Hortic. Environ. Biotechnol. 56(6):821-829. 2015.
DOI 10.1007/s13580-015-0066-5

ISSN (print) : 2211-3452
ISSN (online) : 2211-3460

Research Report

Cloning and Characterization of the *LFY* Homologue from Chinese Cabbage (*Brassica rapa* subsp. *pekinensis*)

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Received May 16, 2015 / Revised October 1, 2015 / Accepted November 24, 2015

Abstract. Flowering is critical to the growth and development of plants, and *LFY* gene homologues play a major role in flowering initiation. To understand the genetic and molecular mechanisms underlying floral initiation and development in *Brassica rapa* subsp. *pekinensis*, *BrpLFY*, a homologue of *LFY*, was cloned using RT-PCR. Sequence analysis showed that the cDNA sequence of *BrpLFY* is 1,341 bp in length, with an ORF of 1,245 bp encoding a predicted protein of 415 amino acids. The predicted protein showed a high degree of identity with LFY homologues from other angiosperm species. Real-time PCR analysis showed that *BrpLFY* mRNA was detected in all tissues during plant development from the vegetative state to fully differentiated flowers, and its expression was highest in the cotyledon and lowest in the root. *BrpLFY* expression in the shoot apex increased gradually during vegetative growth and increased dramatically at stage 1 of flower bud differentiation. The relative expression peaked at stage 5 and then decreased in later stages. Moreover, the trend in *BrpLFY* expression level change in the shoot apex was similar regardless of variety or vernalization method. The relative expression of *BrpLFY* in leaves gradually decreased with leaf development. We overexpressed the gene in *Arabidopsis thaliana* using the floral dip method, and examined flowering time in wild-type and transgenic plants. Overexpression of *BrpLFY* specifically caused early flowering; the transgenic plants flowered 10-14 d earlier than did wild-type plants, and leaf number decreased by 0.5-1 when the plants bolted. Real-time PCR analysis showed that the expression of *BrpLFY* in transgenic Arabidopsis was higher than in wild-type plants. These results indicate that *BrpLFY* plays a role in promoting flowering in Chinese cabbage.

***Additional key words*:** *BrpLFY*, expression, floral promoter, flowering time, function

Introduction

Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) is cultivated in many countries around the world, especially in China, Japan, Korea and other Asian countries, where it is an important vegetable crop in autumn and winter with a large planted area and high yield. Chinese cabbage is a typical crop that requires vernalization. After exposed a period of low temperature, the germinated seed can complete vernalization, and plants will then flower under conditions of high temperature and long day length. However, different varieties have different cold sensitivities. In recent years, because the spring varieties have been widely cultivated, premature bolting of weaker winterness varieties becomes a limiting factor that can affect yield. On the other hand, there can be a problem producing seeds in the strong winterness varieties. Therefore, research on the mechanisms about bolting and flowering in Chinese cabbage will have important scientific and practical value.

The regulation of flowering in plants is a complicated process that is controlled by a complex signaling network. Previous researches have shown that the meristem is regulated by floral meristem identity genes and differentiates to form an inflorescence during the transition stage from vegetative to reproductive development in the model plant Arabidopsis (Weigel and Meyerowitz, 1994). As a necessary gene for determining floral meristem formation, *LFY* is present in both flowering and non-flowering plants (Frolich and Parker, 2000; Gocal et al., 2001). *LFY* is the earliest floral meristem identity gene expressed during the flowering transition, and the LFY protein regulates downstream target genes such as *APETALA1* (*AP1*) (Wagner et al., 1999, 2004), *APETALA3* (*AP3*) (Lamb

et al., 2002), and *AGAMOUS* (*AG*) (Busch et al., 1999) to initiate flowering (Mandelv and Yanofsky, 1995). In addition, the *LFY* gene can maintain the normal function of the floral meristem and prevent its reversion to a vegetative shoot (Shannons et al., 1993; Mizukami and Ma, 1997).

In Arabidopsis, *LFY* expression is weak in the leaf primordium during the vegetative stage and increases gradually before flowering commences (Blázquez et al., 1997). Subsequently, with flower differentiation, expression of *LFY* mRNA in the floral primordia increases and is distributed throughout the entire floral primordium. Shortly after the sepals have started to form, *LFY* expression abates in the center of the incipient flower (Weigel, 1992). In recent years, *LFY* homologues from species such as broccoli (Anthony et al., 1993), rice (Kyozuka et al., 1998), potato (Guo and Yang, 2008), walnut (He et al., 2011), and chrysanthemum (Ma et al., 2014) have been cloned successfully. Studies of *LFY* homologues have shown that the expression patterns can differ, although the structure and function of these genes are highly conserved (Lü et al., 2011; Sayou et al., 2014). The *LFY* homologue from lotus, *NnLFY*, is ubiquitously expressed in the root, stem, leaf and flower in the different periods and the highest expression is detected in the bud period (Liu et al., 2014). The *NLF* from narcissus is expressed in the developing anther and leaf primordium, but not in mature leaves (Porat et al., 2010). This is similar to the *FaLFY* gene from strawberry, which is expressed most strongly in flower buds, but no transcription was detected in vegetative tissues and the mature flower with the exception of a low level in the stolon bud (Liu et al., 2012). In addition, for *Platanus acerifolia* Willd., a diclinous plant, *PlacLFY* was expressed mainly in male inflorescences from May of the first year to March of the next year, with the highest expression level in December, and in female inflorescences from June to April of the second year (Lu et al., 2012).

Although *LFY* homologues have been extensively studied in many plants, little is known about the function and expression pattern of *LFY*-like genes in Chinese cabbage. Differences exist in the expression patterns of *LFY* homologues in different plants. In order to establish a foundation for further study on the mechanism of flowering and vernalization in Chinese cabbage, the *LFY* homologue, *BrpLFY* from Chinese cabbage was cloned and the expression pattern of *BrpLFY* was analyzed by real-time PCR. Furthermore, the role of *BrpLFY* in flowering was investigated by over-expressing the gene in Arabidopsis.

Materials and Methods

Plant Materials

The Chinese cabbage '7#', an inbred line that bolts easily, was used in the spring flowering experiment; '9#', an inbred line that bolts hardly, and '3#', an inbred line with similar characteristics to '7#', were used in the winter storage experiment. These inbredlines were obtained from the Institute of Vegetable Research, Shanxi Academy of Agricultural Sciences.

For the spring flowering experiment, the seeds of '7#' were soaked in water at 45°C for 10 min, then at room temperature for 2 h. The seeds were placed on a double layer of wet filter paper in a Petri dish and put in a growth chamber at 25°C with 16 h light/8 h dark to germinate on June 24, 2014. Two days later, when the cotyledons had expanded, the petri dishes were transferred to 4°C for 30 d. During this time, the filter papers were kept wet enough for the seedlings to grow (Fu, 2014). Following the low temperature treatment, the seedlings were transplanted into a plate with 50 holes on July 26, and managed conventionally until they were sampled at specific stage.

For the winter storage experiment, seeds of lines '3#' and '9#' were treated in the same manner on August 6th, 2014. When the cotyledons had expanded, the seedlings were transplanted into a plate with 50 holes. Then they were transplanted into the field and managed conventionally until the five-leaf stage. The Chinese cabbage were harvested with roots and placed into a cellar for storage on December 1st. The plants of '3#' and '9#' were then transplanted into pots for flowering on January 13 and 29, respectively. During the storage and growth periods, flower bud differentiation states were observed and the shoot apex at specific stage such as rosette stage, heading stage and during storage was sampled.

RNA Isolation and cDNA Synthesis

Total RNA was extracted from the apex of Chinese cabbage '7#' using the RNeasy Plant Mini Kit (QIAGEN, 74903) following the protocol from the instruction. The quality and quantity of each RNA sample was determined by 1% (w/v) agarose gel electrophoresis, and the absorbance was measured at 260 nm and 280 nm. The first strand of cDNA was synthesized using SuperScript® Ⅲ First-Strand Synthesis System for RT-PCR (Invitrogen, 18080-051) according to the manufacturer's instructions.

For isolation of the *LFY* homologue, PCR primers were designed based on the complete sequence of the Chinese cabbage clone KBrH005L20, which was retrieved from GenBank (http://www.ncbi.nlm.nih.gov; accession umber AC 232542.1). Forward and reverse primer sequences were LFY-F_3 5'-AGAAAGAGAATATGGATCCTGAAGG-3' and LFY-R_1 5'-CCAGCCAAAACGAGTAAAAACTAGAGCC-3', respectively. Amplification by touch-down PCR was performed under the following conditions: 94°C for 5 min, followed by 10 cycles of 92°C for 15 s, 60°C → 51°C for 20 s, and 68°C

for 2 min, and then 35 cycles of 92°C for 15 s, 55°C for 20 s, and 68°C for 2 min, with a final extension at 72°C for 5 min. The PCR products were analyzed on a 1% (w/v) agarose gel and purified using the QIAquick Gel Extraction Kit (Qiagen, Houston, Germany). The purified DNA fragments were then cloned into pCR®8/GW/TOPO® (Gateway, Invitrogen, Carlsbad, USA) for sequencing.

Sequence Alignment and Phylogenetic Analysis

Sequence analysis was performed with DNAMAN. The amino acid sequence of *BrpLFY* was aligned with those from various species in the GenBank database using BLAST. Phylogenetic trees were constructed by MEGA5.1 software using the nearest neighbor-joining method (1,000 replicates for bootstrap values) and Jones-Taylor-Thornton (JTT) with Freqs. (+F) substitution matrix model. The complete amino acid sequences of the different LFY homologues were retrieved from GenBank.

Real-time PCR Analysis

To study the expression pattern of *BrpLFY* during floral development, the expression levels in the shoot tips at different developmental stages of 3 varieties were assessed using real-time PCR. Expression levels were also examined in cotyledons, young leaves, mature leaves, flower buds, open flowers and roots of '7#'. In addition, it was also determined in young leaves of transgenic and wild type Arabidopsis. Total RNA used for real-time PCR was extracted from 0.1 g tissue samples, which were collected from six plants. The stage of flower differentiation was determined according to the methods of Li (1964) and Smyth (1990). For reverse transcription, the concentration of each RNA sample was adjusted to 500 ng/μL. Single-stranded cDNA was synthesized with the PrimeScript® RT reagent kit (Perfect Real Time; TaKaRa, DRR037A) as directed by the manufacturer.

Specific primers used for real-time PCR were designed with Primer 3 software based on the *BrpLFY* sequence. The forward primer was 5'-GGCAGGCAAAGATGAAGAAG-3' and the reverse primer was 5'-CCACGGTCTTTAGCAATG GT-3'. The Chinese cabbage *ACTIN* gene was used as internal reference gene, and the primers were 5'-GTTGCTATCCAG GCTGTTCT-3' and 5'-AGCGTGAGGAAGAGCATAAC-3' (Sun et al. 2009). The predicted lengths of the *BrpLFY* and *ACTIN* gene fragments were 286 bp and 118 bp, respectively. For Arabidopsis *AtUBQ10* was used as internal reference gene, and the primers were 5'-CGTTGACTGGGAAAACTA TCACT-3' and 5'-GTCCTGGATCTTGGCTTTCA-3' (Yi et al. 2014). Real-time PCR was carried out using SYBR® *Premix Ex Taq* ™ Ⅱ (Tli RNaseH Plus; TaKaRa, RR820A) in a 25-μl reaction volume which contained 20 ng of cDNA. The amplification program was as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 55°C for 30 s and 72°C for 30 s. Real-time PCR analysis was performed in an ABI 7500 apparatus and the relative expression levels were calculated using the ΔΔCt method. Each sample consisted of two biological replicates and two technical replicates.

Flowering Phenotype Analysis

The *BrpLFY* cDNA was inserted into the plant expression vector pK2GW7, and the resulting plasmid (pK2GW7-BrpLFY) was introduced into *A. tumefaciens* GV3101, which was then used to transform Arabidopsis via the floral dip method (Clough et al. 1998). T_1 generation plants were screened out on 0.5X MS medium supplemented with 50 mg·L^{-1} kanamycin (Kan). The transgenic plants were confirmed by PCR analysis and homogenized so as to get the homozygous T_2 transgenic generation. Seeds of the T_2 generation and wild type Arabidopsis were sown at the same time, and seedlings were transplanted and grown under the same conditions. The flowering time of both transgenic and wild type Arabidopsis were investigated.

Results

Gene Cloning and Sequence Analysis

First-strand cDNA from Chinese cabbage mRNA was amplified by touch-down PCR with the gene specific primers LFY-F_3 and LFY-R_1. Sequence analysis showed that the cDNA sequence of the *LFY* homologue was 1,341 bp in length, and contained an ORF of 1,245 bp. The ORF encoded a predicted protein of 415 amino acids that had a calculated molecular weight of 45.88 kDa and a theoretical pI of 6.93. Alignment by DNAMAN software showed a high degree of similarity with other known *LFY* homologues (Fig. 1). The amino acid sequence similarity was 89% with LFY from *Arabidopsis thaliana* and 100% with BrLFY from *B. rapa*. These results strongly suggest that the gene from Chinese cabbage is a *LFY* homologue, and we named it *BrpLFY* (GenBank accession number: KR190435). Based on the amino acid sequences of *BrpLFY* and 15 other predicted LFY-like proteins, a phylogenetic tree was constructed using MEGA5.1 software. The result revealed that BrpLFY clustered with proteins from the other three crucifer species. Moreover, BrpLFY and BrLFY are the closest, possibly indicating that they maybe have similar or equivalent functions (Fig. 2).

Spatio-temporal Expression Pattern of *BrpLFY*

To clarify the spatio-temporal expression pattern of *BrpLFY* in Chinese cabbage, real-time PCR was carried out with cDNA from different tissues. The results showed that *BrpLFY* was detected in all tissues throughout its development of the plant from the vegetative state to fully differentiated flowers.

In the shoot apex of '7#', an initial low level of *BrpLFY*

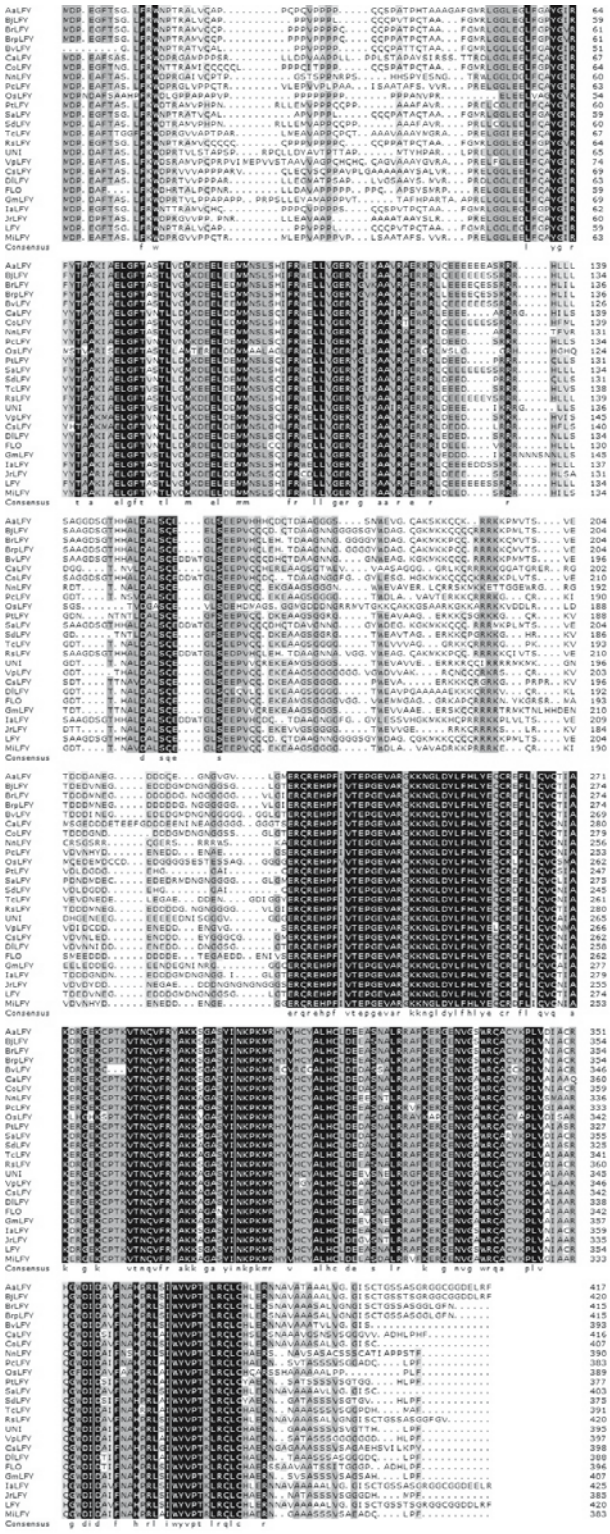

Fig. 1. Multiple amino acid sequence alignment of predicted LFY-like proteins from 25 plant species. Species name and GenBank accession numbers for the sequences are as follows (in parentheses): AaLFY (*Arabis alpina*, KFK27938), BjLFY (*B. juncea*, ABF06559), BrLFY (*B. rapa*, NP_001288996), BrpLFY (*B. rapa* subsp. *pekinensis*, KR190435), BvLFY (*Barbarea vulgaris*, AAW62308), CaLFY (*C. annuum*, ABS18396), CoLFY (*Cochlearia officinalis*, AAW65157), NnLFY (*Nelumbo nucifera*, AGG18259), PcLFY (*Pistacia chinensis*, AGF33326), OsLFY (*Oryza sativa* Japonica Group, AHX83809), PtLFY (*Populus trichocarpa*, XP_002322307), SaLFY (*Selenia aurea*, AAW70552), SdLFY (*Salix discolor*, AAO73539), TcLFY (*Theobroma cacao*, XP_007038161), RsLFY (*R. sativus*, BAH04509), UNI (*P. sativum*, AAC49782), VpLFY (*Viola pubescens*, ABU54075), CsLFY (*Citrus sinensis*, AAR01229), DlLFY (*Dimocarpus longan*, ABP02007), FLO (*Antirrhinum majus* subsp. *majus*, AAA62574), GmLLFY (*Glycine max*, ABE02270), IaLFY (*Ionopsidium acaule*, AAO73066), JrLFY (*Juglans regia*, ADL61865), LFY (*Arabidopsis thaliana*, NP_200993), MiLFY (*Mangifera indica*, ADX97318).

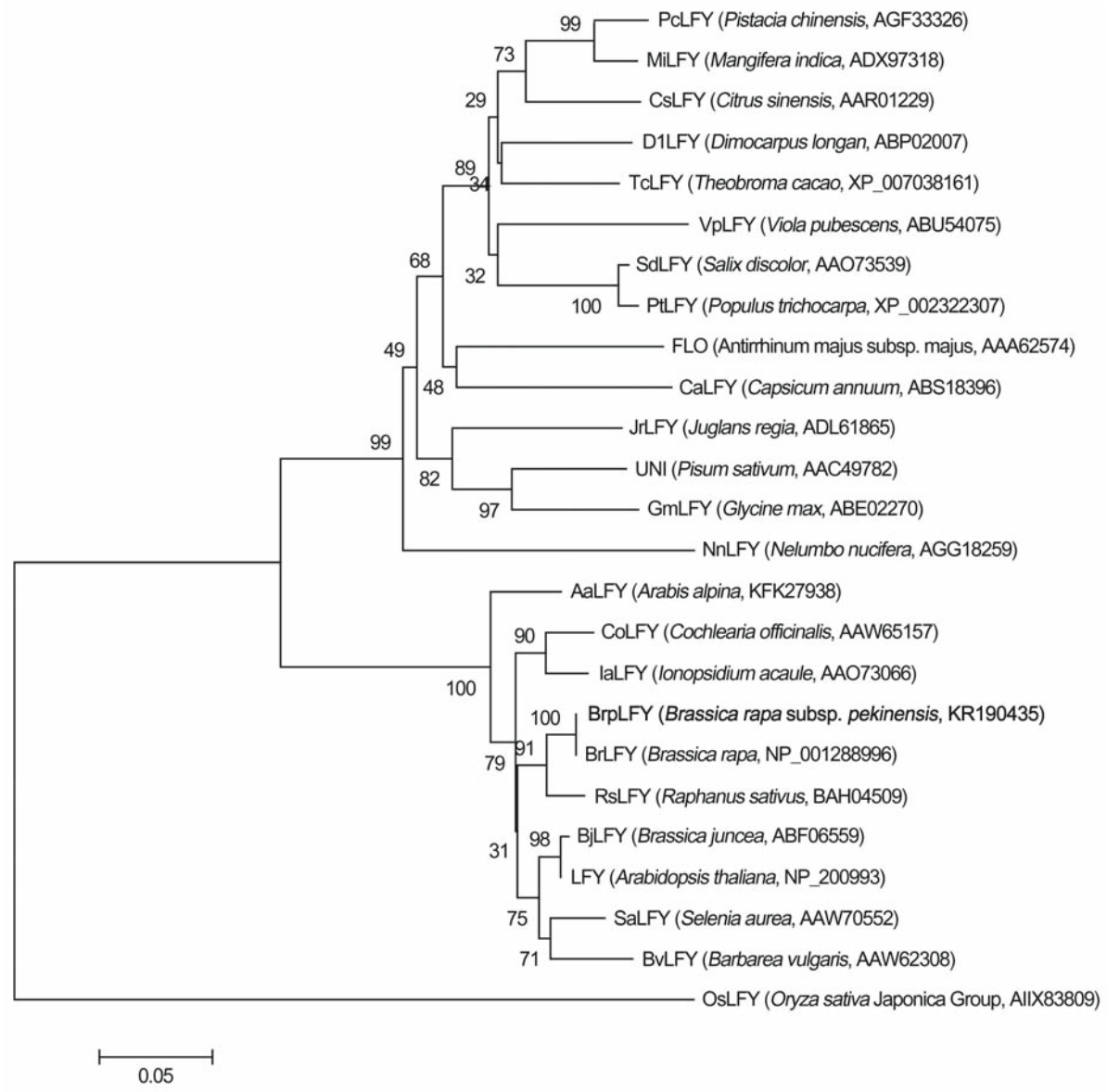

Fig. 2. Phylogenetic analysis of the amino acid sequences of BrpLFY with 24 other FLO/LFY homologues. The scale bar (0.05) indicates a 5% change in amino acids. Numbers on each branch are bootstrap values.

expression that gradually increased was detected at the vegetative stage. Expression increased dramatically in the flower bud at differentiation stage 1, and increased rapidly during the later stages of flower bud differentiation. The relative level expression reached a peak at the stage 5 and then decreased in the inflorescence (Fig. 3A). In the winter storage experiment, *BrpLFY* expression in varieties '3#' and '9#' showed a similar trend with that of variety '7#', gradually increasing with the development of vegetative growth. Expression reached a peak around the flower bud differentiation stage and then decreased in both varieties (Fig. 3B). As '3#' had completed the floral differentiation before storage, while '9#' had not finished by the time they were transplanted into the pots, the peak of *BrpLFY* expression level in variety '3#' appeared earlier than in '9#', but the expression levels in '3#' were lower than in '9#'. These results indicated that the trend of *BrpLFY* expression levelchange was similar and not affected by either the vernalization method or the variety of plant studied. However, the expression levels in the variety that needed stricter vernalization was higher than those in the others.

In the leaves of '7#', *BrpLFY* expression at different stages showed that it was highest in cotyledons, and decreased with the leaf development. At the flower bud stage, the relative level of expression in mature leaves was only ~10% of that in cotyledons (Fig. 3C). These results indicate that *BrpLFY* was more active in younger tissues.

The *BrpLFY* gene expression levels were analyzed in

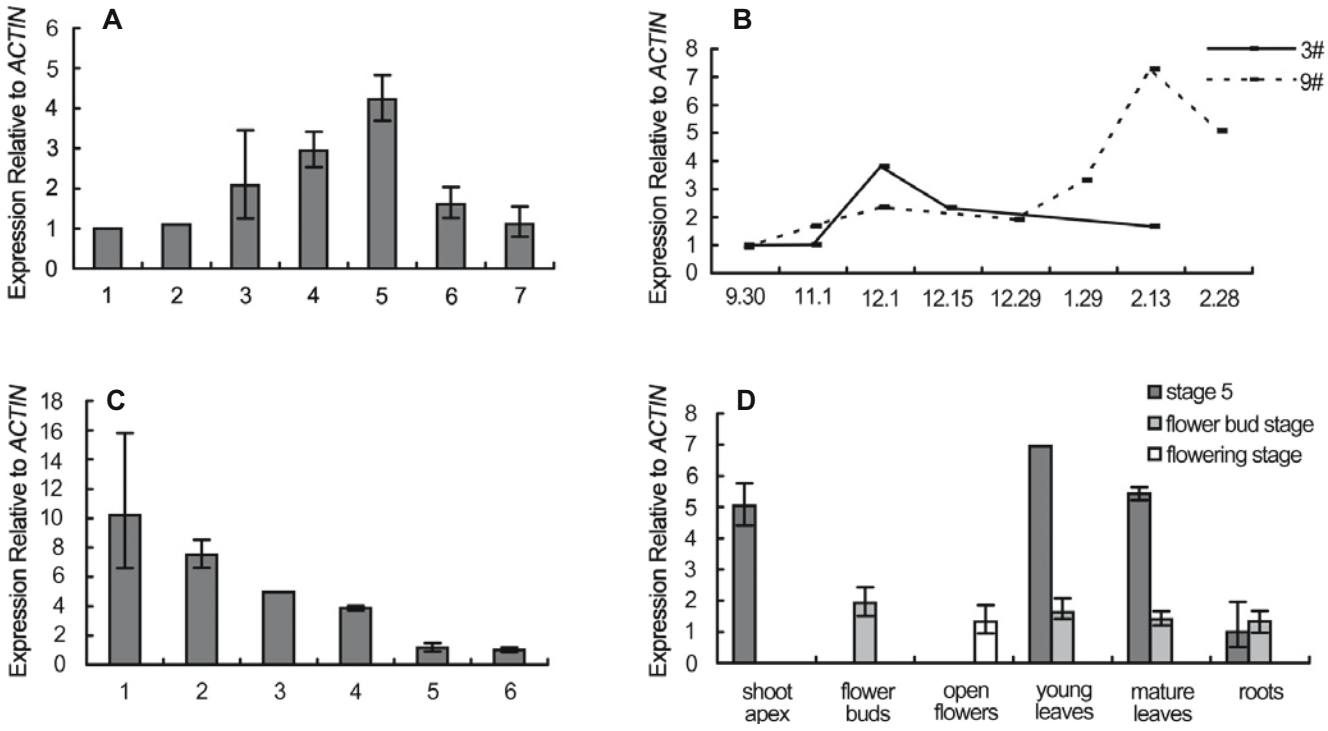

Fig. 3. Spatio-temporal expression patterns of *BrpLFY*. Columns with error bars indicate RQ (Relative Quantification), RQmax, RQmin respectively calculated by ABI 7500 apparatus. A, Expression of *BrpLFY* in the shoot apex of '7#' at different developmental stages (1: 10 d after transplanting; 2: 15 d after transplanting; 3-5: Flower bud differentiation stages 1, 3, and 5; 6: flower buds; 7: flowers). B, Expression of *BrpLFY* in the shoot apex of '3#' and '9#' during winter storage. Numbers on the x-axis are the date sampled. C, Expression of *BrpLFY* in leaves of '7#' at different stages (1: cotyledons at 4°C for 15 d; 2: cotyledons at 4°C for 30 d; 3: young leaves at stage 5; 4: mature leaves at stage 5; 5: young leaves at the flower bud stage; 6: mature leaves at the flower bud stage). D, Expression of *BrpLFY* in different tissues of '7#' during three different stages of flower development.

variety '7#' in the apex, flower buds, open flowers, young and mature leaves and roots during stage 5 of flower bud development, bolting and blossom stage. At stage 5, the expression levels differed greatly between the four tissue types. Expression was highest in the young leaves, somewhat less in the mature leaves and shoot apex, and lowest in the roots. However, when plants underwent bolting, expression levels in the young and mature leaves, roots and flower buds were all lower than that of at the stage 5, with no significant difference between them. The expression level in the open flowers was lower than that in the flower buds (Fig. 3D).

Overexpression of *BrpLFY* in Arabidopsis

A total of 4 transgenic Arabidopsis plants were screened out on 0.5X MS solid medium supplemented with 50 mg·L^{-1} Kan, and were confirmed by PCR for the *Npt*II gene. Seeds of 4 transgenic plants were harvested individually and sown and the seedlings were transplanted at the same time with wild type Arabidopsis. 10 plants were selected randomly from each of the four T_2 lines so as to compare the flowering time with that of wild type. The results showed that overexpression of *BrpLFY* in Arabidopsis resulted in early flowering; the transgenic plants flowered 10-14 d earlier than did the wild type, and the leaf number decreased by 0.5-1 at bolting (Table 1 and Fig. 4A). Real-time PCR analysis showed that the expression levels of *BrpLFY* in young leaves of transgenic Arabidopsis was significantly higher than those in wild type plants (Fig. 4B). These results indicated that *BrpLFY* can promote flowering of the plant.

Discussion

As a key integrator of many floral inductive pathways, the *LFY* gene is crucial for flower formation (Nilsson O et al., 1998). The *LFY* homologous gene *BrpLFY* was cloned from Chinese cabbage. The *BrpLFY* cDNA was found to be 1,341 bp in length with an ORF of 1,245 bp encoding a predicted protein of 415 amino acids. Phylogenetic analysis showed that BrpLFY has a very close evolutionary relationship with BrLFY (from *B. rapa*) and RsLFY (from *Raphanus sativus*), but a more distant relationship to proteins from *Capsicum annuum*, *Citrus sinensis*, and *Pisum sativum*. This result indicated that the structure of *LFY* homologous genes is highly conserved, and that the homology of these genes reflects the phylogenetic relationship, which is consistent with the other plants (An et al., 2012; Wang, 2012; Zhang et al., 2013; Ma

Table 1. Comparison of flowering parameters in transgenic and wild-type Arabidopsis plants

T_1 line	Days to flowering after sowing of T_2	Number of days flowering earlier than the control	Leaf number when bolting	Number of leaves fewer than the control at flowering
1	34.09 ± 4.05bcB	11.04	7.27 ± 0.88bAB	0.86
2	34.88 ± 3.36bB	10.25	7.63 ± 0.96abAB	0.50
3	35.32 ± 4.99bB	9.81	7.40 ± 0.82abAB	0.73
4	30.50 ± 4.51cB	14.63	7.00 ± 0.82bB	1.13
CK	45.13 ± 5.36aA		8.13 ± 0.74aA	

The above data show the average ± SD; the significant differences were tested by Duncan's test, the upper- and lowercase letters represent significant different at $p < 0.01$ and $p < 0.05$ level respectively.

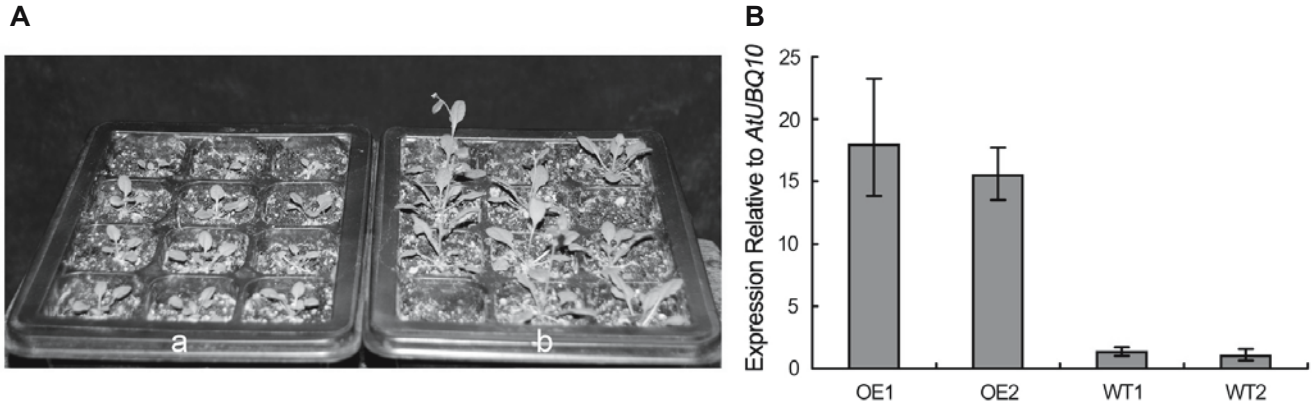

Fig. 4. Overexpression of *BrpLFY* in Arabidopsis results in early flowering. A, Early flowering phenotype of T_2 transgenic Arabidopsis plants overexpressing (OE) *BrpLFY*. a: Wild-type Arabidopsis, b: Transgenic plants; B, Expression levels of *BrpLFY* in the leaf of transgenic and wild-type Arabidopsis. OE1 and OE2: Transgenic plants overexpressing *BrpLFY*; WT1 and WT2: Wild-type Arabidopsis. Expression levels were determined by real-time PCR relative to *AtUBQ10*. Columns with error bars indicate RQ, RQmax, RQmin respectively calculated by ABI 7500 apparatus.

et al., 2014).

LFY plays two key roles in flowering in Arabidopsis: firstly, *LFY* acts as a pathway integrator, regulating the transition to flowering when its expression level reaches a certain threshold in newly formed leaves; secondly, *LFY* affects the specific shape of the floral organ by regulating the expression of the ABC genes after the floral meristem is formed (Jack, 2004; Hamès et al., 2008). The expression of *BrpLFY* in several tissues assayed during different developmental stages, which were analyzed by real-time PCR, showed that *BrpLFY* expressed throughout the vegetative and reproductive stages, similar to the *LFY* homologues from lotus (Liu et al., 2014), tomato (Molinero-Rosales et al., 1999) and alfalfa (Zhang et al., 2013). The expression of *BrpLFY* in the shoot apex increased gradually with the development, but increased dramatically at the beginning of floral bud differentiation, and then increased rapidly in the subsequent differentiation stages until reaching a peak at stage 5. After stage 5, expression in the floral meristem began to decrease and was low in buds and flowers. The *BrpLFY* expression pattern during winter storage in varieties '$3^{\#}$' and '$9^{\#}$' were similar to the plants in spring flowering experiment, which indicated that the change was not related to the varieties or vernalization method. These results suggested that *BrpLFY* induces differentiation when the expression level reaches a certain threshold during the vegetative stage (Coen and Meyerowitz, 1991; Lohmann and Weigel, 2002). The changing trend of *BrpLFY* expression in the shoot apex was in agreement with *LFY* expression in Arabidopsis (Weigel, 1992; Blázquez et al., 1997).

The expression analysis of *BrpLFY* in different tissues during the same stage of development indicated that, like *BjLFY* in *B. juncea* (Roy et al., 2011), the expression of *BrpLFY* in young and mature leaves at stage 5 was significantly higher than in the other tissues. However, during the flower bud stage, this difference was not so significant due to the decrease in expression in leaves. Previous reports have suggested that *LFY* homologues are multifunctional genes, and that they could be involved in the developmental processes of many organs (Neta et al., 2011). For example, the expression of *LFY* homologues was detected in the seeds of radish (Oshima and Nomura, 2008) and soybean (Meng et al., 2007), which could possibly indicate involvement in the regulation of seed development. In the study of Han (2011), the *LFY* homologue gene from Chinese cabbage was expressed

most strongly in young leaves at the shoot apex, with the second highest level detected in proximal leaves and distal leaves. Similarly, in our study the expression of *BrpLFY* was found to be highest in cotyledons, with young leaves showing the next highest level. Also, expression decreased gradually as the leaves matured, which suggests that *BrpLFY* may function in the growth and maturation of leaves.

Transformation of a gloxinia (*Sinningia speciosa*) cultivar with the *CFL*, *LFY* homologous gene from cucumber resulted in bushy growth habits as well as fewer branches, and 71% of the transgenic plants flowered 26-32 d earlier than did the wild type plants (Zhang et al., 2008). Transgenic Arabidopsis plants overexpressing *CmLFY* from chestnut (*Castanea mollissima*) showed accelerated flowering and reduced numbers of rosette leaves (Liu, 2011). This was similar to the situation in transgenic Arabidopsis expressing the *MsLFY* gene from alfalfa (Zhang et al., 2013). In the present work, transgenic Arabidopsis overexpressing *BrpLFY* also showed early bolting and flowering. Transgenic plants of the T_2 lines flowered 10-14 d earlier than the wild type plants, and leaf numbers decreased by 0.5-1 at the time of bolting. Early flowering caused premature cessation of vegetative growth, which resulted in a reduction in the leaf number. High level *BrpLFY* transcripts was detected in transgenic plant, but the expression was also detected in the wild type plants because of high similarity of *LFY* from Arabidopsis and *BrpLFY* from Chinese cabbage in sequences. Our results showed that constitutive expression of *BrpLFY* can promote early flowering in transgenic plants, and strongly indicate that it functions as a floral integrator in Chinese cabbage.

Acknowledgment: This research was financially supported by funding from the Agricultural Science and Technology Research Plan Project of Shanxi Province (No.20140311009-3; 20140311011-4), the Research Project Supported by Shanxi Scholarship Council of China (No. 2013-062) and the College and University Specific Scientific Research Fund (No.2013 1403110006).

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