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Genetic and Environmental Regulation of Flowering and Runnering in Strawberry

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ABSTRACT

Cultivated strawberry (*Fragaria* × *ananassa* Duch.) is one of the most important berry crops worldwide. Its wild relative, woodland strawberry (*Fragaria vesca* L.) is also scientifically important, since recent development of molecular tools including genetic transformation methods, genetic maps and genome sequence is making it as one of the leading perennial model plants. Environmental regulation of strawberry reproductive development including flowering and vegetative reproduction through runners has been studied for almost hundred years and is known quite in detail. Most strawberries require short photoperiod and/or low temperature for the induction of flowering, whereas runnering is activated by opposite environmental signals. On the contrary, everbearing genotypes flower continu-ously in long day conditions. Some groundwork on characterization of molecular pathways controlling runnering and the induction of flowering has been done. In these studies, dozens of candidate flowering genes have been identified, the expression patterns for selected genes have been analyzed, and a marker gene for the induction of flowering has been identified. However, reports on detailed functions of the candidate genes are yet to come. Moreover, the role of gibberellin as a major signal regulating runnering, awaits further characteriza-tion. Two gene loci in *F. vesca* may provide keys to understand underlying regulatory pathways and are therefore major targets of further research. *Runnering locus* (*RL*) makes the difference between runnering/non-runnering phenotypes and different alleles of *Seasonal flowering locus* (*SFL*) cause seasonal and everbearing flowering habits. This review aims at summarizing the recent progress on molecular control of flowering and runnering in strawberry.

Keywords: axillary bud, *Fragaria*, photoperiod, Rosaceae, *Seasonal flowering locus*, *Runnering locus*
Abbreviations: ***AP1***, *Apetala1*; ***CO***, *Constans*; **EB**, everbearing; **EST**, expressed sequence tag; ***FLC***, *Flowering locus C*; ***FT***, *Flowering locus T*; **GA**, gibberellin; **LD**, long day; ***LFY***, *Leafy*; **QTL**, quantitative trait locus; ***RL***, *Runnering locus*; **SD**, short day; ***SFL***, *Seasonal flowering locus*; ***SOC1***, *Suppressor of the overexpression of Constans1*; ***SVP***, *Short vegetative phase*; ***TFL1***, *Terminal flower1*

CONTENTS

INTRODUCTION

Strawberries are perennial rosette plants with high economic value worldwide. Thereby, understanding the regulatory mechanisms controlling strawberry development is of utmost importance to facilitate both breeding and production of this important crop. Recent progress in molecular biological research has also raised academic interest in strawberry and brought it among the most important new model crops as well as led to completely new possibilities to solve many biological questions studied for decades. Most importantly, molecular level studies are expected to provide us new and efficient tools to extend cropping season, to increase berry yields and to improve plant production techniques.

At the vegetative stage of strawberry growth, short internodes produced from the apical meristem of the stem form the "crown". One trifoliate leaf with a long petiole and one axillary bud develops into each node. Axillary buds can differentiate either to runners that are elongated shoots or to new leaf rosettes called "branch crowns". Runner growth involves the formation of successive units of two long internodes followed by a terminal daughter plant that can be used for vegetative reproduction. Inflorescences and consequently flowers are formed by the apical meristem of the crown while the uppermost axillary buds continue vege-

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Invited Mini-Review

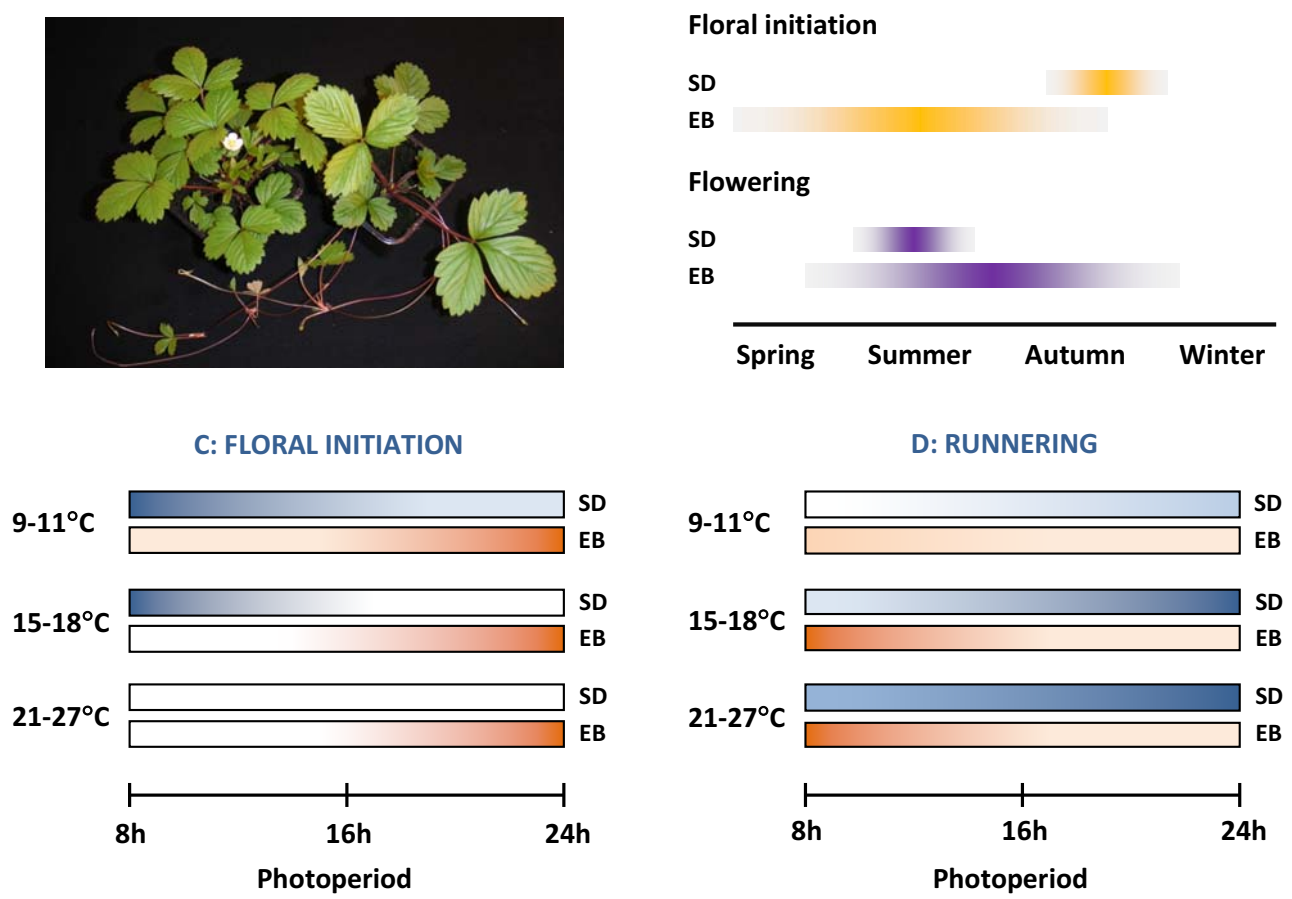

Fig. 1 Schematic illustration of flowering and runnering responses in diploid strawberry *Fragaria vesca* (L.). (**A**) Opposite phenotypes of everbearing (EB) genotype 'Baron Solemacher' (left) and seasonally flowering Finnish short day (SD) genotype (right) grown in long day (LD) conditions. Recessive alleles of a single gene, *Seasonal flowering locus* (*SFL*), cause continuous flowering in 'Baron Solemacher', whereas SD genotype stays vegetative in LD. 'Baron Solemacher' is runner-less because of recessive alleles of another gene, *Runnering locus* (*RL*), whereas SD genotype as well as some other EB genotypes, like Hawaii-4, are able to produce runners. (**B**) Seasonal timing of floral initiation and flowering in SD and EB genotypes of *F. vesca*. In EB genotypes, floral initiation and flowering occurs at the same time, whereas in SD genotype, flower initials are formed only in autumn and flowers emerge during next growing season. (**C**, **D**) Environmental regulation of floral initiation (**C**) and runnering (**D**) in SD and EB genotypes of *F. vesca*. White colour confers to the lack of response and dark colour represents strongest response. The figure highlights the opposite environmental responses of EB and SD genotypes as well as antagonism between flowering and runnering.

tative growth of the rosette by forming branch crowns. Under flowering inducing conditions, also branch crowns may initiate terminal inflorescences that leads to further crown branching. Thus the number of crown branches directly affects the berry yield. Some meristems always remain vegetative, since branch crowns containing less than 2–4 leaf initials are yet not competent to initiate flowers (Arney 1953) enabling successive growth cycles in the perennial life history of *Fragaria*.

The antagonism between vegetative and generative development is a common feature in perennial life history where environmental control, most importantly photoperiod and temperature, have a major role. Several studies on octoploid garden strawberry (*Fragaria* x *ananassa*) as well as on diploid woodland strawberry (*Fragaria vesca*) genotypes have revealed that control of flowering induction and runner formation are almost mutually exclusive although it has been shown that they are genetically separate processes (e.g. Brown and Wareign 1965; Konsin *et al.* 2001; Heide and Sønsteby 2007). Thereby, detailed molecular level analysis is needed to reveal the control mechanisms. Moreover, continuously flowering everbearing (EB) mutants known for both species provide unique material for comparative genetic studies (**Fig 1A, 1B**). As discussed below, most recent molecular studies have especially taken advantage of the simple diploid model *F. vesca* where development of advanced genetic tools including the genome sequence are fast progressing (Shulaev *et al.* 2008, 2011).

ENVIRONMENTAL REGULATION OF STRAWBERRY GROWTH

Photoperiodic and temperature regulation of flowering

Environmental regulation of flowering in strawberry has been extensively explored for several decades, and the early studies focusing mostly on garden strawberry have been reviewed by Guttridge (1985). According to these studies, seasonal flowering cultivars of the garden strawberry are facultative SD plants, in which temperature modifies the photoperiod dependence of flowering. In general, SD is obligatory for flowering induction in temperatures over ~15°C, whereas at lower temperatures, flowering is induced independently of photoperiod. However, large genetic variation is found between different cultivars; the critical day length for flowering induction can vary between 11 to 16 h and the number of SD cycles needed for induction between 7 and 23 (Guttridge 1985). Moreover, photoperiodic flowering induction is highly dependent on temperature in some cultivars, but may be controlled only by photoperiod in other cultivars (Heide 1977; Sønsteby and Heide 2006). Still, according to several studies, temperatures over 24–30°C and under 9°C are inhibitory to flowering (Heide 1977; Guttridge 1985; Sønsteby and Heide 2006).

As in garden strawberry, photoperiod and temperature control flowering induction in *F. vesca*, although the role of

temperature is more pronounced (**Fig. 1C**). This was clearly shown in a recent study, in which the environmental control of flowering induction was tested in Norwegian *F. vesca* populations originating from different latitudes (Heide and Sønsteby 2007). The critical photoperiod for flowering induction did not correlate with the origin of the plants, but the photoperiodic flowering induction was strongly affected by temperature. At 9°C flowering was induced in all photoperiods, at 15–18°C in photoperiods shorter that 16 h, whereas at 21°C flowering induction did not occur at all. The remarkable similarity in the environmental control of flowering induction in a diploid *F. vesca* and octoploid garden strawberry suggests that similar genetic mechanisms are responsible for photoperiodic and temperature regulation of flowering in these species.

After the induction of flowering, the apical meristems of the main crown and branch crowns are turned to inflorescence meristems and flower initials begin to develop. In garden strawberry cvs. 'Korona' and 'Elsanta', photoperiod controls meristem identity (Hytönen *et al*. 2004), whereas the rate of flower initiation is mainly controlled by temperature with an optimum of 18–20°C (Le Mière *et al*. 1996; Sønsteby and Heide 2008a). However, Sønsteby and Heide (2006) showed that also LD promotes floral development after flowering induction in 'Korona' and 'Elsanta' and stated that at least these cultivars are actually SD-LD plants. The dynamic regulation of meristem determination was shown in a study by Hytönen *et al*. (2004), in which plants of 'Korona' were subjected to SD – LD – SD regime. In this study, the first 3-week SD treatment induced crown branching, but floral development took place only in the main crown, probably because the meristems of the branch crowns had not reached the competence for floral initiation during the first SD period. Furthermore, the subsequent LD most likely removed the flowering inducing signals. When the plants were subjected to a second SD treatment 4 weeks later, floral initiation took place in the apices of branch crowns that were formed in response to the first SD treatment. Moreover, plants exposed to continuous SD produced continuously crown branches with flower initials. These data indicate that mobile floral activators and/or inhibitors proposed earlier (Hartmann 1947; Guttridge 1959) are dynamically regulated in strawberry, and contribute to the typical seasonal flowering response. Whether similar flowering responses can be induced by temperature fluctuations remains to be shown.

Although most strawberry genotypes flower seasonally, continuously flowering everbearing (EB) genotypes and cultivars are known. In most studies, these plants are called day-neutral, since the effect of photoperiod on flowering time has been negligible or totally absent (Durner *et al.* 1984; Guttridge 1985; Nicoll and Galletta 1987). This view is now changing after recent findings that show clear LD and high temperature promotion of flowering in several EB cultivars of the garden strawberry and EB genotypes of *F. vesca* (Nishiyama and Kanahama 2002; Sønsteby and Heide 2007b, 2008b; Hytönen 2009). For example, LD grown seedlings of five different EB genotypes of *F. vesca* produced only ~5–8 leaves in the main crown before terminal inflorescence, indicating that flowering induction occurs soon after germination (Hytönen 2009). In contrast, flowering was delayed in plants raised for 5 weeks in SD at 18°C; they produced 4–5 leaves more before flowering than those grown under LD (Mouhu *et al*. 2009). Moreover, low temperature of 11°C caused further delay in flowering in Hawaii-4 genotype. Taken together, these data clearly show that EB genotypes of the garden strawberry and *F. vesca* are LD plants that show opposite flowering response both to photoperiod and temperature than the SD genotypes (**Fig. 1C**).

Environmental regulation of flowering has been characterized also in the parents of the garden strawberry, *F. virginiana* and *F. chiloensis*. In *F. virginiana* flowering is promoted by SD especially in higher temperature, except in the genotype originating from Wasatch mountains (Utah) that was truly day-neutral in temperatures from 9 to 27°C (Sønsteby and Heide 2008c). In contrast, *F. chiloensis* genotypes originating from different latitudes had obligatory SD requirement for flowering at temperatures of 15-21°C, and genotypes collected from Alaska and Chile were day-neutral at 9°C (Sønsteby and Heide 2009), as shown also in Norwegian *F. vesca* (Heide and Sønsteby 2007). Thus, SD and low temperature requirement of flowering induction in the garden strawberry is probably inherited from *F. chiloensis* and LD response of EB cultivars originates from *F. virginiana* (Sønsteby and Heide 2009). The environmental control of flowering in other *Fragaria* species remains to be analysed, although Sargent *et al*. (2004) reported that two diploid species out of eight species tested, *Fragaria nubicola* and *F. viridis*, have remontant flowering habit. The environmental regulation of flowering in diploid species should be carefully characterized and analysed by crossing with *F. vesca*.

Environmental regulation of runnering

Environmental conditions also control the differentiation of strawberry axillary buds to either runners or branch crowns. In SD genotypes of the garden strawberry, LD and high temperature promote runner formation, whereas in SD, axillary buds differentiate into branch crowns increasing the number of meristems capable to initiate inflorescences and, consequently, enhancing the cropping potential of the plants (Heide 1977; Konsin *et al*. 2001; Hytönen *et al*. 2004). Hytönen *et al*. (2009) have studied the control of axillary bud differentiation in detail by using runner axillary buds of 'Korona' (axillary bud #2) as a model system. In this study, the axillary buds differentiated into branch crowns after 8–12 SD cycles in a 12-h photoperiod. Moreover, runner formation occurred when the photoperiod exceeded a critical value that is close/equal to the critical photoperiod for flowering induction (Konsin *et al*. 2001; Hytönen *et al*. 2009). Also in seasonally flowering *F. vesca*, runner formation is similarly controlled by photoperiod and temperature (**Fig. 1D**), but the response is much slower (Battey *et al*. 1998; Heide and Sønsteby 2007). High temperature increases the number of runners also in the EB cultivars of the garden strawberry, but the effect of photoperiod has been variable in different experiments (Sønsteby and Heide 2007a, 2007b). In EB *F. vesca*, the control of runnering is clear-cut. Many genotypes do not form runners at all, but for example in Hawaii-4, SD strongly enhances runner formation (Hytönen 2009). In general, EB genotypes produce less runners than SD genotypes (Sønsteby and Heide 2007b), probably as a consequence of early floral initiation of shoot apices, which enforces the differentiation of uppermost axillary buds to branch crowns. In conclusion, opposite control of flowering induction and runner formation in various *Fragaria* genotypes indicates that these processes are almost mutually exclusive. However, detailed molecular level analysis is needed to confirm this hypothesis.

GENETICS OF FLOWERING AND RUNNERING

Inheritance of EB flowering habit and the presence or absence of runners has been studied in *Fragaria*. Although flowering and runnering seem to be antagonistic processes, Brown and Wareign (1965) showed in their fundamental crossing experiments that they are controlled by different genetic loci in *F. vesca*. They crossed two runnerless EB genotypes with a runnering seasonally flowering genotype and found that all F1 individuals were seasonally flowering and produced runners. Moreover, in F2 and F1 x EB backcross populations, four different phenotypes, EB runnering, EB non-runnering, seasonally flowering runnering and seasonally flowering non-runnering, showed simple Mendelian inheritance. In conclusion, both seasonal flowering and runnering are controlled by separate, dominant single genes, *Seasonal flowering locus* (*SFL*) and *Runnering locus* (*RL*), respectively, and their recessive alleles cause the EB and

non-runnering phenotypes.

Also another gene locus, *Arborea* (*ARB*), has been shown to control runnering in "strawberry tree" mutant, *F. vesca* arborea Staudt. This mutant has long internodes, it continuously produces runners, whereas branch crowns are lacking. In crossing experiments with EB 'Baron Solemacher', *arb* mutation was found to be recessive and epistatic to *RL* (Guttridge 1973). Since the phenotype of *arb* mutant resembles GA treated plants of 'Baron Solemacher', *ARB* gene may encode some negative regulator of the GA pathway.

In contrast to *F. vesca*, the inheritance of EB flowering habit in octoploid *Fragaria* is more complex. In some studies, EB flowering has been proposed to be controlled by a single dominant gene, but most studies favour the multiple gene model (Ahmadi *et al.* 1990; Sakin *et al.* 1997; Hancock *et al.* 2001; Serce and Hancock 2005). For example Weebadde *et al*. (2007) found eight QTLs associated with EB flowering habit in their breeding population. However, they also found considerable variation in the number of EB progenies, when plants were grown in different locations in USA showing that EB flowering was highly dependent on climatic conditions. These data, as well as the presence of several sources of EB genes (Powers *et al.* 1954; Ahmadi *et al.* 1990; Hancock *et al.* 2001), support the multiple gene model in the regulation of EB habit in octoploid *Fragaria*. Thus, it is unlikely that EB flowering habit in octoploid genotypes is controlled by recessive alleles of *SFL*. However, it is tempting to speculate that major EB genes are located in the same genetic pathway with *SFL* in octoploid *Fragaria*. In fact, involvement of a single genetic pathway is also supported by the finding that several EB cultivars with different origin of EB genes show similar flowering response to photoperiod and temperature (Sønsteby and Heide 2007a, 2007b).

GIBBERELLIN REGULATES AXILLARY BUD DIFFERENTIATION

The role of gibberellins (GA) as regulators of strawberry runner development was suggested by Guttridge and Thompson already in 1960's. They showed that exogenous GA application activated runner growth in SD conditions and was able to initiate runner development even in non-runnering strawberry genotypes (Thompson and Guttridge 1959; Guttridge and Thompson 1963). The importance of GA as a regulator of axillary bud differentiation has been shown also by growth regulator applications. For example, the inhibitor of GA biosynthesis, prohexadione-calcium, enhances the formation of branch crowns instead of runners and consequently increases strawberry flowering and yield (Black 2004; Hytönen *et al.* 2008).

Recent studies by Hytönen *et al*. (2009) showed that the changes in the axillary bud fate caused by prohexadione-calcium were associated with a rapid decline in the level of active GA_1. The causality of reduced GA level for changes in axillary bud differentiation was verified by GA_3 application that completely reversed the effect of prohexadione-calcium. GA analyses in SD and LD grown buds revealed that branch crown initiation in SD was associated with about 50% reduction in GA_1 concentration in SD buds compared to LD grown buds. More evidence for GA regulation of axillary bud differentiation came from gene expression studies. It was found that several GA biosynthetic, signaling and target genes including *GA3ox* (*GA3-oxidase*), *GA2ox* (*GA2-oxidase*), *GAI* (*Gibberellic acid insensitive*), *RGA* (*Repressor of ga1-3*), *GID1b*, *SLY1* (*Sleepy1*), *GAST* (*Gibberellic acid stimulated transcript*) and *XERICO*, were affected by reduced GA_1 levels in prohexadione-calcium treated plants, the phenomenon called GA signaling homeostasis (Schwechheimer 2008). These genes were used as markers for the activity of the GA pathway and it was found that most of them were similarly affected by SD in the axillary buds, indicating that GA signaling was reduced in SD grown buds compared to LD. These findings led to the conclusion that GA is one of the key signals mediating the daylength controlled axillary bud differentiation in strawberry (Hytönen *et al.* 2009). However, major regulatory genes of axillary bud differentiation including *RL* remain to be identified.

MOLECULAR STUDIES ON ROSACEAE FLOWERING PATHWAYS

Identification of key genes and understanding the molecular mechanisms regulating growth and development in strawberry or more generally in Rosaceae is needed to enhance breeding of new cultivars and to improve cultivation practises of these important species. Thorough studies on *Arabidopsis thaliana* flowering pathways have facilitated flowering gene discovery in Rosaceae. However, strawberry as a perennial short day plant is fundamentally different from *Arabidopsis* which is an annual, long day plant. To which extend and how the molecular mechanism regulating flowering in these species differ is currently not known.

Major flowering pathways in *Arabidopsis thaliana*

Four major genetic pathways to flowering are known in *Arabidopsis thaliana*. Photoperiodic and vernalization pathways respond to environmental signals and autonomous and GA pathways control floral development according to developmental and hormonal cues (Putterill *et al.* 2004; Simpson 2004; Thomas 2006; Zhou *at al.* 2007; Turck *et al.* 2008; Kim *et al.* 2009). These signals are integrated by a few genes including *FT* (*Flowering locus T*) and *SOC1* (*Suppressor of overexpression of Constans1*), often referred to as floral integrators (Parcy 2005). The floral integrators, in turn, activate the floral meristem identity genes *AP1* (*Apetala1*), *FUL* (*Fruitfull*) and *LFY* (*Leafy*) thereby initiating flowering (Liu *et al.* 2009). *CO* (*Constans*) is a key regulator in the photoperiodic pathway, since it performs seasonal time measurement by integrating endogenous rhythm controlled by the circadian clock and external light signals perceived by phytochrome and cryptochrome photoreceptors (Yanovsky and Kay 2002; Valverde *et al.* 2004). In LD, CO activates the expression of *FT* in the phloem companion cells, and FT protein travels to the meristem and induces flowering in *Arabidopsis* (Corbesier *et al.* 2007; Turck *et al.* 2008). Both autonomous and vernalization pathways culminate in a flowering inhibitor *FLC* (*Flowering locus C*), which in turn represses *FT* and *SOC1* (Searle *et al.* 2006; Li *et al.* 2008). In vernalization pathway, a few protein complexes control the expression of *FLC* by chromatin modifications, and a long period of cold (vernalization) is needed to silence *FLC* and consequently to reach the competence to flower (He 2009; Kim *et al.* 2009). Also the genes of the autonomous pathway are needed to silence *FLC* (Simpson 2004). In addition, a specific thermosensory and light quality pathways has been found (Cerdán and Chory 2003; Lee *et al.* 2007).

Characterization of Rosaceae flowering genes

Mouhu *et al*. (2009) applied EST sequencing of substracted cDNA libraries for identification of candidate genes involved in regulation of flowering. The libraries were constructed from shoot apexes of the SD *F. vesca* and the EB genotype 'Baron Solemacher' (grown under LD conditions) with suppression subtractive hybridization (SSH) method to enrich transcripts that may either promote or inhibit flowering. Altogether 970 SD enriched ESTs and 1184 EB enriched ESTs were sequenced. Some candidate genes such as floral integrator genes *SOC1* and *LFY* were isolated using PCR approaches and, in addition, the sequence search was extended to identify all *Arabidopsis* flowering gene homologs present in the GDR Rosaceae EST database (Jung *et al*. 2004; 2007). In total, 88 candidate flowering genes were identified in Rosaceae and 66 genes specifically from *Fragaria* (Mouhu *et al*. 2009), some of which are presented in

Table 1 Putative flowering time gene homologs identified from strawberry. Sequences corresponding to *Arabidopsis* genes of different flowering pathways are grouped. Biological functions of the proteins are shown and activators and repressors are indicated by + and –, respectively, according to the function of *Arabidopsis* proteins. See Mouhu *et al.* (2009) for more detailed list of genes and their accession numbers. For the genes of the GA pathway, see Hytönen *et al.* (2009).

Gene	Biological function	Activator/ Repressor
Photoperiodic pathway		
phyA	Red light photoreceptor	+
cry2	Blue light photoreceptor	+
LHY	Myb domain transcription factor	-
TOC1	pseudo response regulator	-
CO	putative zinc finger transcription factor	+
FKF1	F-box protein/blue light photoreceptor	+
Vernalization pathway		
VIN3	PHD domain protein	+
VRN1	DNA binding protein	+
SUF4	putative zinc finger containing TF	-
ATX1	putative SET domain protein	-
ELF8	RNA polymerase 2 associated factor -like	-
VIP3	RNA polymerase 2 associated factor -like	-
Autonomous and thermosensory pathway		
FLK	KH-type RNA domain containing	+
FY	mRNA 3' end processing factor	+
LD	DNA/RNA binding homeodomain protein	+
LDL1	histone H3 lysine 4 demetylase -like	+
SVP	MADS-box transcription factor	-
FVE	retinoblastoma associated	+
Gibberellin pathway		
GA20ox	GA 20-oxidase	+
GA3ox	GA 3-oxidase	+
GA2ox	GA 2-oxidase	-
GID1a	Gibberellin receptor	+
RGA	putative transcriptional repressor	-
SPY	O-linked N-acetylglucosamine transf.	-
Light quality pathway		
PFT1	vWF-A domain protein	+
HRB1	ZZ type zinc finger protein	+
Floral integrator and identity genes		
SOC1	MADS box transcription factor	+
LFY	Transcription factor	+
AP1	MADS box transcription factor	+

Table 1. This analysis still failed to identify some central genes such as *FT*, *GI* (*Gigantea*) and *FLC* (Mouhu *et al*. 2009). Also Folta *et al.* (2005) reported few EST sequences corresponding to *Arabidopsis* flowering time genes. Moreover, Stewart (2007) identified several *CO* like sequences and homologs for *Arabidopsis* MADS box genes involved in floral development. The identified genes correspond to all known *Arabidopsis* flowering pathways although their functional roles may eventually be modified or completely different. However, the genome sequence of *F. vesca* will reveal the presence or absence of missing regulators. It will also uncover whether some of the identified candidate genes are located close to flowering related QTLs in cultivated strawberry, since effectively complete co-linearity has been found between the maps of cultivated strawberry and diploid *Fragaria* (Rousseau-Gueutin *et al.* 2008; Sargent *et al.* 2009).

Comparison of 25 selected candidate genes in *Fragaria* SD and EB genotypes by Mouhu *et al*. (2009) did not reveal major differences at the expression level and thus revealed no hints for putative location of *SFL*. However, the expression of *AP1* and *LFY* was correlated with induction of flowering in the meristems of the EB genotype while *AP1* expression was completely lacking from the non-induced SD genotype. Thereby, *AP1* provides a useful marker gene for floral induction (Mouhu *et al*. 2009). Stewart (2007) studied the expression rhythm of strawberry *CO* homolog and found a peak in the morning instead of evening peak typical for other species (Yano *et al.* 2000; Suárez-López *et al.* 2001). However, the function of this CO homolog as a floral regulator remains to be shown.

In addition to strawberry, the high economical impact of Rosaceae has promoted studies on regulation of flowering also in other species, such as peach, apple and rose. The evergrowing mutant (*evg*) of peach (*Prunus persica* L. Batsch) shows non-dormant growth pattern and is not responding to short photoperiod or cold temperatures. Mapping and sequencing of the corresponding genomic region has revealed six clustered MICK-type MADS box genes (so called dormancy-associated MADS-box genes or DAM genes) as candidates for *EVG* (Bielenberg *et al*. 2008; Li *et al*. 2009). The expression of three of these was temporally correlating with seasonal elongation cessation and bud set (Li *et al*. 2009). DAM genes are members of SVP/ StMADS11/AGL24 clade that have been proposed to function as general regulators of development of bud structures in various perennial species under dormancy-inducing conditions (Horvath 2009). Also in another species of Rosaceae, Japanese apricot (*Prunus mume* Sieb. et Zucc.), SVP/ AGL24-type MADS box transcription factor has been identified as a candidate regulator of bud endodormancy (Yamane *et al*. 2008).

Studies in apple (*Malus domestica*) have identified many putative flowering genes and more importantly, first reports demonstrating modification of flowering time in transgenic apple indicate their functional conservation and usability in cultivar improvement (Jeong *et al*. 1999; Yao *et al*. 1999; Sung *et al*. 2000; Kotoda *et al*. 2000; Wada *et al*. 2002; Kotoda and Wada 2005; Hättasch *et al*. 2008; Mimida *et al*. 2009). The two *LFY/FLO* homologs, *AFL1* and *AFL2* as well as the *AP1* homologs *MdMADS5* and *MdMADS2* accelerated flowering by ectopic expression in either *Arabidopsis* or tobacco (Sung *et al*. 1999; Wada *et al*. 2002; Kotoda *et al*. 2002). In contrast to these, ectopic expression of the *TERMINAL FLOWER 1* (*TFL1*) homolog *MdTFL1* delayed flowering in *Arabidopsis* showing that the function of *TFL* genes in repression of flowering and maintenance of inflorescence meristem is conserved between these two species (Kotoda *et al*. 2005; Mimida *et al*. 2009). Consequently, Kotoda *et al*. (2006) were able to substantially promote flowering in transgenic apple trees by suppressing the *MdTFL1* expression using antisense gene constructs. In comparison with controls that did not flower after five years, the juvenile phase in transgenic lines was reduced and they initiated flowering 8-25 months after transfer to a greenhouse.

Perpetual or recurrent blooming is also common among roses (genus *Rosa*). Recurrent roses have a short juvenile phase in contrast to non-recurrent ones, as well as determinate versus in-determinate inflorescences, respectively. Using EST sequencing in combination with gene mining of rose sequences available in public databases, Foucher *et al*. (2008) identified 4765 unigene sequences among which 13 flowering related genes were present. Additional candidate genes representing all major flowering pathways were identified by Remay *et al*. (2009) using degenerate primers resulting in total of 26 flowering genes with those previously identified by Foucher *et al*. (2008). They only failed in identification of *FLC*. Earlier studies by Roberts *et al*. (1999) indicated a major role for GA in regulation of flowering as exogenously applied GA inhibited flowering in non-recurrent roses. Remay *et al*. (2009) showed that the GA signalling gene *RoSPY* was mapped in vicinity of the recessive *RECURRENT BLOOMING* (*RB*) locus. Moreover, comparison of gene expression between non-recurrent rose and its recurrent mutant showed differences in GA signalling gene homolog *RoGID1*. However, the exact functional roles of environmental signals (such as photoperiod) and GA are yet to be verified.

In conclusion, molecular studies and identification of flowering pathways in Rosaceae are under active research in various species and the results obtained so far strongly suggest that the basic flowering gene network is highly con-

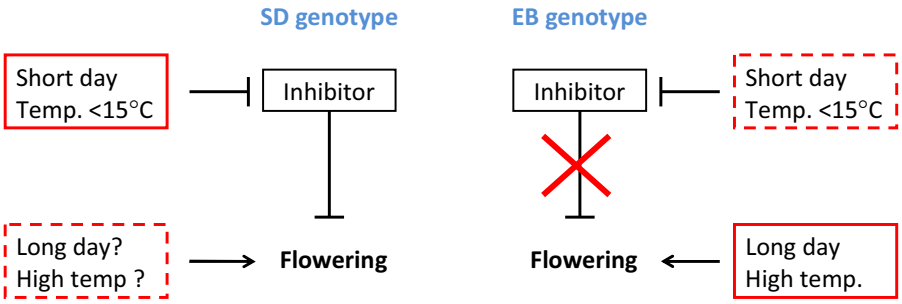

Fig. 2 A hypothetical model of flowering pathways in *Fragaria vesca* (L.). Short day (SD) genotype has a dominant allele(s) of the major inhibitor gene *Seasonal flowering locus*. Short photoperiod or alternatively low temperature is needed to suppress the function of *SFL* and consequently to induce flowering. EB genotype, in contrast, does not require SD or low temperature for flowering, since it has non-functional alleles of *SFL*. In EB genotype, flowering is induced at 1 – 2 leaf stage through a genetic pathway activated by long day (LD) and high temperature conditions. This pathway is expected to be present also in SD genotype, but its role in the induction of flowering is unclear.

served. Thereby, it is reasonable to anticipate that this information provides us tools for modification of flowering in a controlled way. On the other hand, further research is still needed to reveal the detailed molecular mechanisms and to clone the key genes behind the major flowering loci such as *SFL* in strawberry, *RB* in rose and *EVG* in peach.

SFL is a major regulator of flowering in *F. vesca*

The data reported by Mouhu *et al*. (2009) suggest that all known flowering pathways are present in strawberry. However, despite the sequence conservation, the functional roles of different pathways and/or single genes may vary in different species. In *F. vesca*, the yet unknown *SFL* is a famous gene locus that obviously has a novel function. Recessive alleles of this gene cause continuous flowering habit at least in 'Baron Solemacher', an old European EB cultivar (Brown and Wareign 1965; Albani *et al*. 2004), in which flowering is promoted by LD and increasing temperature (**Figs. 1A, 2**) (Sønsteby and Heide 2008b; Mouhu *et al*. 2009). In contrast, seasonal flowering habit of SD genotypes is probably due to dynamic regulation of active *SFL* alleles (Battey *et al*. 1998; Battey 2000). In fall, SD or temperature of 9-15°C is needed to activate floral initiation probably by repressing SFL (**Fig. 1**). However, no further floral initiation takes place in spring (**Fig. 2**), since winter chilling is thought to reactivate SFL. In conclusion, SFL is considered as a major floral repressor in *Fragaria* that makes the difference between opposite flowering responses between the EB and SD genotypes of *F. vesca* and probably contributes to the regulation of perennial growth cycle in this species. As such major gene, *SFL* provides a key for understanding the genetic control of flowering and perennial growth cycle in *F. vesca* and probably in other species of Rosaceae family. Positional cloning effort of *SFL* was presented by Battey *et al*. (1998), and his group reported the development of three SCAR markers located close to *SFL*. Although, one of these markers, SCAR2, was inseparable from *SFL* in the crossing population consisting of 1049 individuals (Albani *et al*. 2004), no advance in the cloning of *SFL* has been reported so far, and the location of these markers in *Fragaria* reference map (Sargent *et al*. 2006) has not been published.

Environmental control of flowering by repressor proteins is a common mechanism in many plant species. The most well-known repressor mechanism is associated to vernalization pathway that has been studied in cereals and characterized in detail in *Arabidopsis* (Kim *et al*. 2009). In winter-annual *Arabidopsis*, vernalization involves the repressor complex with MADS box proteins FLC and SVP. Long period of cool temperatures below 8°C (vernalization) is needed for the silencing of *FLC*, and consequent attainment of competence to flower (Li *et al*. 2008; Kim *et al*. 2009). *FLC* has been proposed to be one candidate for *SFL* (Battey 2000). However, the fact that no *FLC* homologs were found among ~650 000 Rosaceae ESTs (Mouhu *et al*. 2009; Hytönen *et al*. unpublished data), and *FLC* function has been shown only in Brassicaceae family (Searle *et al*. 2006; Wang *et al*. 2009), argues against the hypothesis that *SFL* could be *FLC*-like gene. Moreover, strawberry flowering is induced by cool temperatures above 9°C, whereas lower temperatures (winter chilling) needed for vernalization promote vegetative development and inhibit flowering probably through reactivation of the SFL repressor (Ito and Saito 1962; Battey 2000; Sønsteby and Heide 2006). In contrast, several *SVP-like* genes are present in Rosaceae and contribute at least to the regulation of dormancy in peach (Bielenberg *et al*. 2008). *SVP* homologs have also been identified in strawberry (Mouhu *et al*. 2009), and its function as a floral repressor is currently being tested by transgenic approaches (Mouhu *et al*. unpublished data).

Since photoperiod controls flowering in strawberry, genes belonging to photoperiodic pathway are also candidates for *SFL*. The most obvious candidate is *CO*, the heart of the photoperiodic pathway that in Arabidopsis activates flowering in LD (Suárez-López *et al*. 2001; Yanovsky and Kay 2002). In contrast, in SD plant rice, CO homolog Hd1 represses flowering in LD but activates it in SD (Yano *et al*. 2000). In principal, similar function would match perfectly with the photoperiodic control of flowering in strawberry. However, strawberry *CO* homolog has been cloned and mapped to the *Fragaria* reference map, but it is not located close to *SFL* (Stewart 2007), indicating that other candidates should be searched. Another possibility is that strawberry CO homolog is an activator of flowering, in which case SFL could be transcriptional or post-transcriptional repressor of CO.

Floral initiation in the garden strawberry and *F. vesca* can be suppressed by GA application (Thompson and Guttridge 1959; Guttridge and Thompson 1963) suggesting that SFL could lie in the GA pathway. However, the role of endogenous GA as an inhibitor of flowering has only been analyzed indirectly by GA biosynthetic inhibitor applications. The rapid drop of active GA levels by prohexadione-calcium (Hytönen *et al*. 2009) does not induce flowering and does not have clear effect on flowering time in garden strawberry or *F. vesca* (Hytönen *et al*. 2008, unpublished data). Thus, these results indicate that the function of SFL is not connected to down-regulation of the GA biosynthetic pathway. In fact, no clear differences in the expression of GA biosynthetic gene *GA3ox* and catabolic gene *GA2ox* were found in the shoot apices of SD and EB genotypes before flowering induction, but both genes were clearly down-regulated later during floral development (Mouhu *et al*. 2009). Taken together, it is unlikely that SFL lies in the GA pathway, at least if *SFL* is a ubiquitous repressor gene. To directly test this hypothesis, GA-inducible reporter gene should be expressed in SD and EB genotypes, and local GA activity shown by the reporter should be compared with the expression of floral marker gene *AP1* (Mouhu *et al*. 2009) under various environmental conditions.

Towards high throughput functional studies in strawberry

The octoploid genome of cultivated strawberry is complicating functional studies for identified genes as well as strawberry breeding. However, rapidly developing molecular tools, such as high throughput sequencing technology and improved genetic maps are extending our knowledge on strawberry genomics and consequently facilitate and form the basis for the improvement of agronomically important traits valued by the growers and the consumers. In this respect, molecular studies using more simple models, such as diploid *F. vesca* are of utmost importance. These studies are enhanced by the small genome size of *F. vesca* that was defined to be only 164 Mb (Akiyama *et al*. 2001), only slightly larger than that of *Arabidopsis thaliana* (125 Mb). In fact, genome sequencing of *F. vesca* genotype Hawaii-4 was initiated in the spring 2008 at Virginia Tech, USA (http://strawberry.vbi.vt.edu/tiki-index.php) and recently finalized (Shulaev *et al*. 2011).

Until now, candidate gene mining from EST sequence databases and/or by monitoring transcriptional changes using microarrays has been logical approach for identification of strawberry genes associated with given traits. Still, the total number of ESTs for strawberry in public sequence databases is relatively limited, in fact less than 60 000 (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). This number combines information obtained from conventional cDNA library sequencing of both *F. x ananassa* (Folta *et al*. 2005) and *F. vesca* (Mouhu *et al*. 2009; Brese R., Davis T., Slovin J., unpublished data). However, recent sequencing efforts with efficient pyrosequencing approaches evidently will change the situation in future (Hytönen *et al*. unpublished data; Folta pers. comm.). The publicly available EST data for the whole Rosaceae family including apple, cherry, peach, pear, raspberry, rose and strawberry, is much larger and together with Rosaceae maps and markers, the sequence data is collectively gathered in the Genome Database for Rosaceae (GDR) to promote genomics and genetics research (Jung *et al*. 2004; 2007, http://www.rosaceae.org).

Efficient gene transfer methods are essential for the functional analysis of candidate genes identified by high throughput genomics methods. Since 1990 large number of reports on genetic transformation of both octoploid and diploid strawberry genotypes has been published. Four recent reviews are summarizing this progress in detail (Folta and Dhingra 2006; Debnath and Teixeira da Silva 2007; Quesada *et al*. 2007; Qin *et al*. 2008). Most recent advances were reported by Oosumi *et al*. (2006) who has adapted the *Agrobacterium*-mediated gene transfer method for the diploid *Fragaria vesca*. The method by Oosumi *et al*. (2006) applies very stringent hygromycin selection, a more aggressive strain of *Agrobacterium* as well as green fluorescent protein (GFP) as a visual, selectable marker. Several *F. vesca* accessions showed up to 100% transformation frequency and especially the cv. Hawaii-4 (PI551572) turned out to be most potential with high efficiency in transformation, ease handle in tissue culture and *ex vitro* as well as short life cycle. Such efficiency allows high throughput functional studies using reverse genetic approaches but also development of T-DNA tagged mutant collections for forward genetics. Based on Oosumi *et al*. (2006) 255,000 independent T-DNA transformed lines would be needed to mutate any single gene with the probability of 95%.

Although EB *F. vesca* genotypes are self-fertile and expected to be highly homozygous, Slovin *et al*. (2009) showed that single self-pollinated plants of 'Yellow Wonder' produced progeny that still showed phenotypic variation under uniform growth conditions. Therefore, they developed an inbred line of *F. vesca* f. *semperflorens* 'Yellow Wonder' (YW5AF7) that can also be readily transformed. This line allows the propagation of uniform plant material by self pollination and accurate phenotyping of transgenic seedlings, since the genetic background is void of genotypic variation. Moreover, the use of GFP as a selectable marker allows rapid screening of transgenic seeds after imbibition (Slovin *et al*. 2009).

Folta *et al*. (2006) identified and selected a new, rapid-cycling and transformable octoploid line LF9 (Laboratory Festival #9) for high throughput gene function studies in garden strawberry. LF9 was selected from the segregating progeny obtained by self-pollination of 'Strawberry Festival' cultivar based on its vigorous growth *in vitro*, for its high regeneration capacity and transformability. This freely available experimental genotype is being used for activation-tagging and functional studies but it can also be used to promote functional studies using heterologous genes from other important species in Rosaceae that are not easily transformed themselves or their functional studies are hindered due to long juvenile phases (e.g. tree crops) (Folta *et al*. 2006). Hanhineva and Kärenlampi (2007) applied temporary immersion bioreactors for regeneration of transgenic octoploid strawberry plants after standard *Agrobacterium* cocultivation on semi-solid media. However, transformation frequency and speed was still far from what is needed in a high throughput system. Furthermore, for more rapid functional analyses agroinfiltration methods to introduce RNAi constructs for gene silencing in *F.* x *ananassa* fruits have been developed (Hoffmann *et al*. 2006). In conclusion, recent advances in developing functional genomics tools are truly bringing strawberry among the key model crops both in basic and applied research.

CONCLUDING REMARKS

Our understanding on the physiology of flowering and runnering in *F. vesca* as well as in octoploid species *F.* × *ananassa*, *F. virginiana* and *F. chiloensis* has significantly progressed during last years. Also dozens of putative flowering time genes have been identified in strawberry and other species of Rosaceae, and correlation between some candidate genes and floral initiation has been found. Moreover, physiological and molecular studies have revealed that GA is one of the signals mediating photoperiodic control of axillary bud differentiation to runners and branch crowns. Despite these advances, the knowledge on the molecular mechanisms controlling flowering and runnering is still in its infancy, since neither functional characterization nor map based cloning of responsible genes have been reported in strawberry. However, identification of candidate genes provides groundwork for detailed characterization of regulatory pathways that are expected to be complex and intertwined.

Recent advances in developing molecular tools including efficient transformation methods, a new inbred line, dense genetic maps and the genome sequence, are making *F. vesca* as an attractive model plant for strawberry and for Rosaceae in general. Moreover, short life cycle of *F. vesca* makes it as a transcendent model among most perennials. Combined use of new genetics tools and state-of-the-art sequencing technologies in *F. vesca* will exponentially increase our knowledge about the molecular mechanisms behind important horticultural traits. Ultimately, this information will enhance the cultivar breeding of strawberry and other species of the Rosaceae family through genetic transformation and marker assisted selection breeding.

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