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-
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 (AP1) (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 Ndel and Kpn1 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 Inc., Mountain View, CA, USA) for the analysis are listed in Supplemental Table S1.

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 were listed as a reference.

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
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 FaAP1 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 FaAP1. 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 FaAP1), but up-regulated during the mid- to late phase (from day 20 for FaFT3_Tochi and from day 30 for FaAP1) 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_rscf0000064.1.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-
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 SDLT-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 FaAP1 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 FaAP1, in meri-
| Category                      | Gene ID in FANhybrid_r1.2 | Best-hit in TAIR10 | Annotation/Classification | DEG\(^a\) | DESeq2-normalized counts | \(P\) value | FDR  |
|-------------------------------|---------------------------|--------------------|---------------------------|-----------|--------------------------|-------------|------|
| Flowering related             | FANhyb_icon00017910_a.1.g00001.1 | AT1G65480.1 | FaFT1 nonDEG | 0.000 | 0.000 | 0.000 | 1.165 | 0.000 | 0.712 | 1.000 |
|                              | FANhyb_rscf00001017.1.g00001.1 | AT1G65480.1 | FaFT1 UP | 3.027 | 4.817 | 1.279 | 1102.544 | 359.337 | 0.000 | 0.000 |
|                              | FANhyb_icon00009135_a.1.g00001.1 | 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 | 599.057 | 653.008 | 0.000 | 0.000 |
|                              | FANhyb_rscf00000005.1.g00042.1 | AT2G27550.1 | FaTFL3 UP | 1.009 | 1.376 | 0.000 | 599.057 | 653.008 | 0.000 | 0.000 |
|                              | FANhyb_icon00001611_a.1.g00001.1 | 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 | 22757.678 | 0.496 | 0.841 |
|                              | FANhyb_rscf000000019.1.g00010.1 | AT1G69120.1 | AP1 UP | 21.188 | 4.129 | 10.229 | 32909.670 | 22757.678 | 0.000 | 0.000 |
|                              | FANhyb_rscf00001466.1.g00021.1 | AT4G36920.2 | AP2 DOWN | 7476.355 | 10698.072 | 10116.740 | 1937.028 | 1959.935 | 0.000 | 0.000 |
|                              | FANhyb_icon00006979_a.1.g00001.1 | AT5G61850.1 | LEAFY UP | 1423.635 | 2953.565 | 2364.238 | 17238.621 | 9291.677 | 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 | 0.000 |
| Red, far-red light response   | FANhyb_icon00002013_a.1.g00001.1 | AT1G09570.2 | PHYA DOWN | 1604.238 | 1052.190 | 1222.397 | 423.069 | 432.298 | 0.000 | 0.000 |
|                              | FANhyb_rscf00002431.1.g00011.1 | AT1G09570.1 | PHYA DOWN | 4171.019 | 5031.796 | 4526.449 | 3201.575 | 3074.425 | 0.002 | 0.041 |
|                              | FANhyb_rscf00001791.1.g00008.1 | AT4G15090.1 | FAR1 DOWN | 752.680 | 579.427 | 641.886 | 336.824 | 381.225 | 0.000 | 0.009 |
| Histone                      | FANhyb_rscf00002941.1.g00011.1 | AT5G59970.1 | Histone H4 nonDEG | 28602.859 | 25298.677 | 2292.248 | 35699.830 | 23863.960 | 0.362 | 0.762 |
|                              | FANhyb_rscf00006289.1.g00001.1 | AT5G59970.1 | Histone H4 nonDEG | 20722.928 | 15429.144 | 15842.570 | 29328.149 | 18677.293 | 0.066 | 0.394 |
|                              | FANhyb_rscf00007095.1.g00002.1 | AT5G59970.1 | Histone H4 nonDEG | 26005.809 | 20564.852 | 1970.539 | 35892.134 | 25537.520 | 0.046 | 0.331 |

\(^{a}\) Up- or down-regulated from vegetative to reproductive phase.
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 *FaAP1* 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 *FaAP1* gene expression in the cultivated strawberry ‘Nyoho’. ‘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 *FaAP1* gene expression in the cultivated strawberry ‘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-IMPARED 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 Hilbrunner, 2017; Wang and Wang, 2015). Interestingly, illumination of the crowns of the plants overexpressing the FaTFL1 or FaTFL2 gene.

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