wang R, Cui H, Huang R, Luo Y: **Identification and analysis of glutathione S-transferase gene family in sweet potato reveal divergent GST-mediated networks in aboveground and underground tissues in response to abiotic stresses.** *BMC plant biology* 2017, **17**(1):225.
40. Dong T, Zhu M, Yu J, Han R, Tang C, Xu T, Liu J, Li Z: **RNA-Seq and iTRAQ reveal multiple pathways involved in storage root formation and development in sweet potato (*Ipomoea batatas* L.).** *BMC plant biology* 2019, **19**(1):136.
41. Yang Z, Sun J, Chen Y, Zhu P, Zhang L, Wu S, Ma D, Cao Q, Li Z, Xu T: **Genome-wide identification, structural and gene expression analysis of the bZIP transcription factor family in sweet potato wild relative *Ipomoea trifida*.** *BMC genetics* 2019, **20**(1):41.
42. Li Y, Zhang L, Zhu P, Cao Q, Sun J, Li Z, Xu T: **Genome-wide identification, characterisation and functional evaluation of WRKY genes in the sweet potato wild ancestor *Ipomoea trifida* (H.B.K.) G. Don. under abiotic stresses.** *BMC genetics* 2019, **20**(1):90.
43. Lu Y, Sun J, Yang Z, Zhao C, Zhu M, Ma D, Dong T, Zhou Z, Liu M, Yang D *et al.*: **Genome-wide identification and expression analysis of glycine-rich RNA-binding protein family in sweet potato wild relative *Ipomoea trifida*.** *Gene* 2019, **686**:177–186.
44. Zhu P, Dong T, Xu T, Kang H: **Identification, characterisation and expression analysis of MADS-box genes in sweetpotato wild relative *Ipomoea trifida*.** *Acta Physiologiae Plantarum* 2020, **42**(11):163.
45. Wan R, Liu J, Yang Z, Zhu P, Cao Q, Xu T: **Genome-wide identification, characterisation and expression profile analysis of DEAD-box family genes in sweet potato wild ancestor *Ipomoea trifida* under abiotic stresses.** *Genes Genomics* 2020, **42**(3):325–335.
46. Xu Y, Xu H, Wall MM, Yang J: **Roles of transcription factor SQUAMOSA promoter binding protein-like gene family in papaya (*Carica papaya*) development and ripening.** *Genomics* 2020, **112**(4):2734–2747.
47. Tripathi RK, Bregitzer P, Singh J: **Genome-wide analysis of the SPL/miR156 module and its interaction with the AP2/miR172 unit in barley.** *Scientific reports* 2018, **8**(1):7085.
48. Moore RC, Purugganan MD: **The evolutionary dynamics of plant duplicate genes.** *Current opinion in plant biology* 2005, **8**(2):122–128.
49. Cai C, Guo W, Zhang B: **Genome-wide identification and characterization of SPL transcription factor family and their evolution and expression profiling analysis in cotton.** *Scientific reports* 2018, **8**(1):762.

50. Abdullah M, Cao Y, Cheng X, Shakoor A, Su X, Gao J, Cai Y: **Genome-Wide Analysis Characterization and Evolution of SBP Genes in *Fragaria vesca*, *Pyrus bretschneideri*, *Prunus persica* and *Prunus mume*.** *Frontiers in genetics* 2018, **9**:64.
51. Li J, Gao X, Sang S, Liu C: **Genome-wide identification, phylogeny, and expression analysis of the SBP-box gene family in Euphorbiaceae.** *BMC genomics* 2019, **20**(Suppl 9):912.
52. Gandikota M, Birkenbihl RP, Hohmann S, Cardon GH, Saedler H, Huijser P: **The miRNA156/157 recognition element in the 3' UTR of the Arabidopsis SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings.** *The Plant journal: for cell and molecular biology* 2007, **49**(4):683–693.
53. Ravi V, Naskar SK, Makesh Kumar T, Babu B, Krishnan BP: **Molecular physiology of storage root formation and development in sweet potato (*Ipomoea batatas* (L.) Lam.).** 2009.
54. Tanaka M, Kato N, Nakayama H, Nakatani M, Takahata Y: **Expression of class I knotted1-like homeobox genes in the storage roots of sweetpotato (*Ipomoea batatas*).** *Journal of plant physiology* 2008, **165**(16):1726–1735.
55. Nakatani M: **Changes in endogenous level of zeatin riboside, abscisic acid and indole acetic acid during formation and thickening of tuberous root in sweet potato.** *Jpnjcrop* 1991, **60**.
56. Noh SA, Lee HS, Huh EJ, Huh GH, Paek KH, Shin JS, Bae JM: **SRD1 is involved in the auxin-mediated initial thickening growth of storage root by enhancing proliferation of metaxylem and cambium cells in sweetpotato (*Ipomoea batatas*).** *J Exp Bot* 2010, **61**(5):1337–1349.
57. Huan L, Jin-Qiang W, Qing L: **Photosynthesis product allocation and yield in sweet potato with spraying exogenous hormones under drought stress.** *Journal of Plant Physiology* 2020, **253**:153265.
58. Wang QM, Zhang LM, Wang ZL: **Formation and Thickening of Tuberous Roots in Relation to the Endogenous Hormone Concentrations in Sweetpotato.** *Scientia Agricultura Sinica* 2005.
59. Brasil JN, Costa CNM, Cabral LM, Ferreira PCG, Hemerly AS: **The plant cell cycle: Pre-Replication complex formation and controls.** *Genet Mol Biol* 2017, **40**(1 suppl 1):276–291.
60. Bashline L, Lei L, Li S, Gu Y: **Cell wall, cytoskeleton, and cell expansion in higher plants.** *Molecular plant* 2014, **7**(4):586–600.
61. El-Gebali S, Mistry J, Bateman A, Eddy SR, Luciani A, Potter SC, Qureshi M, Richardson LJ, Salazar GA, Smart A *et al*: **The Pfam protein families database in 2019.** *Nucleic acids research* 2019, **47**(D1):D427–D432.
62. Letunic I, Bork P: **20 years of the SMART protein domain annotation resource.** *Nucleic acids research* 2018, **46**(D1):D493–D496.
63. de Castro E, Sigrist CJ, Gattiker A, Bulliard V, Langendijk-Genevaux PS, Gasteiger E, Bairoch A, Hulo N: **ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins.** *Nucleic acids research* 2006, **34**(Web Server issue):W362–365.
64. Savojardo C, Martelli PL, Fariselli P, Profiti G, Casadio R: **BUSCA: an integrative web server to predict subcellular localization of proteins.** *Nucleic acids research* 2018, **46**(W1):W459–W466.

65. Katoh K, Standley DM: **MAFFT multiple sequence alignment software version 7: improvements in performance and usability.** *Molecular biology and evolution* 2013, **30**(4):772–780.
66. Castresana J: **Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis.** *Molecular biology and evolution* 2000, **17**(4):540–552.
67. Kumar S, Stecher G, Li M, Knyaz C, Tamura K: **MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms.** *Molecular biology and evolution* 2018, **35**(6):1547–1549.
68. Subramanian B, Gao S, Lercher MJ, Hu S, Chen WH: **Evolview v3: a webserver for visualization, annotation, and management of phylogenetic trees.** *Nucleic acids research* 2019, **47**(W1):W270-W275.
69. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS: **MEME SUITE: tools for motif discovery and searching.** *Nucleic acids research* 2009, **37**(Web Server issue):W202-208.
70. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R: **TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data.** *Molecular plant* 2020, **13**(8):1194–1202.
71. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA: **Circos: an information aesthetic for comparative genomics.** *Genome research* 2009, **19**(9):1639–1645.
72. Coordinators NR: **Database resources of the National Center for Biotechnology Information.** *Nucleic acids research* 2018, **46**(D1):D8-D13.
73. Saminathan T, Alvarado A, Lopez C, Shinde S, Gajanayake B, Abburi VL, Vajja VG, Jagadeeswaran G, Raja Reddy K, Nimmakayala P *et al*: **Elevated carbon dioxide and drought modulate physiology and storage-root development in sweet potato by regulating microRNAs.** *Functional & integrative genomics* 2019, **19**(1):171–190.
74. Yang Z, Zhu P, Kang H, Liu L, Cao Q, Sun J, Dong T, Zhu M, Li Z, Xu T: **High-throughput deep sequencing reveals the important role that microRNAs play in the salt response in sweet potato (*Ipomoea batatas* L.).** *BMC genomics* 2020, **21**(1):164.
75. Kuo Y-W, Lin J-S, Li Y-C, Jhu M-Y, King Y-C, Jeng S-T: **MicroR408 regulates defense response upon wounding in sweet potato.** *Journal of experimental botany* 2019, **70**(2):469–483.
76. Liu Y, Su W, Wang L, Lei J, Chai S, Zhang W, Yang X: **Integrated transcriptome, small RNA and degradome sequencing approaches proffer insights into chlorogenic acid biosynthesis in leafy sweet potato.** *PloS one* 2021, **16**(1):e0245266.
77. Bolger AM, Lohse M, Usadel B: **Trimmomatic: a flexible trimmer for Illumina sequence data.** *Bioinformatics* 2014, **30**(15):2114–2120.
78. Kuang Z, Wang Y, Li L, Yang X: **miRDeep-P2: accurate and fast analysis of the microRNA transcriptome in plants.** *Bioinformatics* 2019, **35**(14):2521–2522.
79. Addo-Quaye C, Miller W, Axtell MJ: **CleaveLand: a pipeline for using degradome data to find cleaved small RNA targets.** *Bioinformatics* 2009, **25**(1):130–131.
80. Chen Y, Zhu P, Wu S, Lu Y, Sun J, Cao Q, Li Z, Xu T: **Identification and expression analysis of GRAS transcription factors in the wild relative of sweet potato *Ipomoea trifida*.** *BMC genomics* 2019, **20**(1):911.

81. Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG: **Primer3—new capabilities and interfaces.** *Nucleic acids research* 2012, **40**(15):e115-e115.
82. Park SC, Kim YH, Ji CY, Park S, Jeong JC, Lee HS, Kwak SS: **Stable internal reference genes for the normalization of real-time PCR in different sweetpotato cultivars subjected to abiotic stress conditions.** *PloS one* 2012, **7**(12):e51502.
83. Livak KJ, Schmittgen TD: **Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method.** *Methods* 2001, **25**(4):402–408.
84. National Genomics Data Center M, Partners: **Database Resources of the National Genomics Data Center in 2020.** *Nucleic acids research* 2020, **48**(D1):D24-D33.
85. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR: **STAR: ultrafast universal RNA-seq aligner.** *Bioinformatics* 2013, **29**(1):15–21.
86. Li B, Dewey CN: **RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome.** *BMC bioinformatics* 2011, **12**:323.
87. Langfelder P, Horvath S: **WGCNA: an R package for weighted correlation network analysis.** *BMC bioinformatics* 2008, **9**:559.
88. Smoot ME, Ono K, Ruscheinski J, Wang P-L, Ideker T: **Cytoscape 2.8: new features for data integration and network visualization.** *Bioinformatics* 2011, **27**(3):431–432.
89. Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, von Mering C, Bork P: **Fast Genome-Wide Functional Annotation through Orthology Assignment by eggNOG-Mapper.** *Molecular biology and evolution* 2017, **34**(8):2115–2122.
90. Yu G, Wang LG, Han Y, He QY: **clusterProfiler: an R package for comparing biological themes among gene clusters.** *Omics: a journal of integrative biology* 2012, **16**(5):284–287.

Figures

Figure 1

Comparison of SPL genes in four *Ipomoea* species (a) Comparison of the number and ratio of SPL genes in four *Ipomoea* species, the clades are displayed in different colors. (b-d) Comparison of SPL protein lengths, MWs and pls in four *Ipomoea* species, respectively.

Figure 2

Phylogenetic analysis of SPL proteins from 4 *Ipomoea* species, *C. reinhardtii*, *A. thaliana*, *S. lycopersicum*, and *O. sativa*. The rooted phylogenetic tree was constructed based on the conserved domain of 156 SPLs using neighbor-joining method with 1000 bootstrap replications. CrCRR1 protein from *C. reinhardtii* was used as an outgroup to root the tree. Numbers on the tree indicate bootstrap support (values < 50% not shown). The different-colored arcs indicate different clades of the SPLs. SPL members from the same species are marked with the same colors: Blue, *I. nil*; red, *I. triloba*; green, *I. trifida*; purple, *I. batatas*; grey, *C. reinhardtii*; orange, *O. sativa*; brown, *A. thaliana*; pink, *S. lycopersicum*.

Figure 3

Phylogenetic relationships, gene structures, domain organizations, and motif compositions of SPL genes in *Ipomoea* species (a) Phylogenetic tree of 105 *Ipomoea* SPL proteins. (b) Intron-exon structures of *Ipomoea* SPL genes. Red box indicates coding sequence, and black line represents intron. (c) Domain organizations of *Ipomoea* SPL proteins. SBP domain, ANK repeat and DEXDc domain are exhibited in blue, purple, and orange boxes, respectively. (d) Motif compositions of *Ipomoea* SPL proteins. The ten motifs are displayed in different colored boxes. The length of genes or proteins can be estimated using the scale at the bottom.

Figure 4

Syntenic relationships and selective pressure of *Ipomoea* SPLs (a) The colinear relationship among SPL genes in *I. batatas*. Colored lines indicate syntenic regions. IbSPL genes can be classified into four duplicated types, and marked as different colors. (b) The distribution of SPLs duplication modes in four *Ipomoea* species. (c) The mean Ka/Ks values in each clade.

Figure 5

miR156 target site of Ipomoea SPL genes (a) Multiple alignments of identified miR156 sequences. (b) Summary of miR156-targeted SPL genes in Ipomoea genus. (c) Diagram of IbSPL genes targeted by IbmiR156. The grey box indicates CDS region, the blue box represents SBP domain, and the red box indicates IbmiR156 target sites.

Figure 6

Investigation of cis-acting elements in *Ipomoea* SPL genes (a) Cis-acting elements in the promoter regions of all *Ipomoea* SPL genes. (b) Pie charts of cis-acting elements in all SPL genes. The colored boxes represent different categories or cis-acting elements.

Figure 7

Expression profiles of IbSPL genes in different tissues and developmental stages by transcriptome analysis (a) Heatmap representing the expression patterns of IbSPL genes in 8 different tissues of two sweet potato cultivars. Xuzi3: sweet potato cultivar Xuzi3, Yan252: sweet potato cultivar Yan252; S: shoot, YL: young leaf, ML: mature leaf, T: stem, FR: fibrous root, IR: initial tuberous root, ER: expanding storage root, MR: mature storage root. (b) Heatmap representing the expression patterns of IbSPL genes in eight different stages during root development. 10DAT_TR: undifferentiated root (10 days after transplanting), 20DAT_TR: undifferentiated root (20 days after transplanting), 30DAT_FR: fibrous root (30 days after transplanting), 40DAT40_FR: fibrous root (days after transplanting), 50DAT_FR: fibrous root (50 days after transplanting), 30DAT_SR: storage root (30 days after transplanting), 40DAT_SR: storage root (40 days after transplanting), 50DAT_SR: storage root (50 days after transplanting). Heatmap was generated based on $\log_2(\text{FPKM}+1)$ values for each IbSPL gene.

Figure 8

Expression profiles of 11 IbSPL genes in different tissues and developmental stages by qRT-PCR (a) Different tissues from cultivar Xuyu34 were shown as pictures, including stem, leaf, flower and root. Black bar is scale of 1 cm. (b) Expression patterns of 11 IbSPL genes determined by qRT-PCR. The x-axis indicates different tissues. The y-axis represents the relative expression calculated by the $2^{-\Delta\Delta CT}$ method. The error bars indicate the standard deviations of three biological replicates. Different letters indicate statistically significant difference at $p < 0.05$.

Figure 9

Expression patterns of 6 IbSPL genes under phytohormone treatments (a) Expression patterns of 6 IbSPL genes under IAA treatment. (b) Expression patterns of 6 IbSPL genes under MeJA treatment. (c) Expression patterns of 6 IbSPL genes under ZT treatment. (d) Expression patterns of 6 IbSPL genes under ABA treatment. Samples were collected at 0, 6, 12, 24, and 48 h after treatment. The x-axis indicates different samples. The y-axis represents the relative expression calculated by the $2^{-\Delta\Delta CT}$ method. The error bars indicate the standard deviations of three biological replicates. Different letters indicate statistically significant difference at $p < 0.05$.

Figure 10

Regulatory sub-networks involving IbSPLs and other *I. batatas* genes in the storage root of sweet potato (a) Co-expression sub-network visualization for IbSPLs in the storage root of sweet potato. Only edges with weight above a threshold of 0.05 are displayed. (b) Venn diagram of the number of co-expressed genes for IbSPL1, IbSPL16, IbSPL17, IbSPL21, and IbSPL28 in the turquoise module. (c) GO enrichment analysis of co-expressed genes of IbSPL16, IbSPL17, IbSPL21, and IbSPL28, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTables.xls](#)
- [SupplementaryFigures.docx](#)