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

A R T I C L E   I N F O

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

A B S T R A C T

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.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

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 family that has 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.

Abbreviation: LBD, Lateral Boundary Domain; LOB, Lateral Organ Boundary gene; HMM, Hidden Markov model; NJ, neighbor-joining; CDS, 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
2214-5400/© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).
| Name    | Accession no. | Location  | Gene     | Protein Predictions | 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 6.88  | 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 26,676.7 6.15    | LOB domain-containing protein 29-like: Effecting dedifferentiation of pericycle cells, lateral root formation | (Feng, 2012) |
| MnLBD6  | Morus014552   | Scaffold125: 169,868–169,910              | + 0      | 549 182 20,150.5 6.9  | LOB domain-containing protein 16-like: Lateral Organ Boundaries-like protein | (Shuai et al., 2002) |
| MnLBD7  | Morus014124   | Scaffold127: 242,300–242,671               | + 1      | 693 230 24,924.5 6.7  | LOB domain-containing protein 16-like: Lateral organ development            | (Yordanov, 2010) |
| MnLBD8  | Morus025355   | Scaffold40: 79,612–79,890; 79,972–79,403  | + 1      | 711 236 26,135.9 4.95 | LOB domain-containing protein 33-like: Flower development                  | (Berckmans et al., 2011) |
| MnLBD9  | Morus009789   | Scaffold40: 181,491–183,993                | + 2      | 834 277 30,826.6 7.67 | LOB domain-containing protein 22-like: Leaf development and asymmetric division | (Rubin et al., 2009) |
| MnLBD10 | Morus014124   | Scaffold127: 242,300–242,671               | + 1      | 693 230 24,924.5 6.7  | LOB domain-containing protein 16-like: Lateral organ development            | (Yordanov, 2010) |
| MnLBD15 | Morus013514   | Scaffold40: 269,833–270,199                | + 1      | 534 177 20,389.9 6.9  | LOB domain-containing protein 24-like: Tracheary element differentiation    | (Chalfon-Junior et al., 2005) |
| MnLBD16 | Morus022437   | Scaffold594: 218,060–219,373               | + 2      | 723 240 26,296 9.04   | LOB domain-containing protein 31-like: Flower development                  | (Chalfon-Junior et al., 2005) |
| MnLBD17 | Morus006407   | Scaffold759: 53,703–53,855; 54,737–55,123 | + 1      | 540 179 20,692.5 8.19 | LOB domain-containing protein 24-like: Maintains shoot meristem and flowering | (Uchida, 2003); (Xu B, 2008) |
| MnLBD18 | Morus005911   | Scaffold833: 80,444–80,627; 80,720–81,249 | + 1      | 714 237 25,617.1 7.1  | LOB domain-containing protein 18-like: Tracheary element differentiation    | (Shuai et al., 2002) |
| 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: Secondary woody growth formation     | (Yordanov, 2010) |
| MnLBD21 | Morus008649   | Scaffold1006: 20,558–21,288; 21,390–21,579 | + 0      | 921 306 32,916.4 6.42 | LOB domain-containing protein 41-like: Leaf dorsoventral determination     | (Rubin et al., 2009) |
| MnLBD22 | Morus005283   | Scaffold1065: 73,817–74,757                | + 0      | 759 252 27,518.1 7.75 | LOB domain-containing protein 36-like: Flower development                  | (Chalfon-Junior et al., 2005) |
| MnLBD23 | Morus016263   | Scaffold1154: 391,145–391,480; 394,954–395,133 | + 1      | 516 171 18,920.4 7.7  | LOB domain-containing protein 4-like: Anthocyanin biosynthesis            | (Rubin et al., 2009) |
| MnLBD24 | Morus000899   | Scaffold1198: 190,981–191,532              | + 0      | 552 183 20,024.7 8.69 | LOB domain-containing protein 21-like: Anthocyanin biosynthesis            | (Rubin et al., 2009) |
| MnLBD25 | Morus003679   | Scaffold1252: 33,886–34,404; 34,722–34,360 | + 1      | 627 208 22,356.2 8.9  | 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 6.9  | LOB domain-containing protein 12-like: Flower development                  | (Uchida, 2007); (Xu Li, 2003); (Xu B, 2008) |
| MnLBD27 | Morus003750   | Scaffold1448: 108,282–108,956              | + 0      | 675 224 24,230 8.25   | LOB domain-containing protein 6-like: Maintains shoot meristem and flowering | (Rubin et al., 2009) |
| 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: Leaf development and asymmetric division | (Rubin et al., 2009) |
| MnLBD29 | Morus001015   | Scaffold1681: 56,758–57,467; 57,585–57,726 | + 1      | 852 283 31,686.7 5.7  | LOB domain-containing protein 27-like: Microspore development and asymmetric division | (Rubin et al., 2009) |
| MnLBD30 | Morus009639   | Scaffold2121: 114,567–114,722; 115,086–115,469 | + 1      | 540 179 19,931.7 6.8  | LOB domain-containing protein 12-like: Anthocyanin biosynthesis            | (Rubin et al., 2009) |
| MnLBD31 | Morus000182   | Scaffold11171: 854–1037; 1146–1588        | + 1      | 627 208 19,764 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 LDB 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 isoelectic 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 levels. 

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 le (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, MnLBD10 and MnLBD13 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 le. 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 Ia 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 la and class lc, 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/
Arabidopsis pairwise proteins with a bootstrap value of 99 shown in gray boxes on the phylogenetic tree, namely, MnLBD27/AtLBD6, 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 MnLBDs 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 at specific locations at specific time helps to determine the effect of diversification on the mulberry tree.

Sequence information and gene expression 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 morphogenesis (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. 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 marked 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. cstc2011jijq0010), 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., Kamera, Y., Kato, A., Nasu, R.J., Lee, H.S., Ryu, K.S., 2001. Polyhydroxylalkylated alkaloids isolated from mulberry tree (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.

Berkmann, B., Vassileva, V., Schmid, S.P., Maes, S., Parizot, B., Naramoto, S., Magyar, Z., Alvim Ramei, C.L., Konez, C., Bogre, L., Periasu, G., De Jaeger, G., Frinkl, 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., Pandora, 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., Genten, C.G., 2005. ASYMMETRIC LEAVES2-LIKE 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. CD$S$: 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 EXPAN3/4 promoter in promoting lateral root emergence of Arabidopsis. Plant J. 73, 212–224.

Lee, H.W., Kim, M.J., Lee, D.J., Kim, J., 2009. LBD18/ASL20 regulates lateral root formation in combination with LBD61/ASL19 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 architecture database. Nucleic Acids Res. 36, 115–119.

Majer, C., Hochholdinger, F., 2011. Defining the boundaries: structure and function of LBD 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. Proc. 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., Mistri, J., Tate, J., Bourne, P., 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. 41, 224–230.

Rubin, C., Tohe, 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., Brestel, J., Klapa, M., Currier, T., Thiagarajan, M., Sturn, A., Snuffin, M., Reitz, A., Popov, D., Rytists, A., Kostukovich, E., Borisyovskiy, 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, 741–746.

Singh, A.K., El-Beshbishy, H.A., Yonekawa, M., Nomura, T., Fukui, 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 macrocaulis 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 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.