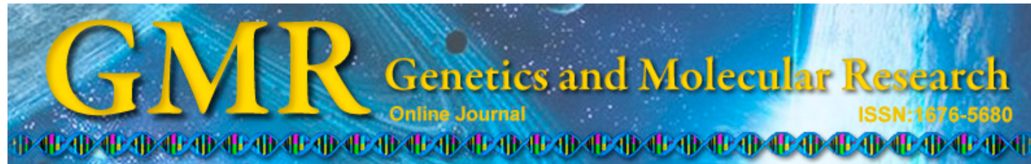

Ectopic expression of the *BoTFL1-like* gene of *Bambusa oldhamii* delays blossoming in *Arabidopsis thaliana* and rescues the *tfl1* mutant phenotype

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ABSTRACT. *TERMINAL FLOWER1* (*TFL1*) homologous genes play major roles in maintaining vegetative growth and inflorescence meristem characteristics in various plant species; however, to date, the function of the bamboo *TFL1* homologous gene has not been described. In this study, a *TFL1* homologous gene was isolated from *Bambusa oldhamii* and designated as *BoTFL1-like*. Phylogenetic analysis of *TFL1* homologous genes revealed that *BoTFL1-like* shared more than 90% identity with the *TFL1* genes of other Gramineae. RT-PCR analysis showed that the expression level of *BoTFL1-like* in floral buds was almost 3.5 times higher than in vegetative buds. In *35S::BoTFL1-like* transgenic *Arabidopsis thaliana* plants, the time of flowering was significantly delayed by 5 to 9 days, and development of floral buds

and sepals was severely affected compared to wild type *Arabidopsis* plants. This suggests that the *BoTFL1-like* gene may play roles in flowering time and flower morphological structure in *B. oldhamii*. The *BoTFL1-like* gene driven by the 35S promoter almost fully rescued the phenotype of the *tfl1* mutant apart from the number of rosette inflorescences, indicating that the function of *BoTFL1-like* was similar to *TFL1* in *Arabidopsis*. We conclude the *TFL1* gene function has been conserved between *B. oldhamii* and *A. thaliana*.

Key words: *Bambusa oldhamii*; Gene cloning; Sequence analysis; *TERMINAL FLOWER1*; Transgenic *Arabidopsis thaliana*

INTRODUCTION

The transition from vegetative to reproductive growth is a crucial developmental switch in flowering plants. Many genetic and molecular components underlying floral transition have been characterized in *Arabidopsis thaliana* (Hayama and Coupland, 2004). The *TFL1* (*TERMINAL FLOWER1*) gene, which has an important role in flowering transition, controls inflorescence meristem identity through inhibition of the expression of flower meristem identity genes (Ohshima et al., 1997; Abdullah et al., 2009; Xi et al., 2010; Tahery et al., 2011; Wang et al., 2011).

TFL1 is a member of the *FT/TFL1* gene family in *Arabidopsis* that includes *FLOWERING LOCUS T* (*FT*) and four related genes, namely, *TWIN SISTER OF FT*, *BROTHER OF FT AND TFL1*, *ARABIDOPSIS THALIANA CENTRORADIALIS HOMOLOGUE*, and *MOTHER OF FT AND TFL1* (Kobayashi et al., 1999). The *FT/TFL1* gene family encodes proteins with similarity to phosphatidyl ethanolamine binding proteins (PEBPs); however, the genes vary in their roles in the regulation of flowering transition and act as either promoters or repressors depending on the key nucleotide of the protein function domain (Banfield et al., 1998; Hengst et al., 2001; Kikuchi et al., 2009).

TFL1 has been proposed to be antagonistic with *LFY* (*LEAFY*), *AP1* (*APETALA1*), and other floral meristem genes, and this antagonism is manifested in their complementary expression patterns and phenotypic effects (Bowman et al., 1993; Parcy et al., 2002). The phenotypes produced by a reduction in *TFL1* activity are similar to the effects of the constitutive expression of *LFY* and *AP1* (Ratcliffe et al., 1999). The expression of the floral meristem identity genes can be inhibited by the shoot meristem identity gene *TFL1*, which encodes a protein that is likely involved in signal transduction, thereby preventing flowering (Shannon and Meeks-Wagner, 1991; Schultz and Haughn, 1993). By contrast, the activity of *TFL1* can be suppressed by *LFY*, and *AP1*. *TFL1* expression is not detected in transgenic *Arabidopsis* carrying either a *35S::LFY* construct or a *35S::AP1* construct (Liljegren et al., 1999; Ratcliffe et al., 1999).

Bambusa oldhamii belongs to the bamboo family, the largest member of the Poaceae. It is well-known for its edible shoots and green timber, and is one of the most economically important forest resources in tropical and subtropical countries (Hsu et al., 2000; Lin et al., 2009). The majority of bamboos do not flower until they are approximately 30- to 60-years-old. Bamboo flowering is unpredictable, gregarious, and the plants usually die after flowering. This not only reduces local income, but also causes ecological and environmental problems (Daniel and Janzen, 1976; Lin et al., 2009). Therefore, there are compelling reasons to study

the genetics of the flowering mechanism in bamboos. A number of factors may influence flowering in bamboo such as environment, nutrition, climate, and physiological status, and several hypotheses have been proposed to explain the pattern of flowering (Daniel and Janzen, 1976; Gielis et al., 1999; Franklin, 2004). In recent years, there has been increasing focus on identifying and characterizing the genes that regulate flowering in bamboo species. Thus, *DIMADS1-18* full-length cDNA sequences were isolated from the panicles of *Dendrocalamus latiflorus* and it was found that overexpression of *DlMADS8* or *DlMADS18* in *Arabidopsis* causes leaf curl and an early flowering phenotype (Tian et al., 2005, 2006). Two *AP1/SQUA-like MADS-box* genes, *PpMADS1* and *PpMADS2* were isolated from *Phyllostachys praecox* during floral transition; in *Arabidopsis*, overexpression of these genes significantly promotes early flowering through upregulation of *AP1* (Lin et al., 2009). Two cDNA libraries have been established from flower buds and vegetative shoots of *B. oldhamii*. From these libraries, 4470 and 3878 ESTs have been annotated, respectively, and many flowering-related genes have been identified (Lin et al., 2010). Recently, a draft sequence of the genome of moso bamboo (*Phyllostachys heterocycla*) was produced and a large number of candidate flowering genes were identified and shown to be transcription factor genes, heat shock protein genes or other stress-responsive genes (Peng et al., 2013). Although substantial advances have been made, a complete description of the genetic regulation of flowering in bamboo species is still not available.

In this study, we isolated a full-length cDNA of a *TFL1-like* gene from *B. oldhamii* and investigated its expression pattern. We generated CaMV *35S::BoTFL1-like* transgenic *Arabidopsis* plants to assay the putative characteristics and function of this gene. Our results provide important insights into the mechanism of flowering in *B. oldhamii*.

MATERIAL AND METHODS

Plant materials and growth conditions

Bamboo plants used for gene cloning and expression analysis were obtained from the bamboo tissue culture room of Zhejiang Forestry Academy, the Nurturing Station for the State Key Laboratory of Subtropical Silviculture, Zhejiang Agriculture and Forestry University; this is the same source as used in a previous study (Lin et al., 2010). Vegetative shoots and floral buds were collected from plants subcultured for two weeks, and used to obtain RNA for the real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis. *A. thaliana* wild type (ecotype Columbia; seeds from ABRC, Ohio State University, Columbus, OH, USA) and transgenic plants were grown on Murashige and Skoog (MS) 0.5X agar medium. Hygromycin B (50 mg/L) was used to select transgenic *Arabidopsis* plants on MS 0.5X agar medium. Wild type and transgenic *Arabidopsis* plants were grown in a temperature-controlled room at 22°C under long day (16 h light) conditions.

Homozygous *tfl1-13* mutant plants (induced by EMS mutagenesis) were purchased from TAIR (serial number CS6237); this mutant carries Lys instead of Glu at position 87.

RNA isolation

The tissues described above were placed in liquid nitrogen, ground into powder, and total RNA was extracted using an RNAiso plus kit (Takara, Japan) according to manufacturer instructions.

Cloning of *BoTFL1-like* from *B. oldhamii*

To amplify *TFL1-like* sequences, a pair of primers (F1 and R1) was designed based on the conserved DNA sequence of *TFL1* homologous genes of graminaceous plants. We performed 3'-rapid amplification of cDNA ends (3'-RACE) and 5'-RACE by using a BD SMART™ RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA) according to the manufacturer instructions. For the 3'-RACE, amplification was performed with the 3' abridged universal amplification primer (3'-AUAP) and two gene-specific primers (F1 and F2). For the 5'-RACE, 5' specific primers (R1 and R2), the abridged anchor primer, and the 5'-AUAP were used. The full-length cDNA of *BoTFL1-like* was obtained by PCR amplification using the forward primer ORFF and reverse primer ORFR. The primers used in this study are listed in Table 1.

Table 1. Sequences of the primers used in this study.

Primer	Sequences (5'→3')	Description
F1	ATGACAGACCCTGATGTGCCA	Forward primer for conserved sequence and 3'-RACE
R1	CCTTCGCTTCTGCTTGAAGAG	Reverse primer for conserved sequence and 5'-RACE
F2	AAAGCCCAACATCGGTATCCAC	Nested gene-specific primer for 3'-RACE
R2	GACAATCCAGTGAAGGTGCTCC	Nested gene-specific primer for 5'-RACE
ORFF	ATGTCTAGGTCTGTGGAGCCTC	Forward primer for ORF
ORFR	TCAGCGTCTCCTGGCAGCAGTC	Reverse primer for ORF
RT-F	CCTTCACATTGGTTATGACAGA	Forward primer for real-time quantitative RT-PCR
RT-R	CAGCAGTCTCTCTCTGAGCATT	Reverse primer for real-time quantitative RT-PCR
actinF	GAGCGAGAAATTGTCAGGGA	Actin primer as internal control, forward
actinR	GATGGCTGGAAGAGGACCT	Actin primer as internal control, reverse

Phylogenetic analysis

The amino acid sequences of *TFL1* homologues from various plant species were obtained from GenBank (http://www.ncbi.nlm.nih.gov/) and aligned with DNAMAN 6.0. A phylogenetic tree based on the complete sequences was generated using PhymL (http://www.atgc-montpellier.fr/phyml/) and constructed using the neighbor-joining method. Bootstrap values were set to 1000.

Real-time quantitative RT-PCR

Real-time quantitative PCR was performed using the SYBR Premix Ex Taq kit (Takara) based on manufacturer instructions. The specific primers were RTF and RTR (Table 1), and the actin primers were actinF and actinR (Table 1). Each cDNA template was used in three replicates. All reactions were performed with the CFX96 Real-Time PCR Detection System (Bio-Rad), and data were analyzed with the CFX96 system software.

Construction of expression vector and *Arabidopsis* transformation

The open reading frame (ORF) of the *BoTFL1-like* cDNA was amplified using Pfu DNA Polymerase (BoCai Biotechnology, Shanghai, China) with forward primer ORFF and reverse primer ORFR, and inserted into a pMD20-T vector (Takara). Both pMD20-T vector and pCAMBIA1301 vector were digested with *Kpn*I and *Xba*I (Takara) and then the *BoTFL1-*

like ORF was cloned into the pCAMBIA1301 vector that contains the cauliflower mosaic virus (CaMV) 35S promoter. The resulting recombinant plasmid was then transformed into *Agrobacterium tumefaciens* strain GV3101 by electroporation using the Gene Pulser MXcell electroporation system (Bio-Rad, USA).

Arabidopsis plant transformation was carried out using the floral dipping method (Clough and Bent, 1998). Transgenic plants were screened on 1/2 MS agar medium supplemented with hygromycin B (50 mg/L) before they were transplanted into soil. The flowering time and rosette leaf numbers of three T3 transgenic lines were counted when the first flower started to open on the main inflorescence, and floral structure was also screened. Each line had 30 repetitions. Insertion of the *BoTFL1-like* gene into the *Arabidopsis* genome was confirmed by PCR analysis using the primers ORFF and ORFR (Table 1).

RESULTS

BoTFL1-like is a homolog of *TFL1*

A 186-bp fragment of the *TFL1* gene was amplified from *B. oldhamii* based on the conserved *TFL1* sequence of graminaceous plants. Using gene-specific primers, a homologue of *TFL1* was isolated from *B. oldhamii* using 3' and 5'-RACE and was designated as *BoTFL1-like*. DNA sequence analysis showed that the entire cDNA length was 833 bp and the genome DNA sequence was 1225 bp containing a complete ORF that encoded a polypeptide of 173 amino acids, and had an 85-bp untranslated 5' region and a 226-bp 3' untranslated region (Figure 1). Comparison of the *BoTFL1-like* gene and cDNA sequences showed that the *BoTFL1-like* gene had 4 exons and 3 introns (Figure 2A). Amino acid sequence alignment revealed that the BoTFL1-like protein had similar conserved regions as TFL1 proteins of other species (Figure 2B). Secondary structure analysis using SOPMA indicated that the putative BoTFL1-like protein contained an alpha helix (16.18%), a rich random coil (53.73%), a beta turn structure (5.78%), and an extended strand (24.28%; Figure 3A). Amino acid sequence alignment with PEBP family members showed that the BoTFL1-like protein contained the key functional site and ligand binding site, and that amino acid residues 71D, 85H, 87H, 110S, 111P, 112K, 118H, and 120F could be involved in the formation of the ligand binding site (Figure 3B).

The sequences of *TFL1* homologs from over 20 plant species were downloaded from NCBI to investigate the phylogenetic relationship of the BoTFL1-like protein with TFL1 proteins of other species; a phylogenetic tree was constructed using PhymL. This tree showed that the BoTFL1-like protein clustered with grass species, and had a 91% identity with barley and ryegrass, and 90% with corn and rice (Figure 4). The data supported the interpretation that the *BoTFL1-like* gene is a *TFL1* homolog.

Expression level of *BoTFL1-like* in floral and vegetative buds

To determine if *BoTFL1-like* transcripts accumulated in vegetative and floral tissues, *BoTFL1-like* RNAs were quantified using RT-PCR. The level of *BoTFL1-like* transcripts in floral buds was almost 3.5-fold higher than in vegetative buds (Figure 5), indicating that *BoTFL1-like* gene expression was lower in vegetative stage tissues and significantly increased in reproductive stage tissues. Thus, the *BoTFL1-like* gene may be involved in maintaining reproductive growth.

```
1    GGGGGGGGGGGGGGGGGGGGGGGGGGGCCCACGGCACATCAAAGTAAATCAGCTTTGCTA
61   CAACTAGTCAGAGCATTGGTTGTGCAAATACAAATACTTGTCCCTCATACCATGTCTAGG
                                                       M  S  R
121  TCTGTGGAGCCTCTCATTGTCGGGCGGGTGATTGGAGAAGTTCTCGACTCCTTTAACCCA
      S  V  E  P  L  I  V  G  T  V  I  G  E  V  L  D  S  F  N  P
181  TGTGTGAAGATGATAGTGACCTACAACTCCAACAGGCTTGCCTTCAATGGCCATGAGCTC
      C  V  K  M  I  V  T  Y  N  S  N  T  L  A  F  N  G  H  E  L
241  TACCCATCAGCGGTTGAATCTAAACCAAGAGTAGAGGTCCAAGGGGGTGACTTGCGGTCT
      Y  P  S  A  V  E  S  K  P  R  V  E  V  Q  G  G  D  L  R  S
301  TCCTTCACATTGGTTATGACAGACCCTGATGTGCCAGGACCAAGTGATCCATACCTAAGG
      S  F  T  L  V  M  T  D  P  D  V  P  G  P  S  D  P  Y  L  R
361  GAGCACCTTCACTGGATTGTCTCTGATATACCTGGGACAACAGATGCTTCTTTTGGACGA
      E  H  L  H  W  I  V  S  D  I  P  G  T  T  D  A  S  F  G  R
421  GAGGTCATAAGCTACGAGAGCCCAAAGCCCAACATCGGTATCCACAGGTTCATTTTTGTG
      E  V  I  S  Y  E  S  P  K  P  N  I  G  I  H  R  F  I  F  V
481  CTCTTCAAGCAGAAGCGAAGGCAGACTGTTATTGTGCCATCCTTCAGGGATCACTTCAAC
      L  F  K  Q  K  R  R  Q  T  V  I  V  P  S  F  T  D  H  F  N
541  ACCCGCCGGTTCGCTGAGGAGAATGACCTTGGCCTCCCTGTGGCTGCTGTCTACTTCAAT
      T  R  R  F  A  E  E  N  D  L  G  L  P  V  A  A  V  Y  F  N
601  GCTCAGAGAGAGACTGCTGCCAGGAGACGCTGAAGAATACAGCTCTGATTGTCCCGTTAT
      A  Q  T  R  T  A  A  R  R  R  *
661  GATAAATAAAGGCCTTCGGATCTTCTCTCTGTGCAGTCAATGCTCCTCTGCTACATTACT
721  TGCTCGTGAACCGATGCTACTTTACAGCATTACTTGCTCGTAAATCTACAAATAAATCAA
781  ATGCATTTTGCAGACTGTATTAGTATTGGATTGTTTTGTAAGCAGTAAGATTGAGAATCA
841  TGTTGACAAAAAAAAAAAAA
```

Figure 1. Nucleotide sequence of the *BoTFL1-like* gene and its predicted amino acid sequence. Initiation and stop codons are marked by black boxes, and PEBP regions are underlined. Important protein functional modules in TFL1 are indicated by gray shading.

A

Exon1 Exon2 Exon3 Exon4

B

Figure 2. Genomic organization of the *BoTFL1-like* gene and protein sequence similarities. (**A**) Genomic organization of *BoTFL1-like* showing untranslated (black boxes) and translated (gray boxes) regions. (**B**) Alignment of partial amino acid sequences of TFL1 homologs. GenBank accession Nos. are: *Hordeum vulgare* (DQ539338), *Lolium perenne* (AF316419), *Oryza sativa* (AF159882), *Zea mays* (NM001112489), *Sorghum bicolor* (XM002450238), *Vitis vinifera* (AF378127), *Cucumis sativus* (AB383155), *Arabidopsis thaliana* (ATU77674), *Glycine max* (EF532597), *Picea abies* (EF633467), *Impatiens balsamina* (AJ888756), *Aquilegia formosa* (DQ286962), *Pisum sativum* (AY343326), *Populus trichocarpa* (DQ310725), *Vicia faba* (ABP73382), *Solanum tuberosum* (DQ307621), *Gossypium hirsutum* (EU026443), *Pyrus communis* (AB162042), and *Eriobotrya japonica* (AB162051).

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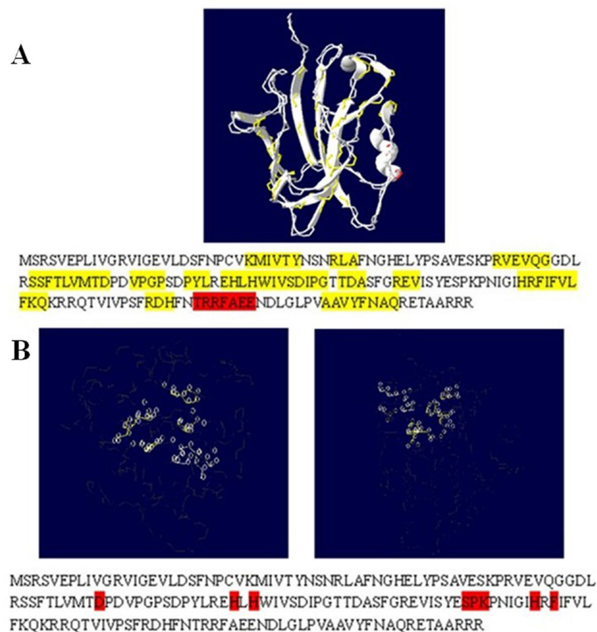

Figure 3. Secondary and tertiary structure predictions for the BoTFL1-like protein. (**A**) Secondary structure prediction. Alpha helix is shown in red and the beta turn in yellow. (**B**) Functional site predictions. Amino acid side chains involved in the formation of functional sites are marked by white circles.

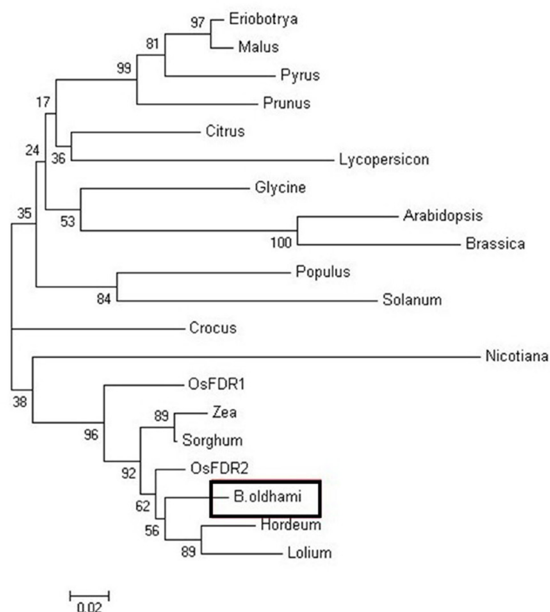

Figure 4. Phylogenetic analysis of TFL1 amino acid sequences from different plant species using PhymL. The BoTFL1-like protein is indicated by the black box.

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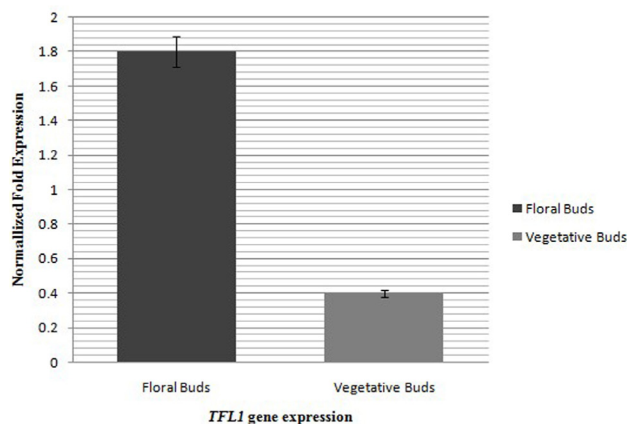

Figure 5. Real-time PCR analysis of the relative expression of *BoTFL1-like* gene. *BoTFL1-like* gene expression in floral buds is shown using black shading, while that in vegetative buds is shown using gray shading.

Ectopic expression of the *BoTFL1-like* gene delays flowering in *Arabidopsis*

To determine the potential role of *BoTFL1-like* gene in floral development, *35S::BoTFL1-like* transgenic *Arabidopsis* plants were produced using *Agrobacterium*-mediated transformation. Twenty independent hygromycin-resistant T1 plants were identified and most showed a delay in flowering time compared to wild type plants. Three independent T3 homozygous transgenic lines were selected for analysis of their phenotypic variations. The flowering time of T3 transgenic plants was significantly delayed by 5 to 9 days compared to the wild type *Arabidopsis* (Figure 6A). In addition, the floral buds and sepals of transgenic *Arabidopsis* were slightly abnormal (Figure 6B, C): the transgenic sepals spread out

Figure 6. Comparison of transgenic and wild type *Arabidopsis* plants. (**A**) Wild type *Arabidopsis* (left side) blossoms at 35 days; *35S::BoTFL1-like* transgenic *Arabidopsis* (right side) begins to bolt after 35 days. (**B**) Wild type *Arabidopsis* flowers have sepals tightly associated with the petals, and the buds are ellipsoidal. (**C**) *35S::BoTFL1-like* transgenic flowers have sepals that grow out like petals, have four grooves in ellipsoid-shaped buds, and smaller buds.

like petals, and the transgenic floral buds were small and four grooves appeared in the original ellipsoid-shaped buds. These effects suggest that the *BoTFL1-like* gene may be involved in the regulation of flowering time and of flower morphological structure in *Arabidopsis*.

Ectopic expression of *BoTFL1-like* largely complements the *tfl1* mutant

To confirm the function of the *BoTFL1-like* gene, the *35S::BoTFL1-like* vector was introduced into plants carrying the *tfl1-13* mutation to determine whether it could complement the mutant phenotype. The *tfl1-13* plants have an early flowering phenotype, a reduced plant height, an increased number of rosette inflorescences, determinate inflorescences, and the whole meristem is divided prior to flower organ determination (Figure 7A and B). Expression of *BoTFL1-like* cDNA in *tfl1-13* mutants caused a prolongation of the vegetative stage to the length expected of wild type *Arabidopsis*; plant height was significantly increased, but the number of rosette inflorescences did not change significantly (Figure 7A). Moreover, the unusual flower structure of *tfl1-13* was restored to normal (Figure 7B and C). These results demonstrated that overexpression of the *BoTFL1-like* gene could largely rescue the effects of this *Arabidopsis TFL1* mutation. Taken together, our data suggest that *BoTFL1-like* may have both similar and divergent functions to *TFL1* in *Arabidopsis*.

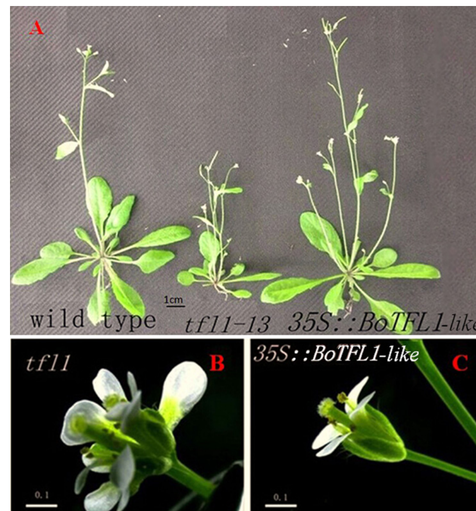

Figure 7. Comparison of floral morphologies of wild type, *tfl1-13* mutant, and *35S::BoTFL1-like* transgenic *tfl1-13* mutant *Arabidopsis* plants at 35 days. (**A**) Wild type *Arabidopsis* (left side); *tfl1-13* mutant (middle) showing early flowering, reduced height and increased number of rosette inflorescences; *35S::BoTFL1-like* transgenic mutant *Arabidopsis* (right side) showing phenotypic rescue for flowering time and height. (**B**) *tfl1-13* mutant plants have an increased number of petals and abnormal flowering pattern. (**C**) *35S::BoTFL1-like* transgenic plant shows phenotypic rescue and resembles the wild type.

DISCUSSION

In this study, the *BoTFL1-like* gene of the *B. oldhamii* was isolated and characterized. As shown in Figure 2B, it shared a high identity with *TFL1* homologues of other species.

Similarly to these *TFL1* homologues, *BoTFL1-like* contained three introns and four exons, and had the same gene structure and intron pattern as the rice homologue *OsCEN1* (Jensen et al., 2001; Lynn et al., 2004; Zhang et al., 2005; Guo et al., 2006; Kotoda et al., 2006; Argiriou et al., 2008; Sato et al., 2009; Esumi et al., 2010) (Figure 2A). *BoTFL1-like* encoded a polypeptide of 173 amino acids that shared high sequence identity with *LpTFL1* and *OsCEN1* proteins. Amino acid sequence alignment with the PEBP family members revealed that the key functional and the ligand binding sites were present, and the amino acid residues 71D, 85H, 87H, 110S, 111P, 112K, 118H, and 120F, needed for formation of the ligand binding site, were present (Banfield and Brady, 2000) (Figure 3B). Phylogenetic analysis showed that the bamboo *BoTFL1-like* gene shared up to 90% identity with genes from barley, rye grass, and rice, indicating that it was highly conserved in evolutionary terms.

TFL1 is a key gene that controls inflorescence meristem characteristics throughout the life cycle; its level of expression is low in early development but increases during the transformation of the inflorescence meristem to a floral meristem (Ratcliffe et al., 1998; Kotake et al., 2003). Real-time PCR analysis showed that the level of *BoTFL1-like* expression was 3.5 times higher in floral buds than in vegetative buds (Figure 5), indicating that *BoTFL1-like* gene expression was at a low level in the vegetative stage but significantly increased in the reproductive stage. Our results suggested that the *BoTFL1-like* gene could play an important role in maintaining reproductive growth. Our observations are similar to a previous study that showed that the *TFL1* gene is expressed at a low level in early development and could inhibit vegetative growth from switching to reproductive growth, and that a higher level of expression could maintain the properties of the inflorescence meristem during flower development (Shannon and Meeks-Wagner, 1991).

Overexpression of the *BoTFL1-like* gene in *35S::BoTFL1-like* transgenic *Arabidopsis* plants caused a significant delay in flowering and a change in inflorescence structure compared to the wild type. This demonstrated that *BoTFL1-like* genes could have a role in the maintenance of vegetative growth and in delaying the phase transition from vegetative growth to reproductive growth in *Arabidopsis*. The *35S::OsCEN1* and *35S::OsCEN2* transgenic *Arabidopsis* plants flowered much later and showed increased branching inflorescence architectures, a similar phenotype as *35S::TFL1* transgenic *Arabidopsis* (Nakagawa et al., 2002; Zhang et al., 2005). Transgenic *Arabidopsis* plants overexpressing the ryegrass *TFL1* homolog *LpTFL1* also have the same phenotype as *35S::TFL1* transgenic *Arabidopsis* plants, with delayed flowering and a dramatic change in structure such as extensive lateral branching and increased growth of all vegetative organs (Jensen et al., 2001). Therefore, the transgenic *Arabidopsis* plants *35S::BoTFL1-like, 35S::OsCEN1*, *35S::OsCEN2* and *35S::LpTFL1* share a similar effect of delayed flowering and altered inflorescence structure or morphology. One possible mechanism for these effects is that the transgenes disrupt the normal function of the endogenous *TFL1* gene by competing for binding sites to specific transcription factors because they all have similar binding sites. Investigation of this possibility is under way.

The complementation experiment demonstrated that *35S::BoTFL1-like* was able to almost fully restore the phenotype of the *tfl1-13* mutant. As shown in Figure 7A, at the vegetative stage, the *tfl1-13* mutant is reduced in size compared to the wild type but overexpression of *BoTFL1-like* cDNA significantly delayed terminal flower development and rescued the height phenotype, although it did not affect the number of rosette inflorescences. Moreover, the abnormal mutant flower structure was also restored (Figure 7B and C). Like *35S::BoTFL1-like*, *35S::LpTFL1* also rescued the phenotype of the severe *tfl1-14* mutant of *Arabidopsis*

(Jensen et al., 2001). This suggested that *BoTFL1-like* may have a similar function to that of *TFL1* in *Arabidopsis*, in regulating or controlling the time of flowering.

Conflicts of interest

The authors declare no conflict of interest.

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