Map-based cloning and promoter variation analysis of the lobed leaf gene BoLMI1a in ornamental kale (Brassica oleracea L. var. acephala)

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Abstract

Background: Leaf shape is an important agronomic trait in ornamental kale (Brassica oleracea L. var. acephala). Although some leaf shape-related genes have been reported in ornamental kale, the detailed mechanism underlying leaf shape formation is still unclear. Here, we report a lobed-leaf trait in ornamental kale, aiming to analyze its inheritance and identify the strong candidate gene.

Results: Genetic analysis of F2 and BC1 populations demonstrate that the lobed-leaf trait in ornamental kale is controlled by a single dominant gene, termed BoL1 (Brassica oleracea lobed-leaf). By performing whole-genome resequencing and linkage analyses, the BoL1 gene was finely mapped to a 127-kb interval on chromosome C09 flanked by SNP markers SL4 and SL6, with genetic distances of 0.6 cM and 0.6 cM, respectively. Based on annotations of the genes within this interval, Bo9g181710, an orthologous gene of LATE MERISTEM IDENTITY 1 (LMI1) in Arabidopsis, was predicted as the candidate for BoL1, and was renamed BoLMI1a. The expression level of BoLMI1a in lobed-leaf parent 18Q2513 was significantly higher compared with unlobed-leaf parent 18Q2515. Sequence analysis of the parental alleles revealed no sequence variations in the coding sequence of BoLMI1a, whereas a 1737-bp deletion, a 92-bp insertion and an SNP were identified within the BoLMI1a promoter region of parent 18Q2513. Verification analyses with BoLMI1a-specific markers corresponding to the promoter variations revealed that the variations were present only in the lobed-leaf ornamental kale inbred lines.

Conclusions: This study identified a lobed-leaf gene BoLMI1a, which was fine-mapped to a 127-kb fragment. Three variations were identified in the promoter region of BoLMI1a. The transcription level of BoLMI1a between the two parents exhibited great difference, providing new insight into the molecular mechanism underlying leaf shape formation in ornamental kale.

Keywords: Ornamental kale, Lobed leaf, Fine mapping, Promoter variation, Enhanced expression

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Background

Leaves are essential organs that play an important role in plants, including carbon assimilation, gas exchange, water transport and nutrient distribution [1]. Leaf shape can significantly affect both leaf function and plant architecture [2, 3]. A typical variation in leaf shape involves the leaf margin, which can be unlobed, serrated or lobed [4]. Lobed leaves can be easily visualized even in the primary leaf stage, which can be used as an indicator trait for hybrid production [5, 6]. Compared to unlobed- or serrated-leaf lines, plants with lobed leaves are better adapted to environmental stresses [7, 8]. With improved heat transfer and light energy absorption, lobed leaves are advantageous for high-density planting and mechanized production [9]. Additionally, lobed leaves are also a graceful decorative trait for ornamental plants such as kale [4].

Ornamental kale (Brassica oleracea L. var. acephala) is an attractive ornamental crop owing to its polymorphic, colorful leaves [10]. Lobed-leaf genes have been genetically analyzed and mapped in some Brassica species. For example, the lobed-leaf trait in B. rapa is controlled by major gene or polygenic effects [11–14]. In B. napus, the incomplete dominant lobed-leaf gene BnLL1 was mapped to the distal end of chromosome A10 [15]. In ornamental kale, some studies have shown that the lobed-leaf trait exhibits incomplete dominance over the unlobed-leaf trait [16]. Genetic analysis of an interspecific hybrid between B. napus and Rorippa indica (L.) Hiern revealed that the lobed-leaf trait is controlled by a dominant gene [9]. Moreover, Ren et al. mapped a quantitative trait locus (QTL) associated with lobed leaves to chromosome 9 of ornamental kale flanked by insertion-deletion (InDel) markers LYIn39 and LYIn40, with genetic distances of 0.17 cM and 0.11 cM, respectively [4].

With the development of high-throughput sequencing technology and the release of B. oleracea draft genomes [17, 18], a growing number of genes that govern important traits have been mapped in this species. Bulk segregant analysis (BSA) is a rapid and accurate gene mapping method that was first developed and performed in plants [19]. This method is characterized by bulk genotyping of a pool of segregants that share the same phenotype. InDel has been considered as an ideal source for marker design due to its high-density distribution and genotyping efficiency. Using InDel markers, many genes/QTLs have been mapped in B. oleracea, including the yellow-green leaf gene ygl-1 [20], the purple leaf gene BoPr [21], QTLs associated with heading traits [22], male sterility genes [23, 24] and the petal color gene BoCCD4 [25].

Lobed-leaf trait is a unique variation in kale that can be produced by infrequent genetic mechanisms. In the present study, we developed F1, F2 and BC1 populations descended from the ornamental kale inbred line 18Q2513 (with lobed leaves) and 18Q2515 (with unlobed leaves). A rare dominant inheritance pattern was identified for lobed-leaf trait using these populations. Furthermore, the lobed-leaf gene BoLL1-1 was fine-mapped to a narrow interval using BSA-seq and linkage analysis. The findings provide new insight into the molecular mechanism underlying leaf shape formation in ornamental kale.

Results

Genetic analysis of leaf shape in ornamental kale

The leaf shape throughout all the F1 plants (comprising 16 individuals) generated by crossing 18Q2513 (lobed-leaf, Fig. 1a) with 18Q2515 (unlobed-leaf, Fig. 1b) was lobed; thus, the lobed-leaf trait is dominant over the unlobed-leaf trait in these two ornamental kale lines. The F2 population comprised 120 individuals, with 92 displaying lobed leaves and 28 unlobed leaves. According to a chi-square test, the segregation ratio is 3:1. The BC1P1 population contained 850 individuals, with 429 displaying lobed leaves and 421 unlobed leaves. According to a chi-square test, the segregation ratio is 3:1. The BC1P1 population contained 850 individuals, with 429 displaying lobed leaves and 421 unlobed leaves.
Lobed-leaf plants and unlobed-leaf plants were determined at the seedling shape segregation in BC and F2 populations. The SNP markers SL4 and SL6 were found to be tightly linked to the reference genome, BoLl-1, with genetic distances of 0.6 cM and BoLl-1 linked to the reference genome, BoLl-1.

To further delineate the location of BoLl-1, 16 Indel and seven SNP markers (by comparing resequencing data of the parents with the sequence of the TO1000 reference genome) within the 1.33-Mb candidate region and its flanking regions (600 kb on each side) were designed. Ultimately, five Indel and three SNP markers showed polymorphisms between the two parents. A total of 429 recessive individuals of the BC1P1 population were subsequently used for BoLl-1 fine mapping.

A linkage map consisting of five InDel and three SNP markers was constructed using MapDraw (Fig. 2b). The SNP markers SL4 and SL6 were found to be tightly linked to BoLl-1, with genetic distances of 0.6 cM and 0.6 cM, respectively. Based on the marker locations in the reference genome, BoLl-1 was ultimately delimited to a 127-kb region (53680797–53,808,289 bp) on chromosome C09.

Table 1 The Chi-square ($\chi^2$) goodness-of-fit test ratios of leaf shape segregation in BC and F2 populations

| Populations | Total plant number | Number of lobed-leaf individuals* | Number of unlobed-leaf individuals* | Expected ratio | $\chi^2$ |
|-------------|--------------------|-----------------------------------|-------------------------------------|---------------|---------|
| F1          | 16                 | 16                                | 0                                   | –             | –       |
| F2          | 120                | 92                                | 28                                  | 3:1           | 0.18    |
| BC1P1       | 850                | 429                               | 421                                 | 1:1           | 0.08    |
| BC1P2       | 200                | 200                               | 0                                   | –             | –       |

* $\chi^2 > \chi^2_{0.05} = 3.84$ was considered significant
* Lobed-leaf plants and unlobed-leaf plants were determined at the seedling stage by visual inspection

and the segregation ratio was confirmed to be 1:1 by a chi-square test. The 200 BC1P2 individuals all had lobed leaves (Table 1). These results indicate that the lobed-leaf trait is controlled by a single dominant gene, which was named BoLl-1.

Fine mapping of the BoLl-1 gene by BSA-seq and linkage analyses

To identify markers associated with lobed leaves, the SNP index and $\Delta$(SNP index) between the two bulks were calculated using high-quality SNPs. The average SNP-index and $\Delta$(SNP-index) of the two bulks across a 1-Mb genomic interval were measured using a 10-kb sliding window and plotted against the genome position. The highest peak region, which was considered to be the candidate interval associated with BoLl-1, contains approximately 1.33 Mb (53.34–54.67 Mb) on chromosome 9 according to the ‘TO1000’ reference genome (Fig. 2a). For the candidate region of BoLl-1, 3280 SNPs between parental lines were identified, 410 of which are effective; 593 InDels were identified, 35 of which are effective (Table S1).

To further delineate the location of BoLl-1, 16 InDel and seven SNP markers (by comparing resequencing data of the parents with the sequence of the TO1000 reference genome) within the 1.33-Mb candidate region and its flanking regions (600 kb on each side) were designed. Ultimately, five InDel and three SNP markers showed polymorphisms between the two parents. A total of 429 recessive individuals of the BC1P1 population were subsequently used for BoLl-1 fine mapping.

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Sequence analysis of the candidate genes

To determine the causal relationship between the candidate genes and leaf shape formation, a comparative sequence analysis of the Bo9g181710 and Bo9g181720 genes body (DNA) and ~ 3-kb promoter region was performed using genomic DNA from 18Q2513 and 18Q2515. No sequence variations (between the parental lines) were detected (Fig. 3).

To analyze the expression patterns of Bo9g181710 and Bo9g181720, qRT-PCR was performed using young leaves from 28-day-old seedlings of the parents. The expression level of Bo9g181710 in lobed-leaf 18Q2513 was significantly higher than that in unlobed-leaf 18Q2515, whereas no significant difference in Bo9g181720 expression between the parental lines was detected (Fig. 3).

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Prediction and expression analysis of the candidate genes

Based on the ‘TO1000’ reference genome [18], 21 genes were identified within the 127-kb interval (Table 2). According to annotations from the Brassica oleracea genome and BLASTX (best hit) to A. thaliana, only two genes Bo9g181710 and Bo9g181720 are related to the formation of leaf shape. These two genes are homologues of the LATE MERISTEM IDENTITY 1 (LMI1) gene in Arabidopsis, which encode a homeodomain leucine zipper class I (HD-Zip I) meristem identity regulator that plays an important role in leaf morphogenesis and bract formation. Thus, we designated that Bo9g181710 and Bo9g181720 were candidate genes controlling lobed leaf shape in ornamental kale.

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BoLI1a and BoLI1b proteins and its close homologues was carried out to evaluate their evolutionary relatedness. The results showed that BoLI1a is closely related to B. napus ATHB-51 and is located in the same clade as other cruciferous plants, indicating that they may be derived from the same ancestor gene (Fig. 5).

**Verification of BoLI1a-specific markers**

Using the co-dominant marker CMLMII and the dCAPS marker DMLMII, we determined whether the variations in the BoLI1a promoter are also present in 118 different cabbage inbred lines (with unlobed leaves) and another ornamental kale inbred line 18Q2523 (with lobed leaves). The results indicated that the insertion, deletion (detected by co-dominant marker CMLMII) and the SNP (detected by dCAPS marker DMLMII) were present only in the lobed-leaf ornamental kale inbred line 18Q2523 (Fig. 6; Fig. S1). These markers exhibited 100% accuracy which can be used for marker-assisted selection. Overall, the analyses strongly indicated that the variations in

![Fig. 2](image-url) Fine mapping of the BoLI1 gene in ornamental kale. a Plot of the Δ(SNP-index) value obtained from the two bulks. The top line indicates the threshold line. The x-axis represents the position of nine chromosomes and the y-axis represents the Δ(SNP-index). b Linkage map of the BoLI1. The left panel is a genetic map of BoLI1 in the target region (units: cM). The right panel is the corresponding physical map of BoLI1 (units: Mb)
the promoter of BoLMIIa exist only in lobed-leaf ornamental kale inbred lines and may be responsible for the change in leaf shape from unlobed to lobed.

Discussion

In previous studies, the lobed-leaf trait was reported to be controlled by an incomplete gene or a QTL in ornamental kale [4, 16, 26]. In the present study, we analyzed the inheritance of leaf shape using F2 and BC populations derived from a cross of lobed-leaf ornamental kale with unlobed-leaf ornamental kale, showing that the lobed-leaf trait is controlled by a single dominant nuclear-encoded gene.

Ren et al. mapped the lobed-leaf gene BoLl to chromosome 9 of ornamental kale flanked by InDel markers LYIn39 and LYIn40, with genetic distances of 0.17 cM and 0.11 cM, respectively [4]. Two candidate genes, Bol010029/Bo9g181710 and Bol010030/Bo9g1181720, were revealed, but no sequence variations were found in their promoter and coding regions according to the B. oleracea '02–12' (cabbage) [17] and 'TO1000' (Chinese kale like) genomes [18]. Therefore, the authors did not conclude which gene controlled the formation of leaf shape in ornamental kale. In our study, based on the 'TO1000' genome, the BoLl-1 gene was finely mapped to a 127-kb (53680797–53,808,289 bp) interval on chromosome 9. SNP markers SL4 and SL6 were tightly linked to BoLl-1, flanking the gene at genetic distances of 0.6 cM and 0.6 cM, respectively. Sequence analysis of the parental alleles revealed no sequence variations in the coding sequence of Bo9g181720, whereas three variations were identified in the promoter region. In contrast, no sequence variations were detected in the promoter and coding regions of Bo9g181710. The expression level of Bo9g181710 in lobed-leaf 18Q2513 was significantly higher compared with unlobed-leaf 18Q2515, though the expression level of Bo9g181720 was similar between the parental lines. Thus, we further confirmed that the Bo9g181710 may control the formation of leaf shape in ornamental kale.

In B. napus, Hu et al. reported that a 2624-bp insertion (317 bp upstream of the transcription start site) and three SNPs were identified in the BnA10.LMI1 promoter sequence, along with 12 SNPs in the 3′ flanking sequence, which were considered to be the cause of the lobed-leaf formation [27]. In ornamental kale, the genes that determine leaf shape are not fully understood. Ren et al. mapped the BoLI gene and found no sequence variations in the promoter and coding regions

### Table 2 The 21 putative gene models in the target mapping region

| Gene ID     | Location       | Homologous gene in A. thaliana | Annotation                                                                 |
|-------------|----------------|-------------------------------|----------------------------------------------------------------------------|
| Bo9g181620  | C9: 53678143–53,680,993 | AT5G03900                    | iron-sulphur cluster biosynthesis family protein                         |
| Bo9g181630  | C9: 53681760–53,682,275 | AT5G03890                    | hypothetical protein                                                      |
| Bo9g181640  | C9: 53685355–53,687,538 | AT5G03880                    | thioredoxin family protein                                               |
| Bo9g181650  | C9: 53697594–53,698,572 | –                            | –                                                                          |
| Bo9g181660  | C9: 53698893–53,699,987 | AT5G03850                    | nucleic acid-binding, OB-fold-like protein                               |
| Bo9g181670  | C9: 53703655–53,704,734 | AT5G03840                    | protein TERMINAL FLOWER 1                                                |
| Bo9g181680  | C9: 53711576–53,712,658 | –                            | –                                                                          |
| Bo9g181690  | C9: 53713320–53,715,156 | AT5G03795                    | probable glycosyltransferase                                              |
| Bo9g181700  | C9: 53717227–53,718,573 | AT5G03795                    | probable glycosyltransferase                                              |
| Bo9g181710  | C9: 53720142–53,721,856 | AT5G03790                    | encodes a homeodomain leucine zipper class I (HD-Zip I) meristem identity regulator |
| Bo9g181720  | C9: 53749509–53,750,894 | AT5G03790                    | encodes a homeodomain leucine zipper class I (HD-Zip I) meristem identity regulator |
| Bo9g181730  | C9: 53755444–53,758,253 | AT5G03770                    | probable 3-deoxy-D-manno-octulosonic acid transferase                    |
| Bo9g181740  | C9: 53760949–53,762,178 | –                            | –                                                                          |
| Bo9g181750  | C9: 53765687–53,769,942 | AT5G03760                    | glucormannan 4-beta-mannosyltransferase 9                                 |
| Bo9g181760  | C9: 53771597–53,773,283 | AT5G03740                    | histone deacetylase 2C                                                    |
| Bo9g181770  | C9: 53777463–53,782,597 | AT5G03730                    | serine/threonine-protein kinase CTR1                                     |
| Bo9g181780  | C9: 53783895–53,785,735 | AT5G03720                    | heat shock transcription factor A3                                       |
| Bo9g181790  | C9: 53793205–53,794,037 | –                            | –                                                                          |
| Bo9g181800  | C9: 53801111–53,802,559 | AT5G03700                    | D-mannose binding lectin protein with Apple-like carbohydrate-binding domain |
| Bo9g181810  | C9: 53803210–53,804,612 | AT5G03690                    | fructose-bisphosphate aldolase 4                                        |
| Bo9g181820  | C9: 53807596–53,810,446 | AT5G03680                    | trihelix transcription factor PTL                                        |
of candidate [4]. In our study, three variations, including an SNP, a 1737-bp deletion, and a 92-bp insertion (765 bp, 1466 bp, and 1466 bp upstream of the transcription start site, respectively) were identified in the BoLMI1a promoter region compared with the ‘TO1000’ reference genome. Through verification analyses of BoLMI1a-specific markers corresponding to the promoter variations revealed that the variations existed only in lobed-leaf ornamental kale inbred lines. These variations may strongly enhance the transcription levels of BoLMI1, thus changing leaf shape from unlobed to lobed.

![Gene structure and protein alignment of BoLMI1a](image)

**Fig. 4** Gene structure and protein alignment of BoLMI1a. **a** The BoLMI1a gene structure as well as promoter variations between 18Q2513 and 18Q2515 are shown; horizontal blue arrows represent specific primers for amplifying the promoter and genomic sequences and detecting the promoter variations of BoLMI1a. **b** Sequence alignment of the BoLMI1a and BoLMI1b proteins and its seven homologues from other cruciferous species. The homeobox domain as well as the leucine zipper domain are indicated.
Leaf shape plays an important role in the reproduction and evolution of plants. Increasing evidence indicates that lobed leaves can improve photosynthesis efficiency and agronomic profitability [7, 28–31]. LMI1-like genes encoding an HD-Zip I transcription factor have been functionally identified in several plants, and they were reportedly involved in leaf shape formation [27, 32–35]. For example, Hu et al. [27] identified the BnA10.LMI1 gene, which was responsible for the lobed-leaf shape in \textit{Brassica napus}. In addition, the BnA10.LMI1 knockout mutations in the HY (with lobed leaves) background were sufficient to produce unlobed leaves. In this study, we identified an \textit{LMI1}-like gene, \textit{BoLMI1a}, which was the strong candidate gene underlying the lobed-leaf trait in ornamental kale. Thus, our findings further strengthen the potential for revealing the molecular mechanism underlying leaf shape formation, and we showed that \textit{BoLMI1a}-specific markers (CMLMI1 and DMLMI1) can...
be used for marker-assisted selection in ornamental kale breeding.

Conclusions
In this study, the lobed-leaf trait is shown to be controlled by a single dominant gene, BoLl-1, in ornamental kale. The BoLl-1 gene was fine-mapped to a 127-kb fragment. A homologue of Arabidopsis LMI1, BoLMI1a was identified as a strong candidate gene. Three variations were identified in the promoter region of BoLMI1a. The expression of BoLMI1a in lobed-leaf parent 18Q2513 was significantly up-regulated compared with unlobed-leaf parent 18Q2515. This study lays a foundation for cloning BoLMI1a and provides new insight into the formation of leaf shape in ornamental kale.

Methods
Plant materials
The 18Q2513 female parent (P₁) is an ornamental kale inbred line with lobed leaves; the 18Q2515 male parent (P₂) is an ornamental kale inbred line with unlobed leaves. 18Q2513 was crossed with 18Q2515 to generate an F₁ population. An F₂ population was generated from self-pollination of the F₁ plants; BC₁P₁ and BC₁P₂ were then generated by BCs of F₁ × 18Q2513, F₁ × 18Q2515, respectively.

Additionally, 118 different cabbage inbred lines (with unlobed leaves) and another ornamental kale inbred line 18Q2523 (with lobed leaves), were screened for BoLl-1 promoter variations. All of the plant materials used in the present study were grown in a 25°C ± 2°C greenhouse (16 h light/8 h dark photoperiod) at the seedling stage and then transplanted to the field after 1 month. Daily watering and fertilization were performed regularly until the plants enter the flowering stage (about 3 months of vernalization from December to February of the next year). All the plant materials are from the Institute of Vegetables and Flowers, Chinese Academy of Agriculture Sciences (IVFCAAS, Beijing, China).

Genetic analysis and whole-genome resequencing
Leaf shape was investigated visually. Segregation ratios for the F₂ and BC₁ populations were analyzed by chi-square (χ²) tests using SAS software.

Fifty lobed-leaf BC₁ and fifty unlobed-leaf BC₁ individuals were selected to construct two bulks. Genomic DNAs were isolated from the individuals within the two bulks and two parental lines using the Plant Genomic DNA Kit (Tiangen, Beijing, China), following the manufacturer’s instructions. The quality of the DNAs was ensured using spectrophotometric analysis and agarose gel electrophoresis. Equally high-quality genomic DNAs from the two bulks and two parental lines were then used to construct paired-end sequencing libraries, which were subsequently sequenced with an Illumina Hi-Seq 2500 sequencer by the Beijing Genomics Institute (BGI) (Shenzhen, China). SNP-index and sliding-window analyses were performed as previously described [36].

Marker development and fine mapping of the BoLl-1 gene
InDel and SNP markers were designed based on candidate region resequencing data for the two parents. Markers were designed with amplicon lengths of 100–180 bp, GC contents of 40–50% and Tm values of 52–58°C. The markers that were polymorphic between the parents were then used to analyze unlobed-leaf individuals in the BC₁P₁ populations.
Genomic DNA was extracted from 28-day-old seedling young leaves of the parents and BC1P1 individuals using a modified cetyltrimethylammonium bromide (CTAB) protocol [37]. The DNA concentration was subsequently determined using a spectrophotometer (BioDrop, UK) and adjusted to 40–50 ng/μL.

The 10-μL PCR reaction mixture consisted of 2 μL DNA template, 1 μL 10× PCR buffer (Mg2+ included), 0.8 μL dNTPs (2.5 mM each), 0.4 μL forward primer (10 μM), 0.4 μL reverse primer (10 μM), 0.2 μL Taq DNA polymerase (5 U/μL), and 5.2 μL ddH2O. The reactions were performed in accordance with the following: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 10 min. The amplification products were analyzed by electrophoresis (160 V for 1.2 h), and the gel was stained with silver nitrate.

For each marker, individuals consistent with the 18Q2513 (lobed-leaf) allele, the 18Q2515 (unlobed-leaf) allele, and the F1 allele were categorized as ‘a’, ‘b’, and ‘h’, respectively. Genetic distances between markers were calculated by the Kosambi map function [38], and a genetic map was constructed using MapDraw [39].

Candidate gene analysis
To identify the lobed-leaf gene BoLl-1, genes located within the candidate interval were analyzed based on annotations for the B. oleracea ‘TO1000’ reference genome (http://plantsensembl.org/Brassica_oleracea/Info/Index) [18]. The expression patterns of candidate genes Bo9g181710 and Bo9g181720 were investigated using quantitative real-time PCR (qRT-PCR). Total RNA was extracted from 28-day-old seedling young leaves of the parents using TRIzol reagent (Invitrogen, United States) according to the manufacturer’s protocol, and PrimeScript™ RT Reagent Kit (Takara, Japan) was used to reverse transcribe cDNA from the total RNA extracted. qRT-PCR was carried out using a CFX96 Real-Time System (Bio-Rad) with SYBR Premix Ex TaqII Reagent Kit (Takara, Japan). Three biological and three technical replicates were included for each experiment. The relative expression level of each gene was calculated using the 2-ΔΔCT method [40]. The qRT-PCR primers used are listed in Table S2, and B. oleracea actin was employed as a control.

Gene-specific markers GL10 (primers GL10-F and GL10-R) and GL20 (primers GL20-F and GL20-R) (Table S2) were used to amplify the promoter and genomic sequences of Bo9g181710 and Bo9g181720, respectively. The resulting PCR products were analyzed by electrophoresis on 1% agarose gels, followed by sequencing and alignment. The co-dominant marker CMLMI1 (primers CMLMI1-F1, CMLMI1-F2 and CMLMI1-R) and the derived cleaved amplified polymorphic sequence (dCAPS) marker DMLMI1 (primers DMLMI1-F and DMLMI1-R) (Table S2) were used to detect variations in the promoter of BoLMI1a in 118 different cabbage and ornamental kale inbred lines.

BLASTP searches were conducted using the amino acid sequence of BoLMI1a to search for homologues within the protein database of the National Center for Biotechnology Information (NCBI) and the B. oleracea reference genome ‘TO1000’. Protein sequence alignment was performed with MAFFT (v7.037) [41]. FastTree (LG+JTT model) was used to construct phylogenetic trees [42].
B. oleracea reference genome 'TO1000' used in this study can be found at the link: http://plants.ensembl.org/Brassica_oleracea/Info/Index. The A. thaliana genome can be found at the link: https://www.arabidopsis.org/. The protein reference genome 'TO1000' used in this study can be found at the link: http://plants.ensembl.org/Brassica_oleracea/Info/Index. The genome can be found at the link: https://www.arabidopsis.org/. All these databases are open to public access.

Declarations

Ethics approval and consent to participate

All the plant materials are from the Institute of Vegetables and Flowers, Chinese Academy of Agriculture Sciences (IVCAAS, Beijing, China). The utilization of these plant materials in this study complies with the guidelines and legislation of China.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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