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Research

The *FveFT2* florigen/*FveTFL1* antiflorigen balance is critical for the control of seasonal flowering in strawberry while *FveFT3* modulates axillary meristem fate and yield

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Summary

- Plant architecture is central in determining crop yield. In the short-day species strawberry, a crop vegetatively propagated by daughter-plants produced by stolons, fruit yield is further dependent on the trade-off between sexual reproduction (fruits) and asexual reproduction (daughter-plants). Both are largely dependent on meristem identity, which establishes the development of branches, stolons and inflorescences.
- Floral initiation and plant architecture are modulated by the balance between two related proteins, FLOWERING LOCUS T (FT) and TERMINAL FLOWER 1 (TFL1). We explored in woodland strawberry the role of the uncharacterised *FveFT2* and *FveFT3* genes and of the floral repressor *FveTFL1* through gene expression analyses, grafting and genetic transformation (overexpression and gene editing).
- We demonstrate the unusual properties of these genes. *FveFT2* is a nonphotoperiodic florigen permitting short-day (SD) flowering and *FveTFL1* is the long-hypothesised long-day systemic antiflorigen that contributes, together with *FveFT2*, to the photoperiodic regulation of flowering. We additionally show that *FveFT3* is not a florigen but promotes plant branching when overexpressed, that is likely to be through changing axillary meristem fate, therefore resulting in a 3.5-fold increase in fruit yield at the expense of stolons.
- We show that our findings can be translated into improvement of cultivated strawberry in which *FveFT2* overexpression significantly accelerates flowering.

Introduction

Flower, fruit and seed production is highly dependent on plant architecture, which is therefore a key breeding target in crop species. Crop architecture has been shaped by selection to support the current high-yielding varieties, for example through branching changes (Park *et al.*, 2014). Plant architecture, which is determined by the number and arrangement of aerial organs (stems, leaves, shoot branches and inflorescences), results from the spatio-temporal activities of stem cells organised in meristems (Wang *et al.*, 2018; Moraes *et al.*, 2019). These include the shoot apical meristem (SAM) at terminal position on the shoot and the axillary meristems (AXM) at the basis of each leaf (Périlleux *et al.*, 2019). The fate of the AXM is governed by the nexus of genetics and environmental conditions, as well as by its position on the axis. SAMs can terminate by a single flower or by an inflorescence or develop into specific organs (McGarry & Ayre, 2012). AXMs can produce inflorescence-bearing side shoots or inflorescence and flowers (Wang *et al.*, 2018). Additionally, in strawberry and potato, which are vegetatively propagated crops, AXM can produce stolons that are elongated and highly specialised side shoots (Tenreira *et al.*, 2017). In many species, the main cue controlling the floral initiation process, that is the meristem transition from vegetative to reproductive state, is the seasonal variation in day length (McGarry & Ayre, 2012). Flowering is induced by reduced daylight in short-day (SD) species or by increased daylight in long-day (LD) species. The acquisition of photoperiod insensitivity in day-neutral crop species was crucial for extending their range and/or duration of the production period (Soyk *et al.*, 2017; Denoyes *et al.*, 2020).

Over the past decade, extensive research has highlighted the role of members of the CENTRORADIALIS/TERMINAL FLOWER 1/SELF-PRUNING (CETS) family in the activation or repression of flowering (Wickland & Hanzawa, 2015). Among the CETS proteins, FT proteins that are expressed in the leaves under inducible floral conditions and that move via the phloem to the

SAM, in which they promote flowering, have been identified as florigen (mobile floral activator) (Lifschitz *et al.*, 2006; Corbesier *et al.*, 2007; Tamaki *et al.*, 2007; Wickland & Hanzawa, 2015). TFL1 proteins are major floral repressors that maintain meristem indeterminacy (Périlleux *et al.*, 2019). The FT/TFL1 balance therefore plays a key role in the pattern of formation of vegetative and reproductive structures and has a considerable impact on plant architecture and yield (Shalit *et al.*, 2009; Lifschitz *et al.*, 2014; Park *et al.*, 2014; Moraes *et al.*, 2019). FT/TFL1 duplication often leads to the neofunctionalisation of one of the paralogous genes (Wickland & Hanzana, 2015). FT proteins may, for example, regulate underground storage organ growth in potato and onion (Navarro *et al.*, 2011; Lee *et al.*, 2013) or even act as antiflorigen in sugar beet (Pin *et al.*, 2010). Functional diversification is also observed in the large TFL1/BFT clade where Arabidopsis and chrysanthemum TFL1-like proteins acquired an antiflorigen function by repressing flowering via long-distance systemic action (Huang *et al.*, 2012; Higuchi *et al.*, 2013; Shalit-Kaneh *et al.*, 2019), a feature normally observed only in FT proteins.

Cultivated strawberry (*Fragaria* × *ananassa*) is a major horticultural species and a berry crop of worldwide importance. In this species, plant architecture and the trade-off between sexual reproduction (via fruits) and asexual reproduction (via daughter-plants) are central in determining two antagonist traits: fruit yield, a major trait for producers; and daughter-plant yield, a major trait for nurseries (Tenreira *et al.*, 2017). Strawberry is an herbaceous perennial plant with sympodial growth. When the SAM becomes floral, the uppermost AXM of the rosette produces an inflorescence-bearing branch crown (BC). Other AXMs can develop into either a BC (Sugiyama *et al.*, 2004) or a stolon that produces daughter-plants for vegetative multiplication (Tenreira *et al.*, 2017). In the wild, most strawberries are seasonal (photoperiodic) flowering ([SF]) plants in which floral initiation takes place in SD conditions when temperature and day-length decrease (Stewart & Folta, 2010; Heide *et al.*, 2013). Mutants exhibiting the perpetual flowering phenotype ([PF]) have been found for the diploid woodland strawberry *F. vesca*, in which [PF] is due to the *tfl1* mutation in the floral repressor *FveTFL1* (Iwata *et al.*, 2012; Koskela *et al.*, 2012). In the octoploid cultivated strawberry *F.* × *ananassa*, perpetual flowering varieties were created by introgressing the uncharacterised *F.* × *ananassa Perpetual Flowering RUnnering* (*FaPFRU*) locus (Gaston *et al.*, 2013; Perrotte *et al.*, 2016) from wild octoploid *F. virginiana* genetic variants (Bringhurst & Voth, 1980).

The molecular mechanisms by which flowering pathway genes modulate floral initiation and plant architecture and yield have only recently begun to be deciphered in strawberry (Hytonen & Kurokura, 2020). In *F. vesca*, the floral repressor *FveTFL1* blocks floral initiation in LD (Iwata *et al.*, 2012; Koskela *et al.*, 2012). In LD, *FveTFL1* expression is induced by *FveFT1,* which nonetheless functions as a LD floral activator in the *tfl1* genetic background (Koskela *et al.*, 2012; Rantanen *et al.*, 2014). So far, the florigen triggering floral initiation in the SD photoperiod remains unknown. FT candidates are *FveFT2* and *FveFT3*, whose role is ill defined in both diploid (Koskela *et al.*, 2012; Darwish *et al.*, 2013; Hawkins *et al.*, 2017) and octoploid strawberry (Nakano *et al.*, 2015; Koembuoy *et al.*, 2020). Seasonal flowering could be additionally controlled by a leaf-produced floral repressor acting at long distance in the SAM, that is an antiflorigen (Guttridge, 1959a,b; Vince-Prue & Guttridge, 1973). Branching is also affected by the *tfl1* mutation (Iwata *et al.*, 2012; Koskela *et al.*, 2012) and is likely to be regulated by the flowering pathway integrator *FveSOC1* (Mouhu *et al.*, 2013). Plant architecture and yield are also affected by mutations in the gibberellin biosynthesis and signalling pathway which dictates AXM decision to make either a stolon or an inflorescence-bearing BC (Tenreira *et al.*, 2017; Caruana *et al.*, 2018).

In this study, we explored the mechanisms by which strawberry CETS family members may control SD floral initiation and modulate plant architecture and yield in strawberry. We demonstrated that the leaf-expressed *FveFT2* acts as a long-distance floral signal and is the long-sought after nonphotoperiodic florigen permitting SD flowering. The closely related *FveFT3*, which is not expressed in the leaves, modulates branching, flower number and fruit yield, is likely to be through the modification of AXM fate. We also demonstrated that the floral repressor *Fve*TFL1 is indeed a mobile signal, a finding that supports old hypotheses about the existence of an antiflorigen in strawberry. Altogether, our results provide a conceptual frame for the control of floral initiation by the *Fve*FT2/*Fve*TFL1 balance in strawberry. We further demonstrated that our findings on *F. vesca* can be translated to cultivated strawberry in which overexpression of the nonphotoperiodic florigen *FveFT2* dramatically advanced the earliness of flowering and increased flower production.

Materials and Methods

Plant material, growth conditions and phenotyping

A genotype with a perpetual flowering [PF] phenotype will from this point forwards be referred to as [PF] genotype, while one with a seasonal flowering [SF] phenotype will be referred to as [SF] genotype. For flowering experiments, two *F. vesca* genotypes with contrasted flowering behaviour, the [SF] 'Norlanska' and the [PF] '815', were observed during a growing season from April to November under glasshouse natural environmental conditions at INRAE Bordeaux (France; 44°47′N, 0°34′W). Excessive high temperatures (>28°C) were reduced by glasshouse ventilation. Each genotype was represented by 30 2-yr-old plants obtained from seedlings. Flowering was fortnightly evaluated by counting the newly emerged inflorescences. In parallel, from mid-July to the end of the experiment, two plants per genotype were maintained under constant noninducible LD in growth chambers (16 h : 8 h, 22°C : 18°C, day : night). For gene expression analyses, leaf discs were regularly picked under natural environment and noninducible LD.

To estimate the time of floral initiation, that is when the SAM becomes floral, dissections of terminal buds were performed under binocular (SZX16; Olympus, Tokyo, Japan) from June to October. Vegetative or floral stage of the SAM was estimated using the scale adapted from Jahn & Dana (1970) and Taylor *et al.* (1997) (Supporting information Fig. S1) after dissecting 11–20 terminal buds for each genotype and sampling date.

For strawberry transgenic experiments, the [PF] *F. vesca tfl1* genotype 'Hawaii-4' (H4) (Mouhu *et al.*, 2009) and the [SF] octoploid genotype 'Sveva' were used (Cappelletti *et al.*, 2015). After *in vitro* culture, transgenic plants were grown in the glasshouse under LD. Numbers of flowers or inflorescences and stolons were regularly counted. Plant architecture, that is the positioning of the vegetative and reproductive development along the axes, was carried out on 6-month-old *F. vesca* H4 (WT) and T1 plants. The fruits of the H4 (WT) and T1 lines were harvested and weighed during the first 4 months of production to evaluate the yield.

For *A. thaliana* transgenic experiments, Landsberg ecotype and late-flowering *ft-1* mutant (NASC ID N56) on a Landsberg background were used. For flowering observation, plants were grown under LD (16 h : 8 h, 22°C : 18°C, day : night). Flowering date was estimated by counting the number of rosette leaves when the first inflorescence emerged.

Transgenic and grafting experiments on tobacco were performed using *Nicotiana tabacum* L. SR1 cv 'Petit Havana' (Maliga *et al.*, 1973) grown under LD. For each construct, three independent lines and five plants per line were used for grafting experiments.

Phylogenetic analysis

Phylogenetic analysis of FT- and TFL1/BFT proteins was performed using *A. thaliana* (At), potato (*Solanum tuberosum*, St), tobacco (*Nicotiana tabacum*, Nt), rose (*Rosa chinensis,* Rc) and strawberry (*Fragaria vesca*, Fve) sequences (Tables S1, S2). Multiple sequence alignments were generated by ClustalW (Thompson *et al.*, 1994) using BLOSUM matrix with default parameter setting. A phylogenetic tree was produced with the Geneious Tree Builder (http://www.geneious.com/) using AtMFT as outgroup with the same parameters as Tenreira *et al.* (2017).

Gene expression studies

Plant material was harvested and immediately frozen with liquid nitrogen or dipped in RNAlater® (Sigma) depending on the experiment. Total RNA was extracted from leaf, petiole, crown, runner tip, flower and root using an adapted protocol from Chang *et al.* (1993). RNA concentrations were determined using a NanoVue spectrophotometer (Biochrom, Cambridge, UK). Reverse transcription was carried out using 1 µg of total RNA and Invitrogen SuperScript IV reverse transcriptase following the manufacturer's instructions. qRT-PCR was performed using 5 µl of the resulting cDNA product (1/50 dilution) and 10 mM of each specific primer (Table S3) in a final volume of 20 µL with GoTaq qPCR Master Mix (Promega) using the CFX96 real-time system (Bio-Rad). Three biological replicates and three technical replicates were used for each sample. Depending on the experiment, two of the following genes, *FvMSI*, *FvEF1* and *FvAct*, were used as an internal standard to calculate the relative expression by the CT method.

Dissection, fixation and *in situ* hybridisation of primary crown from 2-month-old 'Reine des Vallées' (RdV; *tfl1*; *ga20ox4*) were performed as described previously (Tenreira *et al.*, 2017). *FveFT3*_ISH primers used for the synthesis of digoxygenin-UTP-labelled antisense RNAs are indicated in Table S3.

Plasmid constructs and plant transformation

Overexpression vectors were constructed by Gateway® cloning of amplified genomic DNA into donor vectors with Clonase™ II (Thermo Fisher Scientific, Waltham, MA, USA) (see Table S3 for gene-specific primers). Depending on the experiment, different destination vectors were used (Table S4). CRISPR/Cas9 mutagenesis of *FveFT2* (exon1) was carried out as described in Bollier *et al.* (2018) using two sgRNAs (Table S3). Resulting mutations were detected by PCR and sequencing in T1 lines (Table S3). To generate transgenic plants, the constructs were transformed into *Agrobacterium tumefaciens* (strain GV3101 or C58C1), and then either into *Arabidopsis* using the floral dip method (Clough & Bent, 1998), into strawberry by an adapted protocol (Oosumi *et al.*, 2006; Cappelletti *et al.*, 2015) or into tobacco as described in Horsch *et al.* (1986). Seeds or calluses were selected based on antibiotic resistance and/or green fluorescent protein (GFP) fluorescence. For each construct, 10 independent lines were selected, among which three or four lines were characterised in detail.

Tobacco grafting

Wild-type (WT) and transgenic seeds were surface sterilised and grown on selective media containing half-strength Murashige and Skoog (½MS) medium in a sterile chamber for 3 wk. In total, 15 transgenic plants by construct were selected using the appropriate antibiotic and screened for their GFP fluorescence before their transfer to a standard soil mixture. At 2 months after planting, grafting experiments were performed using cleft grafts (Lang *et al.*, 1977). The stock was a WT plant cut at six internodes on which the upper AXM was left as indicator shoot for the flowering response after grafting. The scion was the upper three or four internodes of *35S::FveFT2*^{Nt}, *35S::FveTFL1*^{Nt} or WT plant as control. At 1 and 3 months after grafting, the developmental stage of the indicator shoot on the WT stock was estimated as vegetative, beginning of flowering, full flowering or in fruit production.

Statistical analyses

Statistical analyses were performed using R software (R v.3.5.0) in the interface RSTUDIO (v.1.1.2.1572). A Kruskal–Wallis test (ANOVA on the rank and appropriate post hoc test) was used to compare mean values. Wilcoxon–Mann–Whitney test was used to compare WT to each transformant. Differences were considered significant at a P-value < 0.05.

Results

Floral initiation occurs under SD conditions in [SF] strawberry

When considering a [SF] plant issued from a seed planted in the spring in Year $n-1$, floral initiation takes place in

autumn, that is in inducible SD conditions; the plant remains dormant in winter and flowers in LD in Year *n* (Fig. 1a,b). By contrast, in [PF] plants, floral initiation additionally takes place in the spring after winter dormancy, that is in normally noninducible LD conditions; as a consequence, plants keep flowering all along Year *n* until winter (Fig. 1b). We studied the flowering behaviour of *F. vesca* plants exhibiting either a [SF] phenotype ('Norlanska' genotype) or a [PF] phenotype ('815' *tfl1* genotype) along the growth period from March to November. As indicated by the number of newly emerged inflorescences, the [SF] genotype produced inflorescences during a limited LD period (April to June) whereas the [PF] genotype continuously produced inflorescences throughout the growing season (Fig. 1c), which is consistent with previous observations in octoploid [SF] and [PF] genotypes (Gaston *et al.*, 2013; Perrotte *et al.*, 2016).

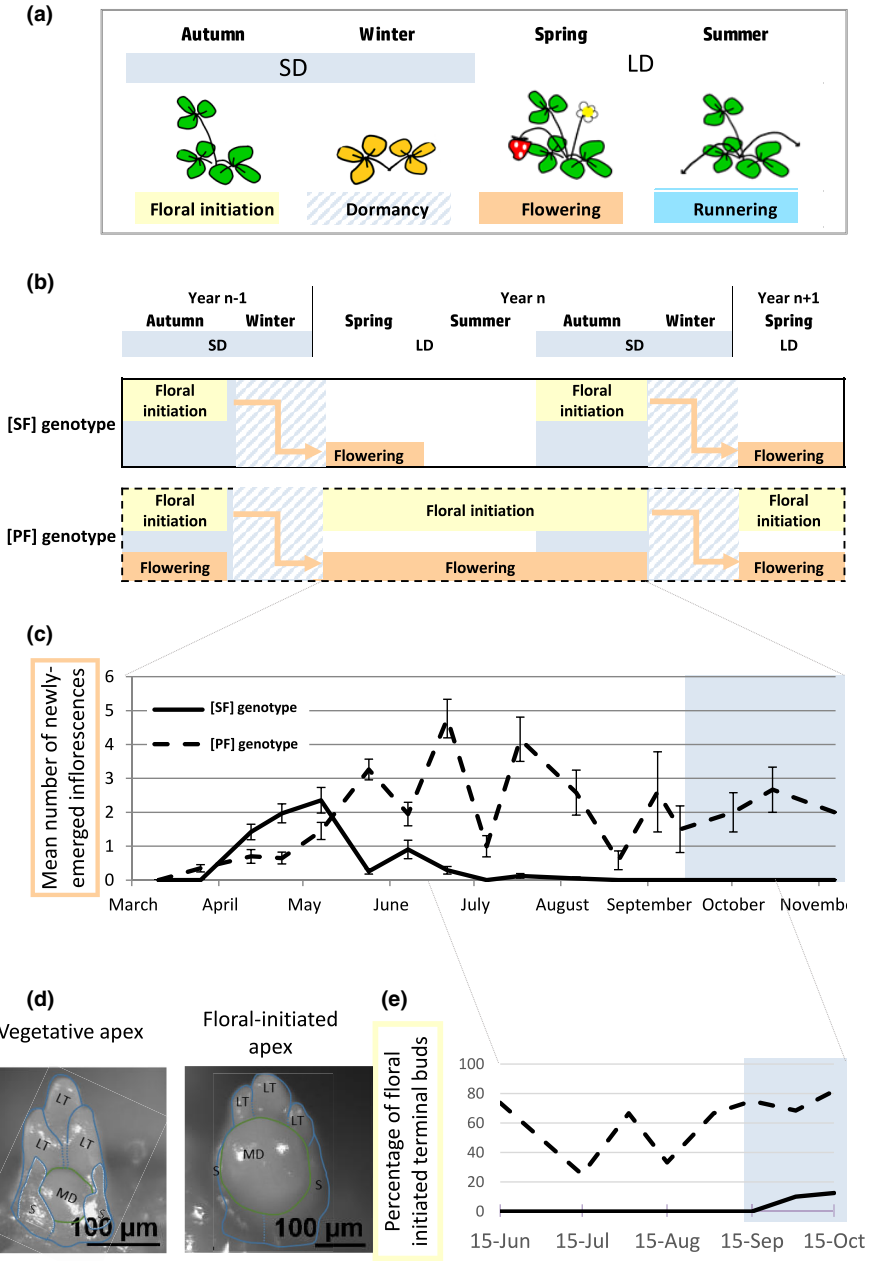

Fig. 1 Life cycle of the seasonal flowering [SF] and perpetual flowering [PF]) *Fragaria vesca* genotypes. (a) Annual life cycle of strawberry in temperate climate. SD, short-day photoperiod; LD, long-day photoperiod. (b) Schematic representation of the floral cycle of [SF] and [PF] genotypes. (c) Flowering period of [SF] and [PF] genotypes is represented by the mean number of newly emerged inflorescences. Error bars, mean ± SE ($n = 30$). (d) Vegetative and floral-initiated shoot apical meristems (SAM). LT, leaflet; MD, meristematic dome; S, stipule. (e) Floral initiation period from June to October of [SF] and [PF] genotypes is represented by the percentage of terminal buds initiated. Percentage of floral-initiated terminal buds is calculated based on the dissection of 11–20 terminal buds for each sampling date and genotype.

Next, we thoroughly characterised the floral initiation in SD, from June to October, by evaluating the SAM stage under stereomicroscope (Fig. 1d). To this end, we considered the meristem as vegetative when the apical dome was relatively flat and tended to be partially enclosed in the developing stipules of the youngest leaf. First indication of floral initiation was when the meristem dome raised above the developing stipules, and became broad and convex in shape (Taylor *et al.*, 1997) (Figs 1d, S1). This stage preceded the first indication of sepal development (Fig. S1). In the [SF] 'Norlanska', floral initiation took place at the beginning of October (11:30 day length and mean temperature of 17°C) (Fig. 1e). In the [PF] '815', initiated terminal buds were observed continuously from spring to late fall. These results indicate that, in the natural environmental conditions studied (South-West France), the SD photoperiod (<12 h) is floral inducible in the *F. vesca* 'Norlanska' [SF] strawberry genotype.

Seasonal flowering is likely to be controlled by the balance between the nonphotoperiodic floral activator *FveFT2* and the LD-expressed floral repressor *FveTFL1*

To gain further insight into the floral initiation in the SD photoperiod and how the floral activator/repressor balance modulates seasonal flowering in strawberry, we investigated the FT/TFL1 proteins from *F. vesca*. Phylogenetic tree analysis of CETS family proteins highlights three *F. vesca* FT-like proteins in a single clade and three TFL1-like proteins, among which was *Fve*TFL1, in two other clades (Iwata *et al.*, 2012) (Fig. 2a; Table S2). The *Fragaria Fve*FT1 grouped with the *Rosa Rc*FTb, and the *Fve*FT2 and *Fve*FT3 grouped with *Rc*FTa, suggesting a possible FT duplication before the separation of these close genera. *FveFT1* is most likely to be orthologous to *RcFTb* and *FveFT2* to *RcFTa* as they are physically located in syntenic regions (Jung *et al.*, 2019). Because *FveFT1* is a LD floral activator (Rantanen *et al.*, 2014), we focused on *FveFT2* and *FveFT3*, which are potential SD floral activators, and showed that they can successfully complement the late-flowering Arabidopsis *ft-1* mutant (Fig. S2a,b).

We then analysed the expression patterns of the three *FveFTs* and of *FveTFL1* in various organs of plants grown under an SD-inducible photoperiod (15 October, autumn) and under LD noninducible photoperiod (5 June, spring) (Fig. S3). In the [SF] genotype under SD, only *FveFT2* was detected in the leaves (Fig. S3), as expected for florigen. Moreover, *FveFT2* was expressed in the leaves regardless of the photoperiod, SD or LD, or the flowering behaviour, [SF] or [PF]. In [SF] leaves, *FveFT1* and *FveTFL1* were also expressed in the leaves but only under LD. *FveFT3* was never detected in the leaves. In both [SF] and [PF] under LD, all three *FveFTs* and *FveTFL1* were additionally detected in flower. Only *FveTFL1* was detected in stolon tip from [SF] (no stolons were produced by [PF] plants). Only *FveFT2* and *FveTFL1* were detected in crown and petiole, their expression being highly dependent on the genotype and photoperiod.

Because cross-talk between photoperiod and the circadian clock may control florigen expression (Andrés & Coupland, 2012), we next monitored the expression in leaves of the three *FveFTs* and of *FveTFL1* over a 24 h period. Leaves from [SF] and [PF] plants grown in natural conditions were sampled every 2 h in SD (September 20, autumn) or in LD (31 May, spring) photoperiods (Fig. 2b,c). The expression of *FveFT1* was prominent during the day in LD-collected leaves from the [SF] genotype. In both [SF] and [PF] genotypes, *FveFT2* expression was low and almost steady all along the day with a small peak of expression past mid-day for SD plants and before sunset for LD plants. *FveFT3* was not detected in the leaves, whatever the time of the day. The expression of *FveTFL1* was restricted to daytime in the LD leaves from the [SF] genotype, almost like *FveFT1,* but was not observed in [PF] whatever the sampling photoperiod or time of the day.

We further explored the *FveFTs* and *FveTFL1* expression patterns along a timeframe spanning the SD and LD photoperiods, from June to November. *FveFT1* expression was restricted to LD in the [SF] genotype. Furthermore, in [PF], *FveFT1* expression was out of phase with that in [SF], which suggests that its expression under SD is somehow deregulated by the *tfl1* mutation. *FveFT2* was weakly but continuously detected all along the growing season, whatever the genetic or environmental context (Fig. 2d). *FveTFL1* was expressed in LD but not in SD in the [SF] genotype. The lack of detection in [PF] plants of *FveTFL1* is likely to be provoked by the transcript instability caused by the *tfl1* mutation (Iwata *et al.*, 2012). To gain additional evidence that *FveFT1* is LD dependent and that *FveFT2* is photoperiod independent, we performed two parallel experiments in which plants grown in the same conditions until 15 July (LD) were either maintained under natural light conditions or transferred to constant controlled LD conditions (16 h : 8 h, day : night) until November (Fig. 2d,e). *FveFT1*, which is not expressed under SD in [SF] genotypes (Fig. 2d), maintained a high expression level under constant LD (Fig. 2e), indicating its dependency on the LD photoperiod. As expected, *FveFT2* proved to be largely insensitive to the photoperiod as it was expressed under both SD and LD (Fig. 2d,e). Unsurprisingly, *FveTFL1*, which was not expressed under SD in [SF] plants (Fig. 2d), like *FveFT1*, was still detected in [SF] plants maintained under LD.

Taken together, these results suggested that *Fve*FT1 is a floral activator restricted to the LD photoperiod in which its action can be counterbalanced by the floral repressor *Fve*TFL1. This activator/repressor balance is likely to be disrupted in [PF] plants lacking *FveTFL1* expression in which *Fve*FT1 can function as an LD floral activator (Koskela *et al.*, 2012; Rantanen *et al.*, 2014). Because the day-length insensitive *FveFT2* is the only strawberry *FT* gene detected both in the leaf and under inducible SD photoperiod, it is an excellent candidate to be the seasonal florigen. Conversely, no clues indicated that *FveFT3*, which was not detected in the leaves whatever the context, was a florigen. The marked expression of *FveTFL1* in the leaves further suggested that it may exert its LD floral repressor function, which was previously established (Iwata *et al.*, 2012; Koskela *et al.*, 2012), by acting as a mobile long-distance signal to the SAM.

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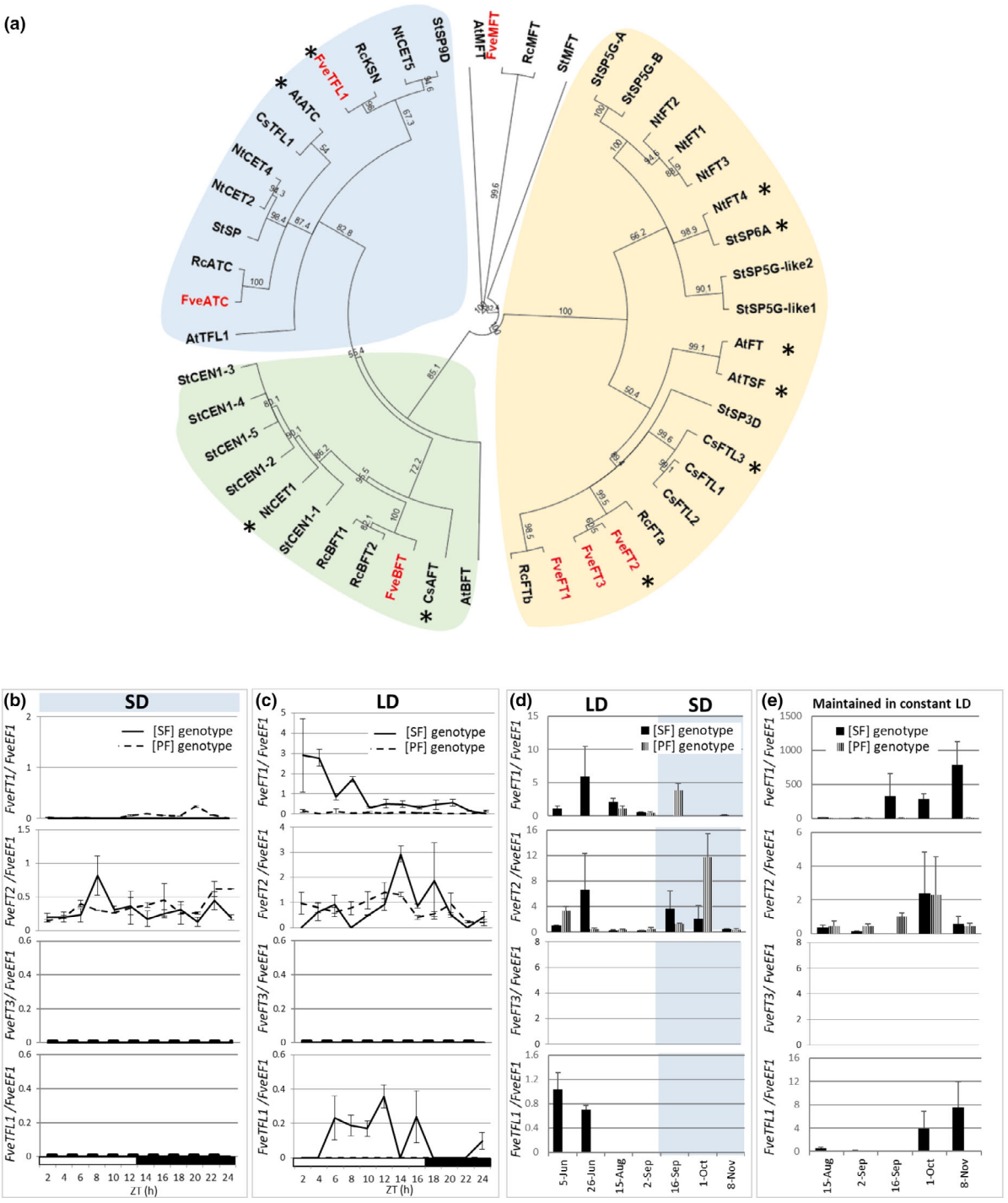

Fig. 2 Expression patterns of *FveFT1*, *FveFT2*, *FveFT3* and *FveTFL1* in seasonal flowering [SF] and perpetual flowering [PF]) strawberry genotypes. (a) Phylogenetic tree of FT and TFL1/BFT protein sequences from *A. thaliana* (At), potato (*Solanum tuberosum*, St), tobacco (*Nicotiana tabacum*, Nt), rosa (*Rosa chinensis,* Rc) and strawberry (*Fragaria vesca*, Fve (v.4.0.a2; Edger *et al*., 2018; Li *et al*., 2019b; Table S1). Strawberry proteins are indicated in red colour. FT, TFL1 and BFT clades are highlighted in yellow, blue and green, respectively. CETS family members with asterisk have been shown to present long-distance mobility capacity. Accession details are provided in Table S2. (b, c) Diurnal rhythm in transcript accumulation of *FveFT1, FveFT2, FveFT3* and *FveTFL1* in seasonal flowering [SF] and perpetual flowering [PF]) strawberry genotypes: (b) under SD (20 September) and (c) under LD (31 May). Day (white) and night (black) are shown on the *x*-axis. SD, short-day photoperiod; LD, long-day photoperiod. (d) Seasonal variations in the expression of the three *FveFT* genes and of *FveTFL1* in leaves of [SF] and [PF] genotypes grown in natural environmental conditions from June to November. SD, short-day photoperiod; LD, long-day photoperiod. (e) Variations in the expression of the three *FveFT* genes and of *FveTFL1* in leaves of [SF] and [PF] genotypes grown in natural environmental conditions from June to mid-July and thereafter maintained in constant noninducible LD conditions until November. Error bars, mean ± SE ($n = 3$), with transcripts normalised to *FveEF1*. LD, long-day photoperiod.

The *FveFT2* floral activator and the *FveTFL1* floral repressor form graft-transmissible signals

To test the possibility of long-distance action of the putative *FveFT2* florigen and of the *FveTFL1* antflorigen, we implemented a tobacco grafting experiment (Lang *et al.*, 1977) based on the stable expression of *FveFT2* and *FveTFL1* in tobacco and grafting of the transgenic shoots onto nontransgenic tobacco stock (from this point forwards named wild-type (WT)) (Fig. 3a). Phenotypes of first generation transgenic plants were consistent with the expected ones: tobacco lines expressing *FveFT2* (*35S::FveFT2*^{Nt}) were early flowering while lines expressing *FveTFL1* (*35S::FveTFL1*^{Nt}) were late flowering (Fig. S4). For each construct, three independent transgenic lines were grafted onto the WT stock. The last AXM on the stock was kept as a control; the shoot it produced was named the indicator shoot. Its role was to indicate if the floral signal could move from the scion to the stock and influence the fate of the AXM on the stock. The development state of the indicator shoot was checked 1 and 3 months after grafting and classified into four categories: vegetative, beginning of flowering, full flowering and fruit production. One month after grafting, indicator shoots were mostly vegetative or began to flower in the control graft (WT/WT) (Fig. 3b). In the *35S::FveFT2*^{Nt}/WT grafted plants, most indicator shoots were in fruiting and the others in the flowering category, thereby indicating an acceleration of the flowering in the indicator shoots. Conversely, the *35S::FveTFL1*^{Nt}/WT grafted plants remained vegetative. Three months after grafting, indicator shoots of WT/WT and *35S::FveFT2*^{Nt}/WT grafted plants all produced fruits, whereas indicator shoots of *35S::FveTFL1*^{Nt}/WT grafted plants were still vegetative and even became necrotic, indicating a strong delay of development. Altogether, these results indicated that *Fve*FT2 and *Fve*TFL1, respectively, are long-distance mobile signals able to accelerate or repress flowering, which is consistent with their florigen and antiflorigen functions in strawberry.

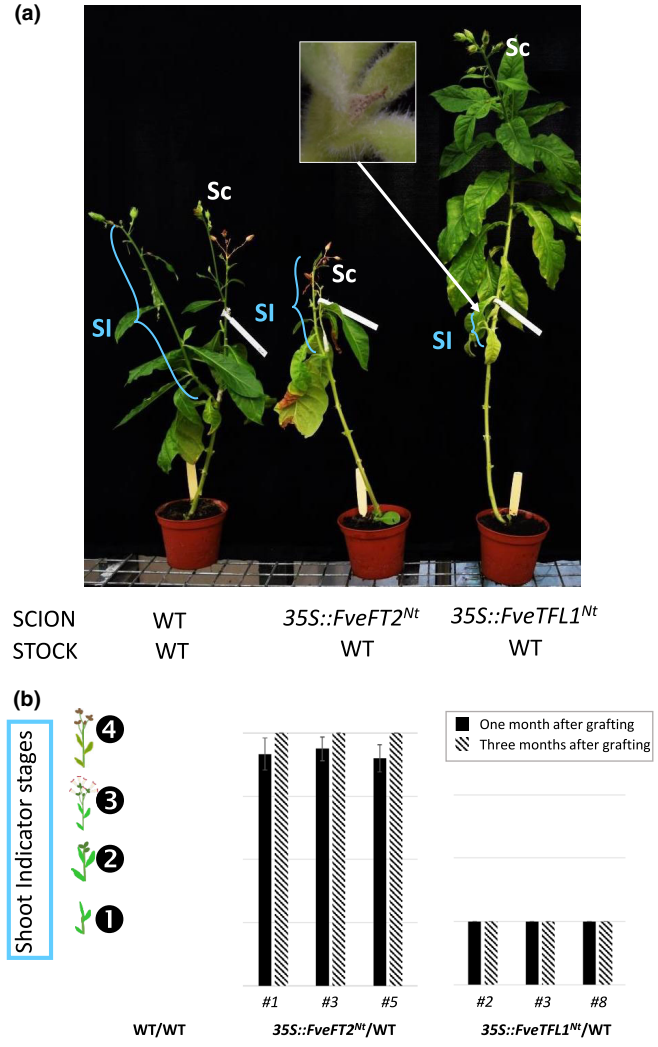

Fig. 3 Long-distance flowering control of *FveFT2* and *FveTFL1* tested by grafting experiments in tobacco (*Nicotiana tabacum*). *(a) Graft* combinations of wild-type (WT), *35S::FveFT2*^{Nt} or *35S::FveTFL1*^{Nt} tobacco transgenic scions on WT tobacco stock. Sc, scion; SI, shoot indicator of the WT tobacco stock. In the box, detail of the necrosis observed on *35S::FveTFL1*^{Nt} shoot indicator at 4.5 months after grafting. (b) Developmental stage of the shoot indicator corresponding to the uppermost axillary bud of the stock. One month (full) or 3 months (hatched) after grafting, the developmental stages of the shoot indicator were evaluated as vegetative (1), beginning of flowering (2), full flowering (3) and fruit production (4). Graft experiments were carried out with three independent lines for each construction. Error bars, mean ± SE (n = 5).

De-regulation of *FveFT2* strongly affects flowering in the diploid strawberry *F. vesca*

To further investigate in strawberry the role of the *Fve*FT2 florigen and of the closely related *Fve*FT3, we generated transgenic *F. vesca* diploid strawberry plants overexpressing *FveFT2* (*35S::FveFT2*^{FveOE}) or *FveFT3* (*35S::FveFT3*^{FveOE}) under the control of the 35S promoter. We used the 'Hawaii-4' genotype, a [PF] *tfl1* mutant commonly used for *F. vesca* genetic transformation that continuously produces inflorescences by SAM (Oosumi *et al.*, 2006). To take into account the potential effect of gene structure on gene regulation and function (Liu *et al.*, 2011; Bollier *et al.*, 2018), we further used genomic DNA for plant transformation.

Overexpression of both *FveFT2* and *FveFT3* (Fig. S5), dramatically affected the balance between flower and stolon production (Fig. 4a,b). The *35S::FveFT2*^{FveOE} T0 plants displayed a strikingly precocious flowering phenotype and could even produce inflorescences *in vitro*, before the transfer of plantlets to soil (Fig. 4a,c). In addition, the *35S::FveFT2*^{FveOE} T0 plants (Fig. 4b), which showed a severe dwarf and runnerless (no stolon) phenotype (Fig. 4d), were characterised by the production of a large number of inflorescences that reached up to 20 inflorescences per plant 10 wk after *in vitro* to soil transfer (Fig. 4c). The continuous production of inflorescences associated with the limited number and small size of the leaves eventually led to the death of the plant. By contrast, *FveFT3* had a very limited impact on flowering regardless of the high amino acid identity (*c.* 85%) it shares with *FveFT2*. The *35S::FveFT3*^{FveOE} T0 plants were similar to 'Hawaii-4' (Fig. 4a) and were not early flowering *in vitro* (Fig. 4c). They flowered slightly sooner (9 d; P = 0.04) than 'Hawaii-4'

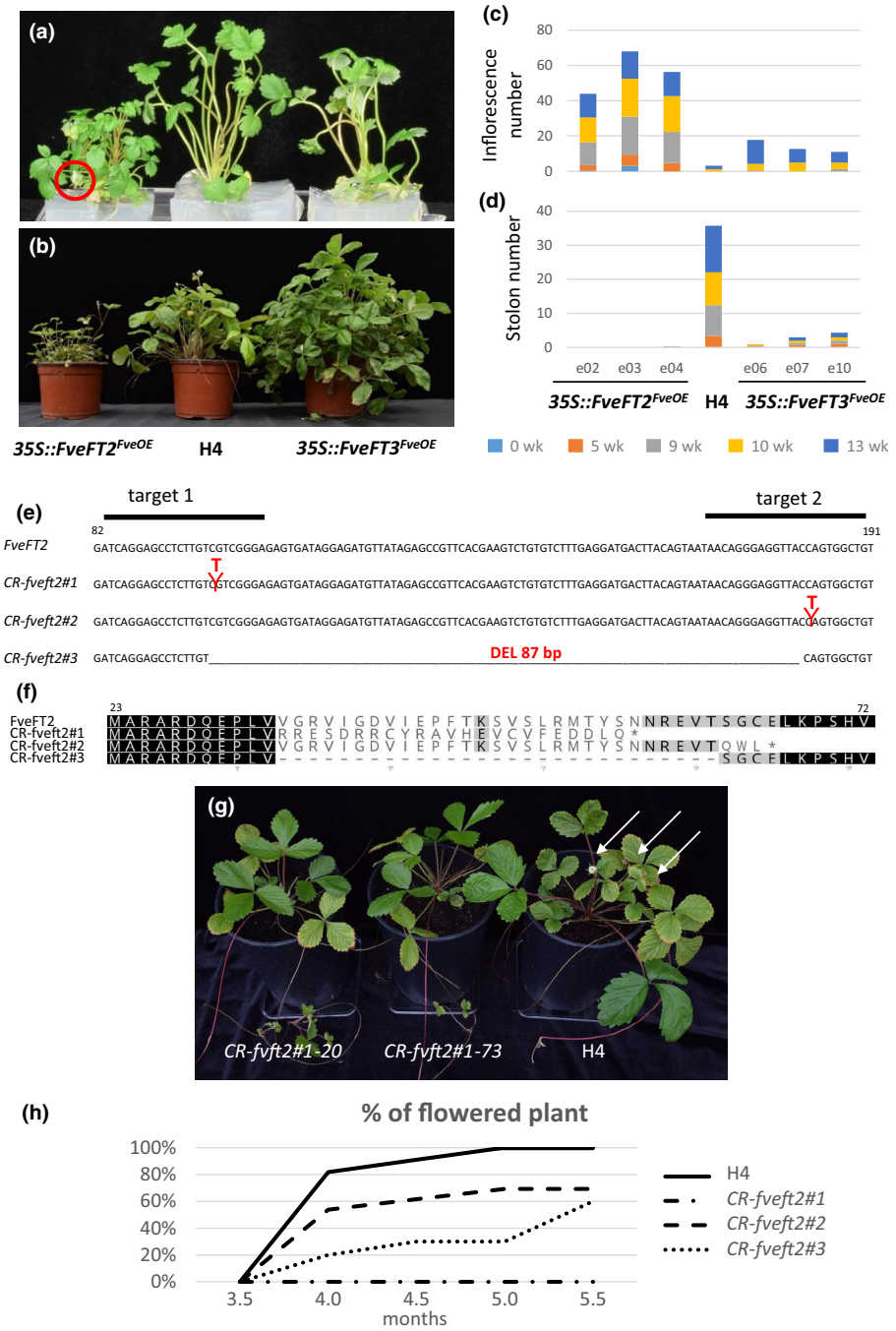

Fig. 4 Overexpression and CRISPR-Cas9 gene editing of *FveFT2* in *Fragaria vesca* have opposite effects on flowering. (a) 'Hawaii-4' H4 (WT) (*tfl1* mutant, [PF] genotype) and of *35S::FveFT2*^{FveOE} and *35S::FveFT3*^{FveOE} T0 plants captured the day of *in vitro* to soil transfer. The red circle indicates the presence of a flower on *in vitro* *35S::FveFT2*^{FveOE} plants. (b) 'Hawaii-4' H4 (WT), *35S::FveFT2*^{FveOE} and *35S::FveFT3*^{FveOE} T0 plants taken 8 months after *in vitro* to soil transfer. (c, d) Cumulative number of (c) inflorescences and (d) of stolons counted regularly from the day of *in vitro* to soil transfer to 13 wk after transfer on 'Hawaii-4' H4 (WT), three independent *35S::FveFT2*^{FveOE} T0 lines and three independent *35S::FveFT3*^{FveOE} T0 lines. Data represent the means of two to six plants per transgenic line or WT. (e) CRISPR/Cas9-induced mutations in the first exon of *FveFT2* obtained in three independent homozygous lines. Black lines indicate the two guide RNA target sequences. Insertions or deletion (DEL) are indicated in red. (f) CRISPR/Cas9-induced frameshift insertions (CR-*fveft2#1* and CR-*fveft2#2*) and deletion (CR-*fveft2#3*) led to truncated FveFT2 protein. (g) 'Hawaii-4' H4 (WT) and CR-*fveft2#1* T1 mutants 6.5 months after sowing. White arrows indicate flowers. (h) Percentage (%) of flowered plants in H4 (WT) and CRISPR/Cas9-induced mutants (T1 lines) from 3.5 to 5.5 months after sowing.

WT (Fig. S6) and had more inflorescences at 10 wk after *in vitro* to soil transfer (Fig. 4c). As shown in Fig. S5, the different flowering behaviours of *35S::FveFT2*^{FveOE} lines and *35S::FveFT3*^{FveOE} lines were not due to the de-regulation of *FveFT1* and *FveFT3* in *35S::FveFT2*^{FveOE} lines or to *FveFT1* and *FveFT2* in *35S::FveFT3*^{FveOE} lines.

Given the strong impact of *FveFT2* on flowering, we further explored its floral activator function by generating a series of *FveFT2* allelic mutants through CRISPR/Cas9 gene editing of 'Hawaii-4'. We selected three of the *CR-FveFT2* lines showing different mutations. Two different T insertions in the *FveFT2* sequence led to the production of different truncated proteins in the *CR-fveft2#1* and *CR-fveft2#2* lines (Fig. 4e,f). A large 87-bp deletion in *CR-fveft2#3* line produced an in-frame 29 amino acid gap in the protein (Fig. 4e,f). As expected, all the *CR-fveft2* mutants analysed displayed a late-flowering phenotype (Fig. 4g, h). While 100% of 'Hawaii-4' WT plants had already flowered at 5 months after sowing, about 40% of plants of *CR-fveft2#2* and *CR-fveft2#3* mutants had not yet flowered at 5.5 months (Fig. 4h). The *CR-fveft2#1* mutant never produced flowers until 5.5 months.

Overexpression of *FveFT3* in *F. vesca* has a strong impact on plant architecture and fruit yield

The impact of *FveFT2* and *FveFT3* overexpression on plant architecture was also very different according to the gene considered. The *35S::FveFT2*^{FveOE} T0 plants showed a stunted phenotype while the *35S::FveFT3*^{FveOE} T0 plants showed a very bushy phenotype (Fig. 5a,b). Both displayed a drastic reduction in stolon production (Fig. 4d). To further investigate the origin of these phenotypic differences, we dissected the architecture of T1 transformants. The *35S::FveFT2*^{FveOE} T1 plants displayed a short primary crown with four or five leaves (Fig. 5a). Almost all the AXMs from the crown developed into a very short BC quickly terminated by a single inflorescence, after producing a single leaf. By contrast, AXMs reprogramming in the *35S::FveFT3*^{FveOE} T1 plants led to the production of new inflorescence-bearing BC after three or four normal leaves, instead of stolons (Fig. 5a). Two new BC emerged from each BC, therefore explaining the bushy phenotype observed in T0 and T1 plants (Figs 4a,b, 5a).

These striking changes in plant architecture were effectively translated into yield variations. To reach this conclusion, we analysed the dynamic of fruit yield during the first 4 months of the production period in T1 plants grown under natural LD conditions (Fig. 5b). The *35S::FveFT2*^{FveOE} plants were early fruiting, as expected, but their total fruit yield was not significantly different from that of 'Hawaii-4' (Fig. 5c). By contrast, while the fruit production peak was reached in April–May for both *35S::FveFT3*^{FveOE} and 'Hawaii-4', the fruit yield of *35S::FveFT3*^{FveOE} increased considerably, being more than 3.5 times higher at the end of the production period (Fig. 5c).

To further explore the function of *FveFT3* in meristem fate and therefore in fruit yield, we investigated its expression in SAM and AXM meristems by *in situ* hybridisation of the primary crown. As the *F. vesca* 'Hawaii-4' AXMs mostly produce stolons (Fig. 5a), we used the *F. vesca* 'Reine des Vallées' (*tfl1*, *ga20ox4*) genotype in which AXMs produce BC because of the *ga20ox4* mutation (Tenreira *et al.*, 2017). *In situ* hybridisation detected *FveFT3* transcript accumulation in the SAM (Fig. S7a,b), which is consistent with published results from RNA-seq analysis of terminal bud from *F. vesca* (Li *et al.*, 2019a) and *F.* × *ananassa* (Koembuoy *et al.*, 2020). Interestingly, *FveFT3* transcripts were also detected in the AXM dome, which is consistent with a role for *FveFT3* in the regulation of AXM fate (Fig. S7c,d). To obtain more insight into the molecular mechanisms by which *FveFT3* may regulate AXM fate, we next investigated by qRT-PCR the expression of the floral promotor *FveFUL* (Ferrándiz *et al.*, 2000) and of the stolon promotor *FveGA20ox4* in axillary bud from *35S::FveFT3*^{FveOE} and 'Hawaii-4' plants (Fig. S7e). *FveFUL* was upregulated in AXM while, conversely, *FveGA20ox4* was downregulated, which is consistent with the promoting or suppressing effects of *FveFT3* overexpression on inflorescence-bearing BC and stolon production, respectively.

FveFT2 also promotes early flowering in octoploid cultivated strawberry

To investigate the potential biotechnological applications of our findings in cultivated octoploid strawberry, we then overexpressed the nonphotoperiodic florigen *FveFT2* in the *F.* × *ananassa* seasonal flowering [SF] genotype 'Sveva' (Fig. S8). In all the four independent *F.* × *ananassa 35S::FveFT2*^{Fa0E} 'Sveva' lines obtained, *FveFT2* overexpression had a substantial impact on flowering and stolon production as these *35S::FveFT2*^{Fa0E} lines displayed precocious *in vitro* flowering and did not produce stolons (Fig. 6), like the *F. vesca 35S::FveFT2*^{Fve0E} lines. Note that 'Sveva' WT did not flower because of the lack of floral initiation. To verify whether this effect was not due to the de-regulation of major floral activator or repressor, we analysed the expression of *FaFT1* and of *FaTFL1* in the *F.* × *ananassa 35S::FveFT2*^{Fa0E} lines. *FaTFL1* expression was not altered while the considerable variations in *FaFT1* expression (Fig. S8) were independent of the very early-flowering phenotype observed in all the lines analysed (Fig. 6).

Discussion

In this paper we present evidence that *FveFT2* behaves as a nonphotoperiodic florigen, regulating the timing of flowering, while *FveFT3* promotes plant branching, that is likely to be through the regulation of AXM fate. We additionally showed that *FveTFL1* is the LD antflorigen hypothesised by Guttridge (1959a,b) and contributes together with *FveFT2* to the photoperiodic regulation of flowering.

FveFT2 is the nonphotoperiodic florigen in strawberry permitting SD flowering

A long-standing question regarding the photoperiodic regulation of flowering in strawberry is the nature of the SD florigen (Hartmann, 1947). FT's role as a florigen, that is a mobile signal produced in the leaf and transmitted to the SAM where it initiates flowering, has been confirmed in a considerable number of plant species (Pin & Nilsson, 2012; Wickland & Hanzawa, 2015). In addition to the *FveFT1* gene previously shown to act as an LD florigen in the flowering de-regulated *F. vesca tfl1* mutant (Koskela *et al.*, 2012), the poorly known *FveFT2* and *FveFT3*

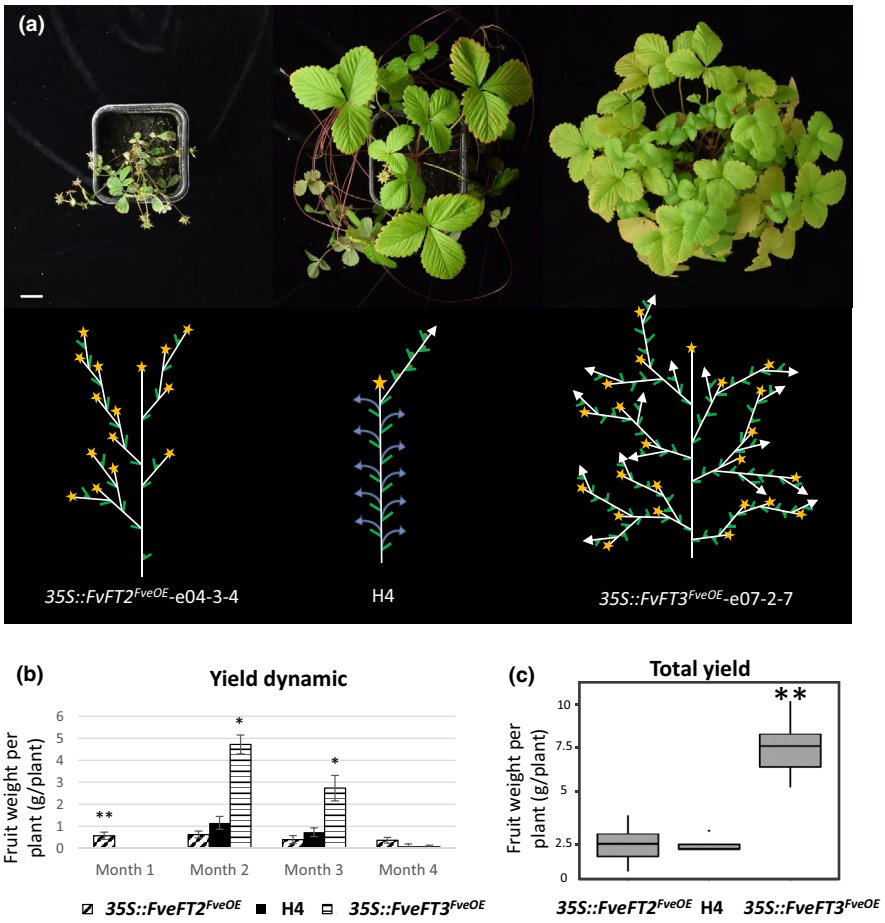

Fig. 5 Overexpression of *FveFT3* in *Fragaria vesca* has a strong impact on plant architecture and fruit yield. (a) 'Hawaii-4' H4 (WT) and of *35S::FveFT2*^{FveOE} and *35S::FveFT3*^{FveOE} T1 plants of 6-month-old and their schematic plant architecture. On each axis (white line), leaves (small green lines) present at their bases an AXM that can either remain latent or give a stolon (blue arrow) or give a new axis or a BC (white arrow), which terminates by an inflorescence (yellow star). White bar, 2 cm. (b) Evolution of fruit production of 'Hawaii-4' H4 (WT) and of *35S::FveFT2*^{FveOE} and *35S::FveFT3*^{FveOE} T1 plants from the first to the fourth month of the production period. Plants were grown under LD natural conditions. For each month, data represent mean of fruit weight (g per plant). A Wilcoxon–Mann–Whitney test showed significant differences (*) between 'Hawaii-4' H4 and *35S::FveFT2*^{FveOE} plants the first month of harvest and between 'Hawaii-4' H4 and *35S::FveFT3*^{FveOE} plants the second and third months of harvest. Error bars, mean ± SE ($n = 4$, H4; $n = 12$, *35S::FveFT2*^{FveOE}; $n = 6$, *35S::FveFT3*^{FveOE}). *P*-value = 0.05* > 0.01**. (c) Total fruit yield at the end of the first 4 months of harvest of 'Hawaii-4' H4 (WT) and of *35S::FveFT2*^{FveOE} and *35S::FveFT3*^{FveOE} T1 plants grown under LD natural conditions. Box plots of fruit yield (g per plant). A Wilcoxon–Mann–Whitney test showed significant differences (*) between 'Hawaii-4' H4 (WT) and *35S::FveFT3*^{FveOE} plants and no difference between 'Hawaii-4' H4 WT and *35S::FveFT2*^{FveOE} plants ($n = 4$, H4; $n = 12$, *35S::FveFT2*^{FveOE}; $n = 6$, *35S::FveFT3*^{FveOE}). **, $P \geq 0.01$.

genes were therefore natural candidates for the control of floral induction in SD photoperiod.

FveFT1 and *FveFT3* were not detected in the leaf under an SD-inducible photoperiod, in agreement with previous studies (Koskela *et al.*, 2012, 2017). *FveFT2* was detected in the leaf under an SD-inducible photoperiod, and is therefore the only strawberry FT that meets the florigen definition (Andrés and Coupland, 2012) for this photoperiod. In agreement with previous studies (Koskela *et al.*, 2012; Nakano *et al.*, 2015; Hawkins *et al.*, 2017), we detected a very low *FveFT2* expression in the leaf in both SD and LD photoperiods, which may explain why *FveFT2* was not previously considered as a likely florigen candidate. The florigen candidate must also be mobile (Chailakhyan, 1936). Using tobacco grafting experiments (Lang *et al.*, 1977; Freiman *et al.*, 2015), we showed that *FveFT2* expression in tobacco accelerates flowering in the indicator shoot, which is evidence that *FveFT2* is responsible for a long-distance signal that activates flowering by moving from scion to stock. The third clue that *FveFT2* functions as a florigen in strawberry is the striking early-flowering phenotype that we observed, even *in vitro*, in *F. vesca* plants overexpressing *FveFT2* and the substantial flowering delay observed in CRISPR/Cas9 mutant lines. Note that these extreme phenotypes were not reported for *FveFT1* (Rantanen *et al.*, 2014). Genetic indications point to the possible role of FveFT2 in the control of flowering. In the octoploid strawberry, *FveFT2* is located in the *FaPFRU* genomic region that oppositely controls perpetual flowering and stolon production (Gaston *et al.*, 2013; Perrotte *et al.*, 2016), while in the *F. vesca* diploid, *FveFT2* is co-located with a flowering QTL controlling AXM differentiation into stolons or BC (Samad *et al.*, 2017).

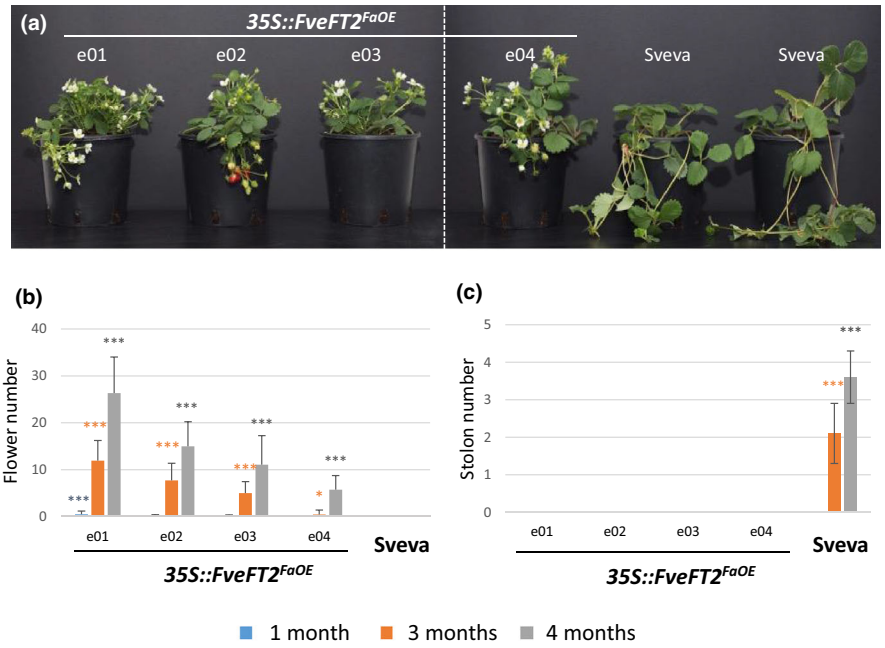

Fig. 6 Overexpression of *FveFT2* in cultivated octoploid strawberry induces early flowering. (a) Sveva (WT [SF] octoploid genotype) and of four independent *35S::FveFT2*^{FaOE} T0 lines 4 months after *in vitro* to soil transfer in a glasshouse under LD. (b) Number of flowers and (c) of stolons counted 1, 3 and 4 months after *in vitro* to soil transfer. Data represent a minimum of 10 plants scored for each line ± SE. Asterisks indicate significant differences (Wilcoxon–Mann–Whitney test) between Sveva (WT) and *35S::FveFT2*^{FaOE} T0 lines: *, $P < 0.05$; ***, $P < 0.001$.

Overexpression of *FveFT2* and *FveFT3* in *F. vesca* additionally revealed that these two close paralogues, which share *c.* 85% amino acid identity, fulfil specific functions in strawberry. While both are able to complement the late-flowering Arabidopsis *ft-1* mutant, only *FveFT2* is a florigen and triggers early flowering in strawberry. Moreover, only *FveFT2* is syntenic with *RcFTa*, a rose floral activator (Randoux *et al.*, 2014; Wang *et al.*, 2017), which is consistent with *FveFT2* having retained a floral activator function and being a florigen. By contrast, *FveFT3* overexpression mostly affects the branching of the plant, which is likely to indicate that the *FveFT3* function in the control of AXM fate was acquired during, or soon after, speciation. An intriguing question is why *FveFT2* and *FveFT3*, which are both under the control of the 35S promoter in the transgenic plants, produce different phenotypic changes in strawberry and Arabidopsis. A possible explanation is that, because we used genomic DNA and that gene regulation depends on the genetic context (Liu *et al.*, 2011), we could discriminate their functions in strawberry but not in Arabidopsis. Small divergences in amino acid sequences may also affect FT function, as shown in Arabidopsis (Ho & Weigel, 2014) and potato (Navarro *et al.*, 2011).

The *FveFT2* florigen and the *FveTFL1* antflorigen contribute to the photoperiodic regulation of strawberry flowering

An additional question is how *FveFT2* triggers floral initiation specifically during the SD photoperiod, whereas its expression is constitutive. At 60 yr ago, Thompson & Guttridge (1960) proposed that photoperiodic flowering in strawberry is regulated by a leaf-produced flowering inhibitor (the antiflorigen). The inhibitory effect was transmitted via the stolon from the mother plant (in LD) to its daughter plant (in SD) (Guttridge, 1959a,b), therefore suggesting the existence of a mobile signal repressing flowering under LD, but not under SD. However, Thompson & Guttridge (1960) did not exclude the existence of a flowering-promoting factor (the florigen) acting independently of the photoperiod, which, in the light of our results, is *FveFT2*.

The question remains as to the nature and properties of the strawberry antiflorigen. Flowering regulation by mobile floral repressors has been documented in few species (Thomas & Vince-Prue, 1997). *TFL1* genes are key floral repressors in many plant species (Wickland & Hanzawa, 2015). In *F. vesca*, depending on the photoperiod, *FveTFL1* is detected in the SAM (Koskela *et al.*, 2012). We additionally detected its expression in the leaf. *FveTFL1* is therefore a possible candidate for being the strawberry antiflorigen providing that it: (i) represses flowering, (ii) shows long-distance mobility, and (iii) is expressed in the leaf in noninducible LD. First, *FveTFL1* represses flowering in both diploid and octoploid strawberries (Koskela *et al.*, 2012, 2016). Second, our results revealed that *FveTFL1* overexpression in tobacco scion represses indicator shoot development, indicating a graft-transmissible floral inhibitor effect. Third, *FveTFL1* is expressed in the leaf under LD. To date, photoperiodic regulation has been much less frequently observed in the *TFL1* clade than in the *FT* clade (Wickland & Hanzawa, 2015). Actually, *FveTFL1* was the first photoperiodic *TFL1* gene reported (Koskela *et al.*, 2012), before the discovery of the chrysanthemum *CsAFT* (Higuchi *et al.*, 2013), which is an antiflorigen like *FveTFL1*.

The expression of *FveTFL1* in the leaf in addition to the SAM raises the question of the mechanisms by which flower initiation is repressed in noninducible conditions. In potato, the FT-like long-distance tuberigenic signal StSP6A migrates from the leaves to the stolon where the tuber-inducing signal is amplified through an autoregulatory mechanism (Navarro *et al.*, 2011). By analogy, we propose that, in noninducible LD, *FveTFL1* is expressed during a short time window in the leaf and migrates to the SAM where the antiflorigenic signal is amplified and maintained. In [SF] genotypes, the role of *FveFT1* in the control loop would be to participate in the maintenance of *FveTFL1* expression in the SAM. Interestingly, while *FveFT1* is a photoperiodic florigen like the canonical *FT* genes, *FveFT2* resembles the nonphotoperiodic FT florigens previously identified in day-neutral plant species, for example the *SFT* gene in tomato and the *StSP3D* gene in potato (Lifschitz & Eshed, 2006; Navarro *et al.*, 2011). *FT* and *TFL1* genes have been shown to dynamically evolve through duplication events over the course of plant evolution (Pin *et al.*, 2010; Wickland & Hanzawa, 2015; Moraes *et al.*, 2019). Taken together, our results support the hypothesis that the balance between the nonphotoperiodic florigen *FveFT2* and the photoperiodic antiflorigen *FveTFL1* controls photoperiodic flowering in strawberry.

FveFT2, *FveFT3* and *FveTFL1* shape plant architecture in different ways

A high FT/TFL1 ratio triggers SAM and AXM conversion into flowers, which results in early-flowering plants (Moraes *et al.*, 2019). In tomato, a sympodial species like strawberry (Gaston *et al.*, 2020), the FT/TFL1 balance coordinates the sympodial cycles (Pnueli *et al.*, 1998; Shalit *et al.*, 2009; Lifschitz *et al.*, 2014). An additional feature of strawberry is that, while the SAM produces flowers as in tomato, the AXM produces either a stolon, as in potato, or an inflorescence-bearing BC, or stays latent (Tenreira *et al.*, 2017). Therefore, the modulation of plant architecture through the control of AXM fate is a means to control the trade-off between fruit yield (through flowering) and daughter-plant yield (through stolons) in strawberry (Tenreira *et al.*, 2017; Gaston *et al.*, 2020).

As shown here, the high *FveFT2/FveTFL1* transcript ratio in transgenic plants causes a striking early-flowering phenotype not only by accelerating the conversion of SAM into flowers but also that of AXM into inflorescences-bearing BC. Consequently, it prevents the AXM from remaining indeterminate or differentiate into a stolon, thereby strongly affecting the plant architecture and the vegetative propagation. Because of the commonalities between the cultivated octoploid *F.* × *ananassa* and the wild diploid *F. vesca*, which is the dominant subgenome of the cultivated strawberry (Edger *et al.*, 2019), we could further demonstrate that our findings on *FveFT2* in *F. vesca* can be readily translated into the improvement of cultivated strawberry. Indeed, expression of the nonphotoperiodic florigen *FveFT2* had also a spectacular impact on flowering (earliness and flower number) in [SF] cultivated strawberry.

FveFT3 transcripts have been previously detected in the strawberry shoot apex under inducible conditions, therefore leading to the hypothesis that *FveFT3* might play a role in floral initiation (Nakano *et al.*, 2015; Koskela *et al.*, 2017; Hytonen & Kurokura, 2020; Koembuoy *et al.*, 2020). However, we showed here that *FveFT3* is not a long-distance florigen and that its overexpression has only a slight effect on flowering. Conversely, shoot branching is strongly affected. Indeed, AXM reprogramming in *F. vesca* plants overexpressing *FveFT3* leads to the production of new BC instead of stolons. This may arise from the release of lateral buds from apical dominance, as observed in tomato when the function in the SAM of the TFL1 homologue, SP (self-pruning), is compromised (Lifschitz & Eshed, 2006). The alteration of *FveFT3* expression in the SAM could generate a systemic signal inducing the production of BC from AXM. This hypothesis involves not only the release from apical dominance but also the reprogramming of AXM, which produces inflorescence-bearing BC instead of stolon. *In situ* hybridisation confirmed that *FveFT3* is indeed expressed in the SAM. Interestingly, *FveFT3* is also expressed in the AXM, therefore suggesting that the change of AXM fate may occur when the function of FveFT3 is locally altered in the AXM. Because each new BC ends up with an inflorescence in the *tfl1* genetic background, the fruit yield of the *35S::FveFT3*^{FvOE} plants is dramatically increased by more than 3.5-fold (Fig. 5c). Such substantial increase in fruit yield can be meaningful for producers for which a few per cent positive or negative variations of total fruit yield may involve the success of or, alternatively, the economic failure of their activity.

Our study additionally sheds light on the mechanisms involved in the determination of strawberry architecture and yield. Until recently, few molecular actors have been shown to affect strawberry branching in addition to the antiflorigen *Fve*TFL1 (Iwata *et al.*, 2012; Koskela *et al.*, 2012). They include the flowering pathway integrator *Fve*SOC1 (Mouhu *et al.*, 2013) and the GA biosynthetic enzyme *Fve*GA20ox4 (Tenreira *et al.*, 2017) and the GA receptor DELLA (Caruana *et al.*, 2018), whose mutations led to a runnerless (no stolon) phenotype. Preliminary evidence supports the idea that *FveFT3* acts as a signal upstream of the molecular machinery controlling AXM fate in strawberry because *FveFT3* overexpression represses *FveGA20ox4* gene expression in the AXM from *35S::FveFT3*^{FveOE} plants and induces the floral promotor *FveFUL*.

Conclusion

We unravelled major mechanisms through which strawberry plants adapt flowering to the photoperiod and control the trade-off between flowering and vegetative propagation. A simplified view of the photoperiodic flowering pathway in strawberry is proposed in Fig. 7a, while the modulation of strawberry plant architecture by the interplay between *Fve*FT2, *Fve*FT3 and *Fve*TFL1 is summarised in Fig. 7b. We bring new insights into the regulation of seasonal flowering by a balance between the photoperiod-insensitive florigen *FveFT2* and the photoperiod-sensitive antiflorigen *FveTFL1*, as well as into the neofunctionalisation of *FveFT3* to control AXM fate. In addition, our findings have

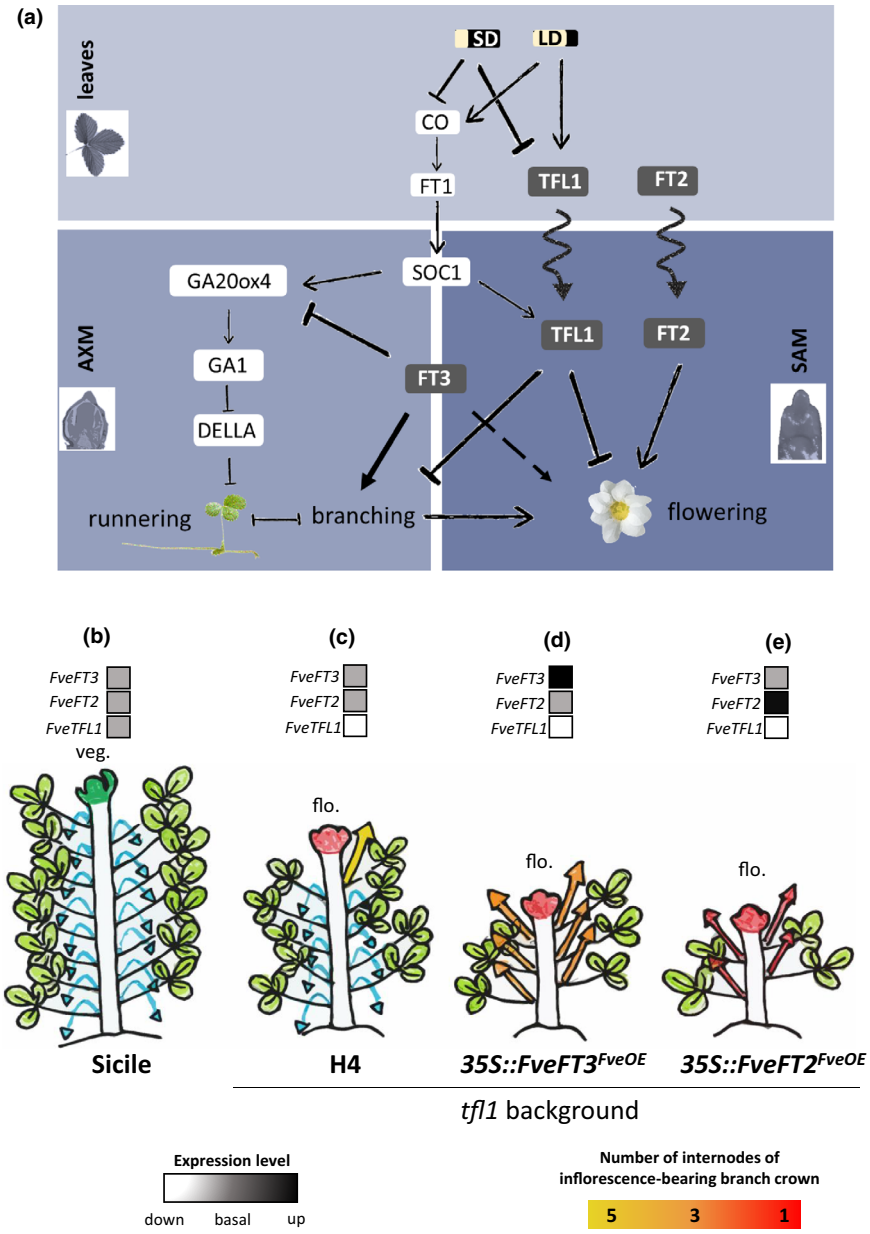

Fig. 7 Photoperiodic flowering pathway in strawberry and impact of *FveFT2*, *FveFT3* and *FveTFL1* on plant flowering and architecture. (a) Hypothetical model for the regulation of flowering, branching and runnering in strawberry. Arrows indicate activation and bars indicate repression. (b–e) Representation of the changes in strawberry plant architecture according to the expression level of *FveFT2*, *FveFT3* and *FveTFL1* under long days (b) In the [SF] 'Sicile' *F. vesca* cultivar, in which the antiflorigen *FveTFL1* gene is active, the fine tuning of the balance between *FveFT2*, *FveFT3* and *FveTFL1* allows the SAM to pursue the vegetative growth and the AXM to produce stolons. (c) In the *tfl1* mutant 'Hawaii-4' H4, in which the antiflorigen *FveTFL1* gene is inactive, the SAM of the primary crown is converted into an inflorescence after 10 leaves and the uppermost AXM produces a new inflorescence-bearing BC after five leaves. This results in a perpetual flowering phenotype. The other AXMs of the primary crown produce stolons. (d) In the transgenic line *35S::FveFT3*^{FveOE} in the 'Hawaii-4' H4 *tfl1* genetic background, a high *FveFT3*/*FveTFL1* ratio induces the conversion of the SAM of the primary crown into an inflorescence after six leaves and of the AXM into a new inflorescence-bearing BC after three leaves. (e) In the transgenic line *35S::FveFT2*^{FveOE} in the 'Hawaii-4' H4 *tfl1* genetic background, a high *FveFT2*/*FveTFL1* ratio triggers the very quick conversion of the SAM of the primary crown into an inflorescence after four leaves, which results in an extreme early-flowering phenotype. All AXMs produce very short new inflorescence-bearing BCs with only one leaf leading to a dwarf phenotype and absence of stolons. AXM, axillary meristem; flo., floral apical meristem; LD, long days; SAM, shoot apical meristem; SD, short days; veg., vegetative apical meristem.

major implications for strawberry improvement because the two traits that we successfully modulated, which are flowering earliness and yield, are major breeding targets (Mezzetti *et al.*, 2018; Gaston *et al.*, 2020). Quantitative tuning of flowering signals can considerably improve productivity of many crop species (reviewed in Eshed & Lippman, 2019), an example of which is tomato (Park *et al.*, 2014; Li *et al.*, 2018; Zsögön *et al.*, 2018). Similarly, in strawberry, future studies aimed at the identification

of natural or edited genetic variants of flowering pathway genes described here (Fig. 7a) should contribute to the creation of superior varieties.

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Author contributions

AG and BD conceived the studied and designed experiments. AG and TT produced and phenotyped the Arabidopsis transgenic lines. AG, TT and NC performed the RNA extraction and expression analyses. AG and AP produced, with the help of KMF, and phenotyped the diploid strawberry transgenic lines. SS and BM produced and phenotyped the octoploid strawberry transgenic lines. MA performed the molecular characterisation of the strawberry transgenic lines. MA and MH performed *in situ* hybridisation. PP took care of the plants and performed the architecture description with ML. ML performed the meristem description. MH and FD produced the tobacco transgenic lines. AG and FD performed the grafting experiments. MA and BD performed the statistical analyses. AG, BD and CR wrote the manuscript. All the authors discussed the results and commented the manuscript.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Development of the shoot apical meristem (SAM) in *F. vesca* from vegetative meristem to inflorescence.

Fig. S2 Functional complementation of the *A. thaliana ft-1* mutant with *F. vesca* strawberry *FveFT2* and *FveFT3* genes expressed under the constitutive 35S promoter.

Fig. S3 Relative level expression of *FveFT* genes and of *FveTFL1* in leaf, petiole, crown, runner tip and flower of [SF] and [PF] genotypes grown under natural environmental conditions and harvested in SD and LD.

Fig. S4 Ectopic expression of *FveFT2* and *FveTFL1* in tobacco.

Fig. S5 Expression pattern of genes-of-interest in leaves of transgenic diploid strawberry T0 lines.

Fig. S6 Effect of *FveFT3* overexpression on flowering.

Fig. S7 Expression of *FveFT3* in AXM of diploid 'Reine des Vallées' RdV (*tfl1*, *ga20ox4*) and of related genes-of-interest in leaf and axillary bud of *35S::FveFT3*^{FveOE} 'Hawaii-4' H4 (*tfl1*).

Fig. S8 Expression pattern of genes-of-interest in leaves of transgenic octoploid strawberry T0 lines.

Table S1 Gene identifiers for CETS family in strawberry.

Table S2 Gene identifiers for phylogenetic analysis.

Table S3 List of primers used in the manuscript.

Table S4 List of constructs used in the manuscript.