Genome-wide Identification, Characterization and Expression profile of TALE gene family in *Juglans regia* L.

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Abstract

Background

The three amino acid loop extension (TALE) are essential transcription factors that regulate the differentiation of meristem in the plant growth and development. there's a lot of journalism of the TALE family in plants, such as Arabidopsis, rice, and others. however, there's no report on the TALE family in walnut.

Results

In this study, the genome-wide identification and expression analysis of the TALE gene family were studied comprehensively in walnut. Based on the specific structure of the TALE domain, a total of twenty-seven walnut TALE genes (*JrTALE*) were identified. According to different domains structure and phylogenetic relationships, the *JrTALE* gene family could be divided into two subfamilies, namely BELL and KNOX. This family was relatively conservative in evolution. Exon-intron structure analysis showed that there were 3 to 6 exons, and they had a wide range of exons length. Analysis of protein conserved motifs showed that *JrTALE* contained similar motif structures in the same phylogenetic branch. Expression pattern analysis of the TALE family genes in walnut showed that *JrTALE41* and *JrTALE42* were differentially expressed in female and male flower buds. We confirmed that *JrTALE4-5*, *JrTALE8-12*, *JrTALE36-37*, *JrTALE46-53*, and *JrTALE14-15* were differentially expressed between different stages of flower bud development by real-time quantitative PCR (qRT-PCR).

Conclusions

Sixty-nine *JrTALEs* were identified in the genome-wide. the physicochemical properties, phylogenetic relationships, gene structure, promoter cis-acting elements, and protein interactions were systematically analyzed by bioinformatics methods. This study provides valuable information about the TALE family in the transitional development of walnut flower buds, which lay the foundation for further understanding of TALE family genes and in walnut.

Background

The Three Amino acid Loop Extension (TALE) transcription factors are widely present in animals, plants, and other eukaryotes, which play important roles in the plant meristem development and other important plant biological processes [1]. Gehring [2] and other scholars isolated an extremely conserved sequence in which some genes were mutated to produce a homologous phenotype, which was later called the homeobox. The family gene contains KNOTTED-like homeodomain (KNOX) and BEL1-like homeodomain (BLH / BELL) proteins [3], which function as heterodimers that are structurally and functionally related. The TALE family gene encodes an atypical homeobox domain containing 63 amino acid residues. There is a three-amino acid extension in the loop connecting the first and second helices of their homeodomain, so the family is called homeobox protein superfamily [4–5]. Therefore, the TALE transcription factors of *Arabidopsis thaliana* are divided into BELL subfamily and KNOX subfamily [5]. At the same time, these two subfamilies have specific interactions [6], which can regulate downstream genes to achieve different biological functions. KNOX subfamily is almost distributed in plants [7], and it has four domains, namely KNOX1, KNOX2, ELK, and Homeobox KN [8–9]. The maize Knotted-1 (Kn1) gene is the first homeobox gene found in plants [10]. Recessive mutations in the SHOOTMERISTEMLESS (STM) gene have resulted in the inability of the stem-tip meristem (SAM) to form or be maintained in *Arabidopsis* [11–12]; KNAT7 is involved in the secondary cell wall biosynthesis [13]; GhKNL1, a member of the TALE family in cotton, regulated the synthesis of cotton fibers [14]; in the TALE family, SHOOTMERISTEMLESS (STM), KNAT2, BREVIPEDICELLUS (BP) / KNAT1, and KNAT6 genes in the KNOX Class I family in *Arabidopsis* mainly regulate the apical meristem, leaf shape control, and hormone effects [15–17]; Class II *KNOX* gene is expressed in the roots, stems, leaves and flowers of angiosperms. This group of genes is mainly involved in regulating the differentiation of plant organs [18–20]; KNOX proteins are also involved in regulating the anabolic metabolism of many substances, including gibberellic acid (GA), cytokinin (CK), and lignin [21–22]. The BELL subfamily has four domains in rice, namely SKY, BEL, Homeobox KN, and VSLTLGL (ZIBEL) [23–24]. Members of the BELL family also have important regulatory roles in the development and transformation of flowers. Overexpression of BLH6 gene delays flowering, and overexpression of the BLH3 gene makes flowering earlier [25]. Different KNOX and BELL selectively combine to form KNOX-BELL heterodimers heteromers, which specifically bind to specific target sequences. It can be concluded that different combinations of two subfamilies can regulate different downstream genes to complete their functions in plants [6, 26]. The dimer of BELL and KNOX StBEL5 / POTH1 can inhibit the expression of the key enzyme *ga20ox1* gene in the biochemical pathway of GA [27]. Members of the KNOX family, STM, and members of the BELL family, ATH1 and PENNYWISE (PNY), have similar functions and regulate the development of plant meristems [28–29]. In

Arabidopsis, BLH1 and KNAT3 proteins form heterodimers, which regulate the expression level of ABA-responsive genes and affect seed germination and growth [30]; KNAT1 interacts with RPL, FUL and AP to regulate the development of pistil marginal tissue [31–32].

Walnut is an important economic tree species cultivated worldwide with its high-quality woody oils and nuts [33]. It belongs to a hermaphrodite, flowers are the most complicated organs in plants. The formation of flower buds means the beginning of reproductive growth, so flowering biology has always been a research hotspot in the field of plants. According to previous research, some members of the TALE family play an important role in meristem development [15, 34]. In order to better understand the molecular mechanism of walnut flower bud transition, it is necessary to research the TALE gene family in walnut. There are few reports on the role of the TALE gene family in walnuts. In this study, members of *JrTALE* gene family were identified in the genome-wide. The physicochemical properties, phylogenetic relationships, gene structure, gene duplication, and protein interactions were systematically analyzed by bioinformatics methods. Transcriptome data were used to explore the ex-pression patterns of the *JrTALE* gene family in different tissues (male and female flower buds) and different development stages, and verified by qRT-PCR. This study lay the foundation for further exploring the regulatory role of the walnut *TALE* gene.

Results

Genome-wide identification of TALE family members in walnut

Sixty-nine TALE gene family members were identified from the walnut genome sequence, which were encoded by twenty-seven genes and located on twenty-five scaffolds. As can be seen from Table 1, the length of the encoded amino acid sequence is between 251-550aa, of which *JrTALE55* encodes the shortest amino acid, and *JrTALE16-JrTALE22* has the longest; the molecular weight of the protein is 28666.7-93611.11 Da, and the isoelectric point is 4.87-9.77. ProtComp 9.0 online software was used to perform subcellular localization analysis on the predicted protein. The results showed that *JrTALE* was located in the nucleus. As shown in Additional file 2: Table S1.

Phylogenetic analysis of TALE gene family

Based on MUSCLE multiple sequence alignment, the MEGA6.0 software was used to construct a neighbor-joining (NJ) phylogenetic tree for the amino acid sequences of 69 walnuts TALE proteins, 33 Arabidopsis TALE proteins, and 21 grape TALE proteins (Fig. 1). Based on the phylogenetic tree and previous studies, all members of the TALE gene family were divided into two subfamilies KNOX and BELL according to different domains. The distribution of *JrTALE* proteins among different subfamilies was as following: KNOX (14) and BELL (55). In each evolutionary branch, there were multiple branches from the same species, which may be caused by gene replication events, and the lack of representatives of certain species may be related to gene loss events in the family. In the KNOX subfamily, *JrTALE35* and grape GSVIVT01007715001 were orthologous genes; In the BELL subfamily, GSVIVT01009779001 and *JrTALE40* were orthologous genes.

Exon-intro Structure Analysis of TALE family members in walnut

The gene structure of *JrTALE* family members was analyzed online by GSDS web server (Fig. 2). The results showed that there were evident differences in the number of exons among all family members, with a maximum of 6 exons. Only *JrTALE55* contains 3 exons; most family members contained four exons. *JrTALE55*, *JrTALE26*, *JrTALE27*, *JrTALE36*, and *JrTALE37* contained 3' UTR (untranslated regions), which helped to bind microRNAs and regulate the expression of transcription factor proteins. the gene structure was combined with the clustering of the phylogenetic tree. the differences in gene structure between each subfamily were small, the result indicated that the structural similarity of the same subfamily was high.

Conserved motifs analyses

Conservative motif analysis can reveal the diversity of TALE protein and its functional differentiation in walnuts. family members containing the same motif may have similar functions; family members contained different motif elements suggesting that may have undergone functional differentiation and have other special functions. the MEME online tool was used to predict the conserved motifs of walnut TALE family members (Fig. 3). All *JrTALE* members contained motif 1, the results showed that it was highly conserved in the TALE gene family; motif 9 was highly conserved at the N-terminus in the KNOX subfamily. Except *JrTALE55*, all BELL subfamily members had seven common conserved motifs were motif8, motif5, motif2, motif4, motif3, motif1, motif7, respectively. The phylogenetic tree shows that closely related members of the TALE family contain the same conserved elements, however, the same subfamily has a proprietary conserved motif, indicating that the same subfamily has similar functions.

Cis-elements prediction in the promoter of *JrTALE* gene

In response to plant hormones, stress factors and light response, the cis element in the promoter region affects gene expression to regulate plant development and adaptability to environmental changes. In order to obtain more information about the function of the TALE gene in walnuts, 26 *JrTALE* gene promoters were analyzed using the online software PlantCARE (of which the *JrTALE55* sequence was too short to be analyzed). The cis-elements were divided into four main subgroups: stress-responsive, hormone-responsive, light-responsive, and MYB binding sites (Fig.4). Most genes were involved in the light-response type promoter (52.51%), followed by hormones (27.41%) and other stress-related genes (13.51%). Among 26 *JrTALEs*, eleven of them possessed MYB binding sites. In addition, we found that there were abundant light-responsive cis-elements in the *JrTALEs*, especially in the *JrTALE41* gene with a number of 19.

Gene Expression Analysis

Based on RNA-seq data, we used the FPKM values of 27 *JrTALE* genes to investigate the expression profile of *JrTALE* family genes. (i) Comparison of *JrTALE* gene expression levels in female flower buds and leaf buds (Fig. 5A). *JrTALE36-37* and *JrTALE46-53* were highly expressed in female and male flower buds, *JrTALE29-31*, *JrTALE55*, *JrTALE32-33*, and *JrTALE54* were lowly expressed in female and male flower buds, and the expression levels of *JrTALE41* and *JrTALE42* in female flower buds were higher than male flower buds. (ii) The expression levels of *JrTALE* gene before, during, and after transformation of female flower buds (FB-1 / FB-2 / FB-3) and before, during, and after transformation of male flower buds (MB-1/ MB-2 / MB-3) were compared (Fig. 5B). *JrTALE55* was not expressed in FB; *JrTALE8-12*, *JrTALE46-53* and *JrTALE14-15* were highly expressed in FB-1 and FB-3; *JrTALE23-25*, *JrTALE55*, *JrTALE29-31* were lowly expressed in female and male flower buds; *JrTALE14-15*, *JrTALE41* and *JrTALE42* were differentially expressed in FB-1, FB-2, FB-3, MB-1, MB-2, MB-3.

Verification of *JrTALE* expression pattern by qRT-PCR

Based on this data comes from the analysis results of *JrTALE* expression pattern, six genes (*JrTALE4-5*, *JrTALE8-12*, *JrTALE14-15*, *JrTALE23-25*, *JrTALE36-37*, *JrTALE46-53*) were further analyzed to detect their expression patterns in different tissues and different development stages using qRT-PCR (Fig. 6). Of these, the expression levels of *JrTALE4-5*, *JrTALE23-25*, *JrTALE36-37* and *JrTALE46-53* were the highest in the male flower buds, consistent with those of the RNA-seq data; As for flower bud transition periods, three genes (*JrTALE4-5*, *JrTALE36-37* and *JrTALE46-53*) were up-regulated in FB / MB-3 than that in FB / MB-2; Moreover, At different developmental stages, the expression of *JrTALE14-15* in female flower buds was higher than that of male flower buds; The suggest that this gene should work as the candidate gene for female and male flower buds difference expression in walnut (Fig. 6).

Protein interaction network of the *JrTALE* family

The function of Arabidopsis TALE has been studied in depth, but the relationship of walnut TALE protein interaction has not been reported. Therefore, we can construct a walnut TALE protein interaction network through the interaction of Arabidopsis TALE protein. twenty-one homologous proteins and five predicted functional proteins were obtained, and the walnut TALE protein corresponding to the Arabidopsis TALE protein was listed below it (Fig. 7). It can be seen from the figure that several *JrTALE* genes (For example, the homologous Arabidopsis BLH2 and KNAT6 corresponding to the walnut TALE proteins) were the core nodes for predicting protein interactions. this result suggests that they may participate in multiple functions by interacting with other proteins.

Discussion

TALE transcription factor is an important transcription factor regulating plant growth and development, meristem maintenance and organ morphogenesis [21, 34, 35]. The TALE gene family included three domains, namely KNOX, ELK and Homeobox. the ELK domain was an amphoteric helix structure, which played an important role in protein-protein interactions; the Homeobox domain was used for binding to target genes. So far, little is known about the regulatory role of the TALE gene family in the development of female and male flower buds in walnut.

Analysis of Phylogenetic and gene structure

Analysis of the physicochemical properties of proteins showed that the protein molecular mass and amino acid quantity of different members of *JrTALE* family were quite different (Table 1), indicating that the family may have complex regulatory modes. It was reported that the MEINOX domain upstream of the KNOX protein homology domain combined with the BELL domain upstream of the BELL protein homology domain to form a KNOX / BELL heterodimer, which was transported from the cytoplasm to the nucleus to play a role in regulate all aspects of plant development [36]. The results of subcellular localization (Additional file 2: Table S1) in the nucleus were consistent with previous studies. Phylogenetic analysis divided the TALE family into BELL and KNOX subfamilies based on evolutionary relationships, and its branches were more obvious, indicating that the functions of these two subfamilies were different and had long been differentiated

(Fig. 1). However, only 5 of the 69 *JrTALE* family members contained a 3' UTR (Fig. 2). This structure served as a possible binding site for miRNA^[21], indicating that it had more abundant regulatory characteristics for downstream genes. All members of the *JrTALE* family contained a motif 1 (Homeobox conserved domain) (Additional file 1: Figure S1 and Fig. 3), and the distribution of motif was highly conserved, which provided a reliable targeting sequence for the following functional verification studies.

Expression analysis of *JrTALE* gene

Gene differential expression analysis identified that the expression levels of *JrTALE14-15*, *JrTALE16-22*, *JrTALE41* and *JrTALE42* in female flower buds were higher than those in male flower buds, which indicated that these *JrTALE* genes may positively regulate the development of female flower buds. Before the flower bud transition (FB / MB-1) compared with during (FB / MB-2) and after (FB / MB-3) transformation of flower buds, the three *JrTALE* genes (*JrTALE36-37*, *JrTALE46-53*, *JrTALE8-12*) were highly expressed before the flower bud transition (FB / MB-1), and expressed at different developmental stages of flower buds, indicating that these *JrTALE* genes may actively participate in the regulation of walnut floral organ development. The KNOX subfamily was involved in the maintenance of meristems and the occurrence of organ-specific patterns. The *JrTALE* genes (*JrTALE41* and *JrTALE42*) differentially expressed in the KNOX subfamily were only expressed in the female flower buds, indicating that these *JrTALE* genes may be involved in the development of pistil, This result consistent with the results of the Arabidopsis study [37–38]. The *BLH8* / *PNF* gene in Arabidopsis was mainly involved in meristem growth maintenance and flowering signal transduction [39–40]. The *JrTALE* gene (*JrTALE16-22*) was identified as *BLH8* (as shown in Fig. 7) and was divided into BELL subfamilies, And the expression level in FB-3 was higher than FB-1 and FB-2, the results show that the *JrTALE* gene is involved in the growth and maintenance of meristematic tissues.

Analysis of *JrTALE* protein-protein interaction

The BELL protein and the KNOX protein specifically recognized and binded to form a heterodimer, and the Homeobox domains of these two proteins recognized and specifically binded to their respective target gene sequences, so TALE gene family may regulate the expression level of downstream genes [21, 36]. So far, the interaction network of the TALE gene family in Arabidopsis has been extensively studied. *JrTALE8-12* / *JrTALE46-53* / *JrTALE26* were homologous to BLH1 / KNAT3. In Arabidopsis, the BLH1 protein of the BELL subfamily can combine with the KNAT3 protein of the KNOX subfamily to form BLH1-KNAT3 dimers, which affected the germination and growth of seeds by regulating the expression of genes related to the ABA signaling pathway [41–42]. *JrTALE43-44* / *JrTALE59-69* / *JrTALE6-7* / *JrTALE34* / *JrTALE41* / *JrTALE45* / *JrTALE35* were homologous to ATH1 / STM / KNAT2, and the dimer formed by the ATH1 protein of the BELL subfamily and the STM protein of the KNOX subfamily can regulate the development of the meristem at the top of the plant [45], while the dimer formed by the ATH1 protein and the KNAT2 protein regulated the development of the inflorescence of the plant [44]. AS1 may cooperate with AS2 to inhibit the expression of KNOX (including STM, KNAT1 / BP, KNAT2, KNAT6) in the heart flap and placenta frame [45–46]. In addition, as the core node of the interaction network, *JrTALEs* (*JrTALE28*, *JrTALE1* and *JrTALE2*) and *JrTALE* protein interaction network also had many interaction partners (Fig. 7), these results illustrated how *JrTALE* family proteins form functional complexes that mediate the expression of genes at different developmental stages of male and female flower buds in walnut.

Conclusions

In this study, 69 *JrTALE* were identified in walnut genome and were classified into two subfamilies. Members of the same subfamily generally had conserved gene structure and protein motif composition. BELL family proteins and KNOX family proteins perform specific binding to form heterodimers and regulate all aspects of plant development. Moreover, many *JrTALE* genes had relatively high expression levels in the female flower buds of walnut. Among them, the expression characteristics of six *JrTALE* genes (*JrTALE4-5*, *JrTALE8-12*, *JrTALE14-15*, *JrTALE23-25*, *JrTALE36-37* and *JrTALE46-53*) in flower buds were confirmed by qRT-PCR measurement. Overall, this study provides valuable information about the gene function of the TALE family in the transitional development of walnut flower buds, and the differential expression in male and female flower buds, which laid the foundation for future functional research.

Methods

Plant materials

All samples of this study were collected from walnut (*Juglans regia* L.), which trees were grown under natural conditions in the southern part of the Xinjiang Uyghur Autonomous Region, China.. male and female flower buds were collected before, during, after the flower transition period (FB / MB-1, FB / MB-2, and FB / MB-3). The samples were sealed with tin foil, immediately frozen in liquid nitrogen and stored at -80°C.

Identification of TALE family members in walnut

Downloaded the latest protein sequence file (GCF_001411555.1_wgs.5d_protein.faa.gz) of the walnut genome from the NCBI website (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/411/555/GCF_001411555.1_wgs.5d/GCF_001411555.1_wgs.5d_protein.faa.gz). The hidden Markov models (HMM) family (PF07526, PF05920, PF03790, PF03791, PF03789) were obtained from the Pfam online (<http://pfam.xfam.org/>) [47]. Based on hmmer3.0, the HMM model was used to retrieve the protein sequence of walnut [48–49] (with a threshold value of $E \leq 1e-20$), redundant sequences were removed, and the TALE gene family of walnut was identified initially. Then the candidate TALE members were uploaded to the CDD (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) and SMART [50] (<http://smart.embl-heidelberg.de/>) online websites to further confirm that if they contained the proper TALE domain (remove sequences without TALE domain). The sequence was submitted to the ProtParam (<https://www.expasy.org/protparam/>) tool to predict its protein information, such as amino acid length, molecular weight, isoelectric point, and so on. the subcellular localization information of *JrTALE* protein was predicted by the online software ProtComp 9.0 (<http://linux1.softberry.com/berry.phtml?topic=protcomppl&group=programs&subgroup=proloc>).

Gene structure analysis and conserved motif

The TALE gene structure information was extracted from the walnut genome information, and its exon and intron structure were analyzed using GSDS (<http://gsds.cbi.pku.edu.cn/>). The TBtools software's BioSequence Structure Illustrator program was used for drawing [51]. MEME program [52] (<http://meme-suite.org/tools/meme>) was used to identify the conserved motifs of *JrTALE* family members with a maximum number of motifs was 15, other parameters were default.

Phylogenetic analysis of TALE members

The domain sequence of the TALE protein was downloaded from PlantTFDB (<http://planttfdb.cbi.pku.edu.cn/>) for walnuts, grapes and Arabidopsis. These sequences were aligned using MUSCLE, and then the neighbor-joining method of MEGA 6.0 software was used for phylogenetic analysis with 1000 bootstrap replicates.

Cis-element analysis in the promoter of *JrTALE* gene

The *JrTALE* gene sequence was submitted to the NCBI website and compared with the walnut genome sequence using the BLASTN program. The 2000 bp sequence upstream of the gene was extracted as the promoter sequence of the *JrTALE* gene, and then the sequence was submitted to the online software PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) database to predict the promoter cis-acting element. the predicted results were visualized using TBtools software [51].

Expression pattern analysis of *JrTALE*

To analyze the expression pattern of *JrTALE* gene, we used RNA-seq data of Biomaker company to analyze the expression of walnut in different tissue parts (female flower buds and male flower buds) and different developmental stages (FB / MB-1, FB / MB-2, FB / MB-3). The FPKM values (Additional file 3: Table S2) were normalized by log10 (FPKM), which was visualized using Heml1.0 [53] software to heatmap.

RNA isolation and qRT-PCR analysis

The male and female flower buds (FB-1, FB-2, FB-3, MB-1, MB-2, MB-3) were collected at different developmental stages. Total RNA was extracted using the EASY spin Plus Plant RNA Rapid Extraction Kit (China, Aidlab) as described by the manufacturer, and Prime Script™ RT reagent Kit with gDNA Eraser Kit (TaKaRa, China) was reverse-transcribed into cDNA. The SYBR Green Prime Script RT-PCR Kit (Toyobo, Osaka, Japan) was used for qRT-PCR amplification in a CFX Connect™ real-time PCR system (BioRad, USA). Walnut 18S-rRNA gene (forward: 5'-GGTCAATCTTCTCGTTCCTT-3' and reverse: 5'-TCGCATTCGCTACGTTCTT-3') was used as the normalizer. The reaction conditions for real-time PCR were follows: initiation with 95°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, 30 s at 55°C, and 30 s at 72°C. A melting curve was included from 65 to 95 °C to verify the specificity of the amplified product. The relative expression levels of candidate genes were determined using the $2^{-\Delta\Delta C_t}$ method [54]. Each treatment consists of three technical replicates. The primers used in this analysis were listed in Additional file 4: Table S3.

Analysis of *JrTALE* Protein Interaction Network

Each walnut TALE protein matched a homologous Arabidopsis TALE protein with the highest score by the blastp program (Additional file 5: Table S4), and thirty-three Arabidopsis TALE proteins which were represent 69 walnut TALE proteins were submitted to the STRING website

(<https://string-db.org>) the program ran with default parameters to predict protein-protein interactions. In addition to 33 input proteins, five predictive functional partners were used to build the network.

Abbreviations

KNOX
KNOTTED-like homeodomain
BLH / BELL
BEL1-like homeodomain
STM
shoot meristemless
UTR
untranslated regions
FB-1 / FB-2 / FB-3
before, during, and after transformation of female flower buds
MB-1 / MB-2 / MB-3
before, during, and after transformation of male flower buds
qRT-PCR
quantitative real-time PCR

Declarations

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Availability of data and materials

Data generated or analyzed during this study are included in this article and its supplementary information files.

Authors' contributions

JXN and CHG designed the research. The materials were collected by CHG and SWQ, and RNA was extracted together with CK and JML. CHG and ZRZ conducted the bioinformatics analysis. GCH wrote the manuscript. All authors have read and approved the final manuscript.

Ehtics approval and consent to participle

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Characterization of the TALE gene family members in walnut.

Gene name	Gene symbol	Scaff	Scaff rename	Related protein	short name	AA [aa]	MV[Da]	IP
<i>JrTALE1-2</i>	LOC108979090	NW_017438852.1	Scaff13	XP_018805223.1	<i>JrTALE1</i>	319	36126.63	4.87
				XP_018805224.1	<i>JrTALE2</i>	319	36126.63	4.87
<i>JrTALE3</i>	LOC108979965	NW_017439513.1	Scaff14	XP_018806314.1	<i>JrTALE3</i>	375	42712.52	5.8
<i>JrTALE4-5</i>	LOC108980836	NW_017440201.1	Scaff15	XP_018807392.1	<i>JrTALE4</i>	685	75261.69	6.13
				XP_018807393.1	<i>JrTALE5</i>	685	75261.69	6.13
<i>JrTALE6-7</i>	LOC108981545	NW_017440575.1	Scaff16	XP_018808307.1	<i>JrTALE6</i>	356	40326.13	5.97
				XP_018808309.1	<i>JrTALE7</i>	356	40326.13	5.97
<i>JrTALE8-12</i>	LOC108982860	NW_017441761.1	Scaff17	XP_018809867.1	<i>JrTALE8</i>	696	75926.63	6.91
				XP_018809868.1	<i>JrTALE9</i>	696	75926.63	6.91
				XP_018809869.1	<i>JrTALE10</i>	696	75926.63	6.91
				XP_018809870.1	<i>JrTALE11</i>	696	75926.63	6.91
				XP_018809871.1	<i>JrTALE12</i>	696	75926.63	6.91
<i>JrTALE13</i>	LOC108986966	NW_017442862.1	Scaff18	XP_018815324.1	<i>JrTALE13</i>	682	74968.47	5.68
<i>JrTALE14-15</i>	LOC108988270	NW_017443011.1	Scaff19	XP_018817027.1	<i>JrTALE14</i>	722	78843.08	6.65
				XP_018817028.1	<i>JrTALE15</i>	722	78843.08	6.65
<i>JrTALE16-22</i>	LOC108990328	NW_017443280.1	Scaff20	XP_018819801.1	<i>JrTALE16</i>	850	93611.11	6.64
				XP_018819802.1	<i>JrTALE17</i>	850	93611.11	6.64
				XP_018819803.1	<i>JrTALE18</i>	850	93611.11	6.64
				XP_018819804.1	<i>JrTALE19</i>	850	93611.11	6.64
				XP_018819805.1	<i>JrTALE20</i>	850	93611.11	6.64
				XP_018819806.1	<i>JrTALE21</i>	850	93611.11	6.64
				XP_018819807.1	<i>JrTALE22</i>	850	93611.11	6.64
<i>JrTALE23-25</i>	LOC108991317	NW_017443495.1	Scaff21	XP_018821045.1	<i>JrTALE23</i>	565	62686.78	5.89
				XP_018821046.1	<i>JrTALE24</i>	565	62686.78	5.89
				XP_018821047.1	<i>JrTALE25</i>	565	62686.78	5.89
<i>JrTALE26-27</i>	LOC108992228	NW_017443527.1	Scaff22	XP_018822263.1	<i>JrTALE26</i>	477	53076.64	6.04
				XP_018822264.1	<i>JrTALE27</i>	476	52920.46	5.98
<i>JrTALE28</i>	LOC108995343	NW_017443578.1	Scaff23	XP_018826438.1	<i>JrTALE28</i>	824	89539.65	6.85
<i>JrTALE29-31</i>	LOC108997442	NW_017443603.1	Scaff24	XP_018829277.1	<i>JrTALE29</i>	676	75710.95	6.01
				XP_018829278.1	<i>JrTALE30</i>	676	75710.95	6.01
				XP_018829279.1	<i>JrTALE31</i>	676	75710.95	6.01
<i>JrTALE32-33</i>	LOC108998188	NW_017443616.1	Scaff25	XP_018830219.1	<i>JrTALE32</i>	846	91749.98	6.6
				XP_018830220.1	<i>JrTALE33</i>	846	91749.98	6.6
<i>JrTALE34</i>	LOC109001756	NW_017388815.1	Scaff1	XP_018834699.1	<i>JrTALE34</i>	321	36251.17	5.24
<i>JrTALE35</i>	LOC109003871	NW_017389148.1	Scaff2	XP_018837747.1	<i>JrTALE35</i>	322	36365.87	5
<i>JrTALE36-37</i>	LOC109008224	NW_017389472.1	Scaff3	XP_018843781.1	<i>JrTALE36</i>	484	53514.16	5.92
				XP_018843782.1	<i>JrTALE37</i>	483	53357.97	5.86

<i>JrTALE38-39</i>	LOC109008282	NW_017389474.1	Scaff4	XP_018843851.1	<i>JrTALE38</i>	682	75108.49	5.5
				XP_018843852.1	<i>JrTALE39</i>	606	66806.45	5.51
<i>JrTALE40</i>	LOC109008283	NW_017389474.1	Scaff4	XP_018843853.1	<i>JrTALE40</i>	511	56856.81	5.92

Table1 Characterization of the TALE gene family members in walnut (Continued)

Gene name	Gene symbol	Scaff	Scaff rename	Related protein	short name	AA (aa)	MV Da	IP
<i>JrTALE41</i>	LOC109008556	NW_017389505.1	Scaff5	XP_018844242.1	<i>JrTALE41</i>	349	39490.4	5.79
<i>JrTALE42</i>	LOC109008843	NW_017389545.1	Scaff6	XP_018844620.1	<i>JrTALE42</i>	376	43255.05	5.87
<i>JrTALE43-44</i>	LOC109008860	NW_017389545.1	Scaff6	XP_018844647.1	<i>JrTALE43</i>	583	65243.78	8.02
				XP_018844648.1	<i>JrTALE44</i>	583	65243.78	8.02
<i>JrTALE45</i>	LOC109011646	NW_017389777.1	Scaff7	XP_018848478.1	<i>JrTALE45</i>	331	37747.19	6.35
<i>JrTALE46-53</i>	LOC109013525	NW_017389916.1	Scaff8	XP_018851194.1	<i>JrTALE46</i>	697	76014.5	7.79
				XP_018851195.1	<i>JrTALE47</i>	697	76014.5	7.79
				XP_018851196.1	<i>JrTALE48</i>	697	76014.5	7.79
				XP_018851197.1	<i>JrTALE49</i>	697	76014.5	7.79
				XP_018851198.1	<i>JrTALE50</i>	697	76014.5	7.79
				XP_018851199.1	<i>JrTALE51</i>	697	76014.5	7.79
				XP_018851200.1	<i>JrTALE52</i>	697	76014.5	7.79
				XP_018851201.1	<i>JrTALE53</i>	697	76014.5	7.79
<i>JrTALE54</i>	LOC109014498	NW_017389990.1	Scaff9	XP_018852535.1	<i>JrTALE54</i>	672	75268.55	6.52
<i>JrTALE55</i>	LOC109017125	NW_017416914.1	Scaff10	XP_018854978.1	<i>JrTALE55</i>	251	28666.7	9.77
<i>JrTALE56-58</i>	LOC109019501	NW_017436672.1	Scaff11	XP_018857333.1	<i>JrTALE56</i>	837	92709.23	6.65
				XP_018857334.1	<i>JrTALE57</i>	825	91487.85	6.77
				XP_018857335.1	<i>JrTALE58</i>	815	90290.68	6.9
<i>JrTALE59-69</i>	LOC109021085	NW_017437773.1	Scaff12	XP_018859168.1	<i>JrTALE59</i>	590	65712.02	6.35
				XP_018859169.1	<i>JrTALE60</i>	590	65712.02	6.35
				XP_018859170.1	<i>JrTALE61</i>	590	65712.02	6.35
				XP_018859171.1	<i>JrTALE62</i>	590	65712.02	6.35
				XP_018859172.1	<i>JrTALE63</i>	590	65712.02	6.35
				XP_018859173.1	<i>JrTALE64</i>	590	65712.02	6.35
				XP_018859174.1	<i>JrTALE65</i>	590	65712.02	6.35
				XP_018859175.1	<i>JrTALE66</i>	590	65712.02	6.35
				XP_018859176.1	<i>JrTALE67</i>	590	65712.02	6.35
				XP_018859177.1	<i>JrTALE68</i>	590	65712.02	6.35
				XP_018859178.1	<i>JrTALE69</i>	590	65712.02	6.35

Note AA: amino acid, MV: molecular weight, IP: iso-electric point

Figures

Figure 1

Phylogenetic analysis and classification of BELL and KNOX subfamily genes in walnut, Arabidopsis and grape. The phylogenetic tree was constructed by MEGA 6.0 software using the neighbor-joining (NJ) method with 1000 bootstrap replicates. The genes for walnuts, Arabidopsis and grapes are marked with stars in purple, blue and red, respectively.

Figure 2

Gene structure analysis of JrTALE family gene. Phylogenetic relationship, Gene structure maps were drawn with the Gene Structure Display Server 2.0. The scale bar is shown at the bottom.

Figure 3

Architecture of conserved protein motifs of JrTALE family members by MEME analysis.

Figure 4

Cis-element analysis of putative JrTALE promoters related to stress responses. Different cis-elements with the same or similar functions are shown in the same color and shape. The up-triangle, rectangle, and eclipse represented the environmental response, hormone responsive and myb-binding site, respectively.

Figure 5

Expression profiles of candidate JrTALE genes in tissues or different developmental stages. A. Heatmap of the JrTALE genes between female flower buds and male flower buds. B. Heatmap of JrTALE genes expressed differently in three development periods of female and male flower buds (FB/MB-1, FB/MB-2, and FB/MB-3).

Figure 6

qRT-PCR analysis of JrTALE genes in different tissues and different development period of flower buds.

Figure 7

Predicted protein-protein interaction network of JrTAL protein. The network nodes represent proteins. The 3D structure of the protein is shown inside the nodes, and the line color indicates the type of interaction evidence. the network was constructed using sixteen input proteins (BEL1, BEL10, BLH1, BLH2, BLH3, BLH4, BLH5, BLH6, BLH7, BLH8, BLH11, ATH1, RPL, KNAT1, KNAT2, KNAT3, KNAT4, KNAT5, KNAT6, KNAT7 and STM) and five predicted functional partners (AS1, AS2, OFP4, MYB75 and WUS).

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