Research paper

**Genome-wide identification and characterization of the lateral organ boundaries domain gene family in Brassica rapa var. rapa**

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**A B S T R A C T**

The Lateral Organ Boundaries Domain (LBD) genes encode highly conserved plant-specific LBD domain proteins which regulate growth and development in various species. However, members of the LBD gene family have yet to be identified in Brassica rapa var. rapa. In the present study, fifty-nine LBD genes were identified and distributed on 10 chromosomes. The BrrLBD proteins are predicted to encode hydrophobic polypeptides between 118 and 394 amino acids in length and with molecular weights ranging from 13.31 to 44.24 kDa; the theoretical pI for these proteins varies from 4.83 to 9.68. There were 17 paralogous gene pairs in the BrrLBD family, suggesting that the amplification of the BrrLBD gene family involved large-scale gene duplication events. Members of the BrrLBD family were divided into 7 subclades (class I a to e, class II a and b). Analysis of gene structure and conserved domains revealed that most BrrLBD genes of the same subclade had similar gene structures and protein motifs. The expression profiles of 59 BrrLBD genes were determined through Quantitative Real-time fluorescent PCR (qRT-PCR). Most BrrLBD genes in the same subclade had similar gene expression profiles. However, the expression patterns of 7 genes differed from their duplicates, indicating that although the gene function of most BrrLBD genes has been conserved, some BrrLBD genes may have undergone evolutionary change.

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**Introduction**

Genes of the Lateral Organ Boundaries Domain/ASYMMETRIC LEAVES2-like (LBD/AS2) protein family encode plant-specific transcription factors that share a highly conserved LOB domain (Iwakawa et al., 2002; Xu et al., 2016). The conserved lateral organ boundaries domain (LBD) consists of 100 amino acids and contains a conserved Cx_{2}Cx_{2}Cx_{2}C zinc finger-like block for DNA-binding activity, a leucine-zipper-like coiled-coil motif for protein–protein interaction and a GAS (Gly-Ala-Ser) block which may also play a role in DNA-binding (Lee et al., 2013; Majer and Hochholdinger, 2011; Shuai et al., 2002; Xu et al., 2016). The variable C-terminal region of the LBD proteins plays a critical role in controlling the expression of downstream target genes (Liu et al., 2005; Majer et al., 2012). LBD proteins have been divided into two clades (class I and class II) according to their structure. In various species, most members of class I have a complete LBD domain and all members of class II have an incomplete leucine-zipper-like coiled-coil motif (Cao et al., 2016; Lu et al., 2017; Majer et al., 2012; Wang et al., 2013; Yang et al., 2006; Yordanov et al., 2010). In barley, mulberry, rice, grape, Arabidopsis, maize and apple, the number of LBD gene family members varies from 24 to 58 (Cao et al., 2016; Guo et al., 2016; Luo et al., 2016; Shuai et al., 2002; Wang et al., 2013; Yang et al., 2006;
The spatio-temporal expression profiles of LBD genes vary, implying that during growth and development LBD proteins function at specific periods and in specific organs (Cao et al., 2016; Shuai et al., 2002; Wang et al., 2013).

Previous studies have demonstrated that LBD proteins play important roles in plant growth, development, signal transduction, and stress response development (Cabrera et al., 2014; Chen et al., 2012; Cortizo and Laufs, 2012; Fan et al., 2012; Feng et al., 2012; Hu et al., 2014; Kim et al., 2015; Lee et al., 2017; Liu et al., 2014; Mangeon et al., 2011; Oh et al., 2010; Okushima et al., 2007; Thatcher et al., 2012; Yordanov et al., 2010; Zhou et al., 2012). For example, Arabidopsis LBD proteins (AtLBD16, AtLBD18, AtLBD29 and AtLBD33) have been shown to act in the auxin-mediated signaling pathway that regulates lateral root development (Okushima et al., 2007; Xu et al., 2016).

LBD proteins were derived from 8-week-old (Cao et al., 2016; Shuai et al., 2002; Wang et al., 2013). The spatio-temporal expression profiles of LBD genes (Zhang et al., 2014). The spatio-temporal expression profile of turnip LBD family genes (Lin et al., 2014) was an important vegetable crop in the Tibetan Plateau. However, no report on the turnip LBD gene family exists. The turnip genome has been sequenced and assembled (Lin et al., 2014), providing the basis for characterizing the LBD gene family in turnip.

In this study, 59 BrrLBD genes were identified in the turnip genome, their phylogenetic relationship, exon/intron structure, protein motifs and chromosome locations were analyzed. Furthermore, we characterized the expression profiles of BrrLBD genes in different tissues.

Material and methods

Plant material and growth conditions

B. rapa var. rapa ‘KTRG-B36’ from Lanping county, Nujiang City, Yunnan Province, China, was used. For root collection, seedlings were grown on a Whatman C14 paper under long-day conditions (16 h light/day). Leaves and shoot apical meristems were derived from 8-week-old plants; floral buds were derived from 10-week-old plants.

Identification of turnip LBD family genes

The whole genome sequence of B. rapa var. rapa (Lin et al., 2014) was downloaded from www.bioinformatics.nl/brassica/turnip. Forty-two Arabidopsis LBD genes were downloaded from TAIR 12.0 (www.arabidopsis.org). All 43 known Arabidopsis LBDs were used as queries to search the turnip genome database at an e-value cutoff of 1e-003 (Du et al., 2017; Lu et al., 2017). The pl (isoelectric points), MW (protein molecular weights) and GRAVY (Grand average of hydropathicity) were obtained by proteomic and sequence analysis tools of the Expasy proteomics server (http://expasy.org/) (Artimo et al., 2012). Chromosomal locations were found in the turnip database by using a Perl-based program.

Gene structure and conserved motif analyses

BrrLBD gene exon and intron structures were identified with Gene Structure Display Server 2.0 (GSDS, http://gsds.cbi.pku.edu.cn/) (Hu et al., 2015). Conserved motifs of the BrrLBD proteins were analyzed using the Multiple Em for Motif Elucidation (MEME) program (http://meme-suite.org/index.html) (Bailey and Elkan, 1994) with the following parameters: (1) the optimum motif width was set from 6 to 200, and (2) the maximum number of motifs was set to identify 20 motifs.

Phylogenetic analysis

All phylogenetic tree of protein sequences were constructed with the neighbor-joining method using the MEGA 7.0 program. The reliability of the trees was tested by the bootstrapping method with 1000 replicates. All images of phylogenetic trees were drawn in MEGA 7.0 (Kumar et al., 2016).

Chromosome distribution, and divergence time estimation of BrrLBD genes

BrrLBD genes were mapped on chromosomes by confirming their detailed chromosome positions supplied by the Turnip Genome Database. To determine their physical location, the starting positions of all BrrLBD genes on each chromosome were confirmed based on a local database of the complete sequence of the turnip genome by searching BLASTn. Segmental and tandem duplication regions were obtained from MCscanX. Analysis of synteny blocks in turnip genes was visualized using Circos (http://circos.ca/). Synonymous (Ks) and non-synonymous (Ka) substitution rates were estimated using the codeml program of the PAML4 package (Yang, 2007). The divergence time (T) of turnip gene pairs was calculated using formula T = Ks/2λ, where λ represents the divergence rate of 1.5 × 10⁻⁸ for Arabidopsis (Gaut et al., 1996).

Quantitative RT-PCR

Total RNA was extracted from KTRG-B36 leaves and shoot apical meristem of 8-week-old plants, floral buds of 10-week-old plants using Easteast™ Universal RNA Extraction Kit (Promega, Shanghai, China). All quantitative real-time PCR primers were designed by the online NCBI program Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) applying the following parameters: 150–200 bp of polymerase chain reaction (PCR) product size, 58 °C–62 °C primer melting temperatures (Tm), and organism (taxid:3711) for B. rapa. At least one of two primers was derived from differential sequences of highly similar genes. qRT-PCR was performed in three technical repetitions with cDNAs synthesized from three biological replicates of KTRG-B36 different tissues. BrrTUB2 was used as reference gene.

Subcellular localization and confocal laser scanning microscopy

The coding region sequence (CDS) of BrrLBD genes were subcloned into pKH101-GFP using the EZ-cloning method. 35S:GFP-BrrLBDs were transferred into Agrobacterium tumefaciens EHA105 using electroporation. Positive transformants were prepared for leaf infiltration according to Du et al. (2017), and laser confocal microscopy was performed.

Results

Identification and annotation of LBD genes in turnips

The release of the complete turnip genome sequences allowed the genome-wide identification of turnip genes (Lin et al., 2014). In the present study, BLAST was used to search BrrLBDs in the turnip genome. The obtained sequences were further verified through the Hidden Markov Model of the SMART and pfam 31.0. A total of 59...
LBD-like sequences were identified from the turnip genome, all of which possessed conserved LOB motifs. The BrrLBD genes were annotated following the nomenclature of Arabidopsis thaliana depending on protein sequence similarities (Fig. 1).

BrrLBD proteins were classified into seven subclades (subclades a to e within class I, and subclades a and b within class II). Among them, most BrrLBDs were clustered into class I. Thirteen BrrLBDs were classified as class Ia; 6 BrrLBDs fell into class Ib; 16 BrrLBDs were included in class Ic, 10 BrrLBDs were clustered into class IId, 4 BrrLBDs were classified as class Ie. Sequence analysis revealed that AtLBD5, 8, 9, 26, 32, 34 and 36 had no orthologs in turnip. AtLOB, AtLBD3, 11, 12, 13, 14, 15, 16, 19, 24, 25, 29, 30, 37, 38, 39 and 41 had more than one ortholog in the turnip genome. The standard annotations of 59 BrrLBD genes are shown in Table 1; accession numbers for sequences were submitted to GenBank.

The BrrLBD proteins were predicted to encode polypeptides of 118–394 amino acids with molecular weights ranging from 13.31 to 44.24 kDa. The theoretical pI ranged from 4.83 to 9.68. All values of GRAVY were less than zero, indicating that all polypeptides of BrrLBD protein are hydrophilic.

BrrLBD genes in turnip are distributed in all 10 turnip chromosomes (Fig. 2). The maximum number of BrrLBD genes per chromosome was found for chromosome A04 with 11 BrrLBD genes. Ten genes were found at chromosome 9, eight genes each were located at chromosome 3 and 5, and six genes each were located at chromosome 2 and 6, respectively. Chromosome 8 and 10 contain the minimum numbers of BrrLBD genes, with only one each. Turnip chromosome 7 has five BrrLBD genes, and the chromosome 1 contains three BrrLBD genes.

In this study, a total of 17 pairs of putative LBD paralog proteins were found in the segmental duplication blocks distributed in different chromosomes. These results demonstrate that the expansion of BrrLBD gene family in turnip was involved in large-scale segmental duplication events.

The divergence time (T) of seventeen pairs of BrrLBD paralog proteins was estimated by measuring the synonymous (Ks) and nonsynonymous (Ka) mutation rates (Table 2). The estimated divergence time (T) for BrrLBD duplicated gene pairs was approximately from 3.9 to 24.30 million years ago (MYA), with an average duplication time of ~10.4 MYA. The ω-values (Ka/Ks) for the BrrLBD paralogs were less than one. However, the duplicated pair BrrLBD19/BrrLBD19a (ω = 0.5624) had a relatively large ω value, which implies that this pair may have evolved rapidly from the last common ancestor.

Gene structure and conserved motifs

To mine information concerning exon/intron organization of BrrLBDs and conserved motifs of BrrLBDs, a new neighbor-joining (NJ) tree was constructed using the protein sequences of BrrLBDs (Fig. 3A). Gene structure analysis revealed that most BrrLBD genes in the same subclade have similar gene structures (Fig. 3B). The exon numbers of BrrLBD gene family members vary from one to three. Fourteen BrrLBD genes have only one exon (24%), most members of BrrLBD gene family have two exons (71%), and only three BrrLBD genes have three exons. The MEME online tool was performed to predict the conserved motif composition of turnip LBD proteins (Fig. 3C). The numbers of BrrLBD protein motifs range from 2 to 8. The motifs of BrrLBDs were annotated using InterProScan. The conserved Cx2CxCx3C zinc finger-like domain sequence was detected in motif 2, which exists in various species in all known LBD proteins. Furthermore, the leucine zipper-like coiled-coil was found in motif 1 and 3, which exists only in majority of the class I LBD proteins (Majer and Hochholdinger, 2011; Shuai et al., 2002).

Fig. 1. Phylogenetic tree of LBD proteins from turnip and Arabidopsis. The evolutionary history of LBD proteins was inferred by using 59 BrrLBD protein and 43 AtLBD protein sequences to construct a Neighbor-Joining (NJ) cluster tree. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Evolutionary analyses were conducted in MEGA7.
### Table 1
Identification and characteristics of LBD genes in turnip.

| Gene Name | Accession Number | CDS length (bp) | Protein size (aa) | MV(kD) | PI | GRAVY | Chr. Location |
|-----------|------------------|-----------------|-------------------|--------|----|-------|---------------|
| BrlLBD42  | MF953658         | 780             | 259               | 27.76  | 8.51 | 0.305 | 0.255 Chr03:9761557..9762501 |
| BrlLBD41  | MF953654         | 711             | 236               | 25.99  | 8.68 | 0.305 | 0.214 Chr03:14495479..14496347 |
| BrlLBD38a | MF953651         | 726             | 241               | 25.99  | 8.68 | 0.305 | 0.119 Chr03:30663003..30663878 |
| BrlLBD33  | MF953649         | 684             | 228               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD31  | MF953647         | 699             | 232               | 25.99  | 8.68 | 0.305 | 0.249 Chr09:1068061..1069446  |
| BrlLBD29  | MF953645         | 711             | 236               | 25.99  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD28  | MF953643         | 757             | 253               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD27  | MF953642         | 798             | 263               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD25  | MF953641         | 817             | 271               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD24a | MF953639         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD24  | MF953638         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD23  | MF953637         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD22  | MF953636         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD21  | MF953635         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD20  | MF953634         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD19  | MF953633         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD18  | MF953632         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD17  | MF953631         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD16  | MF953630         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD15  | MF953629         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD14  | MF953628         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD13  | MF953627         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD12  | MF953626         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD11  | MF953625         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD10  | MF953624         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD9   | MF953623         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD8   | MF953622         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD7   | MF953621         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD6   | MF953620         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD5   | MF953619         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD4   | MF953618         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD3   | MF953617         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD2   | MF953616         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD1   | MF953615         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |
| BrlLBD0   | MF953614         | 777             | 259               | 26.62  | 8.68 | 0.305 | 0.249 Chr09:24819925..24820971 |

MW, molecular weight; PI, isoelectric point; GRAVY, Grand average of hydropathicity.
Expression profiles of turnip LBD genes

The gene expression pattern is always relative to its function (Xu et al., 2015). To investigate possible functions of BrrLBD genes in turnip, quantitative real-time fluorescence PCR (qRT-PCR) was performed to examine expression levels of 59 BrrLBD genes in different turnip tissues (Fig. 4). Except for BrrLBD30a, which is expressed specifically in leaves, 14 of 49 class I BrrLBD genes are highly expressed in roots, 34 are strongly expressed in flower buds, and all class I BrrLBD genes are weakly expressed in leaves.

Tissue-specific expression patterns are similar for genes within each phylogenetic subclade. For instance, all BrrLBD genes in class Ib and class Ie, 12 of 16 BrrLBD genes in class Ic, and 7 of 10 class Id genes are expressed highly in flower buds. However, class Ia genes showed more widespread but less tissue-specific expression patterns; for example, BrrLBD1, 12, 12a, 23, and 24 are highly expressed in flower-buds, and BrrLBD1a, 3, 3a, 4, 11, 11a and 12b are expressed highly in roots. Seventeen duplicated BrrLBD pairs (BrrLBD11/11a, BrrLBD12/12a, BrrLBD13/13a, BrrLBD14/14a, BrrLBD15/15b, BrrLBD23/24, BrrLBD25/25a, BrrLBD38/38a, BrrLBD39/39a and BrrLBD41/41a) have similar tissue-specific expression patterns.

### Table 2

| Seq1       | Seq2       | Identity (%) | Ks   | Ka   | ω   | T (MYA) |
|------------|------------|--------------|------|------|-----|---------|
| BrrLBD12   | BrrLBD12a  | 0.9319       | 0.1616 | 0.0202 | 0.1250 | 5.3867  |
| BrrLBD23   | BrrLBD24   | 0.9008       | 0.1784 | 0.0536 | 0.3004 | 5.9467  |
| BrrLBD3    | BrrLBD3a   | 0.8354       | 0.3331 | 0.0585 | 0.1756 | 11.1033 |
| BrrLBD11   | BrrLBD11a  | 0.8397       | 0.3155 | 0.0245 | 0.0777 | 10.5167 |
| BrrLBD8    | BrrLBD8a   | 0.8798       | 0.2346 | 0.0205 | 0.0874 | 7.8200  |
| BrrLBD25   | BrrLBD25a  | 0.9167       | 0.4132 | 0.0334 | 0.0808 | 13.7733 |
| BrrLBD13   | BrrLBD13a  | 0.8963       | 0.3768 | 0.0102 | 0.0271 | 12.5600 |
| BrrLBD15   | BrrLBD15b  | 0.7832       | 0.3670 | 0.0288 | 0.0785 | 12.2333 |
| BrrLBD19   | BrrLBD19a  | 0.6974       | 0.1161 | 0.0653 | 0.5624 | 3.8700  |
| BrrLBD30   | BrrLBD30a  | 0.8705       | 0.3602 | 0.0332 | 0.0922 | 12.0067 |
| BrrLBD16   | BrrLBD16a  | 0.9024       | 0.2881 | 0.0081 | 0.0281 | 9.6033  |
| BrrLBD14   | BrrLBD14a  | 0.8556       | 0.2311 | 0.0335 | 0.2315 | 7.7033  |
| BrrLBD29   | BrrLBD29a  | 0.8451       | 0.2743 | 0.0323 | 0.1178 | 9.1433  |
| BrrLBD41   | BrrLBD41a  | 0.8491       | 0.2845 | 0.0041 | 0.0144 | 9.4833  |
| BrrLBD39   | BrrLBD39a  | 0.7890       | 0.2530 | 0.0484 | 0.1913 | 8.4333  |
| BrrLBD38   | BrrLBD38a  | 0.8462       | 0.7290 | 0.0165 | 0.0226 | 24.3000 |
| BrrLBD37   | BrrLBD37a  | 0.8272       | 0.3750 | 0.0206 | 0.0549 | 12.5000 |

Ks, synonymous substitution rates; Ka, Nonsynonymous substitution rates; MYA, million years ago.
patterns, respectively. In contrast, \textit{BrrLOB}/\textit{BrrLOBa}, \textit{BrrLBD16}/16a, \textit{BrrLBD19}/19a, \textit{BrrLBD29}/29a and \textit{BrrLBD30}/30a have different tissue expression characteristics. These results imply that most turnip LBD gene functions have been conserved, although some gene functions may have been altered through gene duplication events.

Subcellular localization

The known members of the \textit{LBD} gene family function as transcription factors that regulate plant-specific process. The \textit{GFP} gene was fused with \textit{BrrLBDs} as a reporter. The \textit{GFP} signals of \textit{BrrLBD-GFP} were detected in the nucleus of tobacco leaf epidermal cells (Fig. 5), which suggests that \textit{BrrLBD16}, \textit{BrrLBD18}, \textit{BrrLBD29}, and \textit{BrrLBD33} might function as transcription factors.

Discussion

\textit{LBD} proteins belong to a novel family of plant-specific transcription factors involved in the regulation of processes during plant development and growth in higher plants, such as pollen development, plant regeneration, pathogen response, secondary growth, photomorphogenesis, pulvinus identity and petiole development (Xu \textit{et al.}, 2016). Turnip underwent polyploidization events, such as \textit{γ} triplication (135 MYA) and \textit{β} (90–100 MYA) and \textit{α} (24–40 MYA) duplications. Following these polyploidization events, the Brassica genome underwent chromosome reduction and rearrangement and numerous gene losses took place, resulting in highly complex gene families (Du \textit{et al.}, 2017). Phylogenetic analysis of \textit{LBD} proteins in apple, grape, eucalyptus, rice and \textit{Arabidopsis} have led to the division of \textit{LBD} families into two classes (class I and class II) (Cao \textit{et al.}, 2016; Lu \textit{et al.}, 2017; Wang \textit{et al.}, 2013; Yang \textit{et al.}, 2006). In this study, 59 \textit{BrrLBD} genes were identified from turnip genome sequences, which were distributed on 10 chromosomes. Some \textit{BrrLBD} proteins were detected in the nucleus. An unrooted phylogenetic tree divided \textit{BrrLBDs} into 7 subclades (subclade a to e within class I, and subclade a and b within class II). Alignment analysis revealed that in turnip genome 7 \textit{AtLBD} genes had no orthologs and 17 \textit{AtLBDs} had more than one ortholog. These results demonstrate that the expanded of \textit{BrrLBD} gene family in turnip may have been involved in chromosomal duplication events, containing multiple segmental duplication, tandem duplications, transposition events and genome-wide duplications.

Structural analysis is another effective method to mine the evolutionary history of the gene duplication events and phylogenetic relationship within a gene family. In this investigation, most \textit{BrrLBD} genes had a similar exon/intron structure within the same subclade, which is consistent with \textit{LBD} genes in \textit{A. thaliana}, \textit{Oryza sativa}, \textit{Eucalyptus grandis}, \textit{Vitis vinifera} and \textit{Malus domestica} (Cao \textit{et al.}, 2016; Lu \textit{et al.}, 2017; Shuai \textit{et al.}, 2002; Wang \textit{et al.}, 2013; Yang \textit{et al.}, 2006). Motif analysis demonstrated that most \textit{BrrLBD} proteins possessed similar motif distributions within the same subclade. The characteristic \textit{LOB} domain was discovered in \textit{BrrLBD} proteins, which consists of a CX$_2$CX$_6$CX$_3$C zinc finger-like motif required for the DNA-binding activity within motif 2, a Gly-Ala-Ser (GAS) Block within motif 1, and a leucine-zipper-like coiled-coil motif.
Fig. 4. Expression patterns of *BrrLBD* genes in root, shoot apical meristem (SAM), leaf, and flower buds (Flower). All data obtained from quantitative real-time fluorescence PCR. Constitutive β-tubulin gene served as an internal control. The error bar represents standard deviations for three technical duplications.
different angiosperms. These results suggest that the gene structure and protein motif compositions of 2016). These results suggest that the gene structure and protein motif compositions of LBD genes are relatively conserved in different angiosperms.

In Arabidopsis, previous studies have demonstrated that LBD genes participate in plant growth and development as response factors and regulate nitrogen metabolism and anthocyanin synthesis as repressors (Kim and Lee, 2013; Mangeon et al., 2011; Okushima et al., 2007; Rubin et al., 2009). One important indicator of gene function is its expression profile. In this study, we detected the expression profiles of 59 BrrLBD genes in different tissues using qRT-PCR. These genes displayed distinct expression profiles in different turnip organs. Meanwhile, most genes exhibited similar expression profiles within each subclade. Most (10 of 17) duplicated BrrLBD protein pairs demonstrated similar tissuespecific expression patterns. In contrast, the remaining 7 gene duplicated pairs showed different tissue expression characteristics. These results imply that most gene functions have been conserved, although some gene functions may have changed during gene duplication events. Furthermore, the expression of BrrLBD33 in roots suggests that this gene may play a role in the development of the lateral root in turnip.

Declaration of Competing Interest

The authors declare no conflict of interests.

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Appendix A. Supplementary data

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![Subcellular localization of 35S:BrrLBD-GFP in Nicotiana benthamiana leaves. BrrLBD16-GFP, BrrLBD18-GFP, BrrLBD29-GFP and BrrLBD33-GFP were localized in the nucleus.](Image)
