

FLOWERING NEWSLETTER REVIEW

# The regulation of seasonal flowering in the Rosaceae

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Received 19 April 2013; Revised 19 June 2013; Accepted 24 June 2013

## Abstract

Molecular mechanisms regulating the flowering process have been extensively studied in model annual plants; in perennials, however, understanding of the molecular mechanisms controlling flowering has just started to emerge. Here we review the current state of flowering research in perennial plants of the rose family (Rosaceae), which is one of the most economically important families of horticultural plants. Strawberry (*Fragaria* spp.), raspberry (*Rubus* spp.), rose (*Rosa* spp.), and apple (*Malus* spp.) are used to illustrate how photoperiod and temperature control seasonal flowering in rosaceous crops. We highlight recent molecular studies which have revealed homologues of *TERMINAL FLOWER1* (*TFL1*) to be major regulators of both the juvenile to adult, and the vegetative to reproductive transitions in various rosaceous species. Additionally, recent advances in understanding of the regulation of *TFL1* are discussed.

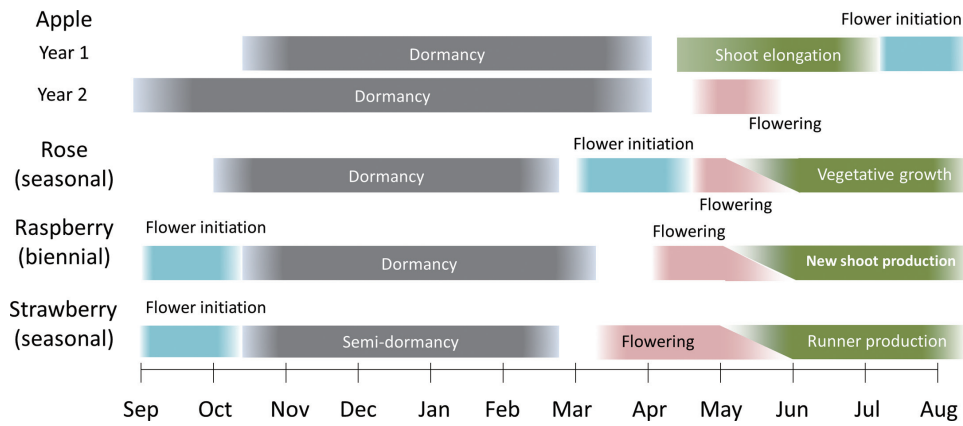
**Key words:** Apple, perennial, raspberry, rose, strawberry, *TFL1*

## Typical yearly growth cycle

The Rosaceae family includes several economically important subfamilies. Rosoideae includes the soft fruits, strawberry (*Fragaria* spp.) and raspberry (*Rubus* spp.), and ornamentals such as rose (*Rosa* spp.). Spiraeoideae contains the genus *Prunus* (tribe Amygdaleae), which includes stone fruits species such as peach (*P. persica*), plum (*P. domestica*), and sweet cherry (*P. avium*). Also in the Spiraeoideae is the subtribe Pyrinae, which contains the pome fruits apple (*Malus* spp.) and pear (*Pyrus* spp.) (Potter *et al.*, 2007). The Rosaceae contains various polycarpic perennial species with different growth forms, from herbaceous rosette plants to deciduous trees. However, many similarities can be found in their yearly growth cycles (Fig. 1). Typically, flower initiation and flowering occur in separate growing seasons in species including strawberry, raspberry, and fruit trees in the genus *Malus*, *Pyrus*, and *Prunus*. However, in roses, the process of flower initiation and flowering can occur within the same season, as it can in perpetual flowering strawberry and annual (primocane) raspberry (Battey *et al.*, 1998; Carew *et al.*, 2000;

Iwata *et al.*, 2012). The length of the juvenile period also varies among the species. For example, strawberry reaches competence to flower in its first season, whereas, in fruit trees, a juvenile period typically lasts for several years (Visser, 1964).

Morphologically, strawberry grows as a herbaceous rosette, whereas raspberry and rose are shrubs. A typical feature of June-bearing strawberry and biennial raspberry cultivars, as well as old rose cultivars, is that they form vegetative shoots during summer and these shoots produce flowers or flower-bearing shoots in the next spring to early summer (Fig. 1). However, each species has specific features in its yearly growth cycle. In strawberry, the vegetative phase is characterized by the formation of asexual reproductive organs, namely stolons (runners), from the axillary buds. In autumn, stolon formation ceases, axillary buds differentiate into axillary leaf rosettes (branch crowns), terminal inflorescence initiation takes place in the shoot apical meristem (SAM) of the main shoot as well as axillary leaf rosettes on upper nodes, and growth is slowed down as winter approaches. In spring, after



**Fig. 1.** A comparison of the typical growth cycles of rosaceous plants in the northern hemisphere. Seasonal flowering strawberry and biennial raspberry initiate flowers from late summer to autumn under short days and cool temperatures, whereas apples and seasonal roses initiate flowers in early summer or in early spring, respectively. Flowering (blooming) takes place in the next spring or summer in all species, except rose in which flower initiation and flowering completes in the same year. Vegetative growth takes place from spring to summer, and plants enter dormancy from autumn, except strawberries in which semi-dormancy is observed. During the dormancy period, visible growth is suppressed whereas flower organs continue to develop within the buds in favourable conditions.

winter chilling, inflorescences grow out and bear fruits. At the same time, the newly formed uppermost axillary shoots initiate the next growth cycle as compressed vegetative branches (crowns) (Guttridge, 1985; Battey *et al.*, 1998; Kurokura *et al.*, 2005).

The growth cycle of raspberry is characterized by the production of elongating above-ground shoots (canes) from roots every year. During the first year, in the biennial floricanes cultivars, vegetative growth of the canes continues until autumn, when flower initiation begins in the axillary buds and plants enter dormancy (Fig. 1; Carew *et al.*, 2000). Chilling temperature in winter breaks dormancy, and flower-bearing shoots burst in the following spring to produce fruits (Carew *et al.*, 2000). After fruit production, these canes die and newly formed canes continue the next biennial cycle.

Seasonal flowering rose species have an indeterminate growth habit, and inflorescences are formed at the top of lateral shoots. Vegetative growth dominates from summer to autumn, when the floral transition begins in the axillary buds of newly developed vegetative shoots; then, after flower initiation in spring, these axillary buds bloom so that the process of flower initiation to flowering is completed within a few months (Fig. 1; Foucher *et al.*, 2008; Iwata *et al.*, 2012; Bendahmane *et al.*, 2013).

In contrast to seasonal flowering, the continuous flowering habit (also called recurrent, perpetual, everbearing, or remontant flowering) is also known in rose and strawberry. The flowering phenotype of roses and strawberries classified in this category may vary from continuous flowering to occasional flowering (Stewart and Folta, 2010; Iwata *et al.*, 2012; Heide *et al.*, 2013). Unlike seasonal flowering types which have months of juvenile phase, perpetual flowering rose and strawberry start to flower in the first growing season and initiate new flowers until late autumn (Sønsteby and Heide, 2007; Foucher *et al.*, 2008). The primocane raspberry cultivars show some similarities with perpetual flowering roses and strawberries. They have annual canes which are induced to

flower in summer, and flowers grow out immediately so that fruiting occurs in the autumn (Carew *et al.*, 2000; Sønsteby and Heide, 2009).

Apple, pear, and various *Prunus* species such as cherry, plum, and peach are perennial woody plants which are adapted to temperate climates. These fruit trees typically bloom in early spring from flower buds developed during the previous growing season (Grainger, 1939; Wilkie *et al.*, 2008). Among these species, the growth cycle has been best characterized in apple (see Wilkie *et al.*, 2008, for example). After bud break in spring, a ‘bourse shoot’ (continuation shoot) starts to elongate as a sympodial branch within the youngest axillary bud on the determinate inflorescence (Foster *et al.*, 2003). The majority of terminal apical meristems on the bourse shoots develop vegetatively (95% in ‘Royal Gala’ in New Zealand; Foster *et al.*, 2003), and these shoots show growth attenuation in late spring. The terminal meristems are converted into inflorescence meristems in early summer and continue to develop flower meristems until October (Foster *et al.*, 2003). In the Japanese climate, deep endodormancy is observed at the beginning of October and continues to early November, and then is released rapidly (Kuroda and Sagisaka, 2001). Finally, after winter, flowers emerge in spring (Fig. 1; Heide and Prestrud, 2005).

## Environmental regulation of flowering in Rosaceae

### *Regulation of flowering by photoperiod*

Strawberry and raspberry can be placed into two groups according to their flowering habits and sensitivity to photoperiod (Carew *et al.*, 2000; Hytönen and Elomaa 2011). June-bearing strawberry cultivars and biennial (‘floricane’) raspberries are facultative short-day (SD) plants, in which flowering is regulated by the interaction of photoperiod and temperature (see, for example, Heide, 1977; Carew *et al.*,

2000; Heide and Sønsteby, 2007). In June-bearing strawberries, there is a large variation in the photoperiodic responses. Typically, the longest photoperiod in which flower induction occurs (i.e. the critical photoperiod) is relatively long, between 12 h and 16 h, and the number of SD cycles needed for flower induction varies between seven and 35 (Heide, 1977; Guttridge, 1985; Heide and Sønsteby, 2007). In biennial raspberry, a critical photoperiod of ~15 h and acceleration of flower initiation by a shorter photoperiod have been reported (Sønsteby and Heide, 2008).

The terminology used in the literature on the photoperiodic responses of perpetual flowering strawberry cultivars is rather confusing. Some reports classify two types of perpetual flowering habit; one is 'everbearing' in which flower initiation takes place mainly under long days (LDs), and another is 'day-neutral' which flower at the same rate irrespective of photoperiod (reviewed by Stewart and Folta, 2010; Heide *et al.*, 2013). It has been reported that LDs advance flower initiation in perpetual flowering *Fragaria vesca* and cultivated strawberry, especially under higher temperatures (Sønsteby and Heide, 2007; Mouhu *et al.*, 2009). However, after flower induction, perpetual flowering *F. vesca* continuously produces new inflorescences irrespective of photoperiod (Koskela *et al.*, 2012). Therefore, 'everbearing' and 'day-neutral' can be used to describe the same plant at different growth stages. Similar to perpetual flowering strawberries, a few studies on annual ('primocane') raspberries suggest that they are also LD plants (Carew *et al.*, 2003; Sønsteby and Heide, 2009).

The photoperiodic regulation of flowering in roses and apples is not well understood, although they are generally considered as day-neutral plants (Zieslin and Moe, 1985; Wilkie *et al.*, 2008). However, careful analysis in controlled conditions should be carried out, since a few studies suggest that photoperiod may affect flower induction in both species. Moe (1972) reported that LDs advanced flower initiation in some perpetual flowering rose cultivars. Also in apple cv. Jonathan, the increase of photoperiod from 8 h to 12 or 14 h increased flower bud formation, whereas a 16 h photoperiod repressed flower induction compared with shorter photoperiods (Stahly and Piringer, 1962).

#### Regulation of flowering by temperature

As mentioned in the previous section, an interaction between photoperiod and temperature determines flower induction in both strawberries and raspberries. In fact, the temperature effect is dominant over photoperiod, and flower induction only occurs within a certain permissive temperature range. In seasonal flowering *F. vesca* as well as biennial raspberries, temperatures above ~18 °C inhibit flower induction, SD conditions are required at intermediate temperatures, whereas at cool temperatures of ~10 °C plants become day-neutral (Heide and Sønsteby, 2007; Sønsteby and Heide, 2008). Similar trends have been shown in seasonal flowering octoploid strawberries, although the effect of temperature seems to be quantitative and there is variation in the photoperiod-insensitive temperature range between cultivars (Heide, 1977; Bradford *et al.*, 2010). It was also found that chilling

temperatures <6 °C repress flowering (Verheul *et al.*, 2006). Additionally, long exposure to chilling contributes to seasonality of flowering, since it changes the physiological status of the plants so that further flower initiation is prevented even under an inductive photoperiod in the spring (Guttridge, 1985).

In perpetual flowering strawberries, the effect of photoperiod on flower induction also depends on temperature. They have an obligatory LD requirement for flower induction at high temperature of ~27 °C. However, at lower temperatures, the effect of photoperiod becomes quantitative, and, at 9 °C, plants are almost day-neutral (Bradford *et al.*, 2010). Similar to perpetual flowering strawberries, high temperature has been shown to promote flowering in annual raspberries under LDs (Carew *et al.*, 2003; Sønsteby and Heide, 2009).

Temperature may affect flower induction in apple. An increase in temperature from 13 °C to 20 °C for 6–7 weeks after bloom enhanced flower bud formation, whereas a higher temperature of 24 °C was found to delay floral development (Tromp, 1980; Zhu *et al.*, 1997). Temperature also controls dormancy in apple and pear: low temperatures induce dormancy, with no effect of photoperiod (SDs or LDs); lack of sufficient chilling during winter can delay release from dormancy and consequently flowering time (Heide and Prestrud, 2005). *Prunus* species show a pronounced photoperiod/temperature interaction in the control of growth cessation and dormancy (Heide, 2008), and, in raspberry, a photoperiod–temperature interaction in the control of dormancy onset was reported by Williams (1959). In seasonal flowering *R. × wichurana*, low temperature (vernalization) during winter is probably needed before flowers can be initiated in the spring (Foucher *et al.*, 2008).

## Molecular control of flowering in model plants

### Photoperiodic flowering

Photoperiodic control of flowering is mediated through a genetic pathway usually called the photoperiodic pathway. In this pathway, the coincidence of a photoperiodic signal perceived by photoreceptors and internal gene expression during a specific phase determines flowering (external coincidence model; reviewed by Searle and Coupland, 2004). In *Arabidopsis*, *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) are key genes directly regulating photoperiodic flowering, and related genes have been found to play similar roles in many other species (see Pin and Nilsson, 2012, for example).

*CO* is a transcription factor which acts as a time keeper. In *Arabidopsis*, *CO* mRNA expression is controlled by the circadian clock so that its expression level oscillates in a diurnal manner, with a peak in the afternoon (Suarez-Lopez *et al.*, 2001). Since *CO* protein degrades in the darkness due to ubiquitinylation, it only accumulates under LDs when its mRNA expression coincides with light (Valverde *et al.*, 2004). As a result, in LDs, accumulated *CO* protein activates *FT* expression and leads to early flowering (Suarez-Lopez *et al.*, 2001; Valverde *et al.*, 2004). The *CO–FT* module also

controls photoperiodic flowering in rice and poplar, but in the SD plant rice flowering is induced when the *CO* homologue *Hd1a* peaks during the night (Kojima *et al.*, 2002; Böhlenius *et al.*, 2006).

FT, a member of the phosphatidylethanolamine-binding protein (PEBP) family, is expressed in the vascular tissues in leaves and transported to the SAM (Lin *et al.*, 2007; Tamaki *et al.*, 2007). In the SAM, FT interacts with a bZIP transcription factor FD, to induce flowering through the floral meristem identity genes *APETALA1* (*API*) and *FRUITFUL* (*FUL*) (Abe *et al.*, 2005; Wigge *et al.*, 2005), and, at least in rice, the interaction of the FT homologue Hd3a with FD also requires 14-3-3 proteins (Taoka *et al.*, 2011).

#### Regulation of flowering by vernalization and ambient temperature

Many plant species require a long period of cold (i.e. vernalization) before they can be induced to flower. In *Arabidopsis*, a MADS-box gene, *FLOWERING LOCUS C* (*FLC*), is the key flowering repressor regulated by vernalization (see Kim *et al.*, 2009, for example). *FLC* represses flowering in a protein complex with another MADS-box protein, SHORT VEGETATIVE PHASE (*SVP*), by binding to the regulatory sequences of flowering time genes *FT*, *FD*, and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) (Searle *et al.*, 2006; Li *et al.*, 2008). In wheat and sugar beet, different repressors, *VRN2* and *BvFT1*, respectively, have been shown to mediate the vernalization requirement by targeting flower-inducing *FT* homologues (Distelfeld *et al.*, 2009; Pin *et al.*, 2010).

In winter-annual *Arabidopsis* accessions, the high expression level of *FLC* is maintained by functional *FRIGIDA* (*FRI*) (Shindo *et al.*, 2005; Geraldo *et al.*, 2009). Winter chilling causes the silencing of *FLC* by modification of chromatin structure in a dose-dependent manner and enables flower initiation to take place in spring (Saleh *et al.*, 2008; Angel *et al.*, 2011). During vernalization, the transcription of the antisense of *FLC*, *COOLAIR*, is up-regulated to mediate the transcriptional silencing of the sense transcripts; then a protein complex, Polycomb repressor complex 2 (*PRC2*), deposits repressive histone marks including histone H3 lysine 27 trimethylation (H3K27me3) on the *FLC* locus, and subsequently *FLC* is stably silenced by LIKE HETEROCHROMATIN PROTEIN1 (*LHP1*) and VERNALIZATION (*VRN2*) (reviewed by Alexandre and Hennig, 2008; Ietswaart *et al.*, 2012).

Not only chilling temperature but also warmer ambient temperature affects flowering time by controlling *FT* mRNA expression levels (Samach and Wigge, 2005). In *Arabidopsis*, genetic studies showed that late flowering at 16 °C, compared with 23 °C, is caused by *FCA*-dependent activation of *SVP* at cooler temperatures. Consequently, *SVP* binds to the CARG-box elements in the *FT* and *SOC1* promoters to repress their expression (Lee *et al.*, 2007; Li *et al.*, 2008). Recent study has revealed that the temperature-dependent change in the proportion of histone variant H2A.Z relative to H2A in chromatin is a key mechanism regulating gene expression

in response to small changes in temperature in the plant (Kumar and Wigge, 2010). H2A.Z is involved in the warm temperature activation of *FT* transcription, since it controls the accessibility of the *FT* promoter to the transcription factor PHYTOCHROME INTERACTING FACTOR 4 (*PIF4*) (Kumar *et al.*, 2012).

#### Regulation of meristem identity by *TERMINAL FLOWER 1*

Flowering is regulated not only by promoters but also by repressors. The *Arabidopsis tfl1* mutant flowers early by making a determinate inflorescence, in contrast to the wild type where the inflorescence is indeterminate (Bradley *et al.*, 1997). *TFL1* is classified as a PEBP similar to the flowering promoter *FT* (Karlgrén *et al.*, 2011). However, unlike *FT*, *TFL1* functions as a flowering inhibitor (Kobayashi *et al.*, 1999). Both *FT* and *TFL1* can bind to *FD*, and the *TFL1*–*FD* complex opposes the function of the *FT*–*FD* complex in the nucleus (Hanano and Goto, 2011). In *Arabidopsis*, *TFL1* is expressed at a low level in the SAM during the vegetative stage, while strong expression in the central zone of the inflorescence meristem represses *API* and *LFY* to maintain the indeterminate meristem (Liljegren *et al.*, 1999; Ratcliffe *et al.*, 1999). In floral meristems, on the other hand, *API* has been shown to repress *TFL1* by direct binding to the CARG-box element downstream of the *TFL1* coding sequence (Kaufmann *et al.*, 2010). In *Arabis alpina*, a perennial relative of *Arabidopsis*, constant expression of *TFL1* in the SAM of young shoots prevents phase transition even after vernalization (Wang *et al.*, 2011). Thus, *TFL1* may have a general role in the control of phase change, but its detailed function may differ, depending on the species (Ordidge *et al.*, 2005; Mohamed *et al.*, 2010).

### TFL1 is the major repressor of flowering in Rosaceae

#### Seasonal flowering versus perpetual flowering

Both seasonal and perpetual flowering cultivars are present in *Fragaria* and *Rosa*. Early genetic studies confirmed that a single dominant gene, *SEASONAL FLOWERING LOCUS* (*SFL*) or *RECURRENT BLOOMING* (*RB*), causes seasonal flowering in both *F. vesca* and *Rosa*, respectively, whereas plants with recessive alleles flower perpetually (Brown and Wareing, 1965; Semeniuk, 1971). Gaston *et al.* (2013) found that in cultivated strawberry, a single major quantitative trait locus (QTL), which is not orthologous to *SFL*, causes perpetual flowering. However, multigenic control has also been proposed (Weebadde *et al.*, 2008).

Recent studies have addressed the molecular mechanisms regulating seasonal flowering in non-rosaceous perennials. In *A. alpina*, dynamic regulation of an *FLC* homologue *PEP1* controls seasonal flowering, whereas, in poplar, the interplay of two *FT* homologues is involved (Böhlenius *et al.*, 2006; Wang *et al.*, 2009; Hsu *et al.*, 2011). Recently, Iwata *et al.* (2012) reported that both *SFL* and *RB* encode the homologue of *TFL1*. They found that a rose *TFL1* homologue, *KSN*,

was not expressed in the perpetual flowering roses because of a retrotransposon insertion. Moreover, they found a 2 bp deletion in the first exon of *FvTFL1* in *F. vesca* (Iwata *et al.*, 2012). Functional characterization of *FvTFL1* confirmed that it is a major floral repressor which causes seasonal flowering, whereas mutation in this gene leads to perpetual flowering (Koskela *et al.*, 2012). In addition, the silencing of *TFL1* homologues in apple and pear also caused perpetual flowering in these species (Flachowsky *et al.*, 2012; Freiman *et al.*, 2012), suggesting that TFL1 is a major floral repressor which contributes to the regulation of seasonal flowering within the yearly growth cycle in the Rosaceae.

### **TFL1 expression and the regulation of the perennial growth cycle**

The following data indicate that down-regulation of *TFL1* homologues in rosaceous species correlates with vegetative to reproductive transitions during the annual cycle.

In *F. vesca*, *FvTFL1* is strongly expressed during the vegetative stage in the shoot apex, young leaf initials, and vascular tissues (Koskela *et al.*, 2012). Experiments in controlled conditions revealed the strong down-regulation of *FvTFL1* in the shoot apex under flower-inducing SDs, and this was followed by the up-regulation of floral meristem identity genes *FvAPI* and *FvFUL1*. After plants were transferred back to non-inductive LD conditions, a high level of *FvTFL1* mRNA expression was again detected in the apices of axillary shoots. Therefore, Koskela *et al.* (2012) proposed that flower initiation only occurs in the autumn when *FvTFL1* is down-regulated by SDs, whereas high *FvTFL1* in the spring may secure the production of new vegetative axillary shoots for the next growth cycle.

In apple, *MdTFL1* expression is detected in the rib meristem zone of the shoot apex throughout the vegetative growth phase in both juvenile and adult plants (Mimida *et al.*, 2011a). In the annual cycle of the adult plant, expression of *MdTFL1* is observed in the bourse bud primordium before bud break in early spring, and its expression is gradually up-regulated in the shoot apex of the bourse shoot throughout the vegetative growth phase. In early summer, when flower initiation occurs, *MdTFL1* is no longer detectable, and the expression levels of *MdAPI*, *AFL* (*LFY* homologue), and *MdFT* are up-regulated in the terminal shoot apex (Hättasch *et al.*, 2008; Mimida *et al.*, 2011a). At the same time, the SAM ceases to produce new leaf primordia, shoot growth stops, and floral differentiation begins.

In *R.×wichurana*, the highest expression of *KSN* was observed in the tip of the vegetative shoots formed after blooming in spring (Iwata *et al.*, 2012). Down-regulation of *KSN* during winter chilling correlated with the activation of rose *FT* and *LFY* homologues during flower initiation the following spring.

Taken together, in three rosaceous species tested so far, the expression maxima of *TFL1* mRNA is detected in the vegetative SAM, whereas its down-regulation precedes flower initiation. These findings suggest that the seasonal regulation

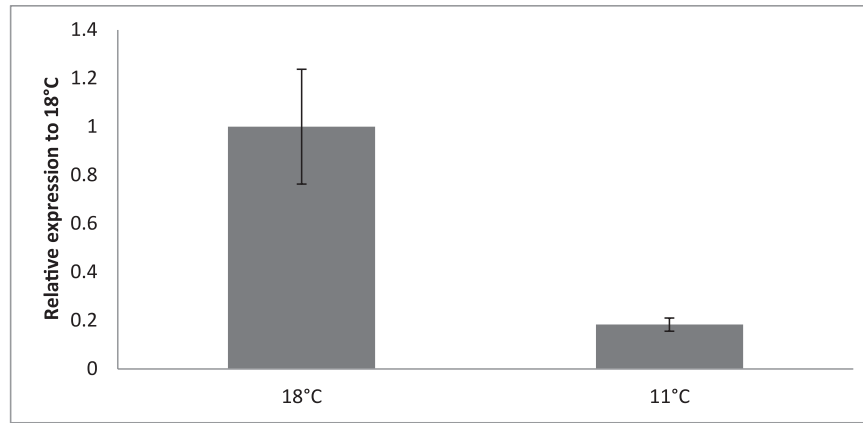
of the *TFL1* mRNA level is a key mechanism, which allows timely floral development in rosaceous species.

### *Role of other genes and plant hormones*

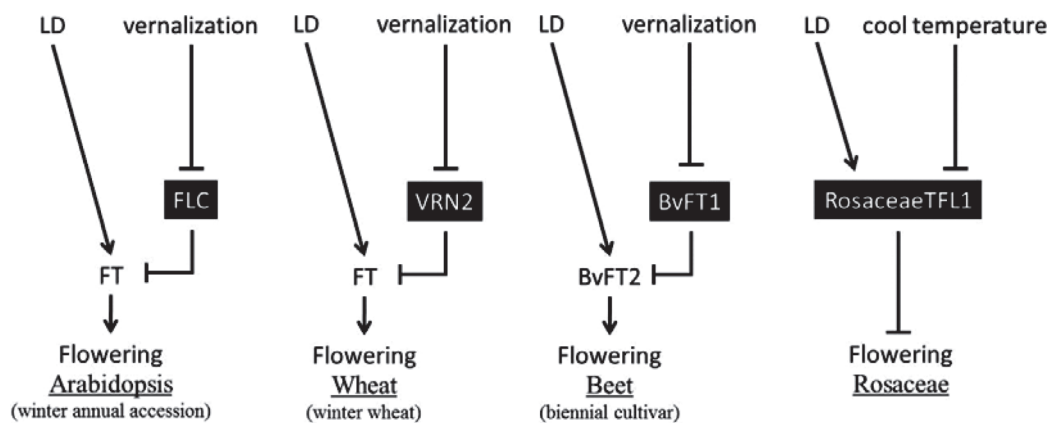
The function of *FvFT1* as a floral promoter was confirmed in a perpetual flowering accession of *F. vesca*, since RNAi (RNA interference) silencing of *FvFT1* delayed the activation of *FvAPI* in the shoot apex and caused late flowering (Koskela *et al.*, 2012). This is consistent with the finding that the overexpression of *AtFT/MdFT* in apple, or of poplar *FTI* in plum, causes precocious flowering (Kotoda *et al.*, 2010; Srinivasan *et al.*, 2012). In both perpetual and seasonal flowering *F. vesca*, *FvFT1* is only expressed in LDs (Koskela *et al.*, 2012), which fits with its role as a floral promoter in perpetual flowering *F. vesca*, where LDs are associated with flower initiation. However, in seasonal flowering *F. vesca*, where flower initiation only occurs in SDs, it means that *FvFT1* expression is negatively correlated with flower initiation. This suggests that *FvFT1* may inhibit flowering in this accession. Consistent with this idea, a recent modelling study suggests that FT may activate *TFL1* in the *Arabidopsis* inflorescence meristem (Jaeger *et al.*, 2013). Further studies are needed to explore whether this occurs in the Rosaceae. An alternative hypothesis is that the strong repressor *FvTFL1* overrides the floral promoter function of *FvFT1* under LDs in seasonal flowering *F. vesca*. This is possible since both FT and TFL1 can bind FD transcription factor to control API in opposite ways (Hanano and Goto, 2011). In addition, the *FT/TFL1* ratio was shown to control flowering time as well as plant architecture in tomato and maize (Shalit *et al.*, 2009; Danilevskaya *et al.*, 2010).

*Fragaria vesca* and apple genomes contain several candidate genes homologous to *Arabidopsis* vernalization and ambient temperature pathway genes, but no homologues for *FLC* have been found (Mouhu *et al.*, 2009; Guitton *et al.*, 2012). However, another repressor, *FvTFL1*, may control temperature-dependent flowering. In fact, a cool temperature of 11 °C down-regulates *FvTFL1* even under LDs in seasonal flowering *F. vesca*, where cool temperature, as well as SDs, promotes flowering (Figs 2, 3). Therefore, the role of the recently identified ambient temperature-sensing mechanism (Kumar and Wigge, 2010; Kumar *et al.*, 2012) should be analysed in relation to the control of *FvTFL1* expression.

Studies in apple and pear indicate that hormone balance may control flowering in the Pyrinae. Bending the lateral branch to a horizontal position can induce an increase in flower bud production on the shoots of apple and Japanese pear (Banno *et al.*, 1985a; Han *et al.*, 2007), possibly owing to the induction of high cytokinin/auxin ratios in the lateral buds (Banno *et al.*, 1985b). Recently, the analysis of transgenic apple containing an *MdTFL1* promoter::*GUS* ( $\beta$ -glucuronidase) construct showed that these two hormones can induce the expression of *MdTFL1* in shoot apices (Mimida *et al.*, 2011b). Application of exogenous gibberellin (GA) has been shown to inhibit flowering in apple, strawberry, and rose (Tromp, 1982; Guttridge, 1985; Roberts *et al.*, 1999; Foucher *et al.*, 2008; Bangerth, 2009), in contrast to



**Fig. 2.** Relative expression of *FvTFL1* mRNA at two temperatures. Samples were taken from the SAM of seasonal flowering *Fragaria vesca* grown in a 18h light/6h dark photoperiod at 18 °C or at 11 °C for 32 d. Only plants grown at 11 °C flowered. Bars indicate  $\pm$ SEM.



**Fig. 3.** A comparison of flowering regulation pathways. Pathways are simplified to highlight the photoperiod and temperature regulation with emphasis on *FLC* and those genes with an equivalent role. *Arabidopsis FLC* and its equivalent genes (black rectangle) in wheat and beet suppress the expression of *FT*-like genes before they are silenced by vernalization. In the rosaceous model *F. vesca*, *TFL1* homologues are expressed under LDs and warm conditions, whereas cool temperature down-regulates *TFL1* expression. Similar regulation may exist in other rosaceous species, based on their growth habits and reported patterns of gene expression. Arrowhead, promotion; bar, suppression.

*Arabidopsis*, in which GA promotes flowering (Blázquez et al., 1998; Moon et al., 2003; Hisamatsu and King, 2008; Li et al., 2008). Although the relationship of GA and apple *FLO/LFY* (*AFL*) remains unclear, the overexpression of either *LFY* or *AFL* was not found to promote flower initiation in apple (Flachowsky et al., 2010; Masato Wada, personal communication). Another possibility is that GA inhibits flowering through *TFL1*-like genes, since, in rose, the inhibitory effect of GA on flowering is observed only in seasonal flowering roses (Roberts et al., 1999) which contain functional *RoKSN* (Foucher et al., 2008; Remay et al., 2009; Iwata et al., 2012). Indeed, Randoux et al. (2012) showed that exogenous GA<sub>3</sub> up-regulates *RoKSN* and suggested that GA may inhibit flowering in roses in spring by promoting the expression of *RoKSN*. However, Mimida et al. (2011b) were unable to activate *MdTFL1* *in vitro* by application of GA in growth media. In addition, QTL analysis showed that the phenomenon of biennial bearing is related to loci of the hormones auxin

and GA, but not to the genes related to flowering such as *MdTFL1/MdFT* (Guitton et al., 2012).

#### Control of juvenility in rosaceous trees

Several studies have suggested that *TFL1* and *FT* may control juvenility in rosaceous fruit trees. Consistent with findings in other perennials (Mohamed et al., 2010; R. Wang et al., 2011), the silencing of *TFL1* in apple and pear causes a short juvenile phase, with flower initiation occurring in small *in vitro* plantlets in some cases (Kotoda et al., 2006; Flachowsky et al., 2012; Freiman et al., 2012). Similar phenotypes have also been observed in apples overexpressing *FT* (Kotoda et al., 2010). These results suggest that *TFL1* and *FT* homologues, or the *TFL1/FT* ratio, have a role in the control of the juvenile to adult transition in rosaceous fruit trees. Also GA is probably involved in the control of juvenility, since dwarf apple trees have a shorter juvenile phase and

exogenous GA application inhibits flowering and promotes vegetative growth in apple (Bangerth, 2009). MiR156 controls juvenility in *Populus* (Wang *et al.*, 2011), and its role in the Rosaceae should be studied.

#### Regulation of dormancy and its effect on flowering time

Flowering time of many rosaceous species is affected by the period of winter dormancy, and especially by the timing of dormancy release, which depends on a genotype-specific chilling requirement (Heide and Prestrud, 2005; Ruiz *et al.*, 2007; Leida *et al.*, 2012). Therefore, dormancy has been intensively studied in a number of rosaceous species. During endodormancy, which lasts 2–3 months in apple, bud break is repressed, but the floral meristems continue to develop (Fig. 1; Kuroda *et al.*, 2001; Dr Komori, Iwate University, personal communication). Strawberries, in contrast, do not have such ‘true dormancy’. However, prolonged exposure to SDs strongly reduces the growth potential, and cold temperature is needed to restore normal growth vigour. Therefore, this state has been described as ‘semi-dormancy’ (Guttridge, 1985; Sønsteby and Heide, 2006).

It has been reported that a high chilling requirement correlates with late flowering in peach (Leida *et al.*, 2012). The breakthrough in this topic was the characterization of a dormancy-insensitive peach (*Prunus persica*) mutant, *evergrowing* (*EVG*; previously known as ‘Evergreen’; Werner and Okie, 1998). The mutant has a deletion of a tandem repeat of six *SVP/AGL24*-like MADS-box genes, called *dormancy-associated MADS-box* (*DAM*) genes (Bielenberg *et al.*, 2008). *DAM*-like genes have also been found in other rosaceous plants such as Japanese apricot (*Prunus mume* Sieb. et Zucc.), raspberry, and Japanese pear (*Pyrus pyrifolia*) (Mazzitelli *et al.*, 2007; Yamane *et al.*, 2008; Ubi *et al.*, 2010). The expression of these *DAM*-like genes is up-regulated by cool temperature and later down-regulated by extensive exposure to chilling so that their down-regulation may correlate with the breakage of endodormancy. The ectopic expression of Japanese apricot *DAM6* in poplar was able to induce growth cessation, terminal bud set, and lateral bud endodormancy after exposure to LD conditions (16/8 h of light/dark, 22 °C) (Sasaki *et al.*, 2011). Recently, it was shown that the silencing of peach *DAM6* is preceded by changes in the methylation status of histone H3K27, as in the silencing of *FLC* and other genes which regulate vernalization in *Arabidopsis* (Leida *et al.*, 2012).

In other trees such as aspen (*Populus* spp.) and Norway spruce (*Picea abies*), the expression of genes homologous to *FT* correlates with growth cessation and bud set in addition to flowering (Böhlenius *et al.*, 2006; Gyllenstrand *et al.*, 2007; Hsu *et al.*, 2011). Moreover, the overexpression of *Populus FT1* in plum trees rendered them unable to enter dormancy (Srinivasan *et al.*, 2012). Further studies are needed to explore possible connections between the *FT* and *DAM* genes and their relationship to *TFL1* in the control of seasonal flowering and the annual growth cycle in the Rosaceae.

## Conclusion and future prospects

Unlike monocarpic annuals, perennial rosaceous plants repeat the cycles of vegetative/reproductive growth every year, by responding to various environmental signals. Among these, photoperiod and temperature are the best studied factors. Recent studies on rosaceous plants suggest that *TFL1* homologues in these plants have important roles in juvenile to adult phase change, and the vegetative to reproductive transition. We propose that *TFL1* is a major repressor of flowering in the Rosaceae, similar to *FLC*, *VRN2*, and *BvFT1* in *Arabidopsis*, wheat, and sugar beet, respectively (Fig. 3). There is evidence that combinations of environmental signals and internal cues (plant hormones) control the expression of *TFL1*, but it is necessary to clarify the mechanisms which regulate its expression in the Rosaceae, for a better understanding of the way in which the perennial growth cycle is controlled in this family.

The role of *FT*-like genes in relation to *TFL1*-like genes is an important issue, since potential inhibitors and promoters of flowering are expressed at the same time in *Fragaria*. To study this, detailed analyses of expression levels of the two genes, as well as the analysis of transgenic plants with different expression levels of each gene, are required. We are currently working on a flowering time analysis of *FT* transgenic *F. vesca* with a seasonal or perpetual flowering background. These studies will reveal the contribution of *FT*-like genes to flowering regulation in this rosaceous model.

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