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Alternative splicing of flowering regulatory gene *LFY* in *Arabidopsis thaliana*

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Abstract: To study the expression of flowering regulatory gene *LFY* gene in *Arabidopsis thaliana*, we used reverse transcription-polymerase chain reaction and isolated three alternative splicing(AS) fragments named as *LFY*1239, *LFY*1263, and *LFY*1275 respectively. Sequence analysis confirmed that fragment *LFY*1263 contained an open reading frame of 1 263 bp, and was identical to the previously reported and predicted fragment, while *LFY*1239 lacked 36bp at the 3′ end of the first exon, and *LFY*1275 had an additional 12bp derived from the 3′ end of the first intron. Expression analysis showed that *LFY*1239 was only detected in rosette during the vegetative stage, while *LFY*1263 and *LFY*1275 presented in both floral organs and rosette during both vegetative and flowering stages. Furthermore, *LFY*1263 appeared to be the most abundant transcript. The expression ratio of *LFY*1275 to *LFY*1263 was higher in floral organs than in rosette leaves, which suggested that such ratio might be associated with the flowering regulation.

Key words: *LFY* gene; floral transition; alternative splicing(AS); differential expression
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拟南芥成花调控 *LFY* 基因的选择性剪接

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摘 要:为研究拟南芥成花调控基因 *LFY*,我们采用 RT-PCR 方法分离克隆了三种选择性剪接的片段,分别命名为 *LFY*1239、*LFY*1263 和 *LFY*1275。序列分析表明 *LFY*1263 包含一个大小为 1 263 bp 的开放阅读框,与之前报道的 *LFY* 基因片段大小相同,而 *LFY*1239 在第一外显子的 3′端缺失了 36 bp,*LFY*1275 在第一内含子的 3′末端插入了 12 bp。对几种片段表达部位的分析显示,*LFY*1239 只能在营养生长期的莲座叶中表达,而 *LFY*1263 和 *LFY*1275 在营养生长期和花期的花器官和莲座叶中都可以检测到,并且,*LFY*1263 呈现出主导地位,*LFY*1275 与 *LFY*1263 表达的比例表现为花器官高于莲座叶,该比例的变化可能预示着与成花调控有关。

关键词:*LFY* 基因;成花转变;选择性剪接;差异表达

LFY gene plays an important role to promote flower formation by interaction and coordination with other genes, such as TFL, AP1, AP2, CAL, FT, AP3, UFO, GA1 *et al*. In the current model of plant flowering development derived from *Arabidopsis*, it has been thought that *LFY* is central to the integration of floral signals and regulates flowering. Since Weigel *et al*. isolated *LFY* gene of *A. thaliana*(Weigel *et al*., 1992), *LFY* gene has been cloned from other species(Maizel *et al*., 2005; Matthew *et al*., 2005; Qingyi *et al*., 2005;

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Siddhartha et al., 2008). Also, *LFY* gene is one of the most well studied genes and its role in flower development has been well established (Weigel & Coupland, 1995; Pena *et al.*, 2001; He *et al.*, 2001). In flower meristems, LFY acts as a master regulator orchestrating the whole floral network (Miguel, *et al.*, 2006; Vivian, 2010). LFY is a plant-specific transcription factor that directly binds to the regulatory region of its target genes through a helix-turn-helix motif buried within a unique protein fold (Cécile Hamès, *et al.*, 2008). To activate AP3 in whorls 2 and 3, LFY binds to an F-box protein, known as UFO in *Arabidopsis*, which is part of an SCF-type ubiquitin ligase (Eunyoung *et al.*, 2008). The expression of *LFY* gene in different plants exist temporality and spatiality. It has been reported that high levels of expression of *LFY* genes are first detected in axillary meristems arising on the flank of the shoot apex in seed plants, from which they thought that LFY still exerts its ancestral role on the regulation of cell division in gymnosperms and angiosperms, but in a territory restricted to axillary meristems.

Alternative splicing (AS) in eukaryotes contributes to the diversity and the complexity of gene expression (Michael *et al.* 2008). It may not only change protein domain organization of activity and localization, but also influence the interaction between protein subunits and protein post-transcription regulation including production of nonfunctional proteins (Craig *et al.*, 2008). AS has been investigated more comprehensively in human and animals with about 70%—80% genes of human with AS shown by microarray assay (Johnson *et al.*, 2003). Although great efforts have been made in recent years (Craig *et al.*, 2008; Kemal, 2003), AS in plants still remain full of challenge. It has been reported that there are several alternative splicing genes in rice, wheat, *Zea mays*, orange, populus, sunflower, *Vitis vinifera* and leafy spurge in these few years (Yamauchi *et al.*, 2008; Terashima & Takumi, 2009; Howitt *et al.*, 2009; Lin *et al.*, 2009; Zhang *et al.*, 2009; Srivastava *et al.*, 2009; Lazarescu *et al.*, 2010; Zenoni *et al.*, 2010; Horvath *et al.*, 2010). And some alternatively spliced genes in *Arabidopsis* thaliana has been reported (Schindler *et al.*, 2008; Yan *et al.*, 2009), including some genes responsible for floral transition, such as SEF, GA5, SPY, MAF4, SVP, FT, TOE1 *et al.* (Reddy & Golovkin, 2008), however, AS of *LFY* gene has not been investigated yet. In this paper, alternative splicing forms of *LFY* gene in *Arabidopsis* thaliana were isolated and characterized, named as *LFY*1239, *LFY*1263 and *LFY*1275, respectively. The expression ratio of three different transcripts in rosette leaves and floral organs was determinated.

1 Material and methods

1.1 Plant materials

The plants used in this study were *Arabidopsis thaliana* (L) *Heynh*, Columbia.

1.2 RNA extraction and synthesis of the first-strand cDNA

RNA was extracted from leaves using RNAsimple Total RNA kit (Tiangen Biotechque Co. Ltd., China). The first-strand cDNA synthesis was carried out using the First-Strand RT-PCR kit (Takara Biotechnology Co. Ltd., China).

1.3 RT-PCR and sequence analysis

PCR was performed in 96-well plates with a Biometer T Gradient thermal cycler. Primers were designed according to the reported cDNA and genomic DNA sequences of *LFY* gene in *Arabidopsis* thaliana (GenBank accession no. NM_125579, M91208). The sequences of primers were as the follows, *LFY* Primer Forward: 5′-ATGGATCCTGAAGGTTTCACGAG-3′, *LFY* Primer Reverse: 5′-CTAGAAACGCAAGTCGTCGCCG-3′ (the full-length coding region of *LFY* cDNA and DNA sequences); LAS Primer Forward: 5′-GAAGAGGAATCTTCTAGACGCCG-3′, LAS Primer Reverse: 5′-CCAGTAACCACTTCCTCCTCCG-3′ (differential fragments of different transcripts) (Shenggong Biological Engineering Technology & Services Co. Ltd., China). The standard PCR conditions were carried out with Taq™ polymerase (Takara Biotechnology Co. Ltd., China). The cycling conditions were with an initial incubation 5 min at 94 ℃ followed by 35 cycles of denaturation lasted 30 s at 94

℃, varied annealing temperatures for 30 s, and polymerization 90 s at 72 ℃. The annealing temperatures included 66 ℃ in the amplification of complete coding sequence and 64 ℃ in the amplification of fragments of 180 bp or so. PCR products were visualized on a 1% agarose gel with 0. 5 μL/mL ethidium bromide. Gel images were captured using a UVP Imaging System and documented by VisionWorksLS. RT-PCR products were purified with a DNA Gel Extraction Mini Kit (Watson Biotechnologies, Inc., China). The purified PCR products were cloned into the pMD18-T Easy vector (Takara Biotechnology Co. Ltd., China) named as pT*LFY*, and then were sequenced in Shenggong Biological Engineering Technology & Services Co., Ltd.

1. 4 Differential expression assay in AS products

Amplifications were carried out under the cycles of 12, 16, 28, 30, 32, 34, 36, 38, 40 and 42 in order to determine the cycle number of linearity stage. cDNA from different tissues during different development stages were used as templates, which were natural competitive templates and the principal was similar to competitive PCR. The primers were LAS primer F and LAS primer R. The products of varied sizes were separated using 3. 5% agarose gel electrophoresis. Optical density assay on RT-PCR products of different AS transcripts of *LFY* gene was carried out by means of VisionWorks LS software of BioSpectrum Imaging System.

2 Results

2. 1 Isolation and characterization of different AS fragments of *LFY*

We isolated the full length of *LFY* in *Arabidopsis* thaliana, which include three transcripts during the vegetative stage and two transcripts during the reproductive stage. Furthermore, in order to attain more visual electrophoresis result of different transcripts, we isolated partial AS fragments of different organs during different stage by using LAS primers, which were shown in Fig. 1. There were three AS fragments in rosette leaves during the vegetative stage (Lane 1, Fig. 1: a), and there were two AS fragments both in floral organs (Lane 1, Fig. 1: b) and in rosette leaves (Lane 2, Fig. 1: b) during reproductive stage. It was obvious that the expression patterns of transcripts were different during different stage.

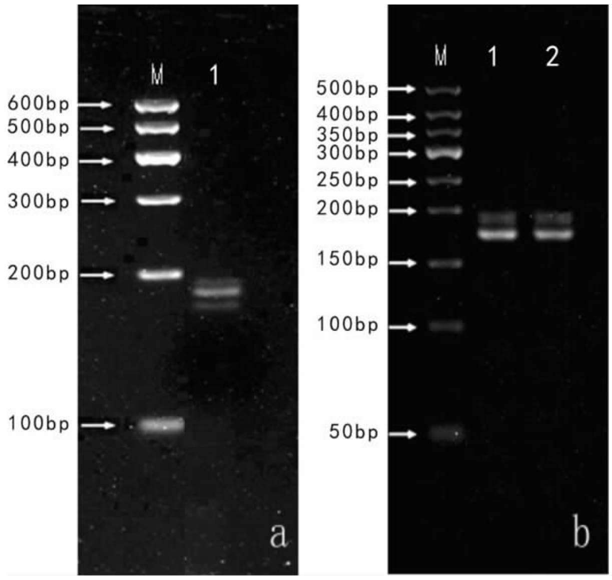

Fig. 1　**a.** AS fragments in rosette during the vegetative stage (**M**: DNA marker); **b.** AS fragments in floral organ & rosette during reproductive stage (**M**: 50 bp DNA ladder; **1**: AS fragments in floral organ; **2**: AS fragments in rosette)

2. 2 Comparison of different transcript sequences

We analyzed these three RT-PCR products of *LFY*. Sequence alignment of these three differential expression fragments showed that there could be three alternative splicing transcripts in *Arabidopsis* thaliana. Compared with *LFY* genomic DNA sequence, LFY1239 lacked of 36bp near the 3′ end of the first exon, *LFY*1263 was identical to what was reported previously with 1 263 bp in length, and *LFY*1275 had additional 12 bp in the 3′ end of the first intron (Fig. 2). The alternative splicing of *LFY* leads to either insertion or deletion of the *LFY* protein (Fig. 3). The alternative spliced transcript, *LFY*1275, displayed four amino acid (DDWT) insertion compared to *LFY*1263. In contrast, the alternatively spliced transcript, *LFY*1239, had twelve amino acid (GTHHALDALSQE) deletions and four amino acid (DDWT) insertion compared to *LFY*1263.

2. 3 Differential expression of AS transcripts

To study if these AS transcripts were differently

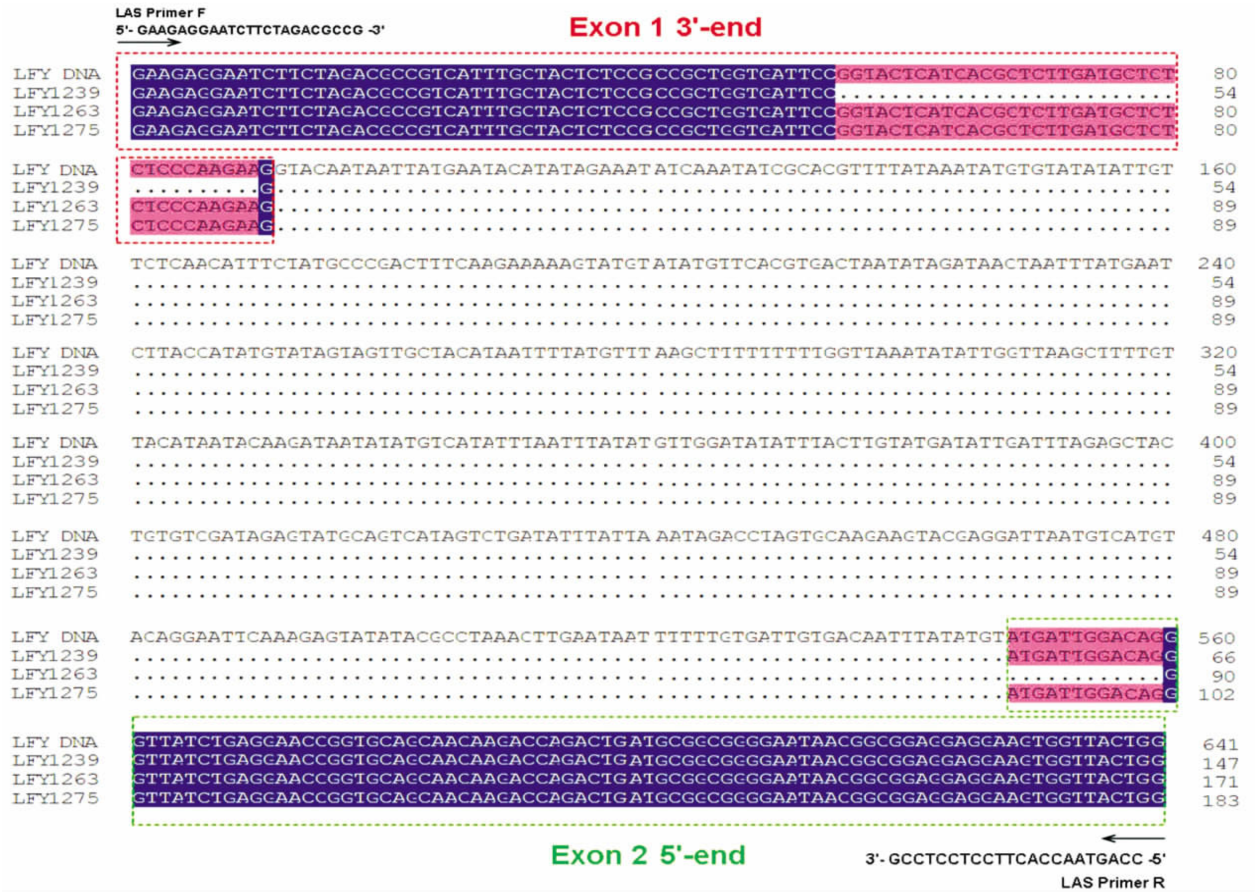

Fig. 2 Sequence alignment of three AS fragments

Fig. 3 Sequence alignment of amino acid deduced from three AS fragments

expressed during different development stage of *Arabidopsis* thaliana, we performed RT-PCR of *LFY* gene transcripts in organs at both vegetative and reproduced stages. The result of optical density assay of these transcripts was shown in Fig. 4. Optical density assay showed that the relative ratio of expression was as follows: a. rosette leaves in the vegetative stage *LFY*1275 ∶ *LFY*1263 ∶

*LFY*1239＝1∶4.784∶1.044, b. rosette leaves in reproduced stage *LFY*1275∶*LFY*1263＝1∶4.652, c. floral organ *LFY*1275∶*LFY*1263＝1∶3.768. Over all, *LFY*1263 and *LFY*1275 expression was consistent in both stages and both rosette leaves and floral organs, with *LFY*1263 in predominant amount. *LFY*1239 transcript was only detectable in rosette leaves at vegetative stage. These data suggested that *LFY* gene alternative splicing was associated with different organs at different development stages of the plant.

3 Discussion

In the current study, we identified two alternatively spliced transcripts of the Flowering Regulatory Gene *LFY*. *LFY*1239 was detected only in rosette leaves at the vegetative stage, which indicated that *LFY*1239 is not associated with floral transition of *Arabidopsis* thaliana. In contrast, *LFY*1275 and *LFY*1263 expressed consistently during the vegetative and reproductive stage, which implied that both transcripts were relevant to floral transition.

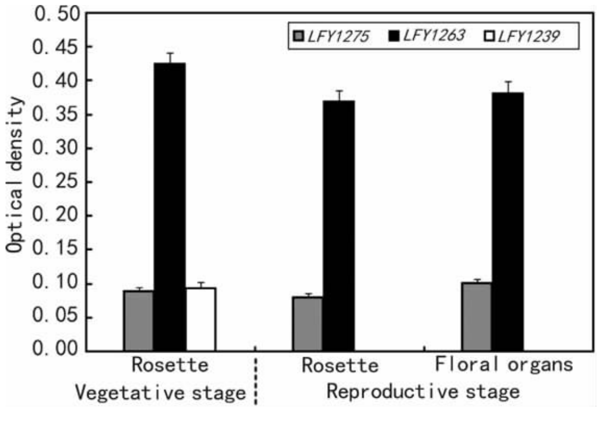

Fig. 4 Optical density assay on RT-PCR products of different AS transcripts expression of *LFY* gene

*LFY*1263 was the predominant transcript at different stage, with the ratio of *LFY*1275 to *LFY*1263 increased slightly in floral organs than that in rosette leaves, suggesting that *LFY*1275 may play an important role in floral transition. Together with the result of the reported studies on the function of *LFY*1263, who thought increasing the copy number of endogenous *LFY*1263 reduced the number of leaves produced before the first flower is formed (Miguel *et al.*, 1997), transcript *LFY*1275 may also regulate the growth and floral transition of *Arabidopsis* thaliana in addition to transcript *LFY*1263. The change of *LFY* expression during the different stage suggested that it played comprehensive effect in plant development process, it not only control floral development including floral initiation but also played an important role in leaf morphogenesis during the vegetative stage. The biological roles of two spliced variants (*LFY*1239 and *LFY*1275) of the *LFY* gene of *Arabidopsis thaliana* remain to be identified, and we will develop it in the subsequent research.

In this research, sequence alignment result of different transcripts showed that they differed in a short stretch of nucleotide base pairs, with most coding region identical and no frame shift mutations. Weigel *et al.* reported that N-terminal (including the first exon) of *LFY* was vital to *LFY* protein function. Transcript *LFY*1239 lack of 36 bp in the 3′ end near the first exon leading to 12 amino acid deletion of the protein, which maybe there just to make up the number and attribute to its interfered with floral initiation, and this consistent with our observation that *LFY*1239 transcript was only detectable in rosette leaves during the vegetative stage. Transcript *LFY*1275 displayed additional 12 bp in the 3′ end of the first intron and result in 4 amino acid insertion in the protein which may enable the gene product to differently influence the growth and floral transition of *Arabidopsis thaliana*.

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