

Article

Genome-wide Identification and Expression Analysis of TALE Gene Family in Pomegranate (*Punica granatum* L.)

Yuying Wang^{1,2}, Yujie Zhao^{1,2}, Ming Yan^{1,2}, Honglian Zhao^{1,2}, Xinhui Zhang^{1,2} and Zhaohe Yuan^{1,2,*}

¹ Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China; wangyuying@njfu.edu.cn (Y.W.); z1184985369@njfu.edu.cn (Y.Z.); punica24@njfu.edu.cn (M.Y.); honglian_zhao@hotmail.com (H.Z.); zhxinhui@hotmail.com (X.Z.)

² College of Forestry, Nanjing Forestry University, Nanjing 210037, China

* Correspondence: zhyuan88@hotmail.com

Received: 1 May 2020; Accepted: 9 June 2020; Published: 11 June 2020



Abstract: The three-amino-acid-loop-extension (TALE) gene family is a pivotal transcription factor that regulates the development of flower organs, flower meristem formation, organ morphogenesis and fruit development. A total of 17 genes of pomegranate TALE family were identified and analyzed in pomegranate via bioinformatics methods, which provided a theoretical basis for the functional research and utilization of pomegranate TALE family genes. The results showed that the *PgTALE* family genes were divided into eight subfamilies (KNOX-I, KNOX-II, KNOX-III, BELL-I, BELL-II, BELL-III, BELL-IV, and BELL-V). All *PgTALEs* had a KNOX domain or a BELL domain, and their structures were conservative. The 1500 bp promoter sequence had multiple cis-elements in response to hormones (auxin, gibberellin) and abiotic stress, indicating that most of *PgTALE* were involved in the growth and development of pomegranates and stress. Function prediction and protein-protein network analysis showed that *PgTALE* may participate in regulating the development of apical meristems, flowers, carpels, and ovules. Analysis of gene expression patterns showed that the pomegranate TALE gene family had a particular tissue expression specificity. In conclusion, the knowledge of the TALE gene gained in pomegranate may be applied to other fruit as well.

Keywords: pomegranate; TALE gene family; bioinformatics; expression analysis

1. Introduction

A homeobox (HB) encodes the transcriptional regulatory factors with homeodomain, which play considerable roles in the development of plants and animals [1]. A typical homeobox domain consists of 60 amino acids, to form three-helix regions, the first and second helices form a loop structure, and the second and third helices form a helix-corner-helical structure [2]. Plant homeobox genes have been divided into distinct subgroups, Bharathan et al. [3] divided them into seven classes, including KNOTTED-like homeobox (KNOX/KNAT), BEL1-like homeobox (BELL/BLH), *Zea mays* homeobox (ZM-HOX), homeobox from *Arabidopsis thaliana* 1 (HAT1), homeobox from *A. thaliana* 2 (HAT2), *A. thaliana* homeobox 8 (ATHB8) and GL2. Mukherjee et al. [4] classified them into 14 classes, containing homeodomain-leucine zipper I to IV (HD-ZIP I to IV), BELL, KNOX, plant zinc finger (PLINC), wuschel homeobox (WOX), plant homeodomain (PHD), DDT, nodulin homeobox genes (NDX), luminidependens (LD), SAWADEE and PINTOX. While Burglin and Affolter [5] classified them into 11 classes, including HD-ZIP I to IV, WOX, NDX, PHD, PLINC, LD, DDT, SAWADEE, PINTOX, KNOX and BELL. In PlantTFDB, the homeobox genes consist of five families: HD-ZIP, TALE, WOX, HB-PHD, and HB-other [6].

Based on protein sequence and evolution, BELL and KNOX belong to the TALE gene family [7,8]. Except for some homeobox genes, TALE encodes an atypical structure forming two helices and three additional amino acid residues (P-Y-P) [9–11]. The TALE family plays a vital role in regulating plant growth and development [12–15], regulating the sporophyte program [16], the formation of plant meristems [7], and the maintenance of organ morphology [17], organ position [18], hormone regulation [19], signal transduction [20] and tuber formation [21]. Studies have shown that BELL and KNOX proteins specifically recognize and bind to form the BELL-KNOX heterodimer protein [22], which is essential for the nuclear localization of two transcription factor proteins and the activity of binding target gene [23,24]. TALE can form complexes to regulate ovule development [25]. After binding to the OVATE family protein (OFP), the BELL-KNOX dimer protein is reversely transferred from the nucleus to the cytoplasm to negatively regulate ovule development [26]. BELL proteins comprise two highly conserved domains: a POX domain (POX is composed of SKY and BEL) and homeodomain. The BELL plays essential roles in ovule development, frond development and fruit development [27,28]. *BEL1* is expressed in the ovule and controlled the ovule integument identity. The dimer formed by the *A. thaliana* homeobox 1 (ATH1) protein and the shoot meristemless (STM) protein participates in the development of plant meristems [29], while the dimer formed by the ATH1 protein and the KNOTTED-like from *A. thaliana* 2 (KNAT2) protein regulates the development of plant inflorescence tissue [30]. The interaction of BLH6 and KNAT7 affects the development of secondary cell walls [31]. The KNOX gene family contains KNOX1, KNOX2, ELK and homeodomain, except for a novel gene *KNATM* without the homeodomain [32,33]. In addition, KNOX1 and KNOX2 domains merge to form a MEINOX domain. KNOX1 is expressed in the meristem, which is necessary for meristem development and maintenance. Studies have shown that the KNOX2 gene is involved in regulating the secondary growth of plant cell walls and plays a crucial regulatory role in the development of roots, stems, seed coats and heartwood [30,34–36].

Myrtales, the myrtle order of flowering plants, is placed in the Angiosperm Phylogeny Group IV (APG IV) botanical classification system [37]. Pomegranate (*Punica granatum* L.) is a considerable economic fruit tree of the *Lythraceae* family and widely cultivated worldwide. It was that pomegranate and the related species *Eucalyptus grandis* H., belonging to the order Myrtales, shared the paleotetraploidy event [38]. Studying the function and regulatory mechanism of TALE genes in pomegranate helps regulate pomegranate growth patterns, flower and fruit development. The completion of pomegranate genome data provided momentous data support for the study of pomegranate gene function [38–40]. In this study, the members of the TALE gene family were identified based on the genome sequence of 'Taishanhong', and their physical and chemical properties, protein structure, cis-elements, phylogenetic relationship, and gene tissue expression were analyzed. Through the systematic identification of PgTALE, the result lays a foundation for further study of the function of TALE genes in pomegranate.

2. Materials and Methods

2.1. Genome and Transcriptome Data Sources

Pomegranate genome sequences (ASM286412v1), protein sequences and transcriptome data were downloaded from NCBI (<http://www.ncbi.nlm.nih.gov/>), and the *A. thaliana* TALE protein sequence was downloaded from the *A. thaliana* database (<http://www.arabidopsis.org/>), *E. grandis*, *Populus trichocarpa*, *Malus domestica*, *Vitis vinifera* and *Solanum lycopersicum* TALE protein sequence were downloaded from the PlantTFDB database (<http://planttfdb.cbi.pku.edu.cn>) [6] (Fasta file S1, Table S1).

2.2. Identification and Sequence Analysis of PgTALE Gene Family Members

The hidden Markov model file of the TALE family (E-value $< 1 \times 10^{-5}$) was constructed by using TALE (PF00046) in the Pfam database [41] (<https://pfam.xfam.org/>) and hmmsearch program in HMMER 3.0 software package (Virginia, USA) [42]. The candidate PgTALE protein conserved domains

were searched, and the BELL domains (POX, homeodomain) or KNOX domains (KNOX1, KNOX2, ELK, homeodomain) were TALE gene family conserved. At the same time, using the published 'Taishanhong' protein sequences [38] and the TALE family protein sequences of 6 species (*A. thaliana*, *E. grandis*, *P. trichocarpa*, *M. domestica*, *V. vinifera* and *S. lycopersicum*) as baits to make a local BLASTP alignment (E-value < $1e^{-5}$, identity >50%), the repetition was removed, the candidate TALE protein sequences were screened. In addition, *A. thaliana* contains a member of the TALE gene family *KNATM* without homeodomain, we added a pomegranate gene homologous to *KNATM* [32], and then the target protein domains were detected by SMART and CDD [43,44]. The sequences without the TALE domain were removed. The online tool ExPASy Proteomics Server (<https://web.expasy.org/protparam/>) was used to predict the physical and chemical properties of PgTALE protein, such as amino acid sequence length, molecular weight, isoelectric point, grand average of hydropathicity [45]. Signal peptide of the PgTALE proteins was performed by SignalP 5.0 Sever (<http://www.cbs.dtu.dk/services/SignalP>). Subcellular localization of the PgTALE proteins was performed using CELLO (<http://cello.life.nctu.edu.tw/>) [46].

2.3. Construction of Phylogenetic tree of PgTALE Gene Family

Multiple sequence alignments of candidate proteins with *A. thaliana*, *E. grandis*, *P. trichocarpa* and *V. vinifera* TALE gene family proteins were performed using MAFFT [47]. The phylogenetic tree was constructed by using RA \times ML -NG [48] with Bootstrap 1000 repeats and the best model of JTT + F + I + G4 selected by ModelFinder [49]. Then, the phylogenetic tree was beautified by using the online software tool EvolView (<http://www.evolgenius.info/>) [50].

2.4. Analysis of PgTALE Conserved Motifs and Gene Structure

The motif type and sequence of the PgTALE family were analyzed by MEME (<http://meme-suite.org/tools/meme>) [51], and the motif characteristics of PgTALE were obtained. According to the protein sequence and gene sequence of the PgTALE gene, the gene structure information of pomegranate TALE was obtained by Perl script (Perl file S1), including intron, exon and upstream and downstream sequence. In addition, a combined figure of phylogenetic tree, conserved motifs and gene structure was drawn by TBtools [52].

2.5. Analysis of PgTALE Protein Structure

Protein sequence similarity of more than 35% as a template, the tertiary structure and homologous modelling of PgTALE proteins were analyzed using the SWISS-MODEL (<https://swissmodel.expasy.org/>) [53], and Ramachandran Plots were used to display protein properties.

2.6. Analysis of Cis-elements and Protein-protein Interaction Network of PgTALE Gene Family

To analyze the cis-elements of the promoter region, the 1500 bp sequence upstream of the start codon was obtained from the pomegranate genome sequence by Perl script (Perl file S2), and the sequence was searched by PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) [54]. The protein-protein interaction network of the TALE family was analyzed by String (<https://string-db.org/>) [55].

2.7. Expression Analysis of PgTALE Gene Family

RNA-Seq data of tissues and organs closely related to pomegranate were downloaded from the NCBI database (Table 1). Subsequently, Kallisto version 0.44.0 software (California, USA) [56] was used to index the sequence with the 'Taishanhong' transcriptome file to calculate further and analyze gene expression. The corresponding expression levels (TPM values) of the TALE family members were obtained, and the obtained TPM values were converted by $\text{Log}_2(\text{TPM} + 1)$. Finally, a heat map of the TALE gene was drawn by using the R package heatmap.

Table 1. RNA-Seq data of pomegranate.

Accession No.	Cultivars	Sample Type	Library	Platform	Reference	Note
SRR5279396	‘Dabenzi’	root	Paired end	Illumina HiSeq 4000	[39]	
SRR5279397	‘Dabenzi’	leaf	Paired end	Illumina HiSeq 4000	[39]	
SRR5279395	‘Dabenzi’	flower	Paired end	Illumina HiSeq 4000	[39]	
SRR5279391	‘Dabenzi’	Inner seed coat (50 days after pollination)	Paired end	Illumina HiSeq 4000	[39]	
SRR5279388	‘Dabenzi’	Outer seed coat (50 days after pollination)	Paired end	Illumina HiSeq 4000	[39]	
SRR5279394	‘Dabenzi’	Pericarp (50 days after pollination)	Paired end	Illumina HiSeq 4000	[39]	
SRR5446598	‘Tunisia’	flower (3.0–5.0 mm)	Paired end	Illumina HiSeq 2500	[57]	Functional male flower
SRR5446595	‘Tunisia’	flower (5.1–13.0 mm)	Paired end	Illumina HiSeq 2500	[57]	Functional male flower
SRR5446592	‘Tunisia’	flower (13.1–25.0 mm)	Paired end	Illumina HiSeq 2500	[57]	Functional male flower
SRR5446607	‘Tunisia’	flower (3.0–5.0 mm)	Paired end	Illumina HiSeq 2500	[57]	Female sterility
SRR5446604	‘Tunisia’	flower (5.1–13.0 mm)	Paired end	Illumina HiSeq 2500	[57]	Female sterility
SRR5446601	‘Tunisia’	flower (13.1–25.0 mm)	Paired end	Illumina HiSeq 2500	[57]	Female sterility
SRR5678820	‘Tunisia’	Inner seed coat (50 days after pollination)	Paired end	Illumina HiSeq 4000	[39]	
SRR5678819	‘Baiyushizi’	Inner seed coat (50 days after pollination)	Paired end	Illumina HiSeq 4000	[39]	
SRR080723	‘Wonderful’	pericarp	Paired end	Illumina HiSeq 2000	[58]	
SRR1055290	‘nana’	Mixed samples of leaves, flowers, fruit and roots	Single end	454 GS FLX Titanium	[40]	
SRR1054190	‘Black127’	Mixed samples of root, leaf, flower and fruit	Single end	454 GS FLX Titanium	[40]	

3. Results

3.1. Identification and Sequence Analysis of PgTALE Gene Family Members

In this study, 74 homebox gene family members were identified by using the hmmsearch method. The homebox family consists of five families (HD-ZIP, TALE, WOX, HB-PHD, and HB-other), and they share a PF number (PF00046). As TALE encodes an atypical structure forming two helices and three additional amino acid residues, 16 candidate members of the TALE gene family were identified, and all candidate proteins were identified to belong to the TALE protein family. 23 candidate members of the TALE gene family were identified by BLASTP. While *Pg001623.1*, *Pg009439.1*, *Pg011532.1*, *Pg017964.1*, *Pg017965.1*, *Pg022249.1* and *Pg027515.1* were removed because they did not contain TALE conserved domain. Our result showed that there were 17 members of the TALE gene family in pomegranate (Table S2). *PgTALE* gene family was renamed, the results as shown in Table 2.

The physical and chemical properties of the PgTALEs was analyzed using the ExpASy online tool. The results showed that the length of the 17 PgTALE gene coding regions ranged from 465 bp (PgTALE17) to 2400 bp (PgTALE16). The amino acid length of the TALE protein ranged from 154 aa (PgTALE17) to 799 aa (PgTALE16), and the protein molecular weight ranged from 17605.66 Da (PgTALE17) to 87381.25 Da (PgTALE16). The pI ranged from 5.13 (PgTALE7) to 8.78 (PgTALE16). Among them, the pI of three PgTALE proteins were higher than 7, suggesting that proteins were slightly alkaline; the other 14 PgTALEs were acidic proteins. The grand average of hydropathicity (GRAVY) was between -0.969 to -0.449 , suggesting that PgTALEs are all hydrophilic proteins. The number of

exons of the PgTALEs was 3–6. Besides, the signal peptide prediction showed that there were no signal peptides in all PgTALE proteins, which belonged to non-secreted proteins. Subcellular localization prediction suggested that all PgTALE proteins were distributed on the nucleus.

Table 2. The basic information of the TALE gene family in pomegranate.

Gene Name	Gene ID	Location	ExonNo.	CDS	AA	MW(Da)	pI	GRAVY	Subcellular Localization
PgTALE1	Pg002952.1	scaffold11:2366550:2369265	4	2097	698	75,172.93	7.80	−0.538	Nuclear
PgTALE2	Pg005682.1	scaffold13:4182457:4188943	5	1035	344	38,810.15	5.25	−0.795	Nuclear
PgTALE3	Pg009001.1	scaffold17:1307284:1312800	4	1959	652	70,713.74	6.51	−0.557	Nuclear
PgTALE4	Pg011533.1	scaffold2:5274088:5277780	4	1932	643	70,086.66	6.33	−0.566	Nuclear
PgTALE5	Pg014946.1	scaffold26:1035323:1039874	5	1065	354	40,403.90	6.15	−0.969	Nuclear
PgTALE6	Pg015766.1	scaffold29:588196:590712	4	1905	634	70,031.46	5.87	−0.717	Nuclear
PgTALE7	Pg022248.1	scaffold49:1377908:1386181	5	1065	354	39,800.27	5.13	−0.751	Nuclear
PgTALE8	Pg024529.1	scaffold6:3685906:3688228	4	1563	520	58,385.62	6.10	−0.449	Nuclear
PgTALE9	Pg024817.1	scaffold6:2019598:2022376	5	1275	424	46,087.05	5.94	−0.635	Nuclear
PgTALE10	Pg026506.1	scaffold7:1735075:1737746	4	2103	700	76,325.21	7.82	−0.647	Nuclear
PgTALE11	Pg027513.1	scaffold73:612910:617019	4	1023	340	37,775.80	6.53	−0.534	Nuclear
PgTALE12	Pg028434.1	scaffold8:2877271:2880879	5	909	302	33,829.16	6.27	−0.635	Nuclear
PgTALE13	Pg028770.1	scaffold81:128287:132801	5	2136	711	78,483.78	6.54	−0.513	Nuclear
PgTALE14	Pg029909.1	scaffold9:754612:757823	4	1851	616	68,481.09	6.27	−0.707	Nuclear
PgTALE15	Pg030082.1	scaffold9:3730507:3733807	6	1203	400	45,378.59	5.30	−0.730	Nuclear
PgTALE16	Pg030621.1	scaffold96:200629:218877	6	2400	799	87,381.25	8.78	−0.549	Nuclear
PgTALE17	Pg005241.1	scaffold13:2266264:2268869	3	465	154	17,605.66	5.18	−0.730	Nuclear

3.2. Phylogenetic Tree Analysis of PgTALE Gene Family

To clarify the evolutionary relationship and possible biological functions of members of the PgTALE gene family, the phylogenetic tree of the TALE gene was constructed based on the amino acid sequences of the pomegranate, *A. thaliana*, *E. grandis*, *P. trichocarpa* and *V. vinifera* (Figure 1). Based on the classification of *A. thaliana* TALE gene family (BELL and KNOX family), the pomegranate BELL proteins were classified into five subfamilies: BELL-I (one member), BELL-II (two), BELL-III (one), BELL-IV (two) and BELL-V (three), and KNOX proteins were classified into 3 subfamilies: KNOX-I (five), KNOX-II (two), KNOX-III (five). In each clade, there are branches from the same species, which may be caused by gene duplications [59].

3.3. Analysis of Conserved Motifs and Gene Structures of PgTALE Gene Family

The conserved motifs of PgTALE were identified. 10 conserved motifs (Figure 2), in which Motif 1 represents the homeodomain (homeobox domain, HOX), Motif 4 represents the ELK domain, and Motif 7 represents SKY domain. The location information of the PgTALE protein domain was analyzed. The results showed that the six members of KNOX subfamily contained KNOX1, KNOX2, ELK and HOX domains, and only PgTALE17 did not contain HOX domains. BELL subfamily all contained POX and HOX domains.

Structural analysis showed that the gene structure of PgTALE was a similarity, and there were little differences in the number of exons and introns among PgTALE genes. The number of exons and introns of the PgTALE genes were 3–6 and 2–5, respectively (Figure 2). Members of the same subfamily of PgTALE showed similar gene structure and protein conserved motif distribution. For example, five members of KNOX group (PgTALE2, PgTALE5, PgTALE7, PgTALE9 and PgTALE12) contained five exons and four introns, and only one member (PgTALE17) contained 3 exons and 2 introns. Seven members of BELL group (PgTALE1, PgTALE3, PgTALE4, PgTALE6, PgTALE8, PgTALE10 and PgTALE14) contained four exons and three introns. The above results indicated that the PgTALE gene family had a certain degree of conservation regardless of its genetic structure or protein conserved motifs.

3.4. Protein Structure Analysis and Protein Interaction Networks of Pomegranate TALE Gene Family

The spatial structure of proteins plays a role in the biological function of proteins. The tertiary structure of the protein was analyzed, which found that the structure of the PgTALE family members was similar (Figure 3), except for PgTALE17 without a template (protein sequence similarity of less

than 35%) that we cannot predict protein tertiary structure. The protein is a multi-chain folded protein, mainly α -helix. The calculation test showed that the Ramachandran Favoured value of the PgTALE family was above 90%, and PgTALE2 and PgTALE15 reached 100%, except PgTALE9 was only 87.27%. The results showed that the PgTALE protein had a sTable Spatial structure.

Protein function prediction suggested that PgTALE2, PgTALE7 and PgTALE15 played roles in meristem function (Figure 4), contributing to the shoot apical meristem (SAM) maintenance and organ separation. They may also be involved in maintaining cells in a meristematic state. In addition, PgTALE14 might involve in the regular pattern of organ initiation. PgTALE11 may be required for SAM formation in embryogenesis. PgTALE12 may be involved in secondary cell wall biosynthesis. PgTALE13 might be required for the SAM to respond appropriately to floral inductive signals.

The protein-protein interaction of PgTALE was analyzed for predicting its potential function, signal transduction and metabolic pathways. It was predicted that there were interactions between PgTALE14 and AG, SEP3, KNAT1, INO and other proteins to regulate ovule development. In addition, BEL1 can form heterodimers with KNAT1, it predicted that PgTALE14 (BELL family) may interact with PgTALE5 (KNOX family) to form heterodimers. PgTALE8 might interact with STM and KNAT6 and enhance the apical meristem of these genes.

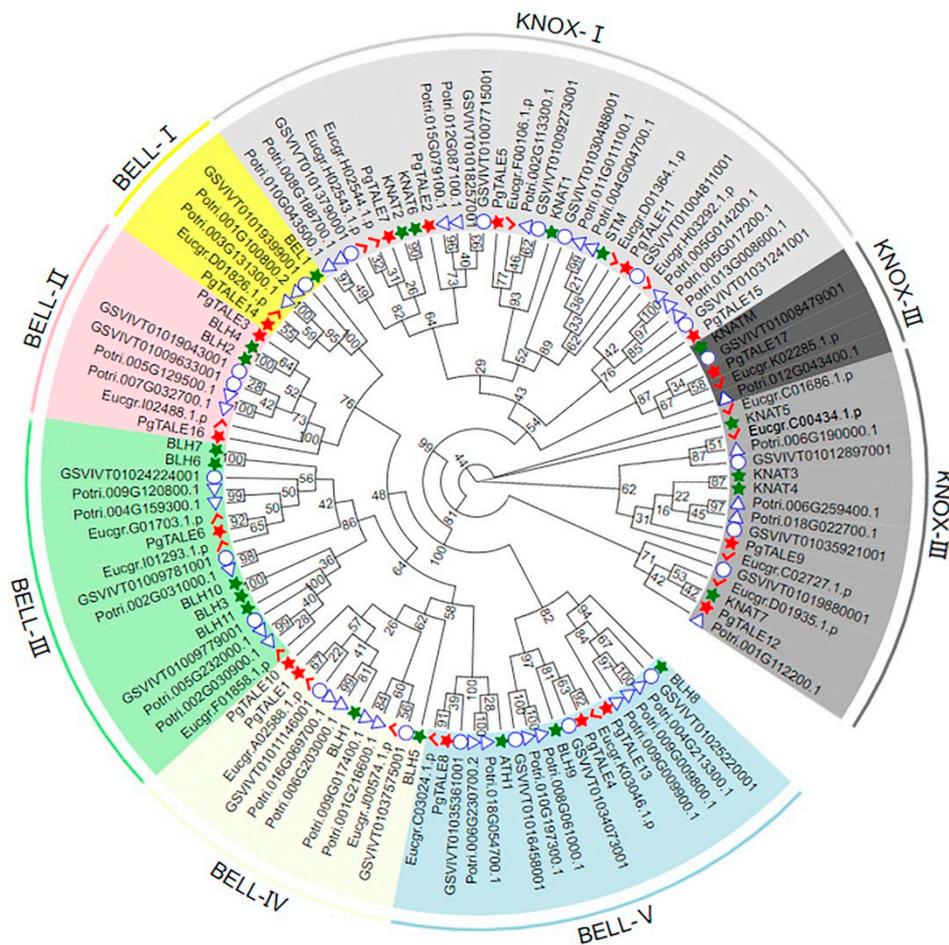


Figure 1. The phylogenetic tree of the three-amino-acid-loop-extension (TALE) gene family in pomegranate, *A. thaliana*, *E. grandis*, *P. trichocarpa* and *V. vinifera*. The phylogenetic tree was constructed by RA \times ML -NG with Bootstrap 1000 repeats and the best model of JTT + F + I + G4. Pomegranate, *A. thaliana*, *E. grandis*, *P. trichocarpa* and *V. vinifera* TALE proteins are marked with a solid red star, solid green star, red check, blue triangle and blue circle, respectively.

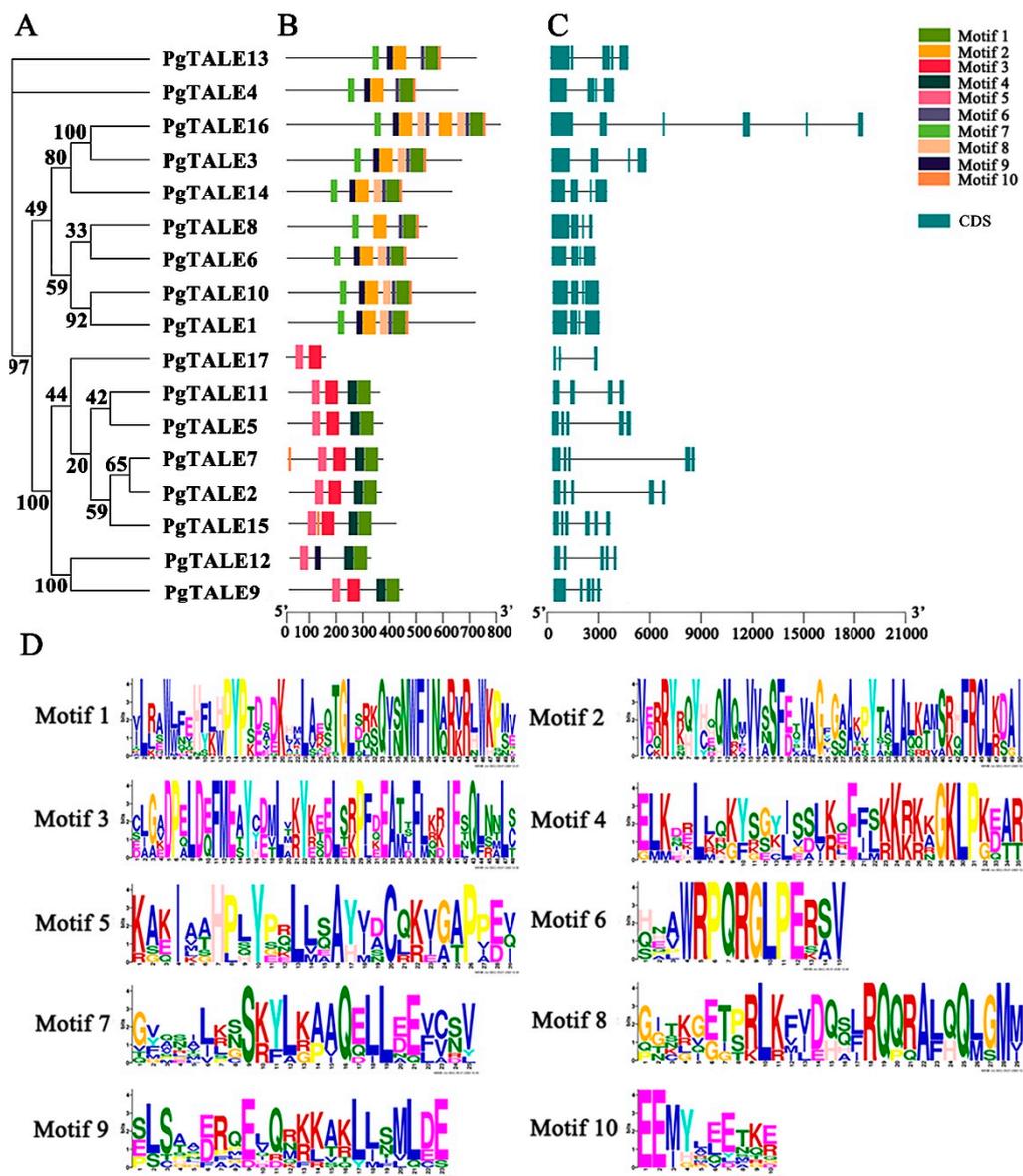


Figure 2. Phylogenetic tree, conserved motifs and gene structures of the PgTALE gene family. (A) The phylogenetic tree of 17 PgTALE proteins. (B) Conserved motifs in the TALE proteins. The motifs were identified by the MEME Suite. Different conserved motifs, numbers 1–10, are displayed in different colored boxes. (C) Gene structures of the TALE genes. The intron/exon structure was mapped by TBtools. The black dotted line represents introns. (D) Sequence logos of 10 conserved motifs were identified.

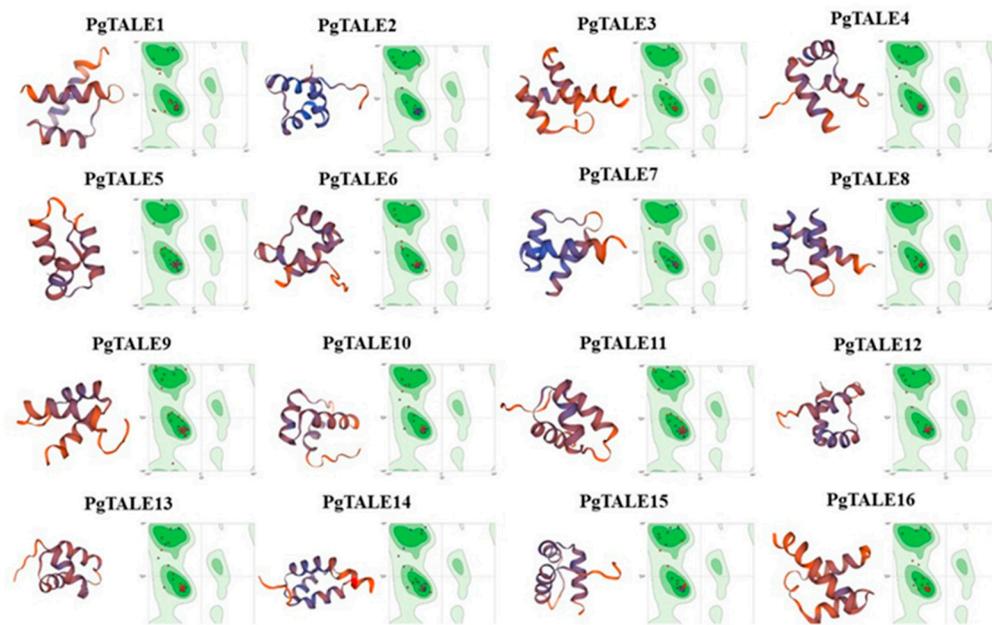


Figure 3. Tertiary structure and Ramachandran plot of PgTALE protein. The tertiary structure of the protein was analyzed through the online SWISS-MODEL.

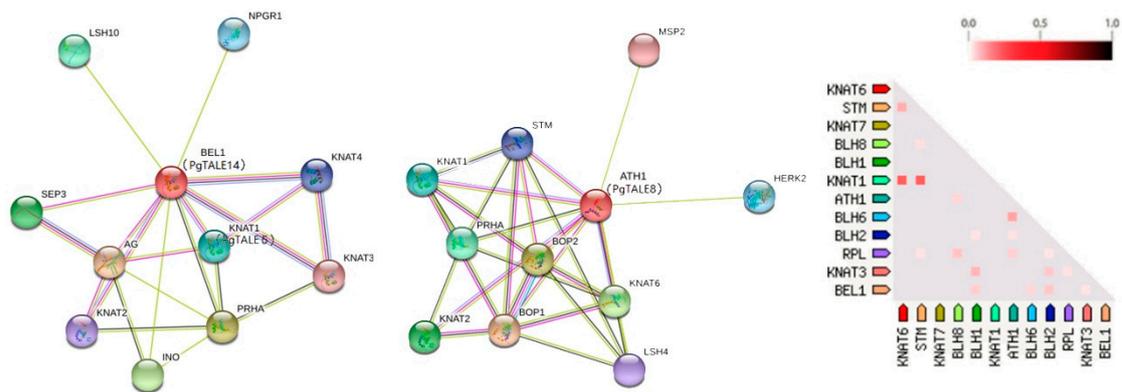


Figure 4. Protein-protein interaction network and co-expression of PgTALE proteins was analyzed by String online software.

From the figure of gene co-expression (Figure 4), we can see the level of co-expression of *KNAT1/KNAT3/KNAT6/STM/BEL1/BLH6/ATH1* and other genes. Among them, *KNAT1* and *KNAT6*, *KNAT1* and *STM* were higher than that of other genes. They may participate in or respond to a biological or abiotic stress process, and it may be inferred that *PgTALE2/PgTALE5/PgTALE11* may also have similar functions.

3.5. Analysis of Cis-elements of PgTALE Gene Family

In this study, the upstream 1500 bp sequence of *PgTALE* gene was extracted, the possible cis-elements in the promoter region were found (Table S3). 13 cis-elements related to abiotic stress were found, which were ABRE, ARE, AuxRR-core, CAAT-box, CGTCA-motif, GARE-motif, LTR, MBS, P-box, TATC-box, TCA-element, TGA-element and TGACG-motif (Figure 5; Table S4). AuxRR-core and TGA-element are auxin-responsive elements. CGTCA-motif and TGACG-motif are MeJA-responsiveness elements, while GARE-motif, P-box and TATC-box are gibberellin response elements. The *PgTALE* genes contain the enhancer response element CAAT-box. 64.7% of the *PgTALE* genes contain ABA response element ABRE and the cold stress response element LTR. 70.6% of the

PgTALE genes contain the antioxidant response element ARE. 41.2% of the *PgTALE* gene contains MeJA-responsiveness response elements CGTCA-motif and TGACG-motif, the salicylic acid response element TCA-element, 29.4%, 23.5%, 35.3% of the *PgTALE* genes contain gibberellin response elements GARE-motif, P-box, TATC-box, 29.4% of the *PgTALE* genes contain the drought stress response element MBS. Besides, only the *PgTALE8* gene contains the auxin response element AuxRR-core, and *PgTALE12* and *PgTALE13* contain the auxin response element TGA-element.

3.6. Expression Analysis of *PgTALE* Gene Family

To further analyze the characteristics and function of the *PgTALE* genes, the tissue-specific expression of the TALE gene was analyzed (Figure 6; Table S5). The results showed that the vast majority of *PgTALE* genes were expressed in different tissues, but *PgBLH8* was expressed in trace or no expression in all tissues.

PgTALE5, *PgTALE12*, and *PgTALE15* are expressed during functional male flower development, indicating that these genes may be involved in the female and male organ differentiation; *PgTALE1* and *PgTALE9* are higher expressed in leaves, bisexual and functional male flower, indicating that they may be related to the differentiation of male and female organs of pomegranate flowers and regulating leaf development. There are also differences in the expression of different *PgTALE* genes in different tissue, such as *PgTALE2* is not expressed in the inner seed coat, outer seed coat and pericarp. The expression of *PgTALE9* is the highest in the functional male flower (5.1 m–13.0 mm), and the expression of *PgTALE10* is the highest in the pericarp. However, there are some differences in the expression of different *PgTALE* genes in different pomegranate varieties, such as *PgTALE7* and *PgTALE14* in the varieties of ‘Dabenzi’, ‘Tunisia’ and ‘Baiyushizi’. In the same pomegranate variety ‘Dabenzi’, there are also significant differences in tissue expression between leaves and outer seed coat. For example, the expression of *PgTALE16* is higher in leaves, but the lowest in the outer seed coat.

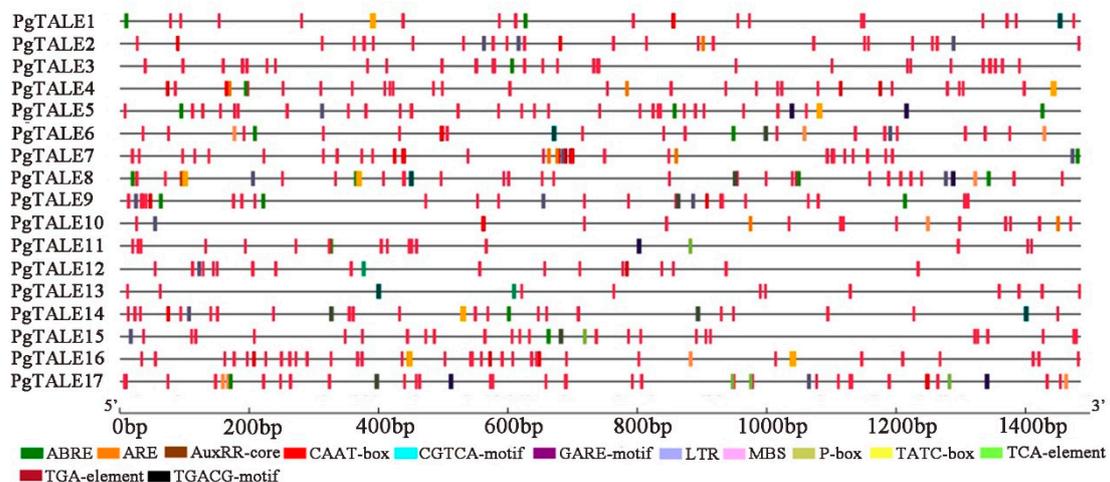


Figure 5. Cis-elements analysis of *PgTALE* genes. Note: ABRE involved in the abscisic acid responsiveness; ARE involved in anaerobic responsiveness; AuxRR-core and TGA-element involved in auxin responsiveness; CAAT-box involved in promoter and enhancer regions; CGTCA-motif and TGACG-motif involved in the MeJA-responsiveness; GARE-motif, P-box and TATC-box involved in gibberellin-responsiveness; LTR involved in low-temperature responsiveness; MBS involved in drought-inducibility; TCA-element involved in salicylic acid responsiveness.

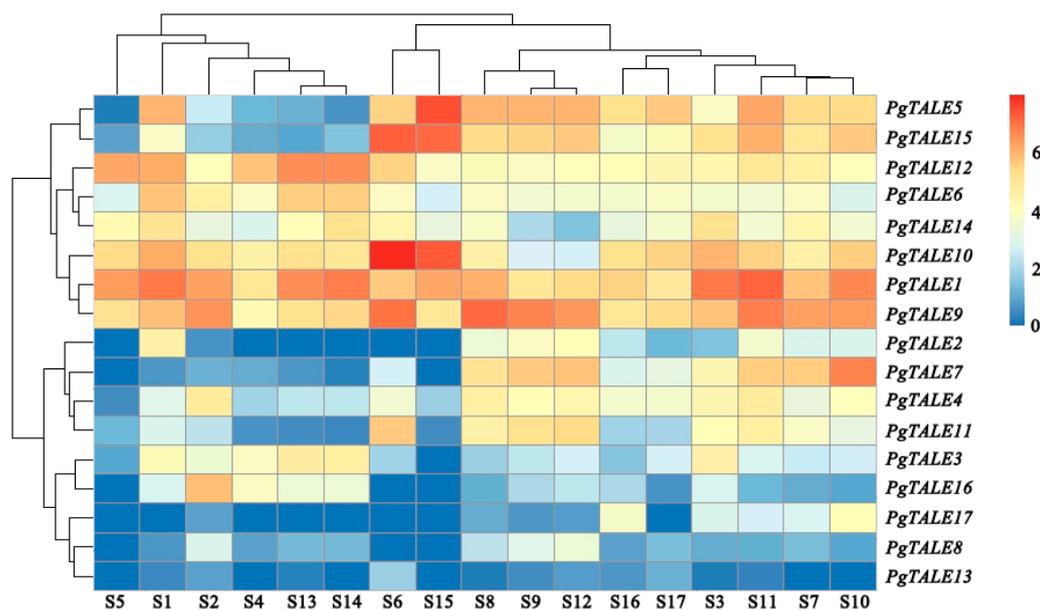


Figure 6. Heatmap of PgTALE gene expression in different tissues. Note: S1: Root; S2: Flesh leaf; S3: Flower; S4: Inner seed coat of ‘Dabenzi’ (50 days after pollination); S5: Outer seed coat (50 days after pollination); S6: Pericarp (50 days after pollination); S7: Functional male flowers (13.1–25.0 mm); S8: Functional male flowers (5.1–13.0 mm); S9: Functional male flowers (3.0–5.0 mm); S10: Female sterility (13.1–25.0 mm); S11: Female sterility (5.1–13.0 mm); S12: Female sterility (3.0–5.0 mm); S13: Inner seed coat of ‘Tunisia’ (50 days after pollination); S14: Inner seed coat of ‘Baiyushizi’ (50 days after pollination); S15: Pericarp of ‘Wonderful’; S16: Mix of leaves, flowers, fruit and roots of ‘nana’; S17: Mix of leaves, flowers, fruit and roots of ‘Black127’ (cultivars S1–S6 are ‘Dabenzi’, cultivars S7–S13 are ‘Tunisia’).

4. Discussion

The TALE gene family is found in plant meristems and is related to the differentiation and signal transduction of meristems, for example, it can inhibit the expression of the critical enzyme gene *ga20ox1* in the GA pathway [60]. In other important fruits belonging to the Rosaceae family, TALE are involved in the rootstock responding to apple cold stress [61], the cherry anthesis [62]. In addition, it regulated tomato fruit development [28]. Currently, the TALE gene family has been found in many plants: 33 *AtTALE* genes in *A. thaliana*, 40 *LjTALE* genes in *Lotus japonicas* K. [63], 46 *GaTALE* genes in *Gossypium arboreum* L., 47 *GrTALE* genes in *G. raimondii* L., 88 *GbTALE* genes in *G. barbadense* L., 94 *GhTALE* genes *G. hirsutum* L. [8], and 35 *PtTALE* genes in poplar [64], 7 *VsTALE* genes in *Vandenboschia speciose* G. [16]. Therefore, the copy number of the TALE gene family in different species is different. At present, the genomic data of three pomegranate varieties have been released in China, but there are no reports on the identification and analysis of pomegranate TALE family genes. In this study, for the first time, 17 TALE genes were identified in the pomegranate. Through the analysis of the physicochemical properties of the protein (Table 1), it was found that pomegranate TALE proteins are all hydrophilic proteins that are consistent with studies in Poplar and *L. japonicas* [63,64]. Domain differences may represent the regulatory effects of promoting or inhibiting. In addition, the *PgTALE* genes are divided into eight subfamilies (Figure 1), which is consistent with the *A. thaliana* and cotton TALE gene subfamily classification [8].

The cis-elements exist at the gene promoter site and specifically binds transcription factors to regulate gene transcription. This study found that the *PgTALE* promoter sequence contained multiple cis-elements related to hormonal response and abiotic stress, which are rich in methyl jasmonate response element, abscisic acid response element and gibberellin response element, which is similar to antecedent studies [8]. It indicated that the promoter of the TALE gene has a certain conservative. Previous studies have found that ABRE is associated with plant drought, ABA induction, and high

salt stress in plants [31,65]. In addition, there are a series of elements related to stress, such as ARE, MBS and LTR. The results indicate that PgTALE plays a role in pomegranate abiotic stress. Gene function prediction and protein-protein network analysis also show that the PgTALE family plays a significant role in regulating ovule and inflorescence development. Gene functional prediction and protein-protein network analysis also showed that there are some interactions between PgTALE14, AG and KNAT1 in floral organs; the results were consistent with the previous study [7,25].

The tissue expression analysis of the *PgTALE* found that most of them were expressed in diverse tissues and varieties, but diverse *PgTALE* genes were expressed in different tissue varieties and showed specific differences. It was similar to the results of TALE genes in *A. thaliana* [66]. It can be speculated that the TALE family of pomegranate has similar functions to this family in other plants. According to the function of *BEL1* in *A. thaliana*, we speculated that its homologous gene *PgTALE14* has important regulatory significance in the development of pomegranate ovules [67,68]; *PgTALE8*, as the homologous gene of *ATH1*, controls inflorescence development [69,70]. The specificity of tissue and variety expression is speculated to be closely related to its gene function. For example, *PgTALE1*, *PgTALE6*, *PgTALE9*, *PgTALE10*, *PgTALE12* and *PgTALE14* had high expression levels in the functional male flowers, bisexual flowers, and fruit tissues. It is predicted that *PgTALE* may have roles in maintaining flower organ and fruit development. However, due to the inconsistency of some sequencing platforms (Illumina and 454) in RNA-seq data, to a certain extent, it may lead to the uneven sequencing depth among tissue samples and the gap in reading length, which has a certain impact on the analysis results, while the difference in pomegranate varieties also has a certain error on the expression analysis results. After the normalization of RNA-seq data, the error may be reduced.

5. Conclusions

In this study, 17 *PgTALE* members were identified in pomegranate and explored their phylogenetic relationships. The *PgTALE* gene structure of all members of the subfamily is very similar. *PgTALE* may participate in the apical meristems, flower organ and fruit development, and the subfamily genes may have the same expression pattern. These conclusions are the foundation for the function research of the *PgTALE* gene and provide a reference for exploring its evolutionary process.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/10/6/829/s1>, Fasta file S1: TALE sequences of 6 species as baits, Perl file S1: Gene structure, Perl file S2: Promoter, Table S1: IDs of TALE sequences of 6 species, Table S2: Domains of 17 *PgTALE*, Table S3: All cis-elements of 17 *PgTALE*, Table S4: 13 cis-elements related to abiotic stress of 17 *PgTALE*, Table S5: RNA-seq data of 17 *PgTALE*.

Author Contributions: Conceptualization, Y.W. and Z.Y.; methodology, Y.W. and Y.Z.; formal analysis, Y.W.; investigation, Y.W., Y.Z. and M.Y.; writing—original draft preparation, Y.W.; writing—review and editing, Y.W., Y.Z., M.Y., H.Z., X.Z. and Z.Y.; supervision, Z.Y.; funding acquisition, Z.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Initiative Project for Talents of Nanjing Forestry University [GXL2014070, GXL2018032], the Priority Academic Program Development of Jiangsu High Education Institutions [PAPD], the Natural Science Foundation of Jiangsu Province [BK20180768].

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

Abbreviations	Full Name
TALE	three-amino-acid-loop-extension
KNOX/KNAT	KNOTTED-like homeobox
BELL/BLH	BEL1-like
HB	homeobox
ZM-HOX	<i>Zea mays</i> homeobox
HAT1	homeobox from <i>Arabidopsis thaliana</i> 1
HAT2	homeobox from <i>A. thaliana</i> 2
ATHB8	<i>A. thaliana</i> homeobox 8
HD-ZIP	homeodomain-leucine zipper
PLINC	plant zinc finger
WOX	wuschel homeobox
PHD	plant homeodomain
NDX	nodulin homeobox genes
LD	luminidependens
OPF	OVATE family protein
ATH1	<i>A. thaliana</i> homeobox 1
STM	shoot meristemless
KNAT2	KNOTTED-like from <i>A. thaliana</i> 2
APG IV	Angiosperm Phylogeny Group IV
HOX	homeobox domain
SAM	shoot apical meristem

References

- Di Giacomo, E.; Iannelli, M.A.; Frugis, G. TALE and shape: How to make a leaf different. *Plants* **2013**, *2*, 317–342. [[CrossRef](#)] [[PubMed](#)]
- Billeter, M.; Qian, Y.Q.; Otting, G.; Müller, M.; Gehring, W.; Wüthrich, K. Determination of the nuclear magnetic resonance solution structure of an *Antennapedia* homeodomain-DNA complex. *J. Mol. Biol.* **1993**, *234*, 1084–1097. [[CrossRef](#)] [[PubMed](#)]
- Bharathan, G.; Janssen, B.J.; Kellogg, E.A. Did homeodomain proteins duplicate before the origin of angiosperms, fungi, and metazoa? *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 13749–13753. [[CrossRef](#)] [[PubMed](#)]
- Mukherjee, K.; Brocchieri, L.; Bürglin, T.R. A Comprehensive classification and evolutionary analysis of plant homeobox genes. *Mol. Biol. Evol.* **2009**, *26*, 2775–2794. [[CrossRef](#)] [[PubMed](#)]
- Bürglin, T.R.; Affolter, M. Homeodomain proteins: An update. *Chromosoma* **2016**, *125*, 497–521. [[CrossRef](#)] [[PubMed](#)]
- Jin, J.; Tian, F.; Yang, D.; Meng, Y.; Kong, L.; Luo, J.; Gao, G. PlantTFDB 4.0: Toward a central hub for transcription factors and regulatory interactions in plants. *Nucleic Acids Res.* **2017**, *45*. [[CrossRef](#)]
- Arnaud, N.; Pautot, V.R. Ring the BELL and tie the KNOX: Roles for TALEs in gynoecium development. *Front. Plant Sci.* **2014**, *5*, 93. [[CrossRef](#)]
- Ma, Q.; Wang, N.; Hao, P.; Sun, H.; Yu, S. Genome-wide identification and characterization of TALE superfamily genes in cotton reveals their functions in regulating secondary cell wall biosynthesis. *BMC Plant Biol.* **2019**, *19*, 432. [[CrossRef](#)]
- Chen, H.; Rosin, F.M.; Prat, S.; Hannapel, D.J. Interacting transcription factors from the three-amino acid loop extension superclass regulate tuber formation. *Plant Physiol.* **2003**, *132*, 1391–1404. [[CrossRef](#)]
- Hay, A.; Tsiantis, M. KNOX genes: Versatile regulators of plant development and diversity. *Development* **2010**, *137*, 3153–3165. [[CrossRef](#)]
- Bertolino, E.; Reimund, B.; Wildtperinic, D.; Clerc, R.G. A novel homeobox protein which recognizes a TGT core and functionally interferes with a retinoid-responsive motif. *J. Biol. Chem.* **1995**, *270*, 31178–31188. [[CrossRef](#)] [[PubMed](#)]
- Mahajan, A.; Bhogale, S.; Kang, I.H.; Hannapel, D.J.; Banerjee, A.K. The mRNA of a Knotted1-like transcription factor of potato is phloem mobile. *Plant Mol. Biol.* **2012**, *79*, 595–608. [[CrossRef](#)] [[PubMed](#)]

13. Sakakibara, K.; Ando, S.; Yip, H.K.; Tamada, Y.; Hiwatashi, Y.; Murata, T.; Deguchi, H.; Hasebe, M.; Bowman, J.L. KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants. *Science* **2013**, *339*, 1067–1070. [[CrossRef](#)] [[PubMed](#)]
14. Lin, T.; Sharma, P.; Gonzalez, D.H.; Viola, I.L.; Hannapel, D.J. The impact of the long-distance transport of a BEL1-Like Messenger RNA on development. *Plant Physiol.* **2013**, *161*, 760–772. [[CrossRef](#)]
15. Furumizu, C.; Alvarez, J.P.; Sakakibara, K.; Bowman, J.L. Antagonistic roles for KNOX1 and KNOX2 genes in patterning the land plant body plan following an ancient gene duplication. *PLoS Genet.* **2015**, *11*. [[CrossRef](#)]
16. Ruiz-Estévez, M.; Bakkali, M.; Martinblazquez, R.; Garridoramos, M.A. Identification and characterization of TALE homeobox genes in the endangered fern *Vandenboschia speciosa*. *Genes* **2017**, *8*, 275. [[CrossRef](#)]
17. Belles-Boix, E.; Hamant, O.; Witiak, S.M.; Morin, H.; Traas, J.; Pautot, V. KNAT6: An Arabidopsis homeobox gene involved in meristem activity and organ separation. *Plant Cell* **2006**, *18*, 1900–1907. [[CrossRef](#)]
18. Aida, M.; Ishida, T.; Tasaka, M. Shoot apical meristem and cotyledon formation during *Arabidopsis* embryogenesis: Interaction among the CUP-SHAPED COTYLEDON and SHOOT MERISTEMLESS genes. *Development* **1999**, *126*, 1563.
19. Shani, E.; Yanai, O.; Ori, N. The role of hormones in shoot apical meristem function. *Curr. Opin. Plant Biol.* **2006**, *9*, 484–489. [[CrossRef](#)]
20. Cnops, G.; Neyt, P.; Raes, J.; Petrarulo, M.; Nelissen, H.; Malenica, N.; Luschnig, C.; Tietz, O.; Ditengou, F.A.; Palme, K. The TORNADO1 and TORNADO2 genes function in several patterning processes during early leaf development in *Arabidopsis thaliana*. *Plant Cell* **2006**, *18*, 852–866. [[CrossRef](#)]
21. Kondhare, K.R.; Vetal, P.V.; Kalsi, H.S.; Banerjee, A.K. BEL1-like protein (StBEL5) regulates *CYCLING DOF FACTOR1* (*StCDF1*) through tandem TGAC core motifs in potato. *J. Plant Physiol.* **2019**, *241*, 153014. [[CrossRef](#)] [[PubMed](#)]
22. Bhatt, A.M.; Etheells, J.P.; Canales, C.; Lagodienko, A.; Dickinson, H.G. VAAMANA-a BEL1-like homeodomain protein, interacts with KNOX proteins BP and STM and regulates inflorescence stem growth in *Arabidopsis*. *Gene* **2004**, *328*, 103–111. [[CrossRef](#)]
23. Kim, D.; Cho, Y.; Ryu, H.; Kim, Y.; Kim, T.; Hwang, I. BLH1 and KNAT3 modulate ABA responses during germination and early seedling development in *Arabidopsis*. *Plant J.* **2013**, *75*, 755–766. [[CrossRef](#)]
24. Smith, H.M.; Boschke, I.; Hake, S. Selective interaction of plant homeodomain proteins mediates high DNA-binding affinity. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 9579–9584. [[CrossRef](#)]
25. Brambilla, V.; Battaglia, R.; Colombo, M.; Masiero, S.; Bencivenga, S.; Kater, M.M.; Colombo, L. Genetic and molecular interactions between BELL1 and MADS Box factors support ovule development in *Arabidopsis*. *Plant Cell* **2007**, *19*, 2544–2556. [[CrossRef](#)] [[PubMed](#)]
26. Hackbusch, J.; Richter, K.; Muller, J.; Salamini, F.; Uhrig, J.F. A central role of *Arabidopsis thaliana* ovate family proteins in networking and subcellular localization of 3-aa loop extension homeodomain proteins. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 4908–4912. [[CrossRef](#)]
27. Byrne, M.E.; Groover, A.; Fontana, J.R.; Martienssen, R.A. Phyllotactic pattern and stem cell fate are determined by the *Arabidopsis* homeobox gene BELLRINGER. *Development* **2003**, *130*, 3941–3950. [[CrossRef](#)]
28. Meng, L.; Fan, Z.; Zhang, Q.; Wang, C.; Gao, Y.; Deng, Y.; Zhu, B.; Zhu, H.; Chen, J.; Shan, W. BEL1-LIKE HOMEODOMAIN 11 regulates chloroplast development and chlorophyll synthesis in tomato fruit. *Plant J.* **2018**, *94*, 1126–1140. [[CrossRef](#)]
29. Rutjens, B.; Bao, D.; Van Eckstouten, E.; Brand, M.; Smeekens, S.; Proveniers, M. Shoot apical meristem function in *Arabidopsis* requires the combined activities of three BEL1-like homeodomain proteins. *Plant J.* **2009**, *58*, 641–654. [[CrossRef](#)]
30. Li, E.; Bhargava, A.; Qiang, W.; Friedmann, M.; Forneris, N.; Savidge, R.; Johnson, L.; Mansfield, S.; Ellis, B.; Douglas, C. The Class II KNOX gene KNAT7 negatively regulates secondary wall formation in *Arabidopsis* and is functionally conserved in *Populus*. *New Phytol.* **2012**, *194*, 102–115. [[CrossRef](#)]
31. Li, P.; Yu, S.; Shen, J.; Li, Q.; Li, D.; Li, D.; Zheng, C.; Shu, H. The transcriptional response of apple alcohol acyltransferase (MdAAT2) to salicylic acid and ethylene is mediated through two apple MYB TFs in transgenic tobacco. *Plant Mol. Biol.* **2014**, *85*, 627–638. [[CrossRef](#)]
32. Magnani, E.; Hake, S. KNOX lost the OX: The *Arabidopsis* KNATM gene defines a novel class of KNOX transcriptional regulators missing the homeodomain. *Plant Cell* **2008**, *20*, 875–887. [[CrossRef](#)] [[PubMed](#)]
33. Hamant, O.; Pautot, V. Plant development: A TALE story. *C. R. Biol.* **2010**, *333*, 371–381. [[CrossRef](#)] [[PubMed](#)]

34. Zhong, R.; Lee, C.; Zhou, J.; McCarthy, R.L.; Ye, Z.H. A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in *Arabidopsis*. *Plant Cell* **2008**, *20*, 2763–2782. [[CrossRef](#)]
35. Bhargava, A.; Mansfield, S.D.; Hall, H.; Douglas, C.J.; Ellis, B.E. MYB75 functions in regulation of secondary cell wall formation in the *Arabidopsis* inflorescence stem. *Plant Physiol.* **2010**, *154*, 1428–1438. [[CrossRef](#)]
36. Li, E.; Wang, S.; Liu, Y.; Chen, J.; Douglas, C.J. OVATE FAMILY PROTEIN4 (OFP4) interaction with KNAT7 regulates secondary cell wall formation in *Arabidopsis thaliana*. *Plant J.* **2011**, *67*, 328–341. [[CrossRef](#)]
37. Byng, J.W.; Chase, M.W.; Christenhusz, M.J.M.; Fay, M.F.; Judd, W.S.; Mabberley, D.J.; Sennikov, A.N.; Soltis, D.E.; Soltis, P.S.; Stevens, P.F.; et al. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. *Bot. J. Linn. Soc.* **2016**, *181*, 1–20.
38. Yuan, Z.; Fang, Y.; Zhang, T.; Fei, Z.; Han, F.; Liu, C.; Liu, M.; Xiao, W.; Zhang, W.; Wu, S. The pomegranate (*Punica granatum* L.) genome provides insights into fruit quality and ovule developmental biology. *Plant Biotechnol. J.* **2018**, *16*, 1363–1374. [[CrossRef](#)]
39. Qin, G.; Xu, C.; Ming, R.; Tang, H.; Guyot, R.; Kramer, E.M.; Hu, Y.; Yi, X.; Qi, Y.; Xu, X. The pomegranate (*Punica granatum* L.) genome and the genomics of punicalagin biosynthesis. *Plant J.* **2017**, *91*, 1108–1128. [[CrossRef](#)]
40. Ophir, R.; Sherman, A.; Rubinstein, M.; Eshed, R.; Sharabi Schwager, M.; Harel-Beja, R.; Bar-Ya'akov, I.; Holland, D. Single-Nucleotide Polymorphism markers from De-Novo assembly of the pomegranate transcriptome reveal germplasm genetic diversity. *PLoS ONE* **2014**, *9*, e88998. [[CrossRef](#)]
41. El-Gebali, S.; Mistry, J.; Bateman, A.; Eddy, S.R.; Luciani, A.; Potter, S.C.; Qureshi, M.; Richardson, L.J.; Salazar, G.A.; Smart, A.; et al. The Pfam protein families database in 2019. *Nucleic Acids Res.* **2019**, *47*, D427–D432. [[CrossRef](#)] [[PubMed](#)]
42. Finn, R.D.; Clements, J.; Eddy, S.R. HMMER web server: Interactive sequence similarity searching. *Nucleic Acids Res.* **2011**, *39*, 29–37. [[CrossRef](#)] [[PubMed](#)]
43. Schultz, J.; Milpetz, F.; Bork, P.; Ponting, C.P. SMART, a simple modular architecture research tool: Identification of signaling domains. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 5857–5864. [[CrossRef](#)] [[PubMed](#)]
44. Marchler-Bauer, A.; Bo, Y.; Han, L.; He, J.; Lanczycki, C.J.; Lu, S.; Chitsaz, F.; Derbyshire, M.K.; Geer, R.C.; Gonzales, N.R.; et al. CDD/SPARCLE: Functional classification of proteins via subfamily domain architectures. *Nucleic Acids Res.* **2017**, *45*, D200–D203. [[CrossRef](#)] [[PubMed](#)]
45. Artimo, P.; Jonnalagedda, M.; Arnold, K.; Baratin, D.; Csardi, G.; De Castro, E.; Duvaud, S.; Flegel, V.; Fortier, A.; Gasteiger, E. ExPASy: SIB bioinformatics resource portal. *Nucleic Acids Res.* **2012**, *40*, 597–603. [[CrossRef](#)]
46. Yu, C.; Lin, C.; Hwang, J. Predicting subcellular localization of proteins for Gram-negative bacteria by support vector machines based on n-peptide compositions. *Protein Sci.* **2004**, *13*, 1402–1406. [[CrossRef](#)] [[PubMed](#)]
47. Katoh, K.; Standley, D.M. A simple method to control overalignment in the MAFFT multiple sequence alignment program. *Bioinformatics* **2016**, *32*, 1933–1942. [[CrossRef](#)]
48. Kozlov, A.M.; Darriba, D.; Flouri, T.; Morel, B.; Stamatakis, A. RAxML -NG: A fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* **2019**, *35*, 1–4453. [[CrossRef](#)]
49. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; von Haeseler, A.; Jermiin, L.S. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **2017**, *14*, 587–589. [[CrossRef](#)]
50. Subramanian, B.; Gao, S.; Lercher, M.J.; Hu, S.; Chen, W.H. Evolview v3: A webserver for visualization, annotation, and management of phylogenetic trees. *Nucleic Acids Res.* **2019**, *47*, W270–W275. [[CrossRef](#)] [[PubMed](#)]
51. Bailey, T.L.; Bodén, M.; Buske, F.A.; Frith, M.; Grant, C.E.; Clementi, L.; Ren, J.; Li, W.W.; Noble, W.S. MEME SUITE: Tools for motif discovery and searching. *Nucleic Acids Res.* **2009**, *37*, W202–W208. [[CrossRef](#)] [[PubMed](#)]
52. Chen, C.; Xia, R.; Chen, H.; He, Y. TBtools, a Toolkit for Biologists integrating various HTS-data handling tools with a user-friendly interface. *BioRxiv* 2018. [[CrossRef](#)]
53. Waterhouse, A.; Bertoni, M.; Bienert, S.; Studer, G.; Tauriello, G.; Gumienny, R.; Heer, F.T.; de Beer, T.A.P.; Rempfer, C.; Bordoli, L.; et al. SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Res.* **2018**, *46*, W296–W303. [[CrossRef](#)] [[PubMed](#)]

54. Lescot, M.; Dhais, P.; Thijs, G.; Marchal, K.; Moreau, Y.; Peer, Y.V.; Rouz, 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 Res.* **2002**, *30*, 325–327. [[CrossRef](#)] [[PubMed](#)]
55. Szklarczyk, D.; Gable, A.L.; Lyon, D.; Junge, A.; Wyder, S.; Huerta-Cepas, J.; Simonovic, M.; Doncheva, N.T.; Morris, J.H.; Bork, P.; et al. STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* **2019**, *47*, D607–D613. [[CrossRef](#)] [[PubMed](#)]
56. Bray, N.; Pimentel, H.; Melsted, P.; Pachter, L. Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol.* **2016**, *34*, 525–527. [[CrossRef](#)]
57. Chen, L.; Zhang, J.; Li, H.; Niu, J.; Xue, H.; Liu, B.; Wang, Q.; Luo, X.; Zhang, F.; Zhao, D.; et al. Transcriptomic analysis reveals candidate genes for female sterility in pomegranate flowers. *Front Plant Sci.* **2017**, *8*, 1430. [[CrossRef](#)]
58. Ono, N.N.; Britton, M.T.; Fass, J.N.; Nicolet, C.M.; Lin, D.; Tian, L. Exploring the transcriptome landscape of pomegranate fruit peel for natural product biosynthetic gene and SSR marker discovery. *J. Integr. Plant. Biol.* **2011**, *53*, 800–813. [[CrossRef](#)]
59. Zhang, T.; Liu, C.; Huang, X.; Zhang, H.; Yuan, Z. Land-plant phylogenomic and pomegranate transcriptomic analyses reveal an evolutionary scenario of CYP75 genes subsequent to whole genome duplications. *J. Plant Biol.* **2019**, *62*, 48–60. [[CrossRef](#)]
60. Chen, H.; Banerjee, A.K.; Hannapel, D.J. The tandem complex of BEL and KNOX partners is required for transcriptional repression of *ga20ox1*. *Plant J.* **2004**, *38*, 276–284. [[CrossRef](#)]
61. Wang, H.; Cheng, L.; He, P.; Chang, Y.; Li, L. Identification of genes encoding transcription factors of apple cold-resistant dwarfing rootstock in response to cold stress. *Plant Physiol. J.* **2017**, *53*, 1468–1478.
62. Wen, B.; Song, W.; Sun, M.; Chen, M.; Mu, Q.; Zhang, X.; Wu, Q.; Chen, X.; Gao, D.; Wu, H. Identification and characterization of cherry (*Cerasus pseudocerasus* G. Don) genes responding to parthenocarpy induced by GA3 through transcriptome analysis. *BMC Genet.* **2019**, *20*, 65. [[CrossRef](#)] [[PubMed](#)]
63. Qiu, R.; Zhang, T.; Yang, S.; Song, L.; Zhao, D. Genome-wide identification and bioinformatics analysis of TALE transcription factor family in *Lotus japonicas*. *J. Plant Genet. Resour.* **2019**, *20*, 466–475.
64. Zhao, K.; Zhang, X.; Cheng, Z.; Yao, W.; Li, R.; Jiang, T.; Zhou, B. Comprehensive analysis of the three-amino-acid-loop-extension gene family and its tissue-differential expression in response to salt stress in poplar. *Plant Physiol. Bioch.* **2019**, *136*, 1–12. [[CrossRef](#)] [[PubMed](#)]
65. Hundertmark, M.; Hincha, D.K. LEA (Late Embryogenesis Abundant) proteins and their encoding genes in *Arabidopsis thaliana*. *BMC Genom.* **2008**, *9*, 118. [[CrossRef](#)]
66. Liberman, L.M.; Sparks, E.E.; Morenorisueno, M.A.; Petricka, J.J.; Benfey, P.N. MYB36 regulates the transition from proliferation to differentiation in the *Arabidopsis* root. *Proc. Natl. Acad. Sci. USA.* **2015**, *112*, 12099–12104. [[CrossRef](#)]
67. Ray, A.; Robinsonbeers, K.; Ray, S.; Baker, S.C.; Lang, J.D.; Preuss, D.; Milligan, S.B.; Gasser, C.S. *Arabidopsis* floral homeotic gene BELL (BEL1) controls ovule development through negative regulation of AGAMOUS gene (AG). *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5761–5765. [[CrossRef](#)]
68. Bencivenga, S.; Simonini, S.; Benkova, E.; Colombo, L. The transcription factors BEL1 and SPL are required for cytokinin and auxin signaling during ovule development in *Arabidopsis*. *Plant Cell* **2012**, *24*, 2886–2897. [[CrossRef](#)]
69. Gómez-Mena, C.; Sablowski, R. *ARABIDOPSIS THALIANA* HOMEODOMAIN GENE1 establishes the basal boundaries of shoot organs and controls stem growth. *Plant Cell* **2008**, *20*, 2059–2072. [[CrossRef](#)]
70. Proveniers, M.; Rutjens, B.; Brand, M.; Smeekens, S. The *Arabidopsis* TALE homeobox gene *ATH1* controls floral competency through positive regulation of *FLC*. *Plant J.* **2007**, *52*, 899–913. [[CrossRef](#)]

