Meta Gene 8 (2016) 44–50

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Identification and characterization of *Lateral Organ Boundaries Domain* genes in mulberry, *Morus notabilis*

Yiwei Luo [1], Bi Ma [1], Qiwei Zeng, Zhonghuai Xiang, Ningjia He *

State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing 400715, People's Republic of China

ARTICLE INFO

Article history:
Received 12 February 2014
Revised 27 March 2014
Accepted 1 April 2014
Available online 2 March 2016

Keywords:
Morus notabilis
LBD family
Phylogeny analysis
Gene expression

ABSTRACT

Genes from the plant specific *Lateral Organ Boundaries Domain* (*LBD*) family encode transcriptional regulators that have a variety of functions in various physiological and developmental processes. In the present study, 31 *LBD* genes were identified in the mulberry genome. The genome features of all *MnLBD* genes and phylogenetic studies with *Arabidopsis* LBD protein sequences, accompanied by the expression analysis of each of the *Morus LBD* genes provide insights into the functional prediction of mulberry LBDs. The genome-wide surveys of the current mulberry genome have resulted in the identification of catalogs of *MnLBD* genes that may function in the development of leaf, root, and secondary metabolism in *Morus* sp.

1. Introduction

The first *Lateral Organ Boundary* (*LOB*) gene was identified in *Arabidopsis thaliana* based on the expression pattern of an enhancer trap insertion and was found to be expressed at the boundaries of lateral organs during vegetative and reproductive plant development (Shuai et al., 2002). An important outcome of the above mentioned work was the discovery of a new conserved protein domain, the LOB domain, a plant specific domain present in 42 other *Arabidopsis* proteins (Shuai et al., 2002). In *A. thaliana*, the conserved LOB domain (LBD) contains approximately 100 amino acids. Furthermore, all 42 LBDs were further divided into two sub classes (Class I and II) according to their sequences. All LBD proteins were previously thought to contain a conserved C block with C-x(2)-C-x(6)C-x(3)-C motif in the N terminus, and it was identified as a zinc finger-like domain, which may play an important role in DNA binding (Shuai et al., 2002; Matsumura et al., 2009). However, Class I LBDs were also found to have an additional GAS motif and a L motif L-x(6)-L-x(3)-L-x(6)-L. The L motif is a leucine zipper-like coiled-coil motif, which is possibly involved in protein dimerization (Shuai et al., 2002). Experimental evidence showed that *LBD* genes encode a class of DNA-binding transcription factors and the LOB domain can interact with the basic helix–loop–helix family of transcription factors (Husbands et al., 2007). Recent studies have suggested that *LBD* genes have a broad range of functions. An *LBD-like* gene in rice, *ARL1*, expressed in lateral and adventitious root primordia, tiller primordia, vascular tissues, scutella, and young pedicels, is an auxin-responsive factor involved in adventitious root formation (Liu et al., 2005). Genes *LBD16*, *29*, *18* in *A. thaliana* mediate lateral root formation (Okushima et al., 2007; Lee et al., 2009; Lee et al., 2013). *LBD33* is involved in lateral root organogenesis by forming a dimer with *LBD18* and the *LBD18*/*LBD33* dimer transcriptionally activated *Arabidopsis E2Fa* to initiate cell cycle in the lateral root (Berckmans et al., 2011). Serrano-Cartagena (1999) found that the gene, *asymmetric leaves 2* (*AS2*, termed *LBD6* later) was relative to leaf development. The abnormal expression of *LBD6* and *LBD36* genes causes hyponastic leaves, downward-pointing flowers, and decreased apical dominance as shown by the gain-of-function mutant by activation tagging and loss-of-function mutant in *Arabidopsis* (Nakazawa et al., 2003; Chalfun-Junior et al., 2005). Members of the LBD proteins are also involved in plant secondary metabolism, such as class II LBDs 37, 38 and 39, which act as negative regulators of anthocyanin biosynthesis in *A. thaliana* (Rubin et al., 2009). The expression patterns of several of the 42 *Arabidopsis LBD* genes change in association with phytohormones. The most prominent among these is *AtLBD40*, whose expression was down regulated by gibberellin (Zentella et al., 2007).

Mulberry (*Moraceae*: *Rosales*) is a perennial woody plant and is widely planted for silkworm rearing (Ganga, 2003). It tolerates of pruning and bud growing well in the following spring (Yamashita and Fujino, 1986). Moreover, mulberry has a strong root system (Olson et al., 2003), which enables it to withstand natural calamities such as desertification, drought, and flood. Numerous active secondary metabolites have been detected in the sorosis, root bark, and leaves of mulberry,

Abbreviation: LBD, *Lateral Boundary Domain*; *LOB*, *Lateral Organ Boundary* gene; HMM, Hidden Markov model; NJ, neighbor-joining; GSDS, Gene Structure Display Server; MEME, Multiple Em For Motif Elicitation; PKM, The reads per kilobase of exon model per million mapped reads.

* Corresponding author at: State Key Laboratory of Silkworm Genome Biology, Southwest University, Beibei, Chongqing 400715, People's Republic of China.
E-mail address: hejia@swu.edu.cn (N. He).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.mgene.2014.04.004

Table 1
List of 31 *LBD* genes identified in mulberry and their sequence characteristics (bp, base pair; aa, amino acids) coupled with the annotation results.

Name	Accession no.	Location	Gene			Protein			Predictions		
			Strand	Introns no.	CDS (bp)	aa	Mw(D)	pI	Annotation	Functions	References
MnLBD1	Morus014650	Scaffold6: 187,049–187,238:187,612–188,168	+	1	747	248	26,920.5	8.31	LOB domain-containing protein 41-like	Leaf dorsoventral determination	(Meng, 2009)
MnLBD2	Morus020555	Scaffold39: 444,787–445,296	−	0	510	169	18,689	6.98	LOB domain-containing protein 25-like	Auxin signaling and photomorphogenesis	(Mangeonet 2011)
MnLBD3	Morus009777	Scaffold40: 77,112–77,405:78,023–78,232	+	1	504	167	18,172.6	8.46	LOB domain-containing protein 16-like	Effecting dedifferentiation of pericycle cells, lateral root formation	(Feng, 2012); (Lee et al., 2009); (T Goh,2012)
MnLBD4	Morus009778	Scaffold40: 86,251–86,655:86,797–87,075	−	1	684	227	24,930.8	6.11	LOB domain-containing protein 29-like	Effecting dedifferentiation of pericycle cells, lateral root formation	(Feng, 2012)
MnLBD5	Morus009779	Scaffold40: 96,469–96,909:97,273–97,551	−	1	720	239	26,676.7	6.15	LOB domain-containing protein 29-like	Effecting dedifferentiation of pericycle cells, lateral root formation	(Feng, 2012)
MnLBD6	Morus014552	Scaffold125: 169,868–170,416	+	0	549	182	20,150.5	6.69	Lateral Organ Boundaries-like protein	Lateral organ development	(Shuai et al., 2002)
MnLBD7	Morus014124	Scaffold127: 242,300–242,671:243,592–243,912	+	1	693	230	24,924.5	6.7	LOB domain-containing protein 1-like	Secondary woody growth	(Yordanov, 2010)
MnLBD8	Morus025355	Scaffold139: 290,612–290,890:290,972–291,403	+	1	711	236	26,115.9	4.95	LOB domain-containing protein 33-like	Lateral root organogenesis	(Berckmans et al., 2011)
MnLBD9	Morus025918	Scaffold276: 181,491–181,850:183,993–184,184	−	1	552	183	19,561	8.56	LOB domain-containing protein 4-like		
MnLBD10	Morus027282	Scaffold329: 591,410–591,509:593,976–594,130:594,532–595,110	−	2	834	277	30,826.6	7.67	LOB domain-containing protein 22-like		
MnLBD11	Morus023484	Scaffold404: 625,335–625,520:638,942–639,277	+	1	522	173	19,816.8	6.06	LOB domain-containing protein 11-like		
MnLBD12	Morus013514	Scaffold410: 269,681–269,833:270,199–270,579	+	1	534	177	20,389.9	6.96	LOB domain-containing protein 24-like		
MnLBD13	Morus022437	Scaffold594: 218,060–218,153:219,373–219,680:222,234–222,554	−	2	723	240	26,296	9.04	LOB domain-containing protein 31-like		
MnLBD14	Morus022439	Scaffold594: 241,631–241,963:244,129–244,527	+	1	732	243	25,276.5	7.1	LOB domain-containing protein 18-like	Tracheary element differentiation; lateral root formation	(Soyano, 2008; (Lee et al., 2009)
MnLBD15	Morus014422	Scaffold710: 370,069–370,467:371,293–371,568	−	1	675	224	24,539.6	5.68	LOB domain-containing protein 1-like	Secondary woody growth	(Yordanov, 2010)
MnLBD16	Morus013233	Scaffold720: 117,943–118,902	−	0	960	319	35,307.6	6.62	LOB domain-containing protein 36-like	Flower development	(Chalfun-Junior et al., 2005)
MnLBD17	Morus015733	Scaffold759: 53,703–53,855:54,737–55,123	+	1	540	179	20,692.5	8.19	LOB domain containing protein 24-like		
MnLBD18	Morus006407	Scaffold778: 83,597–83,720:83,820–84,616	−	1	921	306	34,393.2	5.44	LOB domain-containing protein 27-like	Microspore development and asymmetric division	(Oh, 2010)
MnLBD19	Morus005914	Scaffold833: 80,444–80,627:80,720–81,249	+	1	714	237	25,617.1	8.79	LOB domain-containing protein 38-like	Anthocyanin biosynthesis	(Rubin et al., 2009)
MnLBD20	Morus005107	Scaffold847: 75,061–75,190:75,288–75,943	−	1	786	261	28,747.2	5.31	LOB domain-containing protein 22-like		
MnLBD21	Morus008640	scaffold1006: 20,558–21,288:21,390–21,579	−	1	921	306	32,916.4	8.42	LOB domain-containing protein 41-like	Leaf dorsoventral determination	(Meng, 2009)
MnLBD22	Morus005283	Scaffold1065: 73,817–74,575	+	0	759	252	27,518.1	7.75	LOB domain-containing protein 36-like	Flower development	(Chalfun-Junior et al., 2005)
MnLBD23	Morus016263	Scaffold1154: 391,145–391,480:394,954–395,133	−	1	516	171	18,920.4	7.67	LOB domain-containing protein 4-like		
MnLBD24	Morus009899	Scaffold1198: 190,981–191,532	+	0	552	183	20,024.7	8.69	LOB domain-containing protein 21-like		
MnLBD25	Morus003679	Scaffold1252: 33,898–34,340:34,424–34,607	−	1	627	208	22,356.2	6.89	LOB domain-containing protein 38-like	Anthocyanin biosynthesis	(Rubin et al., 2009)
MnLBD26	Morus011165	Scaffold1300: 320,516–320,893:321,188–321,343	−	1	534	177	19,532	6.39	LOB domain-containing protein 12-like	Leaf development	(Nakazawa et al., 2003)
MnLBD27	Morus003750	Scaffold1448: 108,282–108,956	+	0	675	224	24,230	8.25	LOB domain-containing protein 6-like	Maintains shoot meristem and defines lateral organ boundary; leaf and flower development	(Uchida, 2007); (Xu L, 2003); (Xu B, 2008)
MnLBD28	Morus005793	Scaffold1629: 128,982–129,218:129,876–130,028	−	1	390	129	14,329.5	8.8	LOB domain-containing protein 24-like		
MnLBD29	Morus002082	Scaffold1681: 56,758–57,467:57,585–57,726	+	1	852	283	31,686.7	5.75	LOB domain-containing protein 27-like	Microspore development and asymmetric division	(Oh, 2010)
MnLBD30	Morus006937	Scaffold2113: 114,567–114,722:115,086–115,469	+	1	540	179	19,931.7	6.28	LOB domain-containing protein 12-like	Leaf development	(Nakazawa et al., 2003)
MnLBD31	Morus000182	Scaffold11171: 854–1037:1146–1588	+	1	627	208	22,764	9.06	LOB domain-containing protein 38-like	Anthocyanin biosynthesis	(Rubin et al., 2009)

which have medicinal value especially in traditional Chinese medicine, and are used for the treatment of diabetes, arthritis, and rheumatism (Nomura, 1988; Sun et al., 2001; Singab et al., 2005).

The genome of mulberry was recently sequenced and is available in the *Morus* database (http://morus.swu.edu.cn/morusdb/). This data provides an opportunity to analyze the mulberry *LBD* genes. LBD proteins are plant specific transcription factors and play important roles in almost every aspect of plant development (Majer and Hochholdinger, 2011). Therefore, the identification of mulberry *LBD* genes, revealing their genomic structure, and analyzing their transcriptional profiles will contribute greatly to understanding their role in mulberry development.

2. Materials and methods

2.1. Identification of the mulberry LBD family genes

The *Morus* database was used (http://morus.swu.edu.cn/morusdb/). Forty-two *Arabidopsis LBDs* were downloaded from the Plant Transcription Factor Database (http://planttfdb.cbi.edu.cn/) (Husbands et al., 2007). The Hidden Markov model (HMM) profile for the *LBD* family (DUF260, Pfam number: PF0319) was obtained from the Pfam (http://pfam.sanger.ac.uk/) (Punta et al., 2012). Two methods were used to search against the mulberry peptide database. First, all 42 *Arabidopsis* LBDs were used as queries to search by BLASTP (Altschul et al., 1997) at an e-value of 1e-10. The redundancies were excluded. Secondly, the HMM profile of the LOB domain (Accession no. DUF260) was downloaded from the Pfam database (http://www.sanger.ac.uk). This domain was used as a query to blast against the mulberry peptide database with the BLASTP program. The predicted genes obtained in two methods were examined and corrected by the Simple Modular Architecture Research Tool (http://smart.embl-heidelberg.de/) (Letunic et al., 2012) and GENSCAN Web Server (http://genes.mit.edu/GENSCAN.html) (Burge and Karlin, 1997). Information regarding CDS length, amino acids number, molecular weight, and isoelectric point of protein were downloaded from TIGR release 4. The gene annotations in Table 1 were searched using protein blast on NCBI (http://ncbi.nlm.nih.gov) and they all based on the *Arabidopsis* LBD members. The predicted functions for some of the genes have been described in *Arabidopsis* in previous studies.

2.2. Phylogenetic and gene structure analysis

Multiple alignments of LOB-domain protein sequences were performed using the ClustalW program (Chenna, 2003). Phylogenetic trees were constructed using the MEGA 5.0 software (Tamura et al., 2011) and the neighbor-joining (NJ) method with the p-distance and complete deletion option parameters. The reliability of the trees was tested using a bootstrapping method with 1000 replicates. A diagram of exon–intron structures was generated using the online Gene Structure Display Server (GSDS: http://gsds.cbi.pku.edu.cn) (Guo et al., 2007) with the sites of intron and exon by loading DNA and RNA sequences of mulberry *LBD* gene family (RNA sequences was shown in Supplementary material 1). The conserved sequence logo was obtained using the online Weblogo platform (http://weblogo.berkeley.edu) (Schneider and Stephens, 1990). The conserved motifs were searched on "Multiple Em For Motif Elicitation" (MEME version 2.2, http://meme.nbcr.net/) using the following parameters, —nostatus — time 7200 — maxsize 60,000 — mod zoops — nmotifs 50 — minw 6 — maxw 50 (Bailey and Elkan, 1994).

2.3. Expression analysis of the mulberry LBD family genes

The reads per kilobase of exon model per million mapped reads (RPKM) were used for comparing the differences of gene expression

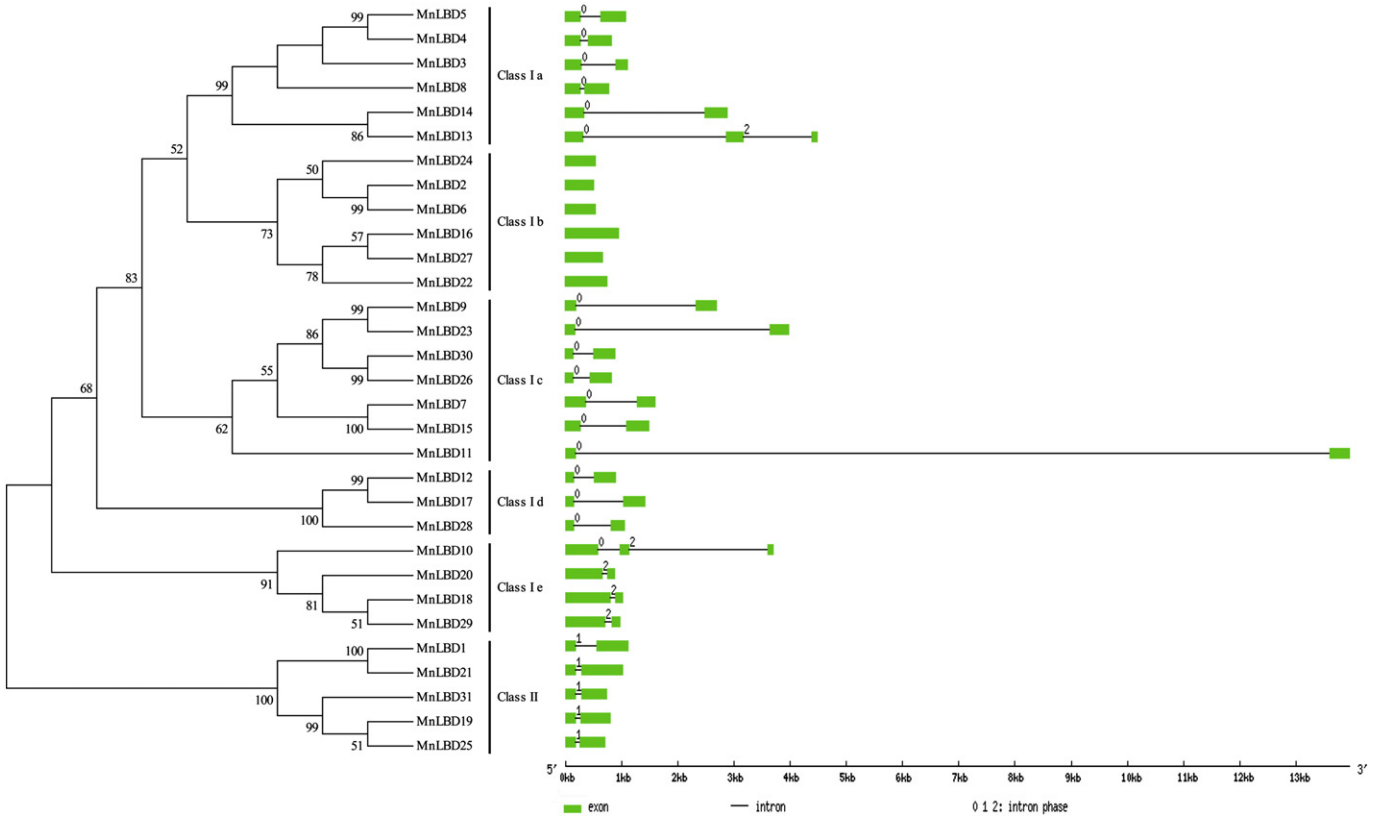


Fig. 1. Phylogenetic analysis (left) and exon intron structures (right) of *MnLBD* genes. Numbers above or below branches of the tree indicate bootstrap values and the values below 50 are hidden. *MnLBD* genes are divided into two classes (Class I and Class II) in which Class I family was further divided into 5 groups and named from class Ia to Class Ie (left). Exons are shown by solid green bars and introns by the connecting lines. The numbers 0, 1, 2 represent the intron phase. The length of the genes can be estimated using the scale on the bottom (right).

among samples (Bullard et al., 2010). The root, bark, bud, flower, and leaf RPKM value of mulberry *LBD* genes were retrieved from RNA sequencing data (http://morus.swu.edu.cn/morusdb/). A heat map was created by the Multi Experiment Viewer (Mev, version 4) (Saeed et al., 2003). Data were adjusted using normalize genes/row. Hierarchical clustering was performed using a default parameter.

3. Results

3.1. Identification of LBD genes in the mulberry genome

BLAST program and HMM analysis resulted in 31 mulberry *LBD* genes. All the 31 MnLBDs contained the LOB domain and the length ranged from 129 to 319 with the average of 220 amino acids (Table 1). Nomenclature of putative *MnLBD* genes was carried out based on the scaffold orders and they were termed *MnLBD1* to *MnLBD31*. The mulberry *LBD* genes were scattered over 28 scaffolds. Of them, *MnLBD3*, *MnLBD4*, and *MnLBD5* were arrayed along the scaffold 40, while *MnLBD13* and *MnLBD14* were located closely on the scaffold 594. As shown in Table 1, the majority of *MnLBD* genes (74.2%) have one intron and six *MnLBDs* are intronless genes. Only two genes, *MnLBD*10 and *MnLBD*13 are intervened by two introns.

3.2. Phylogenetic distribution and gene structure of MnLBD genes

The protein sequences of all *MnLBD* genes were used to build a phylogenetic tree, in which 31 MnLBDs were separated into two classes, class I and class II (Fig. 1 and Supplementary material 2). Class I containing 26 proteins was further divided into five groups named class Ia to Ie. The gene structures of all *MnLBD* genes are illustrated in the right panel of Fig. 1. The data clearly showed that *MnLBDs* in class II have only one phase 1 intron. Most of the *MnLBDs* in class I also have a structure similar to the genes in the same subclass. For example, six genes in class Ib are intronless, seven of class Ic have one phase 0 intron, and three of class Id genes also have one phase 0 intron. However, there were two exceptions. *MnLBD10* and *MnLBD13*, both of which are two-intron genes with atypical structures, are grouped in class Ia and class Ic, respectively.

As shown in Fig. 2, the results of the multiple sequence alignment indicated that a sequence with about 100 amino acids was conserved in all MnLBDs. For the class I MnLBDs, a string of blocks of C, GAS, and L-rich was recognized. Block C in MnLBDs can be summarized as: C-x(2)-C-x(6)-C-x(3)-C. Block GAS beginning with a F-x(2)-(V/A)-H motif and ending with a DP-(V/I)-YG motif. All class II MnLBDs have the conserved C-block similar to Class I and are absent in GAS-block and Leu-zipper like domain.

3.3. Phylogenetic analysis of the LBD proteins

A. thaliana is a model plant species and the functions of some *Arabidopsis LBD* genes have been well-characterized, therefore, we constructed a phylogenetic tree with LBD protein sequences from *A. thaliana* and mulberry to provide insight into the functional prediction of mulberry LBDs. As shown in Fig. 3 (Supplementary material 3), all LBDs were separated into two classes, and most of them belonged to class I. There were 3 MnLBDs that strongly supported mulberry/

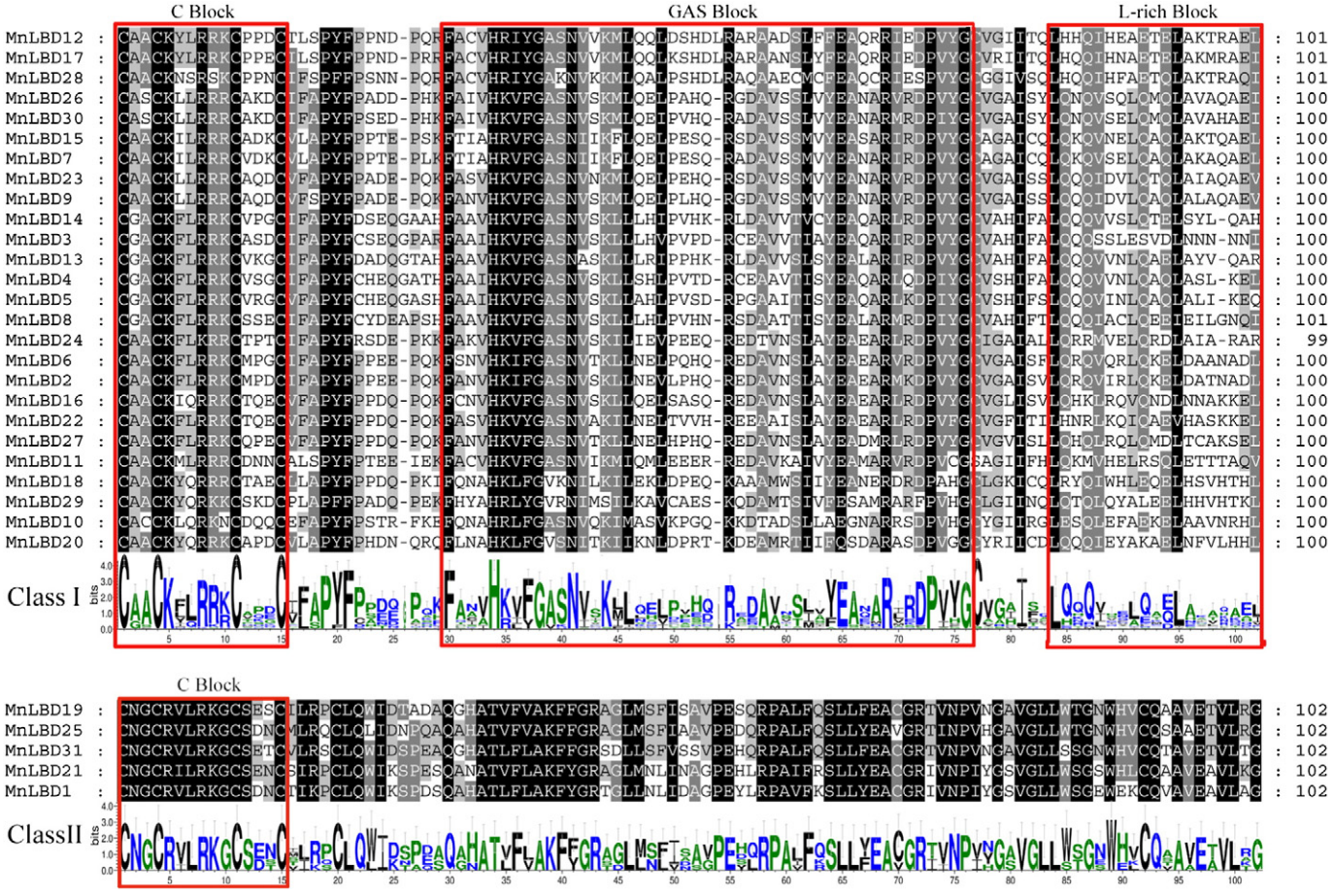
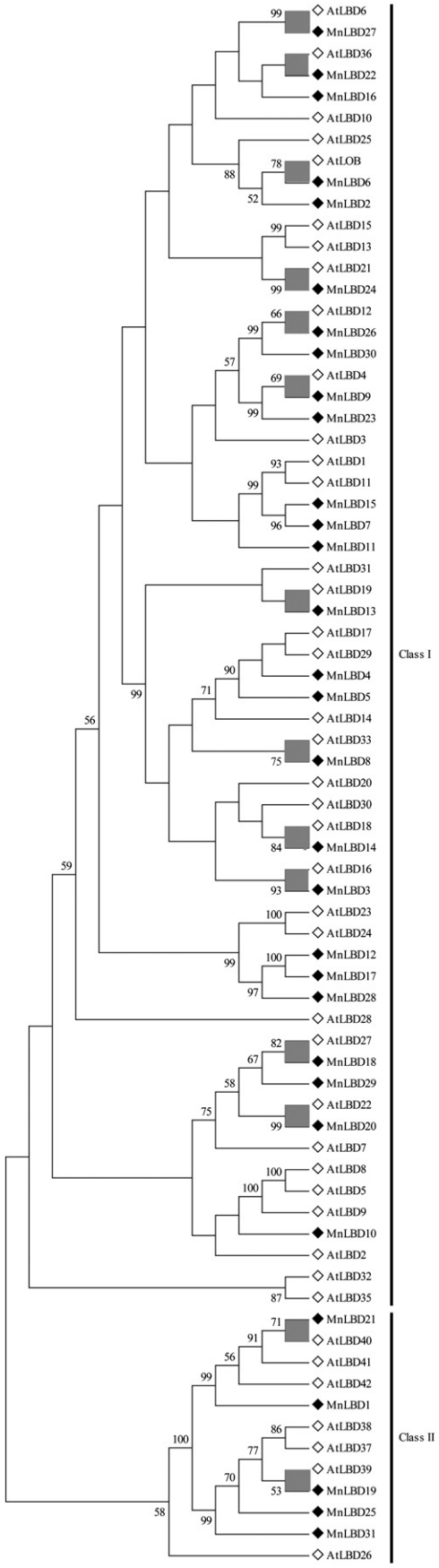


Fig. 2. LBD-domain alignment and corresponding logo of *MnLBDs*. Alignment of *MnLBDs* Class I genes was shown upside, and the conserved C block, GAS block and L-rich block are indicated by a red box. Alignment of *MnLBDs* Class II genes was shown below. The only conserved C block is represented by a red box. Below the alignment are the sequence logos. The overall height of each stack represents the degree of conservation at this position, while the height of the letters within each stack indicates the relative frequency of the corresponding amino acids.



Arabidopsis pairwise proteins with a bootstrap value of 99 shown in gray boxes on the phylogenetic tree, namely, MnLBD27/AtLBD06, MnLBD24/AtLBD21, and MnLBD20/AtLBD22. Furthermore, the phylogenetic relationship of MnLBD3/AtLBD6, MnLBD4/MnLBD5/AtLBD29/AtLBD17, MnLBD13/AtLBD19 and MnLBD14/AtLBD18 were close.

3.4. Expression analyses of putative MnLBDs

A heat map was created to check the expression profiles of various *MnLBD* in leaf, root, bark, bud, and flower. Based on this map, the 31 *MnLBD*s were classified into 5 groups. Group I consisted of 3 genes (*MnLBD14*, *18* and *22*) which showed high transcript accumulation in leaves. Group II comprised of the genes *MnLBD5*, *15*, *23*, *25* and *29*, which had a bark-bias expression. Thirteen genes (*MnLBD3*, *4*, *7*, *8*, *9*, *10*, *11*, *12*, *13*, *17*, *19*, *26* and *28*) in group III were preferentially expressed in the root. In this group, it is worth mentioning that *MnLBD13* was expressed not only in root, but also in the bark and flower. Five genes, *MnLBD6*, *20*, *24*, *30* and *31* in group IV, were expressed at relatively higher levels in the flower, and the expression of *MnLBD1*, *2*, *16*, *21* and *27* in Group V was detected mainly in bud (Fig. 4).

4. Discussion

Our knowledge of plant LBD proteins has increased significantly since 2002. Shuai et al. identified a domain in 42 *Arabidopsis* proteins that are now referred to as LBD domain. The availability of sequenced plant genomes since its discovery has made it possible to isolate and study these genes. For example, through genome-wide analysis, 35 and 58 of *LBD* genes were identified in rice and apple, respectively (Yang et al., 2006; Wang et al., 2013). In the present study, it was revealed that the mulberry genome has 31 genes having LBD domain, in which 23 have a single intron interrupting the coding region while 6 are intronless. Two short genomic regions with clusters of *MnLBD* genes have been sequenced with 2–3 genes in a cluster. Understanding the consequences of gene expansion and diversification of the *MnLBD* genes is compelling. The development of various tissues in specific locations at specific time helps to determine the effect of diversification on the mulberry tree.

Sequence information and gene expressional data of the *MnLBDs* will facilitate future identification of candidate genes. Plant root system has an important role in both their response to soil conditions and tillage of the soil (Russell, 1977). Four genes in class Ia, *MnLBD* 3, 4, 8, and 13 are preferentially expressed in the mulberry root. Phylogenetic analysis revealed that, MnLBD3 was the most identical to *Arabidopsis* LBD16, which functions in lateral root development (Okushima et al., 2007). The other 3 genes, *MnLBD4*, *MnLBD8* and *MnLBD13* may also relate to lateral root development according to the expression profile and the phylogeny relationship with *A. thaliana* counterparts. Further study of these genes will contribute to a better understanding of the mechanism of mulberry in root development. In the same way, gene *MnLBD22* was highly expressed in the leaf and placed in a branch with AtLBD36, which played an important role in leaf morphogeny (Nakazawa et al., 2003; Chalfun-Junior et al., 2005). These data may be used to hypothesize the role of *MnLBD22* in mulberry leaf development.

On the other hand, mulberry has a variety of active secondary metabolites, such as flavonoids (Du et al., 2003), alkaloids (Asano et al., 2001), and terpenoids (Zhi-ming et al., 2012). However, the mechanism of mulberry secondary metabolism has not been well documented. It has been reported that the *Arabidopsis* gene *AtLBD39* is involved in the

Fig. 3. Phylogenetic tree based on the *Arabidopsis* and mulberry LBD protein sequence. The bootstrap values are shown at the nodes and the values less than 50 are hidden. The AtLBDs and MnLBDs are marked with hollow and solid diamond respectively. The scale bar in the circle tree represents 0.5 substitutions per sequence position. The gray boxes indicate pairwise mulberry/*Arabidopsis* orthologous.

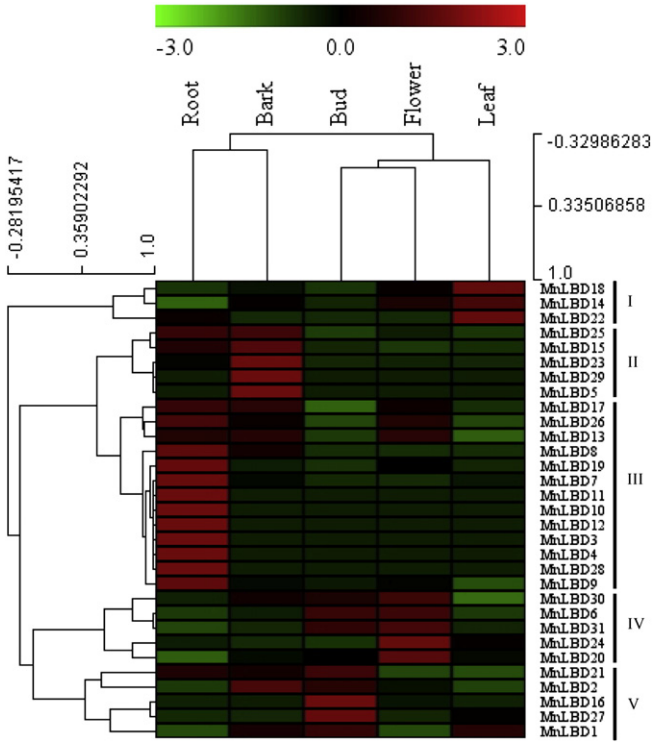


Fig. 4. Expression profiles of *MnLBD* genes. The clustering of genes was done by hierarchical clustering using average linkage clustering as rule with the default option after adjusting data in normalized genes/rows. Differences in gene expression are shown in color according to the scale. Different organs (root, bark, bud, flower and leaf) of mulberry were used for expression profiling, which are mentioned on top of each column listed. On the left side of expression map, clade names were given. Color bar at the top represents log2 expression values, wherein the green color represents low level expression, black shows medium level expression and red signifies a high level of expression. Five groups were divided according to the expression profiles.

biosynthesis of anthocyanins (Rubin et al., 2009). Gene *MnLBD19* in class II is the closest homolog of *AtLBD39* might implicate in the secondary metabolism of mulberry.

5. Conclusions

In the present study, thirty-one putative *MnLBD* genes were identified in the mulberry genome. Data on the expression of each of the *Morus LBD* genes coupled with sequence analysis provides valuable information for functional studies of mulberry *LBD* genes.

Conflict of interest statement

The authors declare that they have no competing interests.

Acknowledgments

This project was funded by the research grants from the National Hi-Tech Research and Development Program of China (No. 2013AA100605-3), the "111" Project (B12006), the Science Fund for Distinguished Young Scholars of Chongqing (Grant No. cstc2011jjjq0010), and the National Science Foundation of China (No. 31201005).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mgene.2014.04.004.

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J.H., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Asano, N., Yamashita, T., Yasuda, K., Ikeda, K., Kizu, H., Kameda, Y., Kato, A., Nash, R.J., Lee, H.S., Ryu, K.S., 2001. Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). J. Agric. Food Chem. 49, 4208–4213.

Bailey, T.L., Elkan, C., 1994. Fitting a Mixture Model by Expectation Maximization to Discover Motifs in Biopolymers. Proceeding of the Second International Conference on Intelligent Systems for Molecular Biology 2 pp. 28–36.

Berckmans, B., Vassileva, V., Schmid, S.P., Maes, S., Parizot, B., Naramoto, S., Magyar, Z., Alvim Kamei, C.L., Koncz, C., Bogre, L., Persiau, G., De Jaeger, G., Friml, J., Simon, R., Beeckman, T., De Veylder, L., 2011. Auxin-dependent cell cycle reactivation through transcriptional regulation of *Arabidopsis E2Fa* by lateral organ boundary proteins. *Plant Cell* 23, 3671–3683.

Bullard, J.H., Purdom, E., Hansen, K.D., Dudoit, S., 2010. Evaluation of statistical methods for normalization and differential expression in mRNA-Seq experiments. BMC Bioinf. 11, 94.

Burge, C., Karlin, S., 1997. Prediction of complete gene structures in human genomic DNA. J. Mol. Biol. 268, 78–94.

Chalfun-Junior, A., Franken, J., Mes, J.J., Marsch-Martinez, N., Pereira, A., Angenent, G.C., 2005. *ASYMMETRIC LEAVES2-LIKE1* gene, a member of the AS2/LOB family, controls proximal–distal patterning in *Arabidopsis* petals. J. Mol. Biol. 57, 559–575.

Chenna, R., 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res. 31, 3497–3500.

Du, J., He, Z.-D., Jiang, R.-W., Ye, W.-C., Xu, H.-X., But, P.P.-H., 2003. Antiviral flavonoids from the root bark of *Morus alba* L. Phytochemistry 62, 1235–1238.

Ganga, G., 2003. Comprehensive Sericulture, Silkworm Rearing and Silk Reeling. 2. IBH publishing house Co. Pvt. Ltd., Oxford.

Guo, A.Y., Zhu, Q.H., Chen, X., Luo, J.C., 2007. GSDS: a gene structure display server. *Yi Chuan* 29 pp. 1023–1026.

Husbands, A., Bell, E.M., Shuai, B., Smith, H.M., Springer, P.S., 2007. *LATERAL ORGAN BOUNDARIES* defines a new family of DNA-binding transcription factors and can interact with specific bHLH proteins. Nucleic Acids Res. 35, 6663–6671.

Lee, H.W., Kim, M.J., Kim, N.Y., Lee, S.H., Kim, J., 2013. LBD18 acts as a transcriptional activator that directly binds to the *EXPANSIN14* promoter in promoting lateral root emergence of *Arabidopsis*. Plant J. 73, 212–224.

Lee, H.W., Kim, N.Y., Lee, D.J., Kim, J., 2009. *LBD18/ASL20* regulates lateral root formation in combination with *LBD16/ASL18* downstream of *ARF7* and *ARF19* in *Arabidopsis*. Plant Physiol. 151, 1377–1389.

Letunic, I., Doerks, T., Bork, P., 2012. SMART 7: recent updates to the protein domain annotation resource. Nucleic Acids Res. 40, D302–D305.

Liu, H., Wang, S., Yu, X., Yu, J., He, X., Zhang, S., Shou, H., Wu, P., 2005. ARL1, a LOB-domain protein required for adventitious root formation in rice. Plant J. 43, 47–56.

Majer, C., Hochholdinger, F., 2011. Defining the boundaries: structure and function of LOB domain proteins. Trends Plant Sci. 16, 47–52.

Matsumura, Y., Iwakawa, H., Machida, Y., Machida, C., 2009. Characterization of genes in the *ASYMMETRIC LEAVES2/LATERAL ORGAN BOUNDARIES* (*AS2/LOB*) family in *Arabidopsis thaliana*, and functional and molecular comparisons between *AS2* and other family members. Plant J. 58, 525–537.

Nakazawa, M., Ichikawa, T., Ishikawa, A., Kobayashi, H., Tsuhara, Y., Kawashima, M., Suzuki, K., Muto, S., Matsui, M., 2003. Activation tagging, a novel tool to dissect the functions of a gene family. Plant J. 34, 741–750.

Nomura, T., 1988. Phenolic compounds of the mulberry tree and related plants. Prog. Chem. Org. Nat. Prod. 53, 87–201.

Okushima, Y., Fukaki, H., Onoda, M., Theologis, A., Tasaka, M., 2007. ARF7 and ARF19 regulate lateral root formation via direct activation of *LBD/ASL* genes in *Arabidopsis*. Plant Cell 19, 118–130.

Olson, P.E., Wong, T., Leigh, M.B., Fletcher, J.S., 2003. Allometric modeling of plant root growth and its application in rhizosphere remediation of soil contaminants. Environ. Sci. Technol. 37, 638–643.

Punta, M., Coggill, P.C., Eberhardt, R.Y., Mistry, J., Tate, J., Boursnell, C., Pang, N., Forslund, K., Ceric, G., Clements, J., Heger, A., Holm, L., Sonnhammer, E.L., Eddy, S.R., Bateman, A., Finn, R.D., 2012. The Pfam protein families database. Nucleic Acids Res. 40, D290–D301.

Rubin, G., Tohge, T., Matsuda, F., Saito, K., Scheible, W.R., 2009. Members of the *LBD* family of transcription factors repress anthocyanin synthesis and affect additional nitrogen responses in *Arabidopsis*. Plant Cell 21, 3567–3584.

Russell, R.S., 1977. Plant Root Systems: Their Function and Interaction With the Soil, *Published by McGraw-Hill Book Company (UK) Limited*. pp. 193

Saeed, A.I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., Braisted, J., Klapa, M., Currier, T., Thiagarajan, M., Sturn, A., Snuffin, M., Rezantsev, A., Popov, D., Ryltsov, A., Kostukovich, E., Borisovsky, I., Liu, Z., Vinsavich, A., Trush, V., Quackenbush, J., 2003. TM4: a free, open-source system for microarray data management and analysis. Biotechniques 34, 374–378.

Schneider, T.D., Stephens, R.M., 1990. Sequence logos: a new way to display consensus sequences. Nucleic Acids Res. 18, 6097–6100.

Serrano-Cartagena, J., 1999. Genetic analysis of leaf form mutants from the *Arabidopsis* information service collection. Mol. Gen. Genomics. 261, 725–739.

Shuai, B., Reynaga-Pena, C.G., Springer, P.S., 2002. The *LATERAL ORGAN BOUNDARIES* gene defines a novel, plant-specific gene family. Plant Physiol. 129, 747–761.

Singab, A.N., El-Beshbishy, H.A., Yonekawa, M., Nomura, T., Fukai, T., 2005. Hypoglycemic effect of Egyptian *Morus alba* root bark extract: effect on diabetes and lipid peroxidation of streptozotocin-induced diabetic rats. J. Ethnopharmacol. 100, 333–338.

Sun, S.G., Chen, R.Y., Yu, D.Q., 2001. Structures of two new benzofuran derivatives from the bark of mulberry tree (*Morus macroura Miq.*). J. Asian Nat. Prod. Res. 3, 253–259.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
Wang, X., Zhang, S., Su, L., Liu, X., Hao, Y., 2013. A genome-wide analysis of the *LBD* (*LATERAL ORGAN BOUNDARIES Domain*) gene family in *Malus domestica* with a functional characterization of *MdLBD11*. PLoS ONE (*e*8, e57044).
Yamashita, T., Fujino, A., 1986. Effects of pruning of young and old shoots on ribulose bisphosphate carboxylase and other constituents in leaves of the mulberry tree (*Morus alba* L.). J. Exp. Bot. 37, 1836–1841.
Yang, Y., Yu, X., Wu, P., 2006. Comparison and evolution analysis of two rice subspecies *LATERAL ORGAN BOUNDARIES* domain gene family and their evolutionary characterization from *Arabidopsis*. Mol. Phylogenet. Evol. 39, 248–262.
Zentella, R., Zhang, Z.L., Park, M., Thomas, S.G., Endo, A., Murase, K., Fleet, C.M., Jikumaru, Y., Nambara, E., Kamiya, Y., Sun, T.P., 2007. Global analysis of della direct targets in early gibberellin signaling in *Arabidopsis*. Plant Cell 19, 3037–3057.
Zhi-ming, L., Hai-ying, W., Shan-shan, L., Nai-xiang, J., 2012. Volatile components of essential oil from mulberry variety “Longsang 1” leaves. Nat. Prod. Res. Dev. 23, 1069–1072.