RNA-seq Analysis of Meristem Cells Identifies the *FaFT3* Gene as a Common Floral Inducer in Japanese Cultivated Strawberry

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In *Arabidopsis thaliana*, the *FLOWERING LOCUS T* (*FT*) gene, acting as a floral promoter, is expressed and translated in leaves, and is then transported to the shoot apical meristem. In contrast, the expression pattern of the *FaFT3* gene in the crown, which contains the shoot apical meristem, is coordinated with the initiation of the floral bud in the June-bearing type of cultivated strawberry (*Fragaria* × *ananassa*) ‘Nyoho’. However, whether the *FaFT3* protein functions as a floral promoter and whether the expression pattern of *FaFT3* in the crown observed in ‘Nyoho’ is conserved in other strawberry cultivars are not known. In this study, we investigated the floral inducer activity of the *FaFT3* gene isolated from the cultivated strawberry ‘Tochiotome’ using *FaFT3*-overexpressing transgenic *Arabidopsis* lines and performed expression analysis on the *FaFT3* gene in the crown tip of ‘Tochiotome’. Transgenic plants overexpressing the *FaFT3* gene exhibited an early-flowering phenotype under both long-day and short-day conditions. Conversely, induction of *FaFT3* expression at the crown tip specifically under floral induction conditions was not observed. However, RNA-seq analysis of laser microdissected meristem cells before and after floral bud initiation clearly revealed that the *FaFT3* gene is specifically expressed in floral meristem cells. These results suggest that the *FaFT3* gene acts as a common floral promoter in June-bearing Japanese cultivated strawberries.

Key Words: floral induction, floral meristem, *Fragaria*, RNA-seq, shoot apical meristem.

Introduction

The cultivated strawberry (*Fragaria* × *ananassa*) is an octoploid plant (2n = 8x = 56) speculated to originate from a cross between the octoploid wild species *F. chiloensis* and *F. virginiana* (Njuguna et al., 2013). Recently, the genome of *Fragaria* × *ananassa* ‘Camarosa’ was determined by combining short-read and long-read sequencing approaches. Phylogenetic analysis using 31 *de novo* assembled transcriptomes, which were comprised of 12 wild *Fragaria* species and four *F. vesca* subspecies, further revealed that four subgenomes of *Fragaria* × *ananassa* originated from

the diploid *Fragaria* species *F. nipponica*, *F. iinumae*, *F. viridis*, and *F. vesca* subsp. *bracteata*. Interestingly, the *F. vesca* subgenome is dominant over the other three subgenomes, as the number of genes on the *F. vesca* subgenome is greater than that on other subgenomes and many dominantly expressed homoeologs have been mapped onto the *F. vesca* subgenome (Edger et al., 2019).

Most cultivated strawberries produced in Japan are June-bearing (or seasonal flowering) types. During the vegetative phase, strawberry plants form vegetative shoots known as runners from a compressed and thickened stem called a crown. When the vegetative to reproductive transition occurs, inflorescence is differentiated from the meristem cells located at the tip of the crown. Generally, in June-bearing strawberry plants, flowering is inhibited under high temperature conditions over 25°C and flowering is promoted under cool temperature conditions between 5°C and 15°C, irrespective of the photoperiod (Heide et al., 2013; Ito and Saito, 1962). When the temperature is between 15°C and 25°C, flowering is induced only under short-

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day conditions (Heide, 1977; Sønsteby and Heide, 2006). A cultivation technology called forcing cultivation, frequently used in Japan to expand the harvesting period, utilizes this flowering behavior in strawberry plants. In this approach, daughter plants at the vegetative phase are grown for about 20 days under short-day and night cooling conditions with a low nitrogen fertilizer in the summer to induce floral transition. However, observation of the floral meristem under a microscope is necessary before transplanting because the treatment has no effect if the treatment period is too short to induce floral initiation. In addition, prolonged treatment causes a negative effect on fruit production because flower numbers in transplanted daughter plants are significantly reduced (Morishita et al., 1992; Ueki et al., 1993). Since determining the floral differentiation stage under a microscope is a skilled and time-consuming job, a simple alternative method is required. Understanding the molecular mechanism of flowering induction in cultivated strawberry is the first step to achieve this goal.

FLOWERING LOCUS T (FT) is well known as a floral inducer. In the facultative long-day plant *Arabidopsis*, *FT* is expressed in the phloem companion cells of leaves under inductive long-day conditions (An et al., 2004). Translated FT protein is then transported to the shoot apical meristem (SAM) and binds to a bZIP transcription factor, FD. This FT-FD complex promotes the expression of the floral meristem identity genes like *APETALA1* (*API*) (Abe et al., 2005). In contrast, TERMINAL FLOWER1 (TFL1) expressed in the SAM acts as a floral repressor by forming a complex with FD, preventing binding of FT to FD (Abe et al., 2005). However, in strawberry, the situation is complicated. Although Rantanen et al. (2014) reported that the ectopic expression of *FvFT1* in the long-day accession of *F. vesca* promoted flowering, expression of the *FvFT1* gene in leaves was observed only under non-flowering-inductive long-day conditions in the short-day accession of *F. vesca* (Koskela et al., 2012). In the June-bearing cultivated strawberry ‘Nyoho’, three *FT* homologues were identified (*FaFT1-3*), but none of them exhibited an expression pattern corresponding to the vegetative-to-reproductive transition in leaves. Instead, *FaFT3* exhibited an expression pattern correlated with floral induction in the shoot tip containing SAM (Nakano et al., 2015). However, whether the expression pattern of *FaFT3* is conserved among other June-bearing strawberry cultivars and whether *FaFT3* functions as a floral inducer are not known.

Previously, we isolated *FaFT1* cDNA from the June-bearing cultivated strawberry ‘Tochiotome’ and confirmed that *FaFT1* was expressed in leaves under non-flowering-inductive long-day conditions (Nakajima et al., 2014). However, we failed to isolate the *FaFT2* and *FaFT3* genes at that time. In the present study, we cloned *FaFT3* from ‘Tochiotome’ with the aid of the

FaFT3 sequence of ‘Nyoho’ and the database of the octoploid strawberry genome (Strawberry GARDEN; <http://strawberry-garden.kazusa.or.jp/index.html>; Hirakawa et al., 2014) and compared its expression pattern between meristem cells at the vegetative or reproductive phases. In addition, floral inducer activity of *FaFT3* was investigated using transgenic *Arabidopsis* plants ectopically expressing *FaFT3*. Our results support the idea that *FaFT3* acts as a common floral inducer in June-bearing Japanese cultivated strawberries.

Materials and Methods

Plant materials

Parent plants of *Fragaria* × *ananassa* ‘Tochiotome’ and ‘Akihime’ were grown in a greenhouse equipped with a misting system under natural day-length conditions at Aichi Agricultural Research Center in 2015. The misting system was activated when the temperature in the greenhouse reached 30°C or higher. ‘Tochiotome’ and ‘Akihime’ runner plants with three expanded leaves were cut off and planted in pots on 30 May 2015. We removed the leaves from the plants except for three to five sets of the youngest fully developed leaves. Plants were grown under short-day and low temperature conditions (SDLT; an 8-h photoperiod and day/night temperatures of average 32.5/18.7°C) or long-day and high temperature conditions (LDHT; average day length and day/night temperatures were 13 hours 49 minutes and 31.2/27.4°C, respectively). SDLT plants were grown in a field in the daytime, but were transferred into a curtained tunnel tent with air cooling from 17:00 to 9:00. We sampled leaves and crowns of ‘Tochiotome’ plants on 0, 5, 15, 20, 25, 30, 35, and the 41st day after SDLT or LDHT treatments. The youngest expanded leaf was sampled from each plant at 17:00. Tissues were stored at –80°C. The developmental stages of the flower buds were defined as: stage 0 – an undifferentiated stage, stage A – an initial to late differentiation stage.

Total RNA extraction from a 5 mm crown tip

Total RNA was extracted from a tip of the strawberry crown (5 mm in length) by the hot borate method, as described in Nakajima et al. (2014).

Isolation of *FaFT3* and *FaFD*

The *FaFT3* and *FaFD* genes of ‘Tochiotome’ were isolated using a standard RT-PCR method described by Nakajima et al. (2014). Briefly, total RNA extracted from the tip of the crown was reverse-transcribed using a PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa Bio Inc., Shiga, Japan). Subsequently, 1 µL of complementary DNA (cDNA) was used for PCR with Ex-Taq (TaKaRa Bio). The *FaFD* gene of ‘Akihime’ was isolated by the same procedure. Primers used for the analysis are listed in Supplemental Table S1.

Vector construction and plant transformation

Full-length coding sequences of *FaFT1* or *FaFT3* were amplified and cloned into a T-Vector pMD20 (TaKaRa Bio). Then, plasmids were digested with *NdeI* and *KpnI* and ligated into the binary vector pRI 101-AN (TaKaRa Bio). The resulting vectors, pRI 101-AN-*FaFT1* and pRI 101-AN-*FaFT3*, were transferred into *Agrobacterium tumefaciens* strain GV3101 by electroporation. *Arabidopsis thaliana* ecotype Columbia were transformed using the modified floral dip method (Narusaka et al., 2010). Transgenic plants were grown under a long-day (16-h photoperiod) or a short-day (8-h photoperiod) at 22°C. We analyzed the flowering phenotype in the second generation.

Total RNA extraction from laser microdissection (LM)-isolated tissues

Laser microdissection was performed according to Takahashi et al. (2015) with the following modifications: tips of the strawberry crown (3 mm in length) were sectioned at a thickness of 12 µm and SAM or the floral meristem were collected from crown cross-sections using a Veritas Laser Microdissection System LCC1704 (Molecular Devices, Sunnyvale, CA, USA). Total RNA was extracted from the LM-isolated tissues using a PicoPure RNA isolation kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. The quantity and value of the RNA Integrity Number (RIN) of extracted total RNA was assessed using the Quant-iT RiboGreen RNA Reagent (Invitrogen, Carlsbad, CA, USA) and an RNA 6000 Pico Kit on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). RNA samples with a RIN value more than 5.6 were used for RNA-seq analysis.

RNA-seq library preparation

cDNA was generated using a SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (TaKaRa Bio USA, Inc., Mountain View, CA, USA) from 3 ng each of RNA samples. Ten cycles of PCR amplification were performed, the quantity of cDNA was analyzed with a Qubit dsDNA HS Assay Kit (Invitrogen), and then the cDNA was fragmented for 10 min by a Covaris sonicator (Covaris, Woburn, MA, USA). Using 5 ng each of cDNA, sequencing libraries were prepared using the Low Input Library Prep Kit v2 (TaKaRa Bio USA) according to the manufacturer's protocol. We used nuclease-free water instead of total RNA as a negative control in cDNA amplification and library preparation, and confirmed that the concentration of the sequencing library of a negative control was below the detection limit. Paired-end 100 bp sequencing was used to sequence the libraries on a HiSeq 2000 sequencer (Illumina, San Diego, CA, USA). All generated sequence data are available from the DNA Data Bank of Japan (DRX178207–DRX178213).

RNA-seq data analysis

Low quality RNA-seq reads were removed by a FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html) with the following options; -t 20 and -l 36. Reads mapped with Bowtie2 (Langmead and Salzberg, 2012) to the rRNA genes of *A. thaliana* were excluded. Quality trimmed and rRNA-contamination-filtered reads were aligned to the FANhybrid_r1.2 genome using Bowtie2. Clustering analysis of sequenced samples was performed using the TCC package in R (Tang et al., 2015). Differentially expressed genes (DEGs) between the SAM (S25-0-1, S25-0-2, and L25-0) and the floral meristem (S41-A-1 and S41-A-2) were identified using DESeq2 (Love et al., 2014). Genes with a FDR < 0.05 were classified as DEGs. For GO enrichment analysis, Arabidopsis homologues of each DEG were obtained from the Strawberry GARDEN and were added to the query list of agriGO v2.0 (Tian et al., 2017). Annotation data from TAIR10 were used as a reference.

Quantitative real-time PCR (qPCR)

Total RNAs extracted from the tip of the crown or LM-isolated tissues were reverse-transcribed using a PrimeScript RT reagent Kit with a gDNA Eraser (Perfect Real Time) (TaKaRa Bio). qPCR was carried out as described in Nakajima et al. (2014). Primers used for the analysis are listed in Supplemental Table S1.

Results

Functional analyses of the *FaFT3* gene isolated from the cultivar 'Tochiotome'

To analyze whether the *FaFT3* gene acts as a floral inducer in June-bearing type cultivated strawberries, we cloned it from the reproductive stage (30 days after SDLT treatment) of 'Tochiotome' crown tissue using primers corresponding to *FaFT3* of 'Nyoho'. The isolated gene *FaFT3_Tochi* showed five nucleotide differences with two amino acid substitutions compared to the amino acid sequence of *FaFT3* in 'Nyoho' (Figs. 1a and S1a). We generated transgenic *Arabidopsis* plants overexpressing *FaFT3_Tochi*, *FaFT1* from 'Tochiotome' or a floral activator of wild strawberry *FvFT1* from the *F. vesca* subsp. *vesca* accession Hawaii-4 under the control of a CaMV 35S promoter and investigated their flowering times under long-day and short-day conditions. As a result, 35S::*FaFT3_Tochi* flowered significantly earlier than wild type plants under both long-day and short-day conditions, whereas 35S::*FvFT1* plants exhibited an early flowering phenotype only under short-day conditions (Fig. 1b, c). Surprisingly, although there is only one amino acid difference at position 80 between *FvFT1* and *FaFT1*, flowering time was significantly delayed in 35S::*FaFT1* plants under short-day conditions (Fig. 1a, c).

Expression pattern of *FaFT3_Tochi* in crown tissue

The early flowering phenotype of 35S::*FaFT3_Tochi*

Fig. 1. Flowering phenotype of transgenic *Arabidopsis* plants ectopically expressing FT homologues from wild and cultivated strawberries. (a) Alignment of FT proteins isolated from a wild strawberry (AEP23098.1 for FvFT1) and the cultivated strawberries ‘Tochiotome’ and ‘Nyoho’ (BAR63708.1 for FaFT1_Nyoho and BAR63710.1 for FaFT3_Nyoho). Black or white circles represent amino acid polymorphisms between FvFT1 and FaFT1s or between FaFT3_Tochi and FaFT3_Nyoho, respectively. (b) and (c) Rosette leaf number at flowering of wild type (WT) and transgenic plants ectopically expressing *FvFT1*, *FaFT1* or *FaFT3* under long-day (b) and short-day (c) conditions. The vertical axis indicates the number of rosette leaves at flowering and the bars represent average values $\pm$ SE ($n = 3-6$). Asterisks indicate significant differences between WT and transgenic plants (Dunnett’s test, $*P < 0.05$, $***P < 0.001$). L1, L2, L3: the transgenic line 1–line 3.

transgenic plants suggests that FaFT3_Tochi acts as a floral inducer in June-bearing cultivated strawberry. To verify this hypothesis, we investigated the expression pattern of *FaFT3_Tochi* in the crown tissue under floral inductive conditions (SDLT) or vegetative conditions (LDHT). The expression pattern of the floral indicator gene *FaAPI* confirmed that the transition from the vegetative to reproductive phase occurred only under the SDLT conditions (Fig. 2). The expression pattern of *FaFT3_Tochi* was highly correlated with that of *FaAPI*. Both genes were down-regulated during the early to mid-phase (from day 0 to day 15 for *FaFT3_Tochi* and from day 0 to day 25 for *FaAPI*), but up-regulated during the mid- to late phase (from day 20 for *FaFT3_Tochi* and from day 30 for *FaAPI*) under the SDLT condition. However, expression of *FaFT3_Tochi* was also up-regulated transiently during the early to mid-phase, but was relatively low during the late phase under the LDHT condition (Fig. 2).

Isolation of *FaFD* and its expression patterns in crown tissue of the June-bearing cultivated strawberry ‘Tochiotome’

As the expression pattern of *FaFT3* could not fully explain the transition from the SAM to the floral meristem, we isolated *FaFD*, a putative binding partner of FaFT3, and investigated its expression pattern in the crown tissue of ‘Tochiotome’. Two FD homologues were predicted in the strawberry GARDEN (FANhyb_icon00001611_a.1.g00001.1 and FANhyb_rscf00000064.1.g00025.1), and we succeeded in isolating two types of cDNA clones, classified as *FaFD_allele_a* and *FaFD_allele_b*. Both these sequences were very similar to FANhyb_icon00001611_a.1.g00001.1, and contained four nucleotide differences that caused two non-synonymous substitutions at the coding region (Fig. S1b, c). Although the total homology of their deduced amino acid sequences and FD proteins from other plants was low, high homology was observed at the basic-leucine zipper domain (Fig. S1c). However, the 14-3-3 protein-binding region that is highly con-

Fig. 2. Time-course expressions of the genes *FaFT3*, *FaFD*, and *FaAPI* in the crown tip of 'Tochiotome' grown under different photoperiods and temperatures for 0 to 41 days. The *MSI* gene was used as an internal control. The means $\pm$ SE from three biological replicates are shown. SDLT, short-day, and low temperature condition; LDHT, long day and high temperature condition.

served in FD homologues (Taoka et al., 2011) was disrupted in the *FaFD* alleles (Fig. S1c). The disruption of the 14-3-3 protein-binding region was also observed in *FaFD* isolated from the cultivated strawberry 'Akihime' (Fig. S1c). From qPCR analysis, *FaFD* was expressed uniformly in the crown tissue under both SDLT and LDHT conditions (Fig. 2).

RNA-seq analysis of meristem cells during the transition from the vegetative to reproductive phase in a June-bearing strawberry

From the time-course expression analysis of *FaFT3*,

it was speculated that *FaFT3* was a floral inducer of the June-bearing type of cultivated strawberry. However, *FaFT3* expression in the crown tissue was observed not only during flower inductive conditions (SDLT), but also during flower non-inductive conditions (LDHT) (Fig. 2). To confirm the function of *FaFT3* in meristem cells of the crown tissue and to identify other genes related to the vegetative-to-reproductive transition in cultivated strawberry, total RNA was extracted from laser-microdissected meristem cells (Fig. S2a) and used for sequencing. We generated six sequence libraries that were then divided into four vegetative phase samples and two reproductive phase samples. For vegetative phase samples, we used a single plant grown under LDHT conditions for 25 or 41 days (LDHT25-0 and LDHT41-0) and two plants grown under SDLT conditions for 25 days (SDLT25-0-1 and SDLT25-0-2). Two plants grown under SDLT conditions for 41 days (SDLT41-A-1 and SDLT41-A-2) were used as the reproductive phase samples. Microscopy observations confirmed that the meristems of all vegetative phase samples had a flat surface, whereas those of all reproductive phase samples had a dome-like structure. However, the number of total reads obtained from LDHT41-0 was about four to five times lower than the other five samples and LDHT41-0 formed a completely different clade by clustering analysis of the RNA-seq samples (Fig. S2b and data not shown). Therefore, we excluded LDHT41-0 from the subsequent analysis.

Using the DESeq2 package, we identified 1,151 DEGs between the vegetative and reproductive phase (Fig. S2c). Among them, 702 genes were up-regulated during the vegetative-to-reproductive transition. Although Kurokura et al. (2006) reported that *Histone H4* mRNA was highly expressed in the central zone of the shoot apex in plants grown under floral inductive conditions, but not in plants grown under non-floral inductive conditions, we did not detect any significant differences in the expression of three *Histone H4* genes between the vegetative and reproductive meristem (Table 1). GO enrichment analysis revealed that the biological processes "response to abiotic stimulus", "response to stimulus", and "response to endogenous stimulus" were the most strongly enriched. Up-regulated genes were also enriched in the biological processes "flower development" and "reproductive structure development" (Fig. 3a and data not shown). Notably, the *FaFT3* gene (FANhyb_icon00009135_a.1.g00001.1; Fig. S3) and the *FaAPI* gene (FANhyb_rscf00000019.1.g00010.1; Fig. S3) were classified as up-regulated genes (Table 1). In contrast, 449 down-regulated genes were strongly enriched in the biological processes "nucleobase-containing compound metabolic process" and "nitrogen compound metabolic process". The GO term "reproductive structure development" was also enriched in down-regulated genes (Fig. 3b). The expression pattern of the three genes, *FaFT3*, *FaFD*, and *FaAPI*, in meri-

Table 1. List of the up- and down-regulated genes during floral bud initiation in the cultivated strawberry ‘Tochtotome’.

Category	Gene ID in FANhybrid_r1.2	Best-hit in TAIR10	Annotation/ Classification	DEG ^z	DESeq2-normalized counts					P value	FDR
					SDLT25-0-1	SDLT25-0-2	LDHT25-0	SDLT41-A-1	SDLT41-A-2		
Flowering related	FANhyb_icon00017910_a.1.g000001.1	AT1G65480.1	FaFT1	nonDEG	0.000	0.000	0.000	1.165	0.000	0.712	1.000
	FANhyb_rscf00000117.1.g00001.1	AT1G65480.1	FaFT1	UP	3.027	4.817	1.279	1102.544	359.337	0.000	0.000
	FANhyb_icon00009135_a.1.g000001.1	AT1G65480.1	FaFT3	UP	0.000	1.376	0.000	3889.208	1067.065	0.000	0.000
	FANhyb_rscf00001426.1.g00001.1	AT5G03840.1	FaTFL1	DOWN	217.934	47.483	1001.189	4.662	12.768	0.000	0.007
	FANhyb_rscf00003474.1.g00001.1	AT2G27550.1	FaTFL2	nonDEG	2.018	9.634	20.459	5.827	1.824	0.299	0.719
	FANhyb_rscf00000005.1.g00042.1	AT2G27550.1	FaTFL3	UP	1.009	0.000	0.000	599.057	653.008	0.000	0.000
	FANhyb_icon00001611_a.1.g000001.1	AT4G35900.1	FD	DOWN	1130.029	706.048	710.934	13.986	13.680	0.000	0.000
	FANhyb_rscf00000064.1.g00025.1	AT4G35900.1	FD	nonDEG	6010.344	15352.071	13113.914	14561.512	12872.275	0.496	0.841
	FANhyb_rscf00000019.1.g00010.1	AT1G69120.1	AP1	UP	21.188	4.129	10.229	32909.670	22757.678	0.000	0.000
	FANhyb_rscf00001466.1.g00002.1	AT4G36920.2	AP2	DOWN	7476.355	10698.072	10116.740	1937.028	1959.935	0.000	0.000
	FANhyb_rscf000006979_a.1.g000001.1	AT5G61850.1	LEAFY	UP	134.191	290.402	180.291	851.966	812.611	0.000	0.000
	FANhyb_rscf00000831.1.g00001.1	AT5G61850.1	LEAFY	UP	1423.635	2953.565	2364.238	17238.621	9291.677	0.000	0.000
Gibberellic acid related	FANhyb_rscf00001850.1.g00003.1	AT3G63010.1	GID1B	UP	995.838	293.843	618.870	7037.171	6723.424	0.000	0.000
	FANhyb_rscf00000478.1.g00007.1	AT5G27320.1	GID1C	UP	17210.751	7358.451	14390.015	27792.046	49361.351	0.000	0.004
Red, far-red light response	FANhyb_icon00002013_a.1.g000001.1	AT1G09570.2	PHYA	DOWN	1604.238	1052.190	1222.397	423.069	432.298	0.000	0.000
	FANhyb_rscf00000243.1.g00011.1	AT1G09570.1	PHYA	DOWN	4171.019	5031.796	4526.449	3201.575	3074.425	0.002	0.041
	FANhyb_rscf00000179.1.g00008.1	AT4G15090.1	FAR1	DOWN	752.680	579.427	641.886	336.824	381.225	0.000	0.009
Histone	FANhyb_rscf00000294.1.g00011.1	AT5G59970.1	Histone H4	nonDEG	28602.859	25298.677	22922.498	35699.830	23863.960	0.362	0.762
	FANhyb_rscf000006289.1.g00001.1	AT5G59970.1	Histone H4	nonDEG	20272.928	15429.144	15842.570	29328.149	18677.293	0.066	0.394
	FANhyb_rscf000007095.1.g00002.1	AT5G59970.1	Histone H4	nonDEG	26005.809	20564.852	19705.395	35892.134	25537.520	0.046	0.331

^z Up- or down-regulated from vegetative to reproductive phase.

Fig. 3. Gene ontology enrichment analysis of the differentially expressed genes during floral bud initiation in the cultivated strawberry 'Tochiotome'. (a) Up-regulated from the vegetative to reproductive phase in meristem cells. (b) Down-regulated from the vegetative to reproductive phase in meristem cells.

stem cells was investigated by qPCR, and the results matched those of the RNA-seq data (Table 1; Fig. S4).

Discussion

In the present study, we isolated the *FaFT3* gene from the cultivated strawberry 'Tochiotome' and examined whether it plays a major role in the transition of meristem cells from the vegetative to reproductive phase. Previously, Nakano et al. (2015) reported that *FaFT3* gene expression was significantly up-regulated at the shoot tip when the plants were grown under short-day or low temperature conditions, and this up-regulation of *FaFT3* occurred prior to the induction of *FaAPI* gene expression in the cultivated strawberry 'Nyoho'. In 'Tochiotome', no clear up-regulation of the *FaFT3* gene specific to the SDLT treatment was observed (Fig. 2). This was in contrast to the induction of *FaAPI* expression that was observed only in plants grown under a flowering-inductive SDLT treatment such as 'Nyoho'. However, in the RNA-seq analysis of meristem cells, the *FaFT3* gene (FANhyb_icon00009135_a.1.g00001.1) was significantly up-regulated during the transition from the vegetative to reproductive phase. This result suggests that the tissue where induction of the *FaFT3* gene occurred was more spatially restricted to the meristem cells in 'Tochiotome' compared to 'Nyoho'. In addition to the similarity of the *FaFT3* expression pattern between 'Tochiotome' and 'Nyoho', ectopic expression of *FaFT3_Tochi* in Arabidopsis promoted flowering, and the amino acid sequences of *FaFT3* from the two cultivars were almost identical (Fig. 1). These results suggest that *FaFT3* acts as a common floral inducer in June-bearing Japanese cultivated strawberries.

Involvement of other flowering-related genes in initiation of the floral bud in cultivated strawberry

During the vegetative to reproductive transition, FANhybrid_r1.2 annotated genes encoding *FaFT1*, *FaTFL3*, *AP1*, and *LEAFY* were up-regulated similar to the *FaFT3* gene, whereas annotated genes encoding *FaTFL1*, one of two FDs (FANhyb_icon00001611_a.1.g00001.1) and *AP2* were down-regulated (Table 1). The expression patterns of *LEAFY* homologues and the *AP2* homologue agree with the previously described function of these two genes in the flowering of Arabidopsis. *LEAFY* plays a pivotal role in floral induction, whereas *AP2* directly induces the transcription of the floral repressor *AGAMOUS-LIKE 15* and represses the expression of floral activators such as *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (Blázquez et al., 1997; Weigel et al., 1992; Yant et al., 2010). Similarly, it was recently shown that FD transcription was reduced soon after the floral transition in Arabidopsis (Abe et al., 2019). However, as the other FD homologue (FANhyb_rscf00000064.1.g00025.1) was stably expressed during the floral transition (Table 1), additional experiments are needed to clarify whether the FT-FD complex functions as a transient stimulus for floral initiation in cultivated strawberries.

We previously reported that a reduction in *FaTFL2* expression in the tip of the crown is a key signal for floral initiation of 'Tochiotome' (Nakajima et al., 2014). However, our RNA-seq data revealed that an annotated gene encoding *FaTFL1*, not *FaTFL2*, was significantly down-regulated in the reproductive meristem compared to the vegetative meristem (Table 1). This result is consistent with the previous study of Nakano et al. (2015) showing that the expression of the *FaTFL1* gene at the shoot tip of 'Nyoho' was significantly higher under

non-floral inductive conditions than under floral inductive conditions. To determine whether either or both FaTFL1 and FaTFL2 act as floral repressors, flowering behavior should be observed in cultivated strawberry plants overexpressing or suppressing the *FaTFL1* or *FaTFL2* gene.

Other gene expression changes in meristem cells during the vegetative to reproductive transition in the cultivated strawberry 'Tochiotome'

In Arabidopsis, five major pathways (i.e., age pathway, autonomous pathway, gibberellin (GA) pathway, photoperiod pathway, and vernalization pathway) are involved in flowering time regulation (Teotia and Tang, 2015). In accordance with this, we detected an up-regulation of the gibberellin receptors *GIBBERELLIN-INSENSITIVE DWARF1B (GID1B)* and *GID1C* homologues and a down-regulation of phytochrome A (phyA)-related genes (Table 1).

GID1 is a GA receptor involved in growth regulation through the formation of the GA-GID1-DELLA complex (Wang and Deng, 2014). In Arabidopsis, flowering is accelerated by exogenous GA treatment. However, the effect of GA treatment on strawberry plants is enigmatic because as Sonam and Singh (2018) reported, GA treatment could promote vegetative growth (i.e., runner production) in some experiments and could induce flowering in others. Hormonome analysis of the crown at the stage before and after floral bud induction could be useful to identify the phytohormones involved in the floral transition in cultivated strawberry.

PhyA is a phytochrome that primarily responds to red and far-red light, and FAR-RED-IMPAIRED RESPONSE1 (FAR1) is a transcription factor positively regulating the phyA-signaling pathway. PhyA exists in two photo-interconvertible forms termed Pr and Pfr, which absorb red light and far-red light, respectively (Sheerin and Hiltbrunner, 2017; Wang and Wang, 2015). Interestingly, illumination of the crowns of the cultivated strawberry 'Strawberry Festival' with red light resulted in a significant decrease in the number of flowering plants (Takeda et al., 2008). Taking into account the fact that the strawberry cultivar 'Carmine' maintained at a high plant density during the vegetative phase flowered under flower-noninductive high temperature and long-day conditions (Takeda and Newell, 2006), Takeda et al. (2008) proposed that a high Pr:Pfr ratio would favor the activation of the flowering pathway in cultivated strawberry. Our finding that phyA-related genes are highly expressed in the vegetative meristem of 'Tochiotome' suggests that cultivated strawberries perceive the photoperiodic stimuli not only in leaves, but also in the crown harboring the vegetative meristem.

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