Relationship between Floral Development and Transcription Levels of **LEAFY** and **TERMINAL FLOWER 1** Homologs in Japanese Pear (**Pyrus pyrifolia** Nakai) and Quince (**Cydonia oblonga** Mill.)

Tomoya Esumi, Ryutaro Tao* and Keizo Yonemori

*Corresponding author (E-mail: rtao@kais.kyoto-u.ac.jp).

Using real-time reverse-transcription polymerase chain reaction (real-time RT-PCR) and *in situ* hybridization, we investigated the temporal and spatial expression of **LEAFY** (**LFY**) and **TERMINAL FLOWER 1** (**TFL1**) homologs in Japanese pear (**Pyrus pyrifolia**) and quince (**Cydonia oblonga**) buds, in order to elucidate their roles in the flower and inflorescence development of fruit trees in the subfamily Maloideae (Rosaceae). Japanese pear and quince were selected because they are very close phylogenetically but develop distinct inflorescence architectures. Floral differentiation in Japanese pear began in late June to early July in Osaka, Japan, forming a raceme inflorescence with about eight flowers, whereas that in quince took place from late October to November in Nagano, Japan, forming a solitary flower in each floral bud. **LFY** homolog expression levels in both species increased at the floral differentiation stage and remained at relatively high levels in flower meristems after the flower organ differentiation stage. In contrast, **TFL1** homolog expression levels in both species were high in vegetative-stage buds, but decreased significantly just before floral differentiation. Japanese pear **TFL1** homologs were expressed in the subepidermal layer of the apical meristem before floral differentiation, whereas those of quince were expressed in the epidermal layer of the apical meristem and leaf primordia. We discuss the possible involvement of maloid **LFY** and **TFL1** homologs in triggering floral differentiation, as well as the involvement of the different spatial expression patterns of **TFL1** homologs in the inflorescence architectures of Japanese pear and quince.

**Key Words:** floral differentiation, floral induction, *in situ* hybridization, real-time reverse-transcription PCR.

**Introduction**

The flowering and inflorescence architectures of fruit trees greatly affect their breeding and production efficiencies. For example, juvenility length influences the fruit tree breeding strategy and efficiency. Since a heavy fruit load in an inflorescence and/or a tree adversely affects productivity and fruit quality, commercial producers usually thin flowers and fruits, a laborious task. Thus, the ability to control and modify flowering and inflorescence architecture by understanding the molecular mechanisms underlying flowering and inflorescence development would improve breeding efficiency and productivity.

The subfamily Maloideae (Rosaceae) contains many fruit tree species, including apples, pears, quinces, and loquats, which show great diversity in phenological and morphological characteristics (Phipps et al., 1990; Robertson et al., 1992; Rohrer et al., 1991, 1994). The origin and diversification of the Maloideae have intrigued botanists, geneticists, and horticulturalists (Campbell et al., 1995; Evans and Campbell, 2002; Phipps et al., 1991; Robertson et al., 1991). Because of the wide variation in the floral morphologies of maloid fruit trees, we compared maloid fruit trees with different floral morphologies to investigate the molecular mechanisms underlying differences in flowering and inflorescence development.

The molecular mechanisms of flowering have been studied extensively in model herbaceous plants, including *Arabidopsis thaliana* (L.) Heynh. and *Antirrhinum majus* L. Numerous flowering-related genes have been isolated from these species, and several models for the genetic pathways of floral induction and differentiation have been proposed (Araki, 2001; Blázquez et al., 2006; Jack, 2004; Parcy, 2005). **LEAFY** (**LFY**) and **TERMINAL FLOWER 1** (**TFL1**) are two...
Arabidopsis flowering-related genes that play important roles in flowering and inflorescence development. *LFY* encodes a transcription regulator that plays a primary role in floral induction and flower meristem identity determination (Blázquez et al., 1997; Weigel et al., 1992). The constitutive expression of *LFY* is sufficient to promote floral induction in Arabidopsis. Furthermore, a change in the inflorescence architecture from indeterminate to determinate has been observed in a transgenic Arabidopsis line that constitutively expresses *LFY* (Weigel and Nilsson, 1995). *LFY* is also a direct activator of floral homeotic genes, such as APETALA 1 (AP1), CAULIFLOWER (CAL), and AGAMOUS (AG) (Busch et al., 1999; Parcy et al., 1998, 2002; Wagner et al., 1999; William et al., 2004).

*TFL1* encodes a protein similar to phosphatidylethanolamine-binding proteins (PEBPs) and Raf kinase inhibitor protein (RKIP), which can bind to membrane protein complexes and may be involved in a signaling process (Yeung et al., 1999). In Arabidopsis, *TFL1* is important in regulating plant growth through the maintenance of shoot apex indeterminacy (Bradley et al., 1997; Oshshima et al., 1997; Shannon and Meeks-Wagner, 1991). In the shoot apex center, TFL1 prevents the expression of flower meristem identity genes, such as *LFY* and *AP1*, and maintains the indeterminacy of the inflorescence meristem. On the shoot periphery, flower meristem identity genes prevent the transcription of *TFL1*, allowing the formation of flower meristems. Ratcliffe et al. (1999) and Liljegren et al. (1999) proposed that the mutual inhibition of *TFL1* and flower meristem identity genes ensures the separation of shoot and flower meristem identity in Arabidopsis.

Information from model plants has led to studies on the molecular mechanisms of fruit tree flowering. Several flowering-related genes, including *LFY*, *TFL1*, and *API* homologs, have been isolated from grapevine (*Vitis vinifera* L.), citrus (*Citrus sinensis* Osbeck), kiwifruit (*Actinidia delicosa* C. F. Liang & A. R. Ferguson), and apple (*Malus domestica* Borkh.) (Boss et al., 2001, 2006; Calonje et al., 2004; Carmona et al., 2002; Jeong et al., 1999; Joly et al., 2004; Kotoda et al., 2000, 2002; Kotoda and Wada, 2005; Pillitteri et al., 2004; Sung et al., 1999, 2000; Wada et al., 2002; Walton et al., 2001). We previously isolated and compared *LFY* and *TFL1* homologs from six maloid fruit trees, including Japanese pear and quince, to elucidate the molecular basis of the differences in inflorescence architecture (Esumi et al., 2005); however, the derived amino acid sequences differed only slightly in their primary structures.

In this study, we investigated the temporal and spatial expression of *LFY* and *TFL1* homologs in Japanese pear and quince buds by real-time RT-PCR and *in situ* hybridization to gain further insight into their roles in flowering and inflorescence development in maloid fruit species. We chose Japanese pear and quince because these species are very close phylogenetically but develop distinct inflorescence architectures, with the former forming a raceme inflorescence and the latter a solitary-flowered inflorescence. Their close relationship can be inferred from artificial intergeneric hybrids of the two, called “Pyronia” (Phipps et al., 1990; Shimura et al., 1983; Trabut, 1916), and the use of quince as a dwarfing rootstock for pear (van der Zwent and Childers, 1982).

**Materials and Methods**

**Plant Materials**

Adult trees of Japanese pear ‘Housui’ (*Pyrus pyrifolia*) and quince (*Cydonia oblonga*) grown in Osaka and Nagano, Japan, respectively, were used. For total RNA extraction, buds on Japanese pear spurs were collected periodically from May to the end of August 2003. Terminal buds on 5- to 10-cm quince shoots were collected periodically from August to the end of November in 2003. For *in situ* hybridization, buds of both species were collected periodically in 2004.

**RNA extraction and cDNA synthesis**

Total RNA was isolated from bud samples using the modified cetyltrimethylammonium bromide (CTAB) method of Kotoda et al. (2000). The total RNA concentration was adjusted to 0.20 ± 0.04 g·L⁻¹ using a NanoDrop ND-1000 spectrometer (NanoDrop Technologies, Wilmington, DE, USA), and further confirmed by electrophoresis on a 1% agarose gel using ethidium bromide staining. A 5-µL aliquot of the sample (1.0 ± 0.2 µg) was treated with five units of DNase I (Takara Bio, Otsu, Japan) and used as a fluorescent reporter to monitor real-time RT-PCR using an ABI PRISM 7900H (Applied Biosystems, Foster City, CA, USA). Gene-specific primers were designed using Primer Express ver. 2.0 software (Applied Biosystems) based on previously isolated cDNA sequences of maloid *LFY* and *TFL1* homologs. In our previous research, we isolated two different types of *LFY* and *TFL1* homologs both from Japanese pear and quince (Esumi et al., 2005). Thus, we designed the primer sets from the conserved regions of the two different *LFY* and *TFL1* homologs, respectively to investigate the sum of the transcripts of two different homologs. As the putative intron sequence is present between primers for each gene, the amplification is supposed to be only from cDNA. Transcription of the *ACTIN* gene was quantified as an internal control. ACT-
F1 and ACT-R1 primers were used to isolate ACTIN cDNAs of Japanese pear and quince (Ushijima et al., 2003). Two ACTIN-specific primers, ACT-F3 and ACT-R3, were designed for real-time RT-PCR analysis (Table 1).

For real-time RT-PCR, 1 µL of cDNA solution, which contained cDNA equivalent to the amount synthesized from 0.025±0.005µg total RNA, was used. The reactions were carried out in a 96-well plate, using a 25-µL reaction volume containing 12.5 µL of 2X SYBR Master Mix (Applied Biosystems), 0.2 µM each of forward and reverse primers, and 1 µL of cDNA sample. Reactions were conducted at least four times for each cDNA sample. The threshold point, giving the best PCR amplification efficiency with the slope coefficient value closest to −3.32 (−1·log2−1), was estimated for each primer set. The threshold cycle value (Ct) of each sample was automatically measured by SDS ver. 2.1 software (Applied Biosystems). After the PCR reaction, dissociation curve analysis was performed to confirm that the fluorescence was only derived from the gene-specific amplification. Based on Ct values, the quantities of LFY and TFL1 homolog cDNAs in each sample were normalized using ACTIN cDNA as an internal control, and the relative expression level was calculated.

RNA probe synthesis

Japanese pear cDNAs of the LFY homolog PpLFY-2 (GenBank Accession AB162035) and the TFL1 homolog PpTFL1-1 (AB162041) were used for RNA probe synthesis. Two conserved regions of PpLFY-2 cDNA, 143–486 (343 bp) and 718–1153 (435 bp), and full-length 651-bp PpTFL1-1 cDNA were separately fused into the pGEM-T Easy plasmid vector (Promega Co., Madison, WI, USA) and used to synthesize the probe. Antisense RNA probes were synthesized by in vitro transcription initiated from T7 or SP6 promoters on the vector using a DIG-labeling RNA synthesis kit (Roche Diagnostics, Basel, Switzerland) following the manufacturer’s instructions. Since the synthesized probes were shorter than 1 kb, hydrolysis treatment to shorten the probe length was omitted, as suggested by Goto (2001). Equal amounts of the two PpLFY-2 antisense probes were mixed and used for hybridization. Since cDNA sequences of PpLFY-1 (AB162029), PpLFY-2, CoLFY-1 (AB162031), and CoLFY-2 (AB162037) are highly conserved (Esumi et al., 2005), the PpLFY-2 antisense probe cross-hybridized to all four of the LFY homologs. Similarly, the PpTFL1-1 antisense probe hybridized to PpTFL1-1, PpTFL1-2 (AB162047), CoTFL1-1 (AB162043), and CoTFL1-2 (AB162049).

In situ hybridization

Buds were fixed in FAA (1.8% formaldehyde, 5% acetic acid, 50% ethanol) for four hours at 4°C. Fixed samples were dehydrated using an ethanol:butanol series, and then embedded in Paraplast Plus (Sigma-Aldrich Co., St Louis, MO, USA). The embedded samples were stored at 4°C until use. The embedded tissues were sliced into 6- to 10-µm sections, and the sections were mounted on silane-coated microscope slides (Matsunami Glass Ind., Kishiwada, Japan). Paraffin was removed by washing twice with xylene, and the tissue sections were rehydrated in an ethanol series. The tissue sections were then incubated in Proteinase K solution (5 mg·L−1 Proteinase K, 50 mM EDTA, 0.1 M Tris-HCl pH 7.5) for 30 min at 37°C, washed twice with distilled water, and fixed in 4% paraformaldehyde for 10 min. After two more washes with distilled water, the fixed tissue sections were acetylated with acetic anhydride (0.25% acetic anhydride in 0.1 M triethanolamine solution) for 10 min, dehydrated in an ethanol series, and then completely vacuum-dried. The probe solution (10% dextran sulfate, 50% formamide, 300 mM NaCl, 10 mM Tris-HCl, pH 8.0, 5 mM EDTA, 1·Denhardt’s solution, 125 mg·L−1 tRNA, and 0.8 mg·L−1 DIG-labeled probe) was applied to the slides and covered with Parafilm (Pechiney Plastic Packaging, Inc., Neenah, WI, USA). Hybridization was performed at 48°C for 16 h. The slides were then washed with 4×SSC and incubated in RNase solution (500 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, 50 mg·L−1 RNase A) at 37°C for 30 min. After RNase treatment, the slides were washed four times with 0.5×SSC at 60°C for 30 min. Blocking solution (25% bovine serum, 100 mM maleic acid, 150 mM NaCl, and 0.5% Tween 20) was applied to the slides, which were then incubated at 30°C for 30 min. An anti-DIG alkaline phosphatase solution
(0.1% anti-DIG-AP Fab fragments, 100 mM maleic acid, 150 mM NaCl) was applied, and the slides were incubated at 30°C for 60 min. The slides were washed twice with washing buffer (150 mM NaCl, 100 mM maleic acid, pH 7.0) and incubated in NBT/BCIP solution (0.35 g·L⁻¹ NBT, 0.18 g·L⁻¹ BCIP, 5 mM MgCl₂, 100 mM NaCl, 100 mM Tris-HCl pH 9.0) at 30°C for at least 8 h. The slides were washed twice with distilled water and dehydrated in an ethanol series and xylene. The samples were then coated with PMMA (Matsunami Glass) for observation. The samples were observed and photographed using an Olympus BX60 microscope equipped with an Olympus DP50 camera (Olympus, Tokyo, Japan). We performed most of the in situ hybridization experiments only with antisense probes because of the limited number of samples. We therefore conducted in situ hybridization experiments with the two different gene-specific probes simultaneously because different detection patterns of the two different genes could serve as reference controls for each other.

Results

Expression levels of LFY homologs in developing buds

Both the Japanese pear and quince LFY homologs were continuously expressed throughout the sampling periods, with the amounts of transcripts fluctuating before and during floral differentiation (Fig. 1). The timing of floral differentiation determined by microscopic observation as described in the previous study (Esumi et al., 2007), is shown in Figure 1.

In Japanese pear, the transcript level began to increase as floral differentiation commenced with doming of the apical meristem, which was observed most frequently on 27 June. By 14 July, when all of the flower meristems were initiated, the transcript levels reached five to eight times the level observed just prior to flower meristem differentiation in June. The transcript level decreased in mid-July, when flower organ differentiation commenced, and then increased again during the flower organ developmental stage in August (Fig. 1A).

In quince, the apical meristems assumed a dome-like structure, and floral differentiation began in late October to early November. In contrast to Japanese pear, the amount of quince LFY homolog transcripts remained low at the beginning of floral differentiation during the doming of the apical meristem; however, at the end of November, after the sepal primordia had differentiated, the transcript amounts increased as the flower organs developed (Fig. 1B). The increase in the transcript level during the floral differentiation stage in Japanese pear was greater than that in quince.

Expression levels of TFL1 homologs in developing buds

The expression levels of TFL1 homologs changed drastically during the observation period (Fig. 2). In Japanese pear and quince buds, the amount of TFL1 homolog transcript decreased considerably before floral differentiation began with apical meristem doming. In Japanese pear, TFL1 homolog transcripts decreased about 1/100-fold from early June to late June, before floral differentiation (Fig. 2A). In quince, the transcripts decreased from September to October (Fig. 2B). The timing of the reduction in the transcript level to the minimum coincided with the apical meristem doming period in both Japanese pear and quince.

Expression of LFY homologs in developing buds detected by in situ hybridization

In Japanese pear, LFY homolog hybridization signals were detected in scale and/or leaf primordia and the

---

**Fig. 1.** Changes in the expression of LFY homologs in Japanese pear and quince buds. The expressions of LFY homologs in Japanese pear (A) and quince (B) floral buds were measured by real-time RT-PCR amplification of two types of LFY homologs in Japanese pear (PpLFY-1 and PpLFY-2) and quince (CoLFY-1 and CoLFY-2). Values represent the summed expression levels of both homologs. Data points represent the mean ± SE of at least eight replications for the relative expression, which were calibrated by the amount of the ACTIN control expression.
T. Esumi, R. Tao and K. Yonemori

Apical meristem in late May and mid-June, prior to floral differentiation (Fig. 3A, B). The apical meristem changed into a dome-like structure at the beginning of floral differentiation in late June, and the signal was continuously observed in the domed meristem and the leaf and/or bract primordia (Fig. 3C). The signal was stronger in newly initiated flower meristems and peripheral cell layers than in other tissues at the inflorescence development stage, a short period that occurs just after meristem doming (Fig. 3D). During the flower organ development stage in July and August, signals were detected only in flower meristems, where flower organ development was in progress (Fig. 3E–H).

In quince, LFY homolog hybridization signals were detected in leaf primordia and apical meristems before floral differentiation (Fig. 4A–C). Signals were continuously observed in the dome-like apical meristem in November (Fig. 4D, E) and in the flower meristem with sepal primordia in December (Fig. 4F).

Expression of TFL1 homologs in developing buds detected by in situ hybridization

In Japanese pear, TFL1 homolog expression was detected in the central region, just below the apical meristem, before floral differentiation. A strong hybridization signal was observed in late May (Fig. 5A), with the signal gradually fading toward mid-June (Fig. 5B–D). The signal was undetectable in all tissues after the apical meristem transformed into the dome-like structure in late June (Fig. 5E–J).

In quince, TFL1 homolog expression was detected in leaf primordia and at the top of the apical meristem in June (Fig. 6A, B). The region of expression differed from that of Japanese pear buds in the vegetative stage before floral differentiation (Fig. 5A). In Japanese pear, TFL1 homologs were expressed in the subepidermal layer of the apical meristem, whereas in quince, the hybridization signal was observed in the epidermal layer. In September, the signal weakened but was still detectable in the apical meristem and leaf primordia (Fig. 6C, D). The signal was barely detectable in the apical and flower meristem after October, although weak signals continued in leaf primordia (Fig. 6E–G).

Discussion

Flowering in Japanese pear and quince

Before discussing the relationship between floral development and transcription levels of the LFY and TFL1 homologs, we describe here our terminology for floral induction and differentiation (Fig. 7). Fruit trees, including Japanese pear and quince, spend several years after germination in the juvenile phase before entering the adult phase. Juvenility is defined as the physiological state of a seedling plant during which it cannot be induced to flower. This state is followed by the adult phase, during which flowering can occur (Westwood, 1993).

The adult tree undergoes both vegetative and reproductive growth each year. Vegetative growth involves the production of vegetative apical meristems, which generate annual shoot growth. Reproductive growth involves floral induction and differentiation, blooming, and fruit development. Floral induction is the transition from the vegetative to reproductive stage, when the apical meristem becomes competent to make flowers. Floral induction is a conceptual term, as there is no visible morphological change in the apical meristem. Following floral induction, floral differentiation begins, with transformation of the apical meristem into a dome-like structure. Doming is the first visible change in the meristem in the reproductive growth stage.
Floral differentiation involves two distinct developmental steps: flower meristem initiation and flower organ differentiation. In Japanese pear, the domed apical meristem develops as an inflorescence by initiating lateral flower meristems. Eventually, a terminal flower meristem is initiated on the inflorescence apex (Banno et al., 1986; Esumi et al., 2007). In quince, the domed apical meristem transforms directly into a single flower meristem (Esumi et al., 2007). After flower meristem initiation, flower organ differentiation begins in the meristem. Flower organ differentiation proceeds from the sepal primordia, which are the first to differentiate, to the inside whorls of petals, stamens, and pistils. The floral bud breaks, and flowers appear the next spring.

**LFY homolog expression**

The amount of LFY homolog transcripts changed more in Japanese pear than in quince after floral differentiation began with the transformation of the apical meristem into a dome-like structure. *As in situ* hybridization showed that LFY homologs were expressed in flower meristems with developed sepals in December (F). Asterisks indicate newly developed scales and/or leaf primordia. SAM, apical meristem; FM, flower meristem; Br, bract; Sc, scale; Sp, sepal. Bars = 200 µm.
expression in Japanese pear and quince flower meristems could also be involved in the regulation of flower organ differentiation and development.

The amounts of LFY homolog transcripts in several fruit tree species have been reported, and in most cases, the expression appears to be involved in flower organ differentiation and development. In citrus, transcription of the LFY homolog CsLFY increased significantly only after floral inductive treatment (Pillitteri et al., 2004). In grapevine, expression of the LFY homolog VFL (Vitis FLO/LFY) increased dramatically at bud break in spring, when flower organ differentiation occurred (Carmona et al., 2002; Joly et al., 2004). In apple, expression of the LFY homolog (AFL1 and AFL2, apple FLO/LFY) was barely detectable by RNA gel blot analysis, but increased during flower organ differentiation and development stages (Kotoda et al., 2000). RT-PCR expression analysis showed that AFL2 was continuously expressed in apple buds throughout the vegetative and reproductive growth stages, whereas AFL1 began to be expressed during floral differentiation (Kotoda and Wada, 2005; Wada et al., 2002). We observed the sum of expressions of the two LFY homologs in Japanese pear and quince that are in substantial agreement with the findings for apple (Kotoda et al., 2000; Wada et al., 2002).

In Arabidopsis, the transcriptional activity of the LFY
promoter increased with plant growth and was enhanced by long-day conditions and the application of the phytohormone gibberellic acid. Thus, LFY expression increased gradually toward floral induction (Blázquez et al., 1997, 1998; Eriksson et al., 2006). Although most of the increase in the amounts of LFY homolog transcripts was in meristems at floral differentiation, a careful look at the transition of the transcript amounts of the LFY homologs reveals that they increase prior to flower meristem differentiation in late May to early June in Japanese pear and in September in quince. Since LFY has been implicated as both a flower meristem identity gene and a flowering signal integrator that controls the timing of floral induction (Araki, 2001; Blázquez et al., 1997; Parcy, 2005), LFY homologs in maloid fruit tree species may be involved in floral induction (Fig. 7).

Reduction of TFL1 homolog expression level as a trigger for floral differentiation

In the past, the amount of transcription of TFL1 homologs in relation to floral induction and differentiation was analyzed solely by non-quantitative or semi-quantitative RT-PCR. In apple, a high concentration of MdTFL1 accumulated in buds before floral differentiation and decreased upon floral differentiation (Kotoda and Wada, 2005). Although the expression patterns of the Japanese pear and quince TFL1 homologs in our study were similar to those of apple, our RT-PCR analysis clearly showed a remarkable reduction in the amounts of TFL1 homolog transcripts just before floral differentiation. The lowest transcript levels coincided with the commencement of floral differentiation, as recognized by the formation of the dome-like apical meristem structure. The in situ hybridization results confirmed the decrease in TFL1 homolog transcript levels before floral differentiation. This sharp decrease in transcript levels suggests that TFL1 homologs are involved in floral induction, probably functioning as floral repressors. Floral induction may be induced by an increase in the amount of LFY homolog transcripts, as discussed above, and a decrease in TFL1 homolog transcripts. If so, floral differentiation may commence when the amount of TFL1 homolog transcripts decreases to its minimum level. This idea is supported by the precocious flowering of transgenic apple in which transcription of the endogenous TFL1 homolog was suppressed (Kotoda et al., 2006), as well as suggestions that TFL1 plays a role in controlling vegetative meristem growth and regulating flowering time (Bradley et al., 1997; Liljegren et al., 1999; Ratcliffe et al., 1998, 1999).

TFL1 and its homologs were shown to be preferentially expressed in undifferentiated meristems, as determined by in situ hybridization in Arabidopsis, Antirrhinum, tobacco (Nicotiana tabacum L.), Impatiens balsamina L., and grapevine (Amaya et al., 1999; Boss et al., 2006; Bradley et al., 1996, 1997; Joly et al., 2004; Ordidge et al., 2005). These findings suggest that TFL1 and its homologs play roles in preventing the termination of meristem development as flower meristems differentiate. This idea is supported by the delayed flowering of transgenic Arabidopsis and tobacco due to the constitutive expression of TFL1 homologs (Amaya et al., 1999; Boss et al., 2006; Jensen et al., 2001; Kotoda and Wada, 2005; Ordidge et al., 2005; Pillitteri et al., 2004; Ratcliffe et al., 1998, 1999; Zhang et al., 2005). Thus, a significant decrease in the levels of TFL1 homolog transcripts could be a crucial indicator of floral induction in Japanese pear and quince buds.

TFL1 homolog expression occurs in different regions in Japanese pear and quince vegetative buds

Our study showed that TFL1 homolog transcripts were
shows indeterminate inflorescence development up to early development of inflorescences. Japanese pear reference to differences in their morphological inflorescence development. Japanese pear solitary-flower inflorescence (Esumi et al., 2007). patterns, one notable morphological difference is in the tree architectures of Japanese pear and quince in VvTFL1 analogous to that of the grapevine TFL1 meristem domed. In Japanese pear inflorescence tissue, differed in Japanese pear and quince in the pattern of Although we have yet to characterize in detail the whole-tree hybridization, but was clearly detected in Arabidopsis inflorescence apices (Ratcliffe et al., 1999). In our study, tissue browning at this stage may have masked hybridization signals in Japanese pear leaf primordia and bracts, as well as inflorescence and pith tissues.

In contrast to Japanese pear, the expression region of quince TFL1 homologs in the vegetative meristem was analogous to that of the grapevine TFL1 homolog VvTFL1. Boss et al. (2006) discussed the expression pattern of VvTFL1, with special reference to sympodial development of the morphological architecture. Although we have yet to characterize in detail the whole-tree architectures of Japanese pear and quince in reference to differences in TFL1 homolog expression patterns, one notable morphological difference is in the early development of inflorescences. Japanese pear shows indeterminate inflorescence development up to the apex, which is terminated by a terminal flower, whereas quince shows determinate development, with a solitary-flower inflorescence (Esumi et al., 2007). Differences in the spatial expression patterns of TFL1 homologs in the Maloideae may control differences in their morphological inflorescence development.

Acknowledgements

We gratefully acknowledge the gift of plant materials from Mr. T. Minemura (Minemura Commercial Orchard, Omachi, Japan).

Literature Cited

Amaya, I., O. J. Ratcliffe and D. J. Bradley. 1999. Expression of CENTRORADIALIS (CEN) and CEN-like genes in tobacco reveals a conserved mechanism controlling phase change in diverse species. Plant Cell 11: 1405–1417.

Araki, T. 2001. Transition from vegetative to reproductive phase. Curr. Opin. Plant Biol. 4: 63–68.

Banno, K., S. Hayashi and K. Tanabe. 1986. Morphological and histological studies on flower bud differentiation and development in Japanese pear (Pyrus serotina Rehd.). J. Japan. Soc. Hort. Sci. 55: 258–265.

Blázquez, M. A. 2006. How floral meristems are built. Plant Mol. Biol. 60: 855–870.

Blázquez, M. A., L. N. Soowal, I. Lee and D. Weigel. 1997. LEAFY expression and flower initiation in Arabidopsis. Development 124: 3835–3844.

Blázquez, M. A., R. Green, O. Nilsson, M. R. Sussman and D. Weigel. 1998. Gibberellins promote flowering of Arabidopsis by activating the LEAFY promoter. Plant Cell 10: 791–800.

Boss, P. K., L. Sreekantan and M. R. Thomas. 2006. A grapevine TFL1 homologue can delay flowering and alter floral development when overexpressed in heterologous species. Funct. Plant Biol. 33: 31–41.

Boss, P. K., M. Vivier, S. Matsumoto, I. B. Dry and M. R. Thomas. 2001. A cDNA from grapevine (Vitis vinifera L.), which shows homology to AGAMOUS and SHATTERPROOF, is not only expressed in flowers but also throughout berry development. Plant Mol. Biol. 45: 541–553.

Bradley, D., R. Carpenter, L. Cospey, C. Vincent, S. Rothstein and E. Coen. 1996. Control of inflorescence architecture in Arabidopsis. Nature 379: 791–797.

Bradley, D., O. Ratcliffe, C. Vincent, C. Carpenter and E. Coen. 1997. Inflorescence commitment and architecture in Arabidopsis. Science 275: 80–83.

Busch, M. A., K. Bombles and D. Weigel. 1999. Activation of a floral homeotic gene in Arabidopsis. Science 285: 585–587.

Calonje, M., P. Cubas, J. M. Martinez-Zapater and M. J. Carmona. 2004. Floral meristem identity genes are expressed during tendril development in grapevine. Plant Physiol. 135: 1491–1501.

Campbell, C. S., M. J. Donoghue, B. G. Baldwin and M. F. Wojciechowski. 1995. Phylogenetic relationships in Maloideae (Rosaceae): evidence from sequences of the internal transcribed spacers of nuclear ribosomal DNA and its congruence with morphology. Amer. J. Bot. 82: 903–918.

Carmona, M. J., P. Cubas and J. M. Martinez-Zapater. 2002. FVI, the grapevine FLORICAULA/LEAFY ortholog, is expressed in meristic regions independently of their fate. Plant Physiol. 130: 68–77.

Eriksson, S., H. Höhlenius, T. Moritz and O. Nilsson. 2006. GA4 is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. Plant Cell 18: 2172–2181.

Esumi, T., R. Tao and K. Yonemori. 2005. Isolation of LEAFY and TERMINAL FLOWER 1 homologues from six fruit tree species in the subfamily Maloideae of the Rosaceae. Sex. Plant Reprod. 17: 277–287.

Esumi, T., R. Tao and K. Yonemori. 2007. Comparison of early inflorescence development between Japanese pear (Pyrus pyrifolia Nakai) and quince (Cydonia oblonga Mill.). J. Japan. Soc. Hortic. Sci. 76: 210–216.

Evans, R. C. and C. S. Campbell. 2002. The origin of the apple
subfamily (Maloideae; Rosaceae) is clarified by DNA sequence data from duplicated GBSSI genes. Amer. J. Bot. 89: 1478–1484.

Goto, K. 2001. *In situ* hybridization. p. 184–198 (In Japanese). In: K. Shimamoto and K. Okada (eds.) Moderu-shokubutsu no jikken protocol. Shujunsha, Tokyo.

Guo, X., Z. Zhao, J. Chen, X. Hu and D. Luo. 2006. A putative *CENTRORADIALIS/Terminal Flower 1*-like gene, *Ljcen1*, plays a role in phase transition in Lotus japonicus. Plant Physiol. 163: 436–444.

Jack, T. 2004. Molecular and genetic mechanisms of floral control. Plant Cell 16: 51–17.

Jensen, C. S., K. Salchert and K. K. Nielsen. 2001. *A Terminal Flower 1*-like gene from perennial ryegrass involved in floral transition and axillary meristem identity. Plant Physiol. 125: 1517–1528.

Jeong, D. H., S. K. Sung and G. An. 1999. Molecular cloning and characterization of CONSTANS-like cDNA clones of the Fuji apple. J. Plant Biol. 42: 23–31.

Joly, D., M. Perrin, C. Gertz, J. Kronenberger, G. Demangeat and J. E. Masson. 2004. Expression analysis of flowering genes from seedling-stage to vineyard life of grapevine cv. Riesling. Plant Sci. 166: 1427–1436.

Kotoda, N., H. Iwanami, S. Takahashi and K. Abe. 2006. Antisense expression of *MdTFL1*, a *TFL1*-like gene, reduces the juvenile phase in apple. J. Amer. Soc. Hort. Sci. 131: 74–81.

Kotoda, N. and M. Wada. 2005. *MdTFL1*, a *TFL1*-like gene of apple, retards the transition from the vegetative to reproductive phase in transgenic *Arabidopsis*. Plant Sci. 168: 95–104.

Kotoda, N., M. Wada, S. Komori, S. Kidou, K. Abe, T. Masuda and J. Soejima. 2000. Expression pattern of homologues of floral meristem identity genes *LFY* and *API* during flower development in apple. J. Amer. Soc. Hort. Sci. 125: 398–403.

Kotoda, N., M. Wada, S. Kusaba, Y. Kano-Murakami, T. Masuda and J. Soejima. 2002. Overexpression of *MdMADS5*, an *APETALA1*-like gene of apple, causes early flowering in transgenic *Arabidopsis*. Plant Sci. 162: 679–687.

Liljegren, S. J., C. Gustafson-Brown, A. Pinyopich, G. S. Ditta and M. F. Yanofsky. 1999. Interactions among *APETALA1*, *LEAFY*, and *Terminal Flower 1* specify meristem fate. Plant Cell 11: 1007–1018.

Ohshima, S., M. Murata, W. Sakamoto, Y. Ogura and F. Motoyoshi. 1997. Cloning and molecular analysis of the *Arabidopsis* gene *Terminal Flower 1*. Mol. Gen. Genet. 254: 186–194.

Ordidge, M., T. Chirrugwi, F. Tooko and N. H. Battey. 2005. *LEAFY*, *Terminal Flower 1* and *AGAMOUS* are functionally conserved but do not regulate terminal flower flowering and floral determinacy in *Impatiens balsamina*. Plant J. 44: 985–1000.

Parcy, F. 2005. Flowering: a time for integration. Int. J. Develop. Biol. 49: 585–593.

Parcy, F., K. Bomblies and D. Weigel. 2002. Interaction of *LEAFY*, *AGAMOUS* and *Terminal Flower 1* in maintaining floral meristem identity in *Arabidopsis*. Development 129: 2519–2527.

Parcy, F., O. Nilsson, M. A. Busch, I. Lee and D. Weigel. 1998. A genetic framework for floral patterning. Nature 395: 561–566.

Phipps, J. B., K. R. Robertson, J. R. Rohrer and P. G. Smith. 1991. Origins and evolution of subfam. Maloideae (Rosaceae). Syst. Bot. 16: 303–322.

Phipps, J. B., K. R. Robertson, P. G. Smith and J. R. Rohrer. 1990. A checklist of the subfamily Maloideae (Rosaceae). Can. J. Bot. 68: 2209–2269.

Pillitteri, L. J., C. J. Lovatt and L. L. Walling. 2004. Isolation and characterization of *Terminal Flower* homolog and its correlation with juvenility in citrus. Plant Physiol. 135: 1540–1551.

Ratcliffe, O. J., I. Amaya, C. A. Vincent, S. Rothstein, R. Carpenter, E. S. Coen and D. J. Bradley. 1998. A common mechanism controls the life cycle and architecture of plants. Development 125: 1609–1615.

Ratcliffe, O. J., D. J. Bradley and E. S. Coen. 1999. Separation of shoot and floral identity in *Arabidopsis*. Development 126: 1109–1120.

Robertson, K. R., J. B. Phipps and J. R. Rohrer. 1992. Summary of leaves in the genera of Maloideae (Rosaceae). Ann. Miss. Bot. Gard. 79: 81–94.

Robertson, K. R., J. B. Phipps, J. R. Rohrer and P. G. Smith. 1991. A synopsis of genera in Maloideae (Rosaceae). Syst. Bot. 16: 376–394.

Rohrer, J. R., K. R. Robertson and J. B. Phipps. 1991. Variation in structure among fruits of Maloideae (Rosaceae). Amer. J. Bot. 78: 1617–1635.

Rohrer, J. R., K. R. Robertson and J. B. Phipps. 1994. Floral morphology of Maloideae (Rosaceae) and its systematic relevance. Amer. J. Bot. 81: 574–581.

Shannon, S. and D. R. Meeks-Wagner. 1991. A mutation in the *Arabidopsis* *TFL1* gene affects inflorescence meristem development. Plant Cell 3: 877–892.

Shimura, I., Y. Ito and K. Seike. 1983. Intergeneric hybrid between Japanese pear and quince. J. Japan. Soc. Hort. Sci. 52: 243–249 (In Japanese).

Sung, S. K., G. H. Yu and G. An. 1999. Characterization of *MdMADS2*, a member of the *SQUAMOSA* subfamily of genes, in apple. Plant Physiol. 120: 969–978.

Sung, S. K., G. H. Yu, J. Nam, D. H. Jeong and G. An. 2000. Developmentally regulated expression of two MADS-box genes, *MdMADS3* and *MdMADS4*, in the morphogenesis of flower buds and fruit in apple. Planta 210: 519–528.

Trabut, L. 1916. PYRONIA, A hybrid between the pear and quince—Many new combinations might be made among the relatives of the pear. J. Hered. 7: 416–419.

Ushijima, K., H. Sassa, A. M. Dandekar, T. M. Gradziel, R. Tao and H. Hirano. 2003. Structural and transcriptional analysis of the self-incompatibility locus of almond: identification of a pollen-expressed F-box gene with haplotype-specific polymorphism. Plant Cell 15: 771–781.

van der Zwert, T. and N. F. Childers. 1982. The pear, cultivars to marketing. Horticultural Publications, Gainesville.

Wada, M., Q. F. Cao, N. Kotoda, J. Soejima and T. Masuda. 2002. Apple has two orthologues of *FLORICAULA/LEAFY* involved in flowering. Plant Mol. Biol. 49: 567–577.

Wagner, D., R. W. M. Sablowski and E. M. Meyerowitz. 1999. Transcriptional activation of *APETALA1* by *LEAFY*. Science 285: 582–584.

Walton, E. F., E. Podivinsky and R. M. Wu. 2001. Bimodal patterns of floral gene expression over the two seasons that kiwifruit flowers develop. Physiol. Plant. 111: 396–404.

Weigel, D., J. Alvarez, D. R. Smyth, M. F. Yanofsky and E. M. Meyerowitz. 1992. *LEAFY* controls floral meristem identity in *Arabidopsis*. Cell 69: 843–859.

Weigel, D. and O. Nilsson. 1995. A developmental switch sufficient for flower initiation in diverse plants. Nature 377: 495–500.

Westwood, M. N. 1993. Temperate-zone pomology physiology and culture. Timber Press Inc., Portland.
William, D. A., Y. Su, M. R. Smith, M. Lu, D. A. Baldwin and D. Wagner. 2004. Genomic identification of direct target genes of LEAFY. Proc. Natl. Acad. Sci. USA 101: 1775–1780.

Yeung, K., T. Seitz, S. Li, P. Janosch, B. Mcferran, C. Kaiser, F. Fee, K. D. Katsanakis, D. W. Rose, H. Mischak, J. M. Sedivy and W. Kolch. 1999. Suppression of Raf-1 kinase activity and MAP kinase signaling by RKIP. Nature 401: 173–177.

Zhang, S., W. Hu, L. Wang, C. Lin, B. Cong, C. Sun and D. Luo. 2005. TFL1/CEN-like genes control intercalary meristem activity and phase transition in rice. Plant Sci. 168: 1393–1408.