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ARTICLE

Identification and Expression Analysis of the TALE Gene Family Across the Genome of Tree Peony (*Paeonia ostii*)

Huiyun Li*, Caimin Kuang, Zixin Liu, Yujian Song, Xue Guo, Jinbo Li and Yanzhao Zhang*

College of Life Science, Luoyang Normal University, Luoyang, China
*Corresponding Authors: Huiyun Li. Email: huiyunli2008@163.com; Yanzhao Zhang. Email: yzhao_zhang@163.com

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ABSTRACT: TALE transcription factors are essential for plant growth, development, and abiotic stress responses. In *P. ostii*, a species of high ornamental and economic value, heat and drought stress severely limit productivity, yet systematic identification of the TALE family has been lacking. Here, 30 TALE transcription factors were identified via bioinformatics, with protein lengths of 89–774 aa and molecular weights (Mws) of 10,182.91 Da–84,550.84 Da. Comprehensive analyses covered physicochemical properties, phylogenetics, chromosomal localization, promoter *cis*-elements, conserved motifs, synteny, and tissue-specific expression. Based on homeodomain features, genes were classified into KNOX and BELL subfamilies, with similar structural features within each subfamily. All PoTALE proteins were predicted to be nuclear-localized. Genes were unevenly distributed across five chromosomes, enriched on chromosome 1. Additionally, *PoTALE-25* and *PoTALE-10* were mapped to uncharacterized scaffolds 3 and 2294, respectively. Promoter analysis revealed *cis*-elements related to development, hormones, and abiotic stress, suggesting roles in stress adaptation and development. Intron numbers varied: 15 genes had 1–4 introns, and 15 had 5–15 introns. Collinearity analysis showed 12 homologous pairs between *P. ostii* and each of *P. ludlowii*, *S. lycopersicum*, and *S. tuberosum*, versus 6 with *A. thaliana* and 1 with *O. sativa*, consistent with higher syntenic retention among dicots. The highest retention between the two peony species reflects their closest phylogenetic relationship. Tissue-specific expression profiling revealed pronounced transcript accumulation in leaves, pink and red petals, pistils, seeds, shoots, and flower spots, with the highest levels in leaves, petals, and spots. Among these, *PoTALE-3*, *PoTALE-14*, and *PoTALE-15* were prioritized as candidates for functional studies on spot formation and vegetative organ development. Collectively, these findings highlight the functional diversity of the PoTALE family and its potential involvement in stress responses and developmental regulation.

KEYWORDS: Tree peony; TALE gene family; KNOX; BELL; bioinformatics

1 Introduction

Homeobox genes constitute a large family of transcription factors (TFs) found throughout eukaryotes and are indispensable for the growth and development of animals and plants [1,2]. Homeobox genes were originally discovered in the fruit fly (*Drosophila melanogaster*) [3]. KNOTTED-1, the first homeobox gene, was identified by Vollbrecht et al. in 1991 [4]. The typical homeobox domain features a characteristic triple-helix region of 60 amino acids. In this region, the first and second helices form a loop, whereas the second and third create a motif of helix-turn-helix [5].

Researchers have categorized plant homeobox genes using two distinct classification methods. In the early classification of homeobox genes, seven distinct groups were identified: ZM-HOX, ATHB8, HAT1,

HAT2, GL2, the BEL1-like homeodomain (commonly referred to as BELL or BLH), and the KNOTTED-like homeodomain (known as KNOX or KNAT) [6]. Subsequent studies categorized homeobox genes into 11 classes: HD-ZIP (classes I through IV), BLH/BELL, DDT, LD, KNOX/KNAT, NDX, PINTOX, PHD, SAWADEE, PLINC, and WOX [6]. On the basis of protein sequence analysis and evolutionary relationships, the KNOX and BEL1-like homeobox genes are classified within the three-amino-acid-loop-extension (TALE) superfamily. The TALE family directly functions in a wide array of plant processes, ranging from growth and development to abiotic stress resistance [7–9], also affecting organ morphology [9,10], hormone levels [10], signal transduction [11], and tuber formation [11]. Four domains are typically found in KNOX proteins: KNOX1, ELK, KNOX2, and a homeodomain. Based on gene structure and expression profiles, the KNOX family was originally grouped into two distinct classes. KNOX proteins that lack homeodomains have been identified in dicots, thereby falling into three distinct groups [12]. *STM*, *KNAT1*, *KNAT2*, and *KNAT6* fall into the first category. *KNAT3*, *KNAT4*, *KNAT5*, and *KNAT7* belong to the second category. The third category is represented solely by *KNATM*, a gene that is unique to dicotyledonous plants. Genes such as *ATH1*, *BEL1*, and *BLH1* through *BLH10* belong to the BELL family. However, they have not been systematically categorized [6]. Extensive studies have been conducted on *KNOX* genes within the TALE superfamily. KNOX class I genes show diverse expression patterns and functions, with predominant expression in meristematic tissues [13,14]. In *Arabidopsis*, for instance, *KNAT2* expression in apical meristems converts nucellar tissue into carpel-like structures and interferes with normal AGAMOUS (AG) expression in the ovule center and the carpel [15]. The *STM* gene is necessary for normal shoot apical meristem maintenance in *Arabidopsis* [16], whereas the *KNAT1* gene contributes to root tilt. In *knat1* mutants, auxin transport is notably decreased in basal leaves, whereas auxin accumulation is elevated in the roots. In *Arabidopsis*, this change in auxin transport is associated with reduced levels of the auxin efflux carrier PIN2 in the root apex region. *KNAT1* therefore appears to exert a negative regulatory effect on root tilt by modulating auxin transport [17]. As opposed to the KNOXI subfamily, the KNOXII subfamily is characterized by broad expression across diverse tissues. *KNAT7* is the most studied among all KNOXII members. It functions as a pivotal regulator of a negative regulatory cycle that curbs the overcommitment of metabolic resources to SCW (secondary cell wall) formation, thereby preserving metabolic homeostasis. Emerging evidence has revealed that *KNAT7* and *KNAT3* are capable of forming heterodimers. Additionally, *KNAT3* interacts with *NST1* and *NST2*, two key transcription factors that regulate SCW formation, thereby forming a *KNAT3-NST1/2* heterodimer complex. This complex is essential for regulating *F5H* expression and promoting syringyl lignin biosynthesis [18]. This finding suggested that *KNAT3* and *KNAT7* collaboratively facilitate the biosynthesis of SCW [18]. In *Medicago truncatula*, *KNAT3/4/5*-like transcription factors may restrict additional rhizobial infections and nodule development by activating the EFD/RR4 pathway, which locally suppresses cytokinin signaling. This mechanism helps regulate the border and shape of nodular organs. KNATM proteins are categorized as members of the third KNOX class, and they are found only in dicotyledonous plants. Through binding to other TALE proteins, KNATM modulates their functions and thus directs leaf polarity and development [12]. BELL-like proteins have received less research attention than KNOX-like proteins. Functional roles have been identified for several BELL family members, including BEL1, ATH1, PNY/BLH8, PNF/BLH9, SAW2/BLH4, and SAW1/BLH2 [19]. The roles of other BELL-like proteins remain unknown. ATH1, a BELL-family protein, represses flowering by regulating FLC (FLOWERING LOCUS C) expression levels [20]. Furthermore, *BLH3* and *BLH6* played opposing roles during floral transformation. Plants with elevated expression of *BLH6* exhibited delayed flowering, whereas those with increased expression of *BLH3* bloomed earlier compared to *BLH6* [21]. PNY represses flowering with ATH1 but promotes it with PNF [22]. BLH4/SAW2 and BLH2/SAW1 shape leaves by repressing certain *KNOX* genes, thereby limiting growth

within particular leaf zones [23]. As a transcriptional repressor, BLH6 enhances its repressive activity through physical interaction with *KNAT7*. By binding to the REV promoter, the BLH6-KNAT7 complex directly represses REV expression to regulate SCW biosynthesis in interfascicular fibers [24]. Each of the 13 *Arabidopsis* BELL proteins was shown by large-scale yeast two-hybrid assays to bind to at least one KNOX partner. For example, AtBLH1 and AtKNAT3 act together to regulate *Arabidopsis* seed germination and seedling development [25].

Extensive genomic characterization of the TALE transcription factor family has been performed across diverse plant species, including *Arabidopsis* [8], poplar [26], soybean [27], pomegranate [28], radish [29], and tomato [30], with members from poplar and soybean shown to be closely associated with salt and drought tolerance [26,27]. In addition, the TALE genes in *A. thaliana*, such as *KNAT3* and *BLH4*, are capable of responding to abiotic stresses including drought, low temperature, high temperature, and salt stress [8]. Tree peony (*P. ostii*) is a woody plant species belonging to the genus *Paeonia* in the family *Paeoniaceae*, order *Saxifragales*. It holds substantial ornamental, medicinal, and oil-related value [31,32]. However, under global climate change, enhancing its growth and abiotic stress tolerance has become increasingly critical, as it is highly sensitive to high temperature and drought in production practice—stresses that inhibit vegetative growth, reduce the duration of flowering, and degrade flower quality, thereby severely diminishing its ornamental and commercial value [33]. Given the pivotal role of TALE transcription factors as key regulatory nodes in plant development and stress responses, they may hold considerable potential for improving stress tolerance in tree peony. Nevertheless, systematic identification and functional characterization of this family in *P. ostii* remain lacking [34], representing a critical bottleneck for molecular breeding. Therefore, a comprehensive investigation of the TALE gene family in *P. ostii* is urgently needed to bridge this knowledge gap and facilitate the development of stress-tolerant varieties.

In this study, we performed a genome-wide systematic identification of the TALE family in tree peony, identifying 30 *PoTALE* genes, and conducted comprehensive bioinformatics analyses covering physicochemical properties, phylogeny, chromosomal distribution, gene architecture (including conserved motifs and coding/non-coding regions), *cis*-regulatory elements, and tissue-specific expression profiles. This work provides a thorough structural and functional characterization of the PoTALE family and establishes a valuable foundation for future functional studies and genetic improvement of stress tolerance and developmental regulation in tree peony.

2 Materials and Methods

2.1 Characterization of the PoTALE Gene Family

The genome, protein (pep), coding (cds), and annotation (gff) files for tree peony were retrieved from the National Gene Bank, download the *Arabidopsis* TALE protein sequence file from the PlantTFDB website. Using *Arabidopsis* TALE protein sequences as queries, we ran BLASTP searches against the *P. ostii* protein sequences via TBtools-II (v2.476) with default settings (tab format, e-value < $1e^{-5}$, 500 hits and 250 alignments per query).

The TALE protein sequences of tree peony were then obtained by preliminary screening.

NCBICDD, InterPro and SMART were used to screen the primary proteins. Finally get 30 members of the tree peony TALE gene family and name them *PoTALE-1*, *PoTALE-2*, *PoTALE-3*, …, *PoTALE-30*.

The molecular weights and pI (isoelectric points) of each PoTALE family member were determined using Expasy-ProtParam, while their subcellular localizations were predicted with Cell-PLoc.

2.2 Phylogeny and Evolution of the PoTALE Gene Family

TALE protein sequences from tree peony and *A. thaliana* were aligned via MEGA7.0, and the phylogenetic tree of *Arabidopsis* and tree peony was constructed and exported after beautification on Evolview website. To comprehensively identify PoTALE proteins, a local BLAST search was performed using all AtTALE amino acid sequences as queries. A minimal evolutionary tree was constructed using MEGA7.0 software to conduct phylogenetic analysis aimed at elucidating the evolutionary relationships among TALE protein sequences.

2.3 Chromosomal Localization of the Tree Peony TALE Gene Family

Using the annotation file of tree peony, the chromosomal locations of the *PoTALEs* were identified. These positions, along with chromosome length data, were then submitted to the MG2C website for map visualization and saving.

2.4 Cis-Element Analysis of PoTALEs

The tree peony genome was used to extract promoter sequences using TBtools-II (v2.476). First, the Gff file was initialized in the "Gtf/Gff3 Sequences Extract" tool, followed by loading the genome file. The 2 kb (kilobases) promoter region (upstream of the initiation codon) of each gene was extracted and saved in txt format. Subsequently, the txt file was uploaded to the PlantCARE website to generate an Excel file containing *cis*-element annotations. After necessary formatting, the final visualization was achieved on the "Simple BioSequence Viewer" page of TBtools-II (v2.476) and Microsoft Office Professional Plus 2016. Putative *cis*-elements in the 1.5 kb promoter were predicted using PlantCARE.

2.5 Structural and Sequence Conservation Analysis of PoTALEs

TALE protein sequences from tree peony were aligned using MEGA7.0. Conserved motifs were identified using the MEME website based on TALE protein sequences. Information on the TALE gene family was screened from the tree peony annotation file and saved as a txt file. Gene structure maps were subsequently generated using TBtools-II (v2.476). Finally, visualization was performed and saved via the Gene Structure View (Advanced) component of TBtools-II (v2.476).

2.6 Syntenic Relationship Analysis of PoTALEs

Collinearity between tree peony and *Arabidopsis* was analyzed using TBtools-II (v2.476) (One Step MCScanX and Dual Synteny Plot). The CTL file was simplified to highlight the TALE gene family. The same workflow was applied to generate collinearity files for rice, tomato, potato and *P. ludlowii* against *P. ostii*.

2.7 Tissue Expression Specificity Analysis of Tree Peony TALE Gene Family

To examine the tissue-specific expression profiles of *PoTALE* genes in tree peony, we obtained FPKM (Fragments Per Kilobase per Million mapped reads) values from our previously published transcriptome data [35] and calculated fold-changes as the ratio of FPKM values between target and control tissues. A heatmap was constructed using $\log_2^{(\mathrm{FPKM}+1)}$-transformed values via TBtools-II (v2.476), facilitating the prediction of candidate *PoTALE* genes associated with tree peony growth and development.

3 Results and Analysis

3.1 Systematic Genome-Wide Characterization and Classification of PoTALEs

To investigate the conserved domains present in tree peony TALE proteins, conserved domain analysis was executed with the aid of the NCBI CDD search tool (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). To verify whether the candidate TALE protein sequences from tree peony possess the conserved domain features typical of plant TALE gene families, further analysis was conducted employing the Pfam and SMART online tools. Through this comprehensive screening process, all protein sequences belonging to the TALE gene family in tree peony were successfully obtained. The tree peony pep file and the *Arabidopsis* TALE protein file were subjected to preliminary screening using TBtools-II (v2.476), yielding 31 candidate TALE protein members. These candidates were further verified through NCBI CDD, InterPro, and SMART. One member, Pos.gene82579.mRNA-1, showed no detectable conserved domains and was consequently excluded. Ultimately, 30 TALE protein sequences from tree peony were retained for subsequent analyses (Table 1).

PoTALE-3 possessed the maximum amino acid length (774 aa) among tree peony TALE family members; the minimum amino acid number of PoTALE-18 was 89 aa; PoTALE-3 had the highest Mw (84,550.84 Da); PoTALE-18 had the smallest molecular mass (10,182.91 Da); the maximum isoelectric point (pI) of PoTALE-8 is 8.98, and the minimum pI of PoTALE-22 is 5.01. PoTALE-8, PoTALE-20, PoTALE-23, PoTALE-28, and PoTALE-29 are basic amino acids, all having pI values > 7, whereas the remaining members are acidic amino acids. All TALE family proteins are localized in the nucleus. All TALE family proteins are found in the nucleus.

Table 1: Summary of PoTALE gene family characteristics.

Gene ID	Gene Naming	Amino Acid Number (aa)	Relative Molecular Mass (Da)	Isoelectric Point (pI)	Subcellular Localization
Pos.gene81134	*PoTALE-1*	741	81,381.49	6.25	Nucleus
Pos.gene31078	*PoTALE-2*	690	75,544.03	6.96	Nucleus
Pos.gene82447	*PoTALE-3*	774	84,550.84	6.54	Nucleus
Pos.gene69357	*PoTALE-4*	436	48,746.56	6.29	Nucleus
Pos.gene62230	*PoTALE-5*	692	77,293.82	6.00	Nucleus
Pos.gene37760	*PoTALE-6*	655	72,620.94	6.25	Nucleus
Pos.gene8916	*PoTALE-7*	626	68,984.51	6.41	Nucleus
Pos.gene34193	*PoTALE-8*	452	51,005.94	8.98	Nucleus
Pos.gene21801	*PoTALE-9*	567	63,229.65	5.68	Nucleus
Pos.gene28130	*PoTALE-10*	312	35,879.62	5.06	Nucleus
Pos.gene56470	*PoTALE-11*	392	45,036.12	6.41	Nucleus
Pos.gene54739	*PoTALE-12*	295	33,546.96	6.20	Nucleus
Pos.gene22091	*PoTALE-13*	342	38,904.66	5.16	Nucleus
Pos.gene48263	*PoTALE-14*	297	33,664.68	5.12	Nucleus
Pos.gene48054	*PoTALE-15*	405	45,487.37	5.84	Nucleus
Pos.gene56562	*PoTALE-16*	328	37,229.15	6.46	Nucleus
Pos.gene72847	*PoTALE-17*	449	50,641.03	6.04	Nucleus

Table 1: *Cont.*

Gene ID	Gene Naming	Amino Acid Number (aa)	Relative Molecular Mass (Da)	Isoelectric Point (pI)	Subcellular Localization
Pos.gene1192	*PoTALE-18*	89	10,182.91	5.27	Nucleus
Pos.gene53145	*PoTALE-19*	251	28,437.03	5.98	Nucleus
Pos.gene39064	*PoTALE-20*	248	28,334.92	8.94	Nucleus
Pos.gene46808	*PoTALE-21*	426	48,759.53	6.10	Nucleus
Pos.gene3973	*PoTALE-22*	242	27,456.38	5.01	Nucleus
Pos.gene74381	*PoTALE-23*	267	30,625.00	7.76	Nucleus
Pos.gene11760	*PoTALE-24*	375	42,381.73	6.85	Nucleus
Pos.gene65813	*PoTALE-25*	182	20,383.23	5.63	Nucleus
Pos.gene66068	*PoTALE-26*	265	29,755.35	6.45	Nucleus
Pos.gene58413	*PoTALE-27*	125	13,878.98	6.43	Nucleus
Pos.gene20997	*PoTALE-28*	193	22,106.47	8.73	Nucleus
Pos.gene47758	*PoTALE-29*	247	28,698.70	8.87	Nucleus
Pos.gene44272	*PoTALE-30*	275	31,312.54	6.31	Nucleus

3.2 Molecular Phylogenetic Analysis of PoTALE Family Members

Using MEGA7.0 software, we constructed a neighbor-joining (NJ) phylogenetic tree for the PoTALE transcription factor family. The Bootstrap method with 1000 replicates and the pairwise deletion parameter were applied. Visualization and editing of the resulting tree were performed using FigTree. TALE proteins from tree peony and *A. thaliana* were aligned using ClustalX2. A phylogenetic tree was built with MEGA 7.0 using NJ, incorporating the bootstrap method and the pairwise deletion parameter. Bootstrap support was assessed with 1000 replicates to infer the evolutionary relationships among TALE members across various plant species. Their evolutionary tree was then visualized using iTOL (https://itol.embl.de) [36]. According to the classification in *Arabidopsis* [8], the PoTALE family members were assigned to two subfamilies (KNOX and BELL). PoTALE-10 to PoTALE-30 (21 members) belong to the KNOX subfamily, whereas PoTALE-1 to PoTALE-9 (9 members) belong to the BELL subfamily (Fig. 1).

3.3 Chromosome Analysis of Tree Peony TALE Gene Family

TALE gene family members in tree peony are distributed across multiple chromosomes. The 30 PoTALE gene family members are distributed across Chr01–Chr05 and two scaffolds. Chr01 harbors 12 members (*PoTALE-5*, *-6*, *-12*, *-14*, *-16*, *-17*, *-19*, *-20*, *-21*, *-22*, *-23*, *-24*); Chr02 harbors six (*PoTALE-2*, *-4*, *-7*, *-11*, *-13*, *-26*); Chr03 harbors three (*PoTALE-1*, *-15*, *-28*); Chr04 harbors four (*PoTALE-3*, *-18*, *-27*, *-30*); and Chr05 harbors three (*PoTALE-8*, *-9*, *-29*). *PoTALE-25* and *PoTALE-10* reside on unchr_scaffold_3 and unchr_scaffold_2294, respectively (Fig. 2).

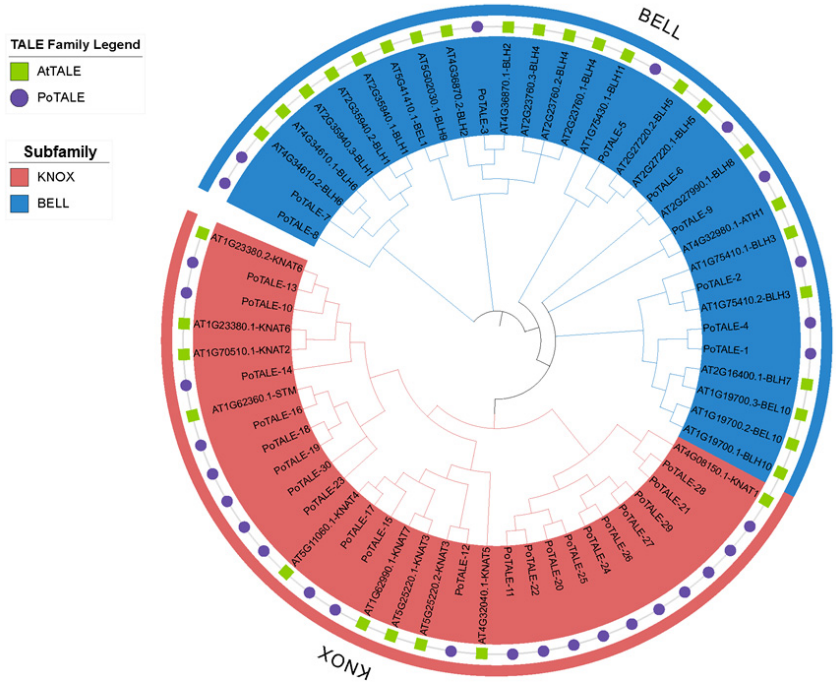

Figure 1: Evolutionary tree of TALE gene family in tree peony and *Arabidopsis*. Green square: *A. thaliana*; purple circle: *P. ostii*. The two subfamilies were color-coded in distinct colors.

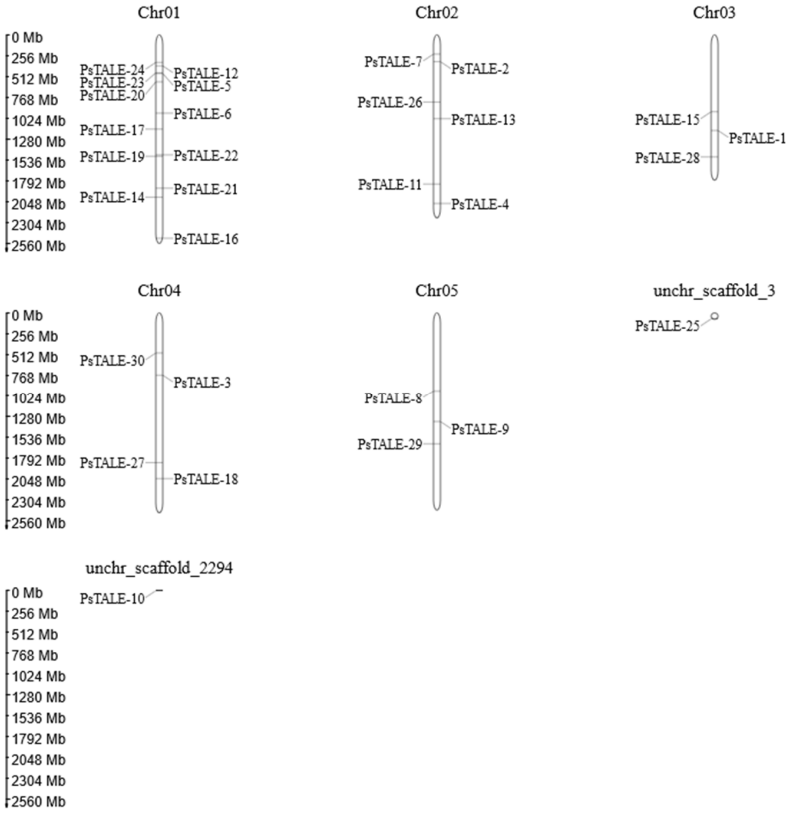

Figure 2: Chromosomal localization of TALE gene family.

3.4 Cis-Regulatory Analysis of PoTALE

Cis-elements are essential for transcriptional regulation. The TALE gene promoter contains diverse *cis*-elements, including those for light, hormones (e.g., gibberellin, auxin, abscisic acid), and stress (e.g., low temperature, defense) [37]. A 2000 bp sequence upstream of the *PoTALE* start codon was taken as the promoter. PlantCARE analysis of this promoter revealed multiple *cis*-elements in this region.

The promoter regions of both KNOX and BELL subfamily genes harbor diverse *cis*-elements, with most basic motifs (e.g., light- and hormone-responsive elements) widely distributed across almost all phylogenetic branches. KNOX subfamily promoters are enriched in stress- (MBS, ARE), light- (GT1-motif, BOX 4), hormone- (O2-site, GA-motif), and development-related (TCT-motif, CAT-box, WUN-motif) elements, while BELL subfamily promoters are similarly enriched in development- (TCT-motif, WUN-motif), light- (G-box, BOX 4), hormone- (TGACG-motif, P-box, ABRE), and stress-responsive (MBS, ARE) elements (Fig. 3A).

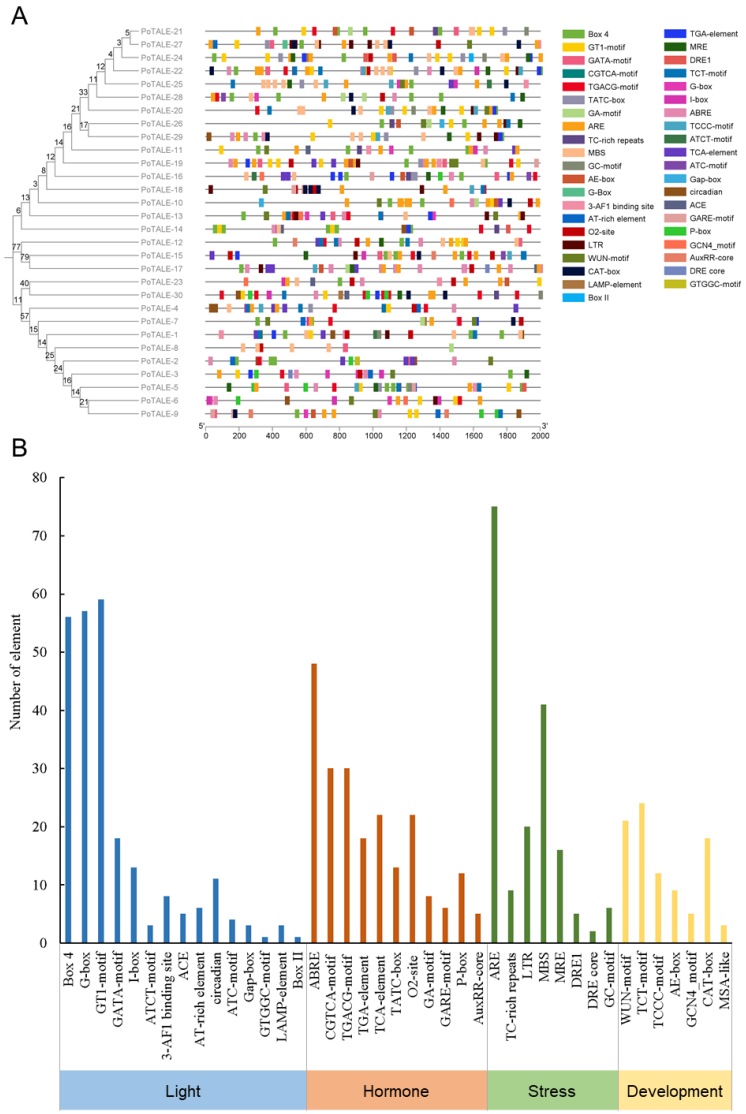

Figure 3: *Cis*-acting elements in PoTALE gene family promoters. (**A**) Distribution of *cis*-elements on each *PoTALE* gene, (**B**) the numbers of four categories of *cis*-elements (light-, hormone-, stress-, and development-related) in PoTALE gene family members.

Quantification of these four categories revealed that among stress-responsive elements, ARE was the most frequent (75 copies), followed by MBS (41 copies), with LTR and MRE present at 20 and 16 copies, respectively. Among light-responsive elements, GT1-motif, G-box, and Box 4 exhibited comparable abundance (59, 57, and 56 copies, respectively). For hormone-responsive elements, ABRE was the most abundant (48 copies), while CGTCA-motif and TGACG-motif were equally represented (30 copies each). Regarding development-related elements, TCT-motif (24 copies), WUN-motif (21 copies), and CAT-box (18 copies) were relatively evenly distributed (Fig. 3B).

Collectively, these findings suggest that both subfamilies likely play broad regulatory roles in plant growth and development, stress adaptation, and light/hormone signaling.

3.5 Analysis of Conserved Motifs in the PoTALE Gene Family

To clarify the evolutionary affinities among PoTALE members, we constructed a Neighbor-Joining phylogenetic tree (Fig. 4A). Analysis of the 12 conserved motifs predicted via MEME (Fig. 4B,D) showed that the BELL subfamily predominantly contained motifs 1, 4, 5, and 6. In the KNOX subfamily, motif 6 was present in all members except PoTALE-18, PoTALE-19, PoTALE-23, PoTALE-27, PoTALE-28, PoTALE-29, and PoTALE-30; notably, PoTALE-30 contained only motif 3, while PoTALE-18 contained only motif 2. PoTALE-12, PoTALE-15, and PoTALE-17 were tightly clustered in the same branch and all contained motifs 1, 4, 6, 8, and 12. Together, these findings suggest that motifs 1, 4, 5, and 6 appear to be specifically conserved in the BELL subfamily, whereas motifs 2 and 6 appear to be specifically conserved in the KNOX subfamily. In contrast, motifs 1, 2, 4, 6, and 8 may be relatively conserved in Class Ia, with the exception of PoTALE-18, PoTALE-19, and PoTALE-30.

The gene structure map (Fig. 4C) was drawn on the TBtools-II (v2.476), and it can be seen that the gene coding region and non-coding region of the tree peony TALE gene family were significantly different. *PoTALE-18*, and *PoTALE-20* through *PoTALE-30* lack non-coding regions, among which *PoTALE-21* contains the highest number of coding regions. In the BELL subfamily, intron numbers range from 3 to 8, with *PoTALE-2* having the most (8). In the KNOX subfamily, intron numbers range from 1 to 15, with *PoTALE-18* having the fewest (1) and *PoTALE-21* the most (15).

Taken together, the divergent motif compositions and gene structural patterns indicate that PoTALE family members potentially play diverse regulatory roles in the growth and development of tree peony.

3.6 Collinearity Analysis of PoTALEs

To investigate the evolutionary history of the PoTALE gene family, we performed a collinearity analysis with four representative species (*Arabidopsis*, rice, tomato, and potato) (Fig. 5). The results revealed a striking gradient in the number of collinear gene pairs between tree peony and these species: 10 shared orthologs were identified with both tomato and potato, whereas only four and one were found with *Arabidopsis* and rice, respectively.

This gradient is largely consistent with phylogenetic distances—tomato and potato are core eudicots more closely related to tree peony, whereas *Arabidopsis*, though also a eudicot, belongs to the *Brassicaceae* family with an earlier divergence time, and rice, as a monocot, is the most distantly related. Thus, the abundance of collinear gene pairs likely serves as a proxy for evolutionary distance, reflecting the degree of genomic synteny retention. Notably, the 10 genes shared between tomato and potato were identical (*PoTALE-2*, *-3*, *-5*, *-7*, *-8*, *-9*, *-12*, *-13*, *-14*, and *-17*), suggesting the existence of a conserved syntenic block within the *Solanaceae* lineage that has been maintained since its divergence from tree peony. Intriguingly, the four orthologs identified in *Arabidopsis* (*PoTALE-3*, *-4*, *-12*, and *-15*) are all included within this *Solanaceae*-shared

set, implying that these may represent a more ancient core of collinear genes, while others (e.g., *PoTALE-2* and *-5*) may have arisen through lineage-specific expansions or rearrangements after the eudicot radiation.

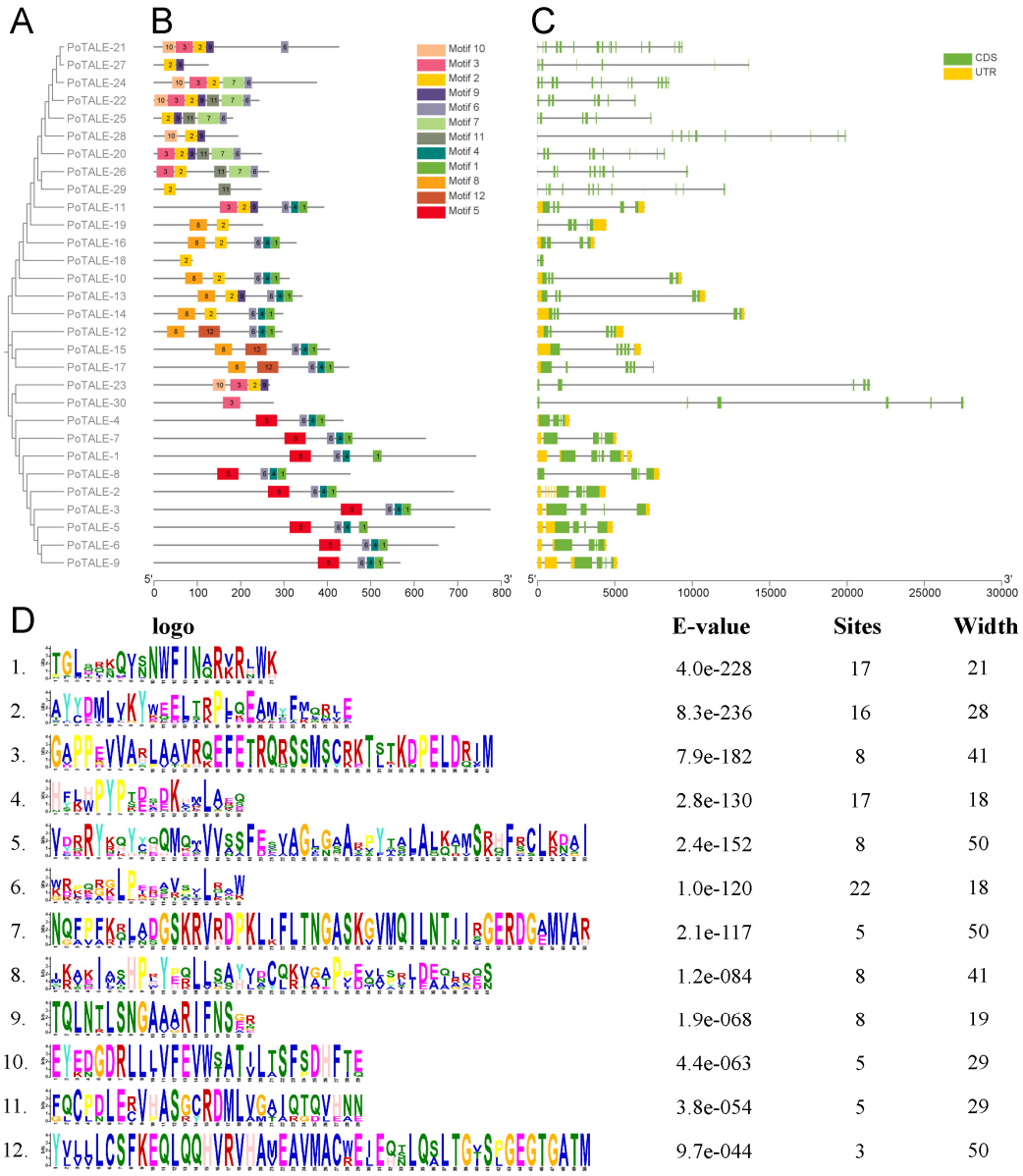

Figure 4: Phylogenetic relationships, conserved motifs, and gene structure of the PoTALE gene family in *P. ostii*. (**A**) Phylogenetic relationships of the PoTALE proteins, (**B**) conserved motifs of the PoTALE proteins, (**C**) distribution of coding (CDS) and non-coding (UTR) regions within the *PoTALE* genes, (**D**) the twelve conserved motifs in the PoTALE gene family.

Collectively, these collinearity patterns not only provide insights into the evolutionary history of *PoTALE* genes but also suggest the coexistence of core conserved functions and lineage-specific differentiation within this gene family.

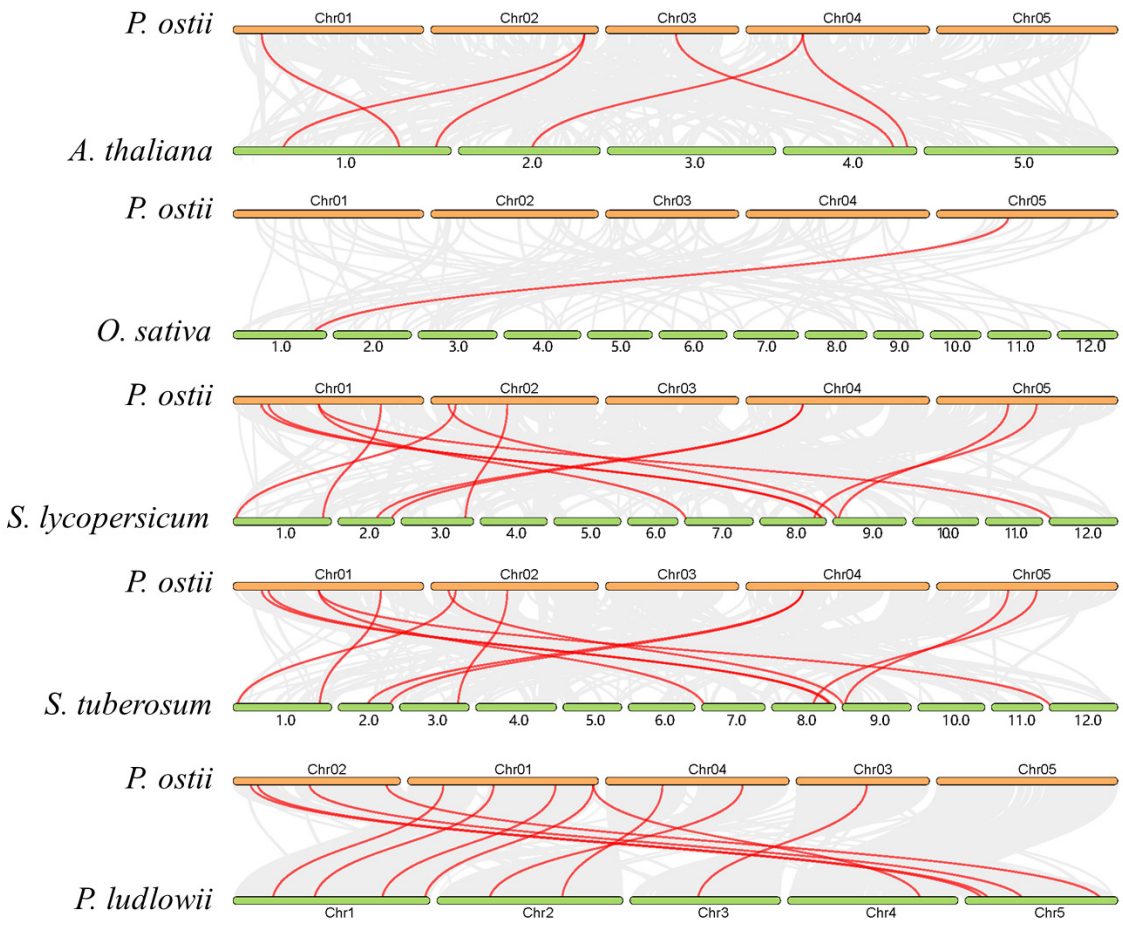

Figure 5: Synteny analysis of the *TALE* gene family between *P. ostii* and five other plant species. Note: red lines indicate collinear gene pairs between the *P. ostii TALE* gene family and the *TALE* gene families of *Arabidopsis thaliana*, *Oryza sativa* (rice), *Solanum lycopersicum* (tomato), *Solanum tuberosum* (potato), and *Paeonia ludlowii*.

3.7 Tissue-Specific Transcriptional Analysis of the PoTALEs

To investigate the tissue-specific expression of *PoTALE* genes in tree peony, we retrieved FPKM values from our transcriptome dataset reported previously [35] and generated expression heatmaps across various tissues, including leaves, pink petals, pistils, red petals, seeds, shoots, and spots (Fig. 6). Within the KNOX subfamily, *PoTALE-14* exhibited highly restricted expression in spots, with transcript levels approximately 66 times those in leaves, suggesting its potential role in spot formation and development. Meanwhile, *PoTALE-20*, *-22*, *-25*, *-26*, and *-29* all showed markedly elevated expression in shoots, with average transcript levels approximately 59 times those in seeds, implying their possible involvement in shoot development.

Both *PoTALE-3* (BELL subfamily) and *PoTALE-15* (KNOX subfamily) displayed markedly higher expression in leaves than in seeds, with transcript levels approximately 112 times and 19 times those in seeds, respectively. Similarly, another KNOX member, *PoTALE-17*, also exhibited specific high expression in leaves (approximately 6 times that in red petals), further supporting its potential role in leaf development. In contrast, the other members (*PoTALE-2*, *-4*, *-5*, *-8*, *-10*, *-11*, *-18*, *-23*, *-24*, *-28*) showed universally low expression across all seven tissues, suggesting that they may have undergone functional degeneration or may be expressed only under specific conditions.

Based on a comprehensive evaluation of tissue-specific expression (FPKM values from dataset [35]) (Fig. 6), phylogenetic position (representative genes from each major clade) (Figs. 1 and 4A), motif composition (canonical TALE domain features) (Fig. 4B), and promoter elements (enriched in light- and hormone-responsive *cis*-elements) (Fig. 3), *PoTALE-3*, *PoTALE-14*, and *PoTALE-15* were prioritized as candidate genes for subsequent functional validation to further elucidate their regulatory roles in spot formation and vegetative organ development.

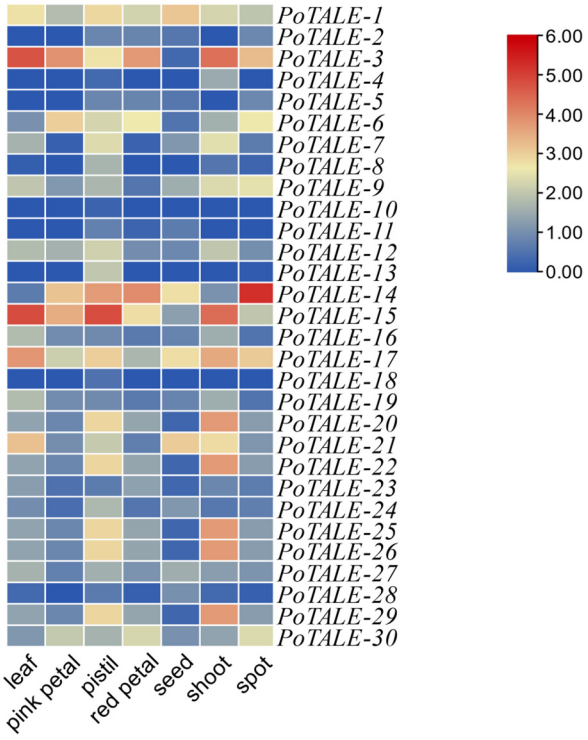

Figure 6: Tissue-specificity of *PoTALEs* transcripts. Note: expression levels of *PoTALEs* in leaf, pink petal, pistil, red petal, seed, shoot, and spot were normalized using a $\log_2^{(FPKM+1)}$ transformation.

4 Discussion

TALE transcription factors are known to play pivotal roles in plant development and abiotic stress responses, as demonstrated by their involvement in drought, temperature, and salt tolerance in *Arabidopsis* [8]. Tree peony (*P. ostii*) holds substantial ornamental and economic value but is highly sensitive to heat and drought stress, which severely compromise its growth and flower quality [31–33]. However, systematic identification of the TALE family in this species remain lacking, representing a critical bottleneck for molecular breeding and stress-tolerance improvement [34].

Whole-genome analysis provides a powerful approach for uncovering gene function and evolutionary history [38]. Through bioinformatics analysis, 30 *PoTALE* genes were identified in *P. ostii* and phylogenetically classified into two subfamilies, KNOX and BELL, based on their relationships with *Arabidopsis* orthologs, mirroring the KNOX/BELL classification established in soybean [27]. The number of *TALE* genes identified in *P. ostii* (30) is comparable to that in radish (33) [29] and poplar (35) [26], but markedly lower than in wheat (70) [39], likely reflecting species-specific adaptations to growth environments [30]. Considerable variation was observed among PoTALE members in amino acid length, Mw, and theoretical pI, largely consistent with previous reports in poplar [26], soybean [27], and cotton [38]. Most PoTALE members

exhibit acidic isoelectric points (pI < 7), a common feature of transcription factors. In addition, subcellular localization analysis revealed that all PoTALE proteins are targeted to the nucleus, consistent with previous findings in *Arabidopsis* [8], suggesting their potential involvement in genetic metabolic regulation.

The chromosomal distribution of *PoTALE* genes was highly uneven. Chr01 exhibited the highest density, harboring nearly 50% of the family members (e.g., *PoTALE-24*, *-12*, *-23*, *-5*, *-20*, *-6*, *-17*, *-22*, *-19*, *-21*, *-14*, and *-16*). However, these genes were scattered across the entire length of Chr01 rather than forming a tight tandem array, suggesting that they did not originate from recent tandem duplications. In addition, the complete absence of *PoTALE* genes on Chr03 implies potential chromosome-specific gene loss during species divergence. Two KNOX-subfamily members, *PoTALE-25* and *PoTALE-10*, were localized to the unanchored scaffolds unchr_scaffold_3 and unchr_scaffold_2294, respectively.

Notably, no tandem or segmental duplication events were detected within the TALE gene family in *P. ostii* in this study, suggesting that these duplication modes have not been the primary driver of their expansion. This finding aligns with the genomic evolutionary history of this species, in which the massive genome size is largely attributable to recent transposable element bursts rather than lineage-specific whole-genome duplication (WGD) [40]. Accordingly, large-scale duplication can be excluded as a major contributor to the expansion of this clade. Furthermore, the extensive proliferation of transposable elements may have facilitated the dispersed chromosomal distribution and regulatory diversification of these loci through mechanisms such as transduction or ectopic recombination, potentially driving their functional divergence. Taken together, it is plausible that the current PoTALE gene family members are mainly derived from more ancient duplication events, such as the γ hexaploidization event shared by core eudicots, rather than through recent small-scale duplications [41–43].

Furthermore, the evolutionary dynamics of the PoTALE gene family may also be linked to the major geological events that shaped the distribution of *Paeonia* species. Hong [44] highlighted that the uplift of the Qinghai–Tibet Plateau and the subsequent Quaternary glaciation significantly influenced the biogeography and speciation of woody peonies, including the lineage to which *P. ostii* belongs. Against this paleoclimatic background, the differential retention of *PoTALE* genes on Chr01 and Chr03 may reflect adaptive genomic responses of *P. ostii* to ecological shifts. Given the established roles of the TALE gene family in plant development and stress responses [7,9,45], the retention of these genes may have provided *P. ostii* with a selective advantage for survival in fluctuating montane habitats.

Synteny analysis revealed 12 homologous gene pairs between *P. ostii* and each of *P. ludlowii*, *S. lycopersicum*, and *S. tuberosum*, but only 6 with *A. thaliana* and 1 with *O. sativa*. This gradient of syntenic retention reflects both phylogenetic relatedness and differential evolutionary rates among lineages, with the highest conservation observed between the two congeneric species, *P. ostii* and *P. ludlowii*. Despite the ancient evolutionary divergence between *Solanaceae* and *Paeoniaceae* [43,46], the robust 12-pair synteny retained between *P. ostii* and *solanaceous* species suggests that this gene family may reside within a highly conserved genomic block, which is inferred to have undergone minimal chromosomal rearrangement. The reduced synteny between *P. ostii* and *A. thaliana* (only six pairs) can be largely explained by the distinctive genomic evolution of the *Brassicaceae* lineage, where extensive restructuring of ancestral genomic blocks has occurred via chromosome fusions and other chromosomal rearrangements [47]. The single retained pair with *O. sativa* likely represents the most ancestral and functionally indispensable core component of this family, predating the monocot–eudicot divergence. Collectively, these findings suggest that the gene family in *P. ostii* has maintained an evolutionarily conservative structural framework, and that functional inference may be more reliably drawn from *solanaceous* than from *Arabidopsis* models.

Analysis of *cis*-elements indicated that *PoTALE* genes respond to diverse signaling molecules, such as: Light, plant hormones, developmental regulation, environmental stress, especially light response, abscisic acid, gibberellin elements, these findings are broadly consistent with those in pomegranate [28], indicating that *PoTALEs* gene may be involved in hormone signaling and stress regulation. Light-responsive *cis*-elements are found in all PoTALE family members, suggesting that *PoTALE* genes are critical for various plant developmental processes, particularly those involving light regulation and drought stress response, potentially via ABA or MeJA pathways, this is consistent with the findings in maize and tomato [7,8].

Sequence conservation analysis revealed that introns are present in all PoTALE family members. The study of introns is very important for the study of gene family evolution. Further motif analysis of the tree peony *TALE* gene showed that BELL and KNOX subfamilies had similar motifs, and the motifs of different subfamilies were markedly different. Motif 5 existed predominantly in the BELL subfamily, while Motif 2 existed only in KNOX subfamily, suggesting that some motifs might play subfamily-specific functions, this aligns with the findings in soybean [27].

The tissue-specific expression patterns provided key clues for functional differentiation among *PoTALE* genes. *PoTALE-14* exhibited an expression level in spots that was approximately 66 times that in leaves, suggesting its potential involvement in spot formation and development. In KNOX subfamily, five members (*PoTALE-20*, *-22*, *-25*, *-26*, and *-29*) all showed coordinately high expression in shoots (approximately 59-fold that in seeds), implying potential functional redundancy or synergistic regulation in shoot development, with members of the same branch displaying parallel expression signatures and tissue distributions. These findings are consistent with previous reports in soybean, switchgrass and melon [27,48,49]. In leaves, *PoTALE-3* (BELL) and *PoTALE-15* (KNOX) showed expression levels approximately 112 and 19 times those in seeds, respectively, whereas two other BELL members (*PoTALE-5* and *PoTALE-8*) exhibited universally low expression across tissues. This suggests a specific role for *PoTALE-3* and *PoTALE-15* in leaf development, while other members of the BELL subfamily may have undergone functional degeneration. Based on these pronounced expression divergences, *PoTALE-3*, *PoTALE-14*, and *PoTALE-15* were prioritized as candidate genes for subsequent functional validation to further elucidate their regulatory mechanisms in spot formation and vegetative organ development.

5 Conclusion

In this study, we systematically characterized 30 *PoTALE* genes from tree peony through comprehensive bioinformatics analyses, including physicochemical properties, phylogenetic relationships, chromosomal localization, *cis*-acting elements, conserved motifs, and syntenic relationships. Phylogenetic analysis categorized the PoTALE family members into two subfamilies, and members within each group exhibited highly conserved gene structures and protein motifs. Gene duplication may have played a limited role in the expansion of the PoTALE family. Tissue-specific expression profiling further revealed their potential roles in growth and development, providing a solid foundation for future functional validation.

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analysis and discussion of the findings: Huiyun Li, Yanzhao Zhang, Jinbo Li; manuscript drafting and revision: Huiyun Li, Yanzhao Zhang. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: The genome sequencing data for *P. ostii* were obtained from CNGBdb (https://db.cngb.org/data_resources/assembly/CNA0050666). Genome sequencing data of *P. ludlowii* were retrieved from the figshare repository (https://figshare.com/articles/dataset/Paeonia_ludlowii_genome_annotation/23537670). Protein and genome sequences of *A. thaliana* were obtained from Plant TFDB (https://planttfdb.gao-lab.org/family.php?sp=Ath&fam=HSF) and TAIR (www.arabidopsis.org), respectively. Genome sequences of *S. lycopersicum*, *O. sativa*, and *S. tuberosum* were obtained from Ensembl Plants (http://plants.ensembl.org/index.html). The expression data of *TALE* genes in various tissues of tree peony were obtained from the Genome Sequence Archive (BIG Data Center, Chinese Academy of Sciences) under accession number CRA001327 (http://bigd.big.ac.cn/gsa).

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