Plant Biotechnology 33, 297–307 (2016)
DOI: 10.5511/plantbiotechnology.16.0620a

Original Paper

The *FT-like* gene *PehFT* in petunia responds to photoperiod and light quality but is not the main gene promoting light quality-associated flowering

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Received February 2, 2016; accepted June 20, 2016 (Edited by K. Aoki)

Abstract In *Arabidopsis*, flowering is delayed under red light and induced under far red light and blue light. Studies suggest that the florigen, *FLOWERING LOCUS T*, is involved in the control of light quality-associated flowering in *Arabidopsis.* In petunia, similar to *Arabidopsis*, flowering is delayed under red light and induced under blue light, however its mechanism still remains unknown. Here we isolated a gene which has 75% amino acid sequence similarity with *Arabidopsis FT* (*AtFT*), named *PehFT*. By overexpressing *PehFT* in *Arbidopsis* and petunia, we tested its ability to induce flowering. Also, by conducting expression analyses of *PehFT* under different light quality treatments, we tested its response to light quality. We concluded that *PehFT*, like *AtFT*, is a gene which responds to photoperiod and light quality, but unlike *AtFT*, is not the main gene controlling the light quality-associated flowering.

Key words: blue and red light, floral bud formation, LED, petunia.

Light is one of the most important environmental factors determining a flowering time. The effect of photoperiod on flowering was first discovered in 1920 by Garner and Allard who experimentally demonstrated that Bixloxi soybean and Maryland Mammoth tobacco plants only flower when photoperiod is under a certain threshold. Ever since, there has been many studies on photoperiod and flowering, and the results obtained from the studies are applied in commercial cut flower production.

In *Arabidopsis*, red light is known to delay flowering, whereas far red light and blue light are known to induce flowering (Eskins 1992; Goins et al. 1998; Lin 2000). This phenomenon is thought to occur mainly because light having different wavelengths are received by different photoreceptors, namely *Phytochrome A*, *Phytochrome B* and *Cryptochromes*, and each of these receptors sends different signals to the flowering genes controlling flowering time (Lin 2000; Mockler et al. 2003). For instance, *Phytochrome B* is known to control the expression of the flower induction gene, *FLOWERING LOCUS T* (*FT*) by sending signals to *PHYTOCHROME AND FLOWERING TIME1* (*PFT1*) (Cerdán and Chory 2003). *CRYPTOCHROME2* (CRY2) is found to induce flowering by stabilizing the flowering inducing, CONSTANS (CO) protein, through CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1), and also by binding to CRYPTOCHROME-INTERACTING BASIC-HELI-LOOP-HELIX1 (CIB1) protein and directly inducing the expression of *FT* gene (Liu H et al. 2008; Liu LJ et al. 2008). In addition, not only CRY2, ZEITLUPE (ZTL) also mediate a signal of blue light to prevent the CIB1 protein degradation in *Arabidopsis* (Liu et al. 2013). The expression of *FT* and *SUPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*) genes are indeed observed to be low under red LED and high under blue LED in Arabidopsis (Fukuda et al. 2011). On the contrary, floral inhibitor, anti-florigen was shown to control flowering timing corresponded

Abbreviations: *FT*, FLOWERING LOCUS; T, LED light emitting diode; LD, long day; SD, short day.

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This article can be found at http://www.jspcmb.jp/
Published online September 29, 2016

to light quality environment. Higuchi et al. (2013) revealed that anti-florigen *FT*/*TFL1* family protein from a wild chrysanthemum (*CsAFT*) is induced under non-inductive condition on flowering with a night break and long day condition. In the case of short day type *Fragaria vesca*, treatments of end of day (EOD) by far-red and blue light that could induce a higher expression of *FvTFL1*, the repressor of floral induction (Rantanen et al. 2014).

Previously, we found that the flowering of petunia (*Petunia*×*hybrida*) are delayed in plants grown under red LEDs and accelerated in plants grown under blue LEDs (Fukuda et al. 2012; Fukuda et al. 2016). Gibberellic acid is known as a signal inducing floral initiation in some plant species (Bernier and Perilleux 2005; Bonhomme et al. 2000). Furthermore, it was shown that blue light are strong signals to enhance shoot elongation in petunia, through an increase in contents of active GAs (Fukuda et al. 2016). However, in petunia, GA application did not induce floral bud initiation under red light conditions. On the contrary, cytokinin application induced floral initiation in petunia grown under red light conditions (Fukuda et al. 2012), but the effect of cytokinin application was less strong compared to a signal from blue light irradiation. From those results, we can speculate that petunia could have a direct signal transduction system to promote floral bud formation from blue light receptors such as cryptochrome compared to *Arabidopsis*, the genome of petunia is not well studied. However, a floral inducer, *PETUNIA FLOWERING GENE* (*PFG*) (Immink et al. 1999), and homologues of *Arabidopsis* flowering genes such as *UNSHAVEN* (*UNS*), *FBP21* and *FBP28* of *SOC1* like gene of *Arabidopsis* (Ferrario et al. 2004; Preston et al. 2014), and *ABERRANT LEAF AND FLOWER* (*ALF*) of homologue gene of *LEAFY* of *Arabidopsis* that promotes the floral meristem identity genes (Souer et al. 2008) have been found to be involved in the transition from the vegetative to the reproductive phase in petunia. In our previous study, although its relation to controlling the flowering time still remains unclear, we found that the expression of *FBP28*, a *SOC1-like* gene, is reduced and induced in petunia plants grown under red and blue LEDs, respectively (Fukuda et al. 2012). In *Arabidopsis*, *SOC1* has a role to transduce a signal from the *FT* gene to induce floral bud formation. *FT* homologous genes have been found in various plant species, monocotyledonous and dicotyledonous, such as rice (Kojima et al. 2002) , barley (Faure et al. 2007), tomato (Lifschiz et al. 2014) and chrysanthemum (Oda et al. 2012). Recently, Fukuda et al. (2011) reported an *FT* like gene in lettuce. In *Phalaenopsis* orchids an *FT*-like gene was reported to induce novel early flowering in the *Arabidopsis ft-1* mutant (Li et al. 2014). However, in petunia plants, a *FT* gene has yet not been described.

In this study, to gain a better understanding of the mechanisms of how light quality controls flowering in petunia, we isolated an *FT* gene in petunia (*PehFT*) which has 75% amino acid sequence similarity with *Arabidopsis FT*. We tested whether *PehFT* has ability to induce flowering like *AtFT*, and also determined its relationship to light quality by measuring the expression pattern of *PehFT* in petunia plants grown under different light qualities. We also tested the hypothesis that flowering of petunia is delayed under red LEDs due to reduce a *PehFT* expression. The results of this study suggested that *PehFT* is a gene which expression is influenced by photoperiod and light quality, but that it is unlikely to be the major gene promoting the light quality-associated flowering in petunia.

Materials and methods

Plant materials

Seeds of *Arabidopsis thaliana* ecotype 'Landsberg erecta' wild type, *ft* mutants, and the transgenic plants were sown on plug trays containing a commercial soil mix (Sakata Seed Corporation, Yokohama, Japan) and were grown in a growth cabinet with fluorescent lamps (5N-EX, Toshiba Lighting & Technology Co., Tokyo, Japan) as the main light source. The air temperature was kept at 25°C and irradiance was set to 70 μmol m^{-2} s^{-1}. Photoperiod was kept at 16 h or 14 h for long day (LD) and 8 h for short day (SD). The seedlings were fertilized with a commercial liquid fertilizer (Hyponex, N : P : K = 6 : 10 : 5, Hyponex Japan, Osaka, Japan). Petunia (*petunia*×*hybrida* Vilm.) 'Michelle' (cv. W115) was used for transgenic experiments, and 'Bacarat Blue' (Sakata Seed Corporation, Yokohama, Japan) was used for all other petunia experiments. The seeds of petunia were sown on a 6 : 1 mixture of a commercial soil mix (Sakata Seed Corporation, Yokohama, Japan) and vermiculite, and were grown in a growth cabinet with fluorescent lamps as the main light source. The air temperature was kept at 25°C and irradiance was set to 70 μmol m^{-2} s^{-1}. Photoperiod was kept at 14 h, and the seedlings were fertilized with the commercial liquid fertilizer described above.

Light quality treatments

When five true leaves were fully expanded, the petunia plants were transferred into a growth cabinet equipped with white fluorescent lamps, red LED panels (peak wave length: 660 nm, ISL150x150RR, CCS Inc., Kyoto, Japan) or blue LED panels (peak wave length: 470 nm, ISL150x150BB, CCS Inc., Kyoto, Japan) (Figure 1). The air temperature was kept at 25°C and the irradiance was maintained at a photosynthetic photon flux density (PPFD) of 70 μmol m^{-2} s^{-1} around the shoot apices. The irradiance was measured using a quantum sensor (LI-190 SA, Li-Cor, Lincoln, Neb., USA). The photoperiod was kept at 14 h.

Isolation of PehFT

Total RNA was extracted by an RNA extraction kit (RNeasy Plant Mini kit, QIAGEN, Hilden, Germany)

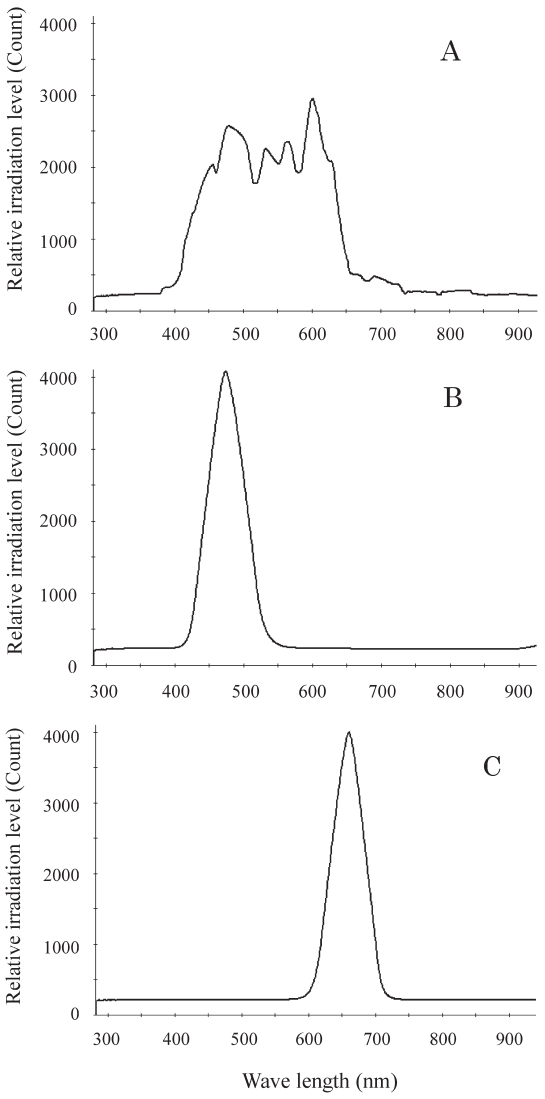

Figure 1. Intensities and spectral distribution of lights used in this study. (A) White fluorescent lamp. (B) Blue LED. (C) Red LED.

from the leaves of petunia plants sampled at 1 h in the dark period. cDNA was transcribed using 5 μg of the extracted total RNA, SuperScriptIII (Invitrogen, Carlsbad, CA), and oligo (dt) primers. Degenerate primers (forward 5′-ATG GTIGAYCCIGAYGTICC-3′; reverse 5′-GTG TTG AAA TTC TGA CGC CAT CC-3′) were used to isolate fragments of *PehFT* through PCR. To obtain the full length cDNA of *PehFT*, first, mRNA was isolated using OligotexTM-dt30 (Super) mRNA purification kit (from total RNA) (TaKaRa, Kusatsu, Japan). Next, Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA) was used to create an adaptor-ligated cDNA library. Then, adpotor primer (5′-CCA TCC TAA TAC GAC TCA CTA TAG GGC-3′) and two *PehFT* primers (5′-GGA GTA TTT ACA TTG GTT GGT CAC AG-3′ and 5′-CTG TGA CCA ACC AAT GTA AAT ACT CC-3′) were used to amplify, through PCR, the sequences of the 5′ and 3′ ends respectively.

PehFT vector construction

The coding sequences of *PehFT* were amplified through PCR, and the PCR products were cloned into the pENTR D-TOPO entry vector (Invitrogen, Carlsbad, CA). The inserts were then transferred to pBIDAVL-GWR1 (Inplanta Innovations Inc., Yokohama, Japan) through LR reaction and *PehFT*/pBIDAVL-GWR1 was constructed. The *PehFT*/pBIDAVL-GWR1 was transferred into *Agrobacterium tumefaciens* GV2260 using the method of Wen-jun and Forde (1989).

Arabidopsis thaliana plant transformation

A vector containing *PehFT* was transformed into *Agrobacterium tumefaciens* competent cells (GV3101) using a pulse controller. After checking the vector insertion through colony PCR, *Arabidopsis thaliana* wild type and *ft* mutant plants were infected by *Agrobacterium* containing the *PehFT* vector, through the Floral Dip Method (Clough and Bent 1998). After the infection, the plants were grown in a growth cabinet with fluorescent lamps as the main light source, described above, and T_1, T_2, and T_3 seeds were collected.

To check the gene insertion in transgenic plants, 2 to 3 young leaves were sampled from T_3 plants for DNA extraction. The sampled leaves were frozen in liquid nitrogen and kept in a deep freezer at −80°C. A DNA extraction liquid was made from 1 ml Tris HCl (pH 7.5, 1 M), 0.25 ml NaCl (5 M), 0.25 ml EDTA (0.5 M), 0.25 ml SDS (10%), and 3.25 ml sterile water. The sampled leaves were grinded using a pulverizer (TissueLyserII, QIAGEN, Hilden, Germany), and 100 μl of the DNA extraction liquid and 200 μl of Ethanol were added and well mixed. The samples were centrifuged for 10 min (13,200 rpm at 4°C), and the precipitate was dissolved in 100 μl Tris-EDTA Buffer. Genomic PCR was done using the extracted DNA with 35S forward primer (5′-GAT AAA GGA AAG GCC ATC GTT GAA G-3′), *PehFT* reverse primer (5′-GGA TAT CTG TGA CCA ACC AAT G-3′) and *ACTIN8* primers (forward 5′-TGT CCC TAT CTA CGA GGG TTA TGC-3′; reverse 5′-GTT AGG TCA CGG CCA GCA A-3′). Bands were checked by gel electrophoresis.

Phenotyping of Arabidopsis transgenic lines

Flowering time of *Arabidopsis* transgenic lines was measured using the T_3 plants. 25 plants each of wild type, *ft* mutants and T_3 from 3 different transgenic lines were grown in the growth cabinet described above, under LD and SD conditions, and the number of rosette and cauline leaves were counted at the time of flowering.

Petunia gene expression analyses

RNA was extracted from 2 to 3 young leaves of petunia plants sampled at the time schedules below.

a) *PehFT* gene expression analysis at different growth stages of petunia wild type plants grown under fluorescent lamps 0, 7, 14, 21, 28, 35 days after the five true leaves stage, at 12 h after the start of daytime.
b) *PehFT* gene expression analysis at different growth stages

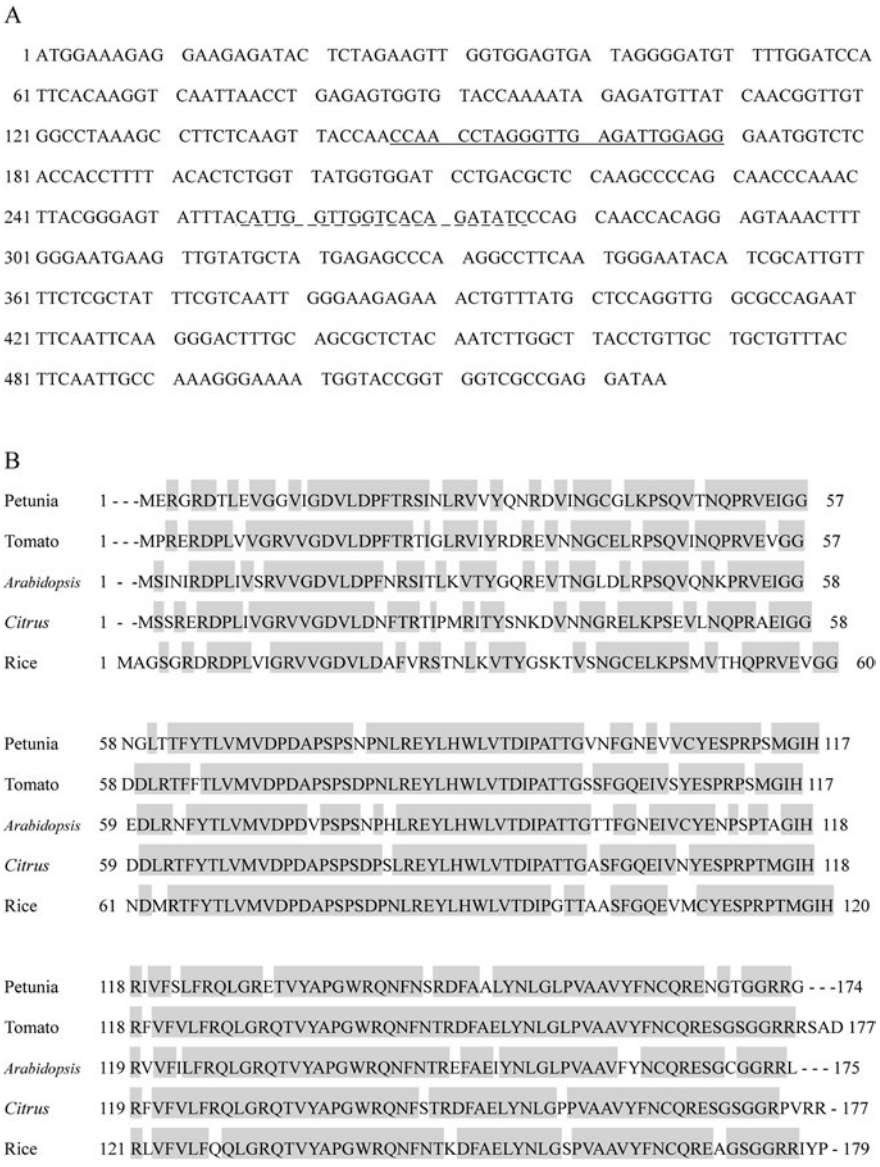

Figure 2. cDNA sequences of *PehFT* and sequence alignment of *PehFT* and FT-like proteins. (A) The underlines indicate the binding sites of forward (—) and reverse primers. (- - -). (B) The amino acid sequences alignment of *PehFT* and FT-like proteins of tomato, *Arabidopsis*, *Citrus*, and rice.

of petunia wild type plants grown under red and blue LED panels

0, 7, 14, 21 days after the five true leaves stage at 12 h after the start of daytime.

c) *PehFT* gene expression analysis at different time period of petunia wild plants grown under different light quality environments

0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 h after the start of the light quality treatment at the five true leaves stage.

d) *PehFT* gene expression analysis of petunia transgenic genes

12 h after the start of daytime at the five true leaves stage. Sampled plant shoots were frozen in liquid nitrogen and kept in a deep freezer at −80°C. Total RNA was extracted using an RNA extraction kit (RNeasy Plant Mini Kit, QIAGEN, Hilden, Germany). Using the extracted total RNA as the template, cDNA was synthesized with a cDNA synthesis kit (PrimeScript II 1st strand cDNA Synthesis Kit, TaKaRa, Kusatsu, Japan). Real-time quantitative PCR expression analysis of *PehFT* and the house keeping gene *ACTIN8* were performed using the quantitative PCR kit (Brilliant II SYBR Green QPCR Master Mix, STRAGA GENE, La Jolla, CA, USA), *PehFT* primers (forward 5′-CCA CCT AGG GTT GAG ATT GGA G-3′; reverse 5′-GGA TAT CTG TGA CCA ACC AAT G-3′) and *ACTIN8* primers described above. All quantitative PCR analyses were performed on Mx3000p (STRATA GENE, La Jolla, CA, USA). 3 biological samples were analyzed each time.

Petunia plant transformation

Petunia transgenic lines were prepared using the *Agrobacterium*-containing *PehFT* vector, through the Leaf Disk Method (Khan et al. 2012). Culture medium was made by adding 30 g Sucrose,

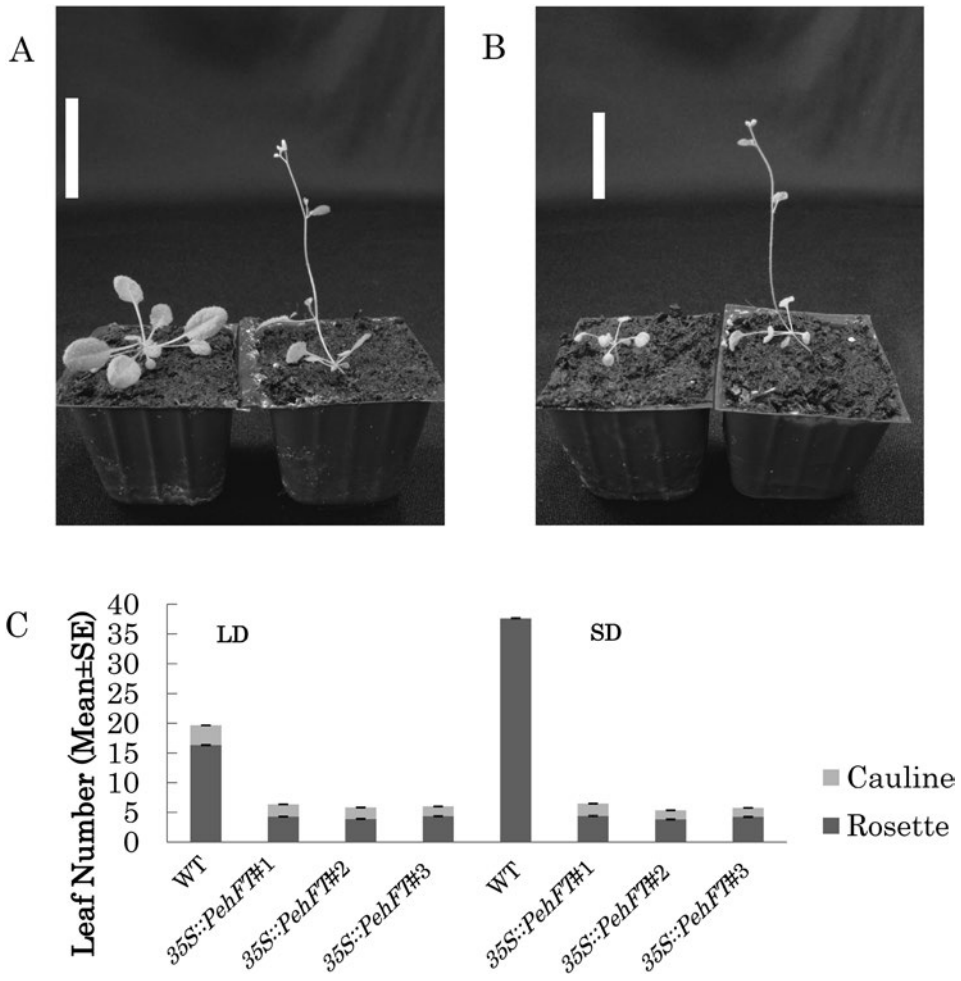

Figure 3. Phenotype and flowering time of *Arabidopsis 35S::PehFT* transformants. (A) *Arabidopsis* wild type (left) and *35S::PehFT* plant (right) grown under long day (LD) conditions (16 h light/8 h dark). (B) *Arabidopsis* wild type (left) and *35S::PehFT* plant (right) grown under short day (SD) conditions (8 h light/16 h dark). White bars show 5 cm length in each figure (A) and (B). (C) Flowering time of *Arabidopsis* wild type (WT) and *35S::PehFT* plants (*35S::PehFT* #1, #2, #3) grown under LD (16 h light/8 h dark) and SD (8 h light/16 h dark) conditions was measured as the mean number (±SE) of cauline and rosette leaves at the time of flowering ($n>20$).

0.1 mg NAA, 0.8 mg BA and 8 g Agar to 1 l Murashige and Skoog medium. After the infection, the transgenic lines were selected using media containing 30 mg L^{-1} Meropenem and 100 mg L^{-1} Kanamycin. The gene insertion was checked through genomic PCR, as described above for *Arabidopsis* transgenic lines.

Phenotyping of petunia transgenic lines

Apical shoots including 5 young leaves were cut from the transgenic petunia plants and were rooted in a pot filled with vermiculite. The plants were grown in the growth chamber with fluorescence light or red LED as the main light source, and the number of leaves at the time of flowering were counted.

Genomic Southern blot analysis

Genomic DNA was extracted using the CTAB method (Rogers and Bendich 1985). The genomic DNA was digested with *Eco*RI, *Xho*I, *Dra*I, *Hin*dIII, *Sac*I and *Pst*I, the digested genomic DNA was electrophoresed in 1% agarose gels, then transferred to a positively charged nylon membrane (GE Healthcare, Buckinghamshire, UK) by capillary transfer. Transferred membranes were hybridized with the DIG-labeled *PehFT* full length probe for 16 h at 45°C. The hybridization signals were detected using an LAS4000 mini Image Analyzer (GE Healthcare, Buckinghamshire, UK).

Results

Isolation of PehFT, a FT homolog from petunia

The *PehFT* gene isolated from petunia had 75% DNA sequence similarity with *Arabidopsis FT (AtFT)* (Figure 2, *PehFT* was registered as LC167156 in DDBJ). *PehFT* was found to have a conserved phosphatidylethanolamine binding domain (PEBP) which is also found in *AtFT* (Data note shown). Furthermore, *PehFT* had a high amino acid sequence similarity with FT-like proteins isolated from tomato (80%), citrus (77%) and rice (76%) (Figure 2).

Overexpression of PehFT in Arabidopsis

To evaluate the function of *PehFT* in *Arabidopsis*,

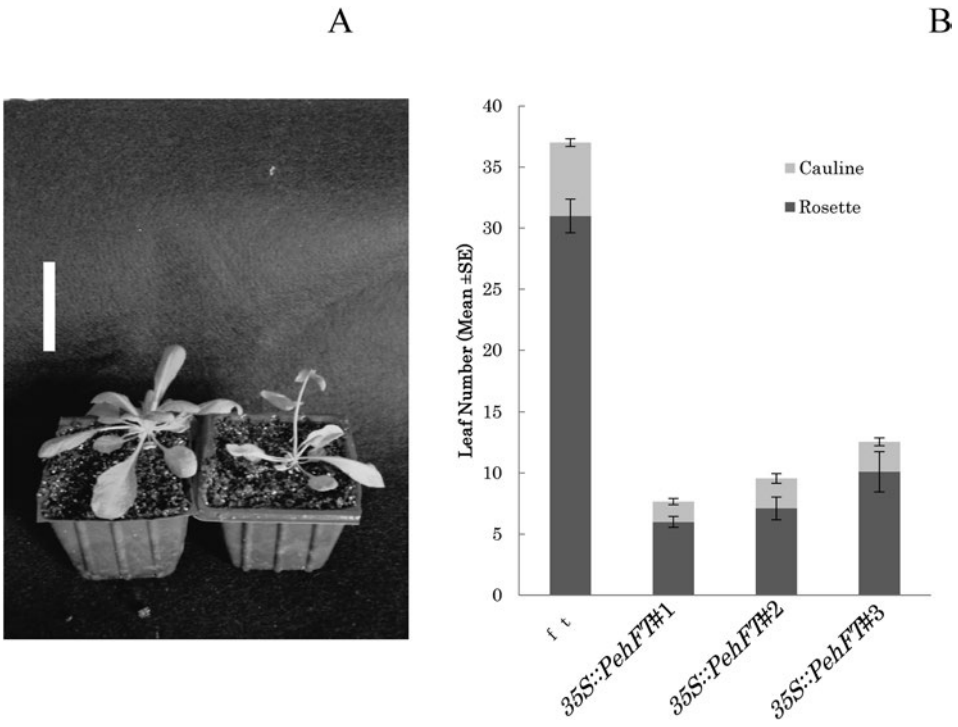

Figure 4. Phenotype and flowering time of *Arabidopsis 35S::PehFT;ft* transformants. (A) *Arabidopsis ft* mutant (left) and *35S::PehFT*; ft transformants (right) grown under LD conditions (16 h light/8 h dark). White bar shows 5 cm length. (B) Flowering time of *Arabidopsis ft* mutant (*ft*) and *35S::PehFT ;ft* transformants (*35S::PehFT;ft* #1, #2, #3) grown under LD conditions was measured as the mean number (±SE) of cauline and rosette leaves at the time of flowering ($n>3$).

transgenic lines overexpressing *PehFT* in *Arabidopsis* wild-type background were prepared, and flowering time was measured under both long day (LD) and short day (SD) conditions, by counting the number of rosette and cauline leaves at the time of flowering. *PehFT* gene insertion in the transgenic lines was checked by conducting a genomic PCR (Data not shown). Under both LD and SD conditions, the transgenic lines flowered significantly earlier than the wild-type plants (Figure 3). At the time of flowering of the transgenic lines, under both photoperiod conditions, wild type plants kept the rosette plant shape without any flowering. In the transgenic lines, the number of rosette leaves when the first flowering was observed was approximately 4, while the wild type had 16 leaves and 38 leaves under LD and SD conditions, respectively.

To confirm the function of *PehFT* in flowering, transgenic lines overexpressing *PehFT* in a *ft* mutant background were prepared and flowering time was measured under LD conditions. The transformed plants flowered earlier with a lower number of rosette leaves than the *ft* mutant plants under LD conditions (Figure 4).

The expression pattern of PehFT and effect of light quality on the expression pattern of PehFT at different developmental stages of petunia

The expression levels of *PehFT* were also measured in petunia plants grown under white fluorescent lamp, red LEDs and blue LEDs. In petunia grown under blue LEDs, the *PehFT* expression level was almost the same as those grown under white fluorescent lights; the expression level was the highest on the first day of experiment followed by the depletion of expression levels on the 7th day of the experiment (Figure 5). In petunia grown under red LEDs, the *PehFT* expression was kept at relatively high level for 7 days since the start of the experiment, but it decreased significantly on the 14th day of the experiment.

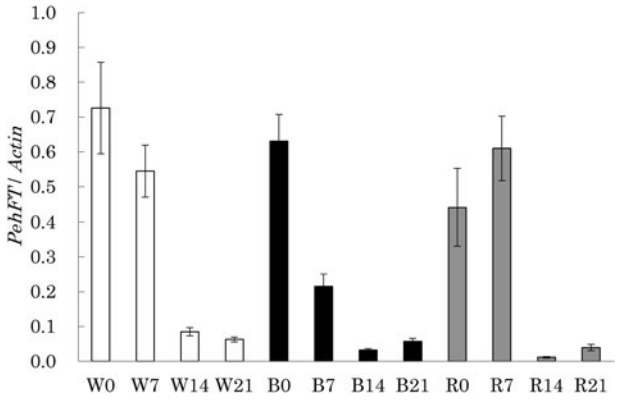

Figure 5. Expression of *PehFT* in petunia plants grown under different light treatments at different developmental stages. Real-time PCR was performed to quantify *PehFT* mRNA extracted from young leaves of petunia plants grown under white fluorescent light (W), blue LEDs (B) and red LEDs (R) in LD (14 h light/10 h dark) conditions. The numbers on the x-axis represent days after the start of the light treatments. The error bars show SE ($n=3$).

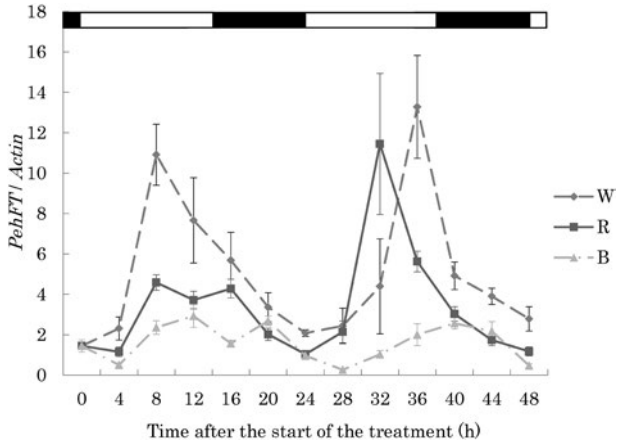

Figure 6. Expression of *PehFT* in petunia plants grown under different light treatments. Real-time PCR was performed to quantify *PehFT* mRNA extracted from young leaves of petunia plants grown under white fluorescent light (W), blue LEDs (B) and red LEDs (R) in LD (14 h light/dark) conditions. White and black bars upward line graph indicate light and dark periods, respectively. The numbers on the x-axis represent hours after the start of the light treatments. The error bars show SE (n=3).

The expression pattern of PehFT and effect of light quality on the diurnal and nocturnal expression pattern of PehFT in petunia

The expression levels of *PehFT* were measured in petunia wild type plants grown under white fluorescent lamps, red LEDs and blue LEDs, during daytime and nighttime on the first and 2nd day of the experiment. Under fluorescent lamps, the expression level was the highest at 8 h after treatment, during daytime, and we could observe the expression peak again 36 h after treatment, at the end of the daytime of the 2nd day of treatments (Figure 6). On the contrary, *PehFT* expression level decreased during nighttime. Under red LEDs, although there was some variation, the expression levels seemed to also increase during daytime and decrease during nighttime. However, the peak expression level was lower than under white fluorescent lamps, and the peak expression level was unclear on the first day, but observed at 32 h after start of the treatment on the 2nd day of the experiment. Under blue LEDs, the *PehFT* expression levels during daytime were lower than other light treatments, and the diurnal change in *PehFT* expression levels was unclear.

Overexpression of PehFT in petunia

Transgenic lines overexpressing *PehFT* in petunia (wild type background) were prepared. *PehFT* gene insertion was confirmed in two transgenic lines by genomic PCR (data not shown), and the expression levels of *PehFT* in the transgenic lines were measured by real-time qRT-PCR (Figure 7). The expression levels in Line #1 and Line #2 were approximately 40 and 120 times higher, respectively, than in the untransformed wild type and the

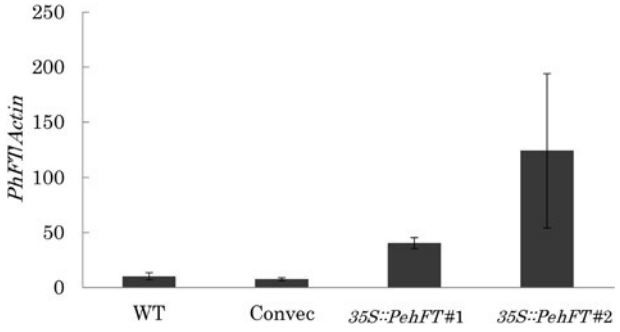

Figure 7. Expression of *PehFT* in petunia *35S::PehFT* transformants. Real-time PCR was performed to quantify *PehFT* mRNA extracted from young leaves of petunia wild type (WT), control vector-inserted transformant (Convec) and 2 different lines of *35S::PehFT* transformants (*35S::PehFT* #1, #2) grown under white fluorescent lights in LD (14 h light/10 h dark) conditions. The young leaves were sampled at 12 h after the start of daytime at five true leaves stage. The error bars show SE (n=3).

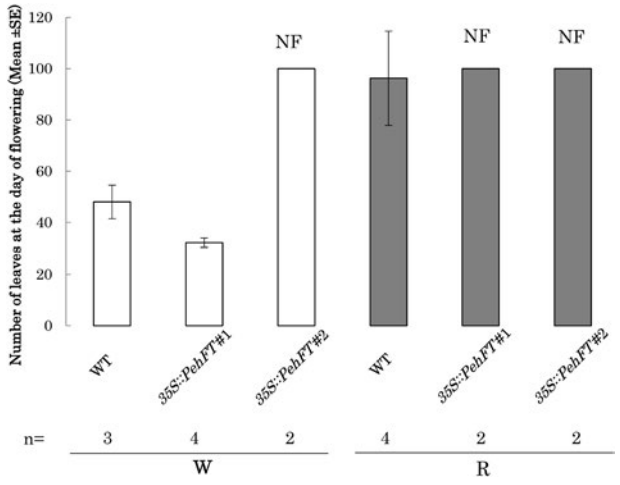

Figure 8. Flowering timing of petunia*35S::PehFT* transformants. Flowering timing of petunia wild type (WT) and *35S::PehFT* transformants (*35S::PehFT* #1, #2) grown under white fluorescent light (W) and red LED (R) in LD conditions (14 h light/10 h dark) was measured as the mean number (±SE) of leaves at the day of flowering. Replicate numbers were shown below each line. NF shows no flowering during experiment and those plants had more than 100 leaves.

control vector-transformed line. Under white fluorescent lights, Line #1 flowered 16 leaves earlier than the wild-type, but Line #2 did not flower even after 100 leaves had developed (Figure 8 and 9A, B, C). Line #2 had abnormal phenotype with increased numbers of nodes and leaves, and shorter plant height.

The transgenic lines were grown under red LEDs, and the flowering time was compared to those grown under white fluorescent lights. Both Line #1 and Line #2 did not flower even after 100 leaves had developed (Figure 8 and 9D, E, F).

PehFT homologs in petunia

To determine if any other *PehFT* homologs exist in the petunia genome, genomic Southern blot was performed.

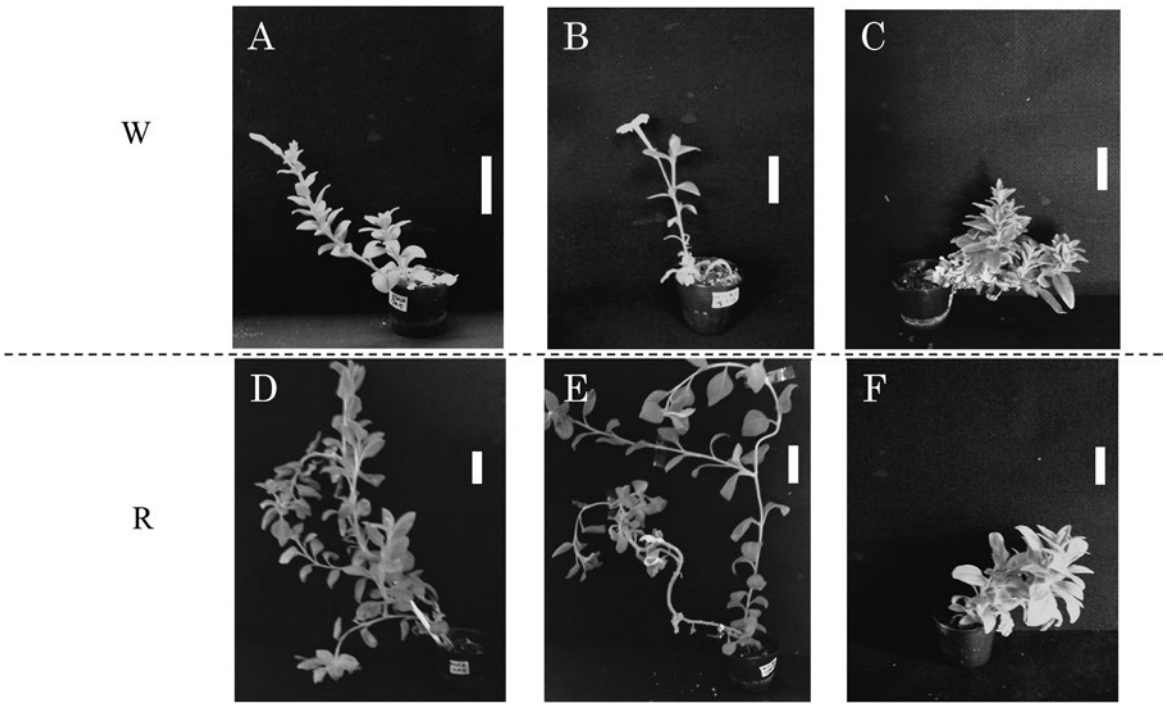

Figure 9. Phenotype of petunia wild type and *35S::PehFT* transformants grown under white fluorescent lights and red LEDs. Petunia wild type (A, D), *35S::PehFT* transformant Line #1(B, E), and *35S::PehFT* transformant Line #2(C, F). A, B, C are grown under white fluorescent lights (W) and D, E, F are grown under red LEDs (R) in LD (14 h light/10 h dark) conditions. White bars show 10 cm length in each figure.

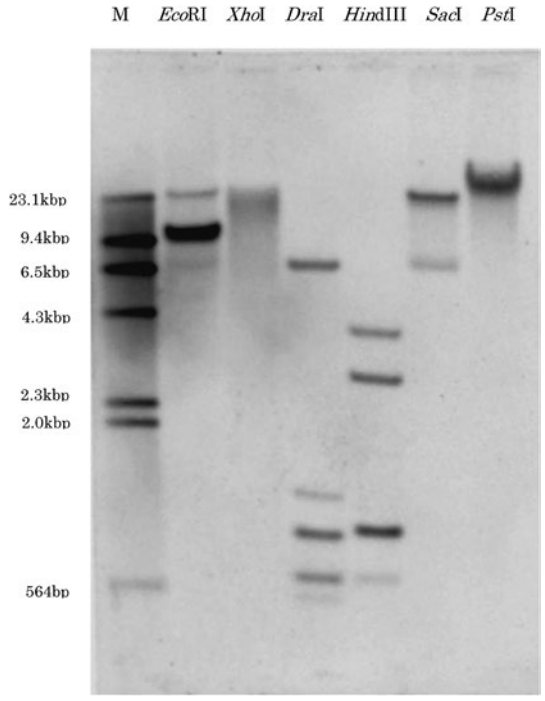

Figure 10. Genomic Southern blot analysis of *PehFT* in petunia. Petunia genomic DNA was digested with *Eco*RI, *Xho*I, *Dra*I, *Hin*dIII, *Sac*I, and *Pst*I. *PehFT* was used as the probe. M represents a size marker.

From well digested lanes which used *Dra*I and *Hin*dIII, 3 to 5 bands were detected, suggesting the existence of 3 to 5 homologs of *PehFT* in the genome (Figure 10).

Discussion

Isolation and overexpression of PehFT accelerated flowering in Arabidopsis

Since the transgenic *Arabidopsis* plants overexpressing *PehFT* in both wild-type and *ft* mutant backgrounds flowered early compared to the control, *PehFT* is most likely to accelerate flowering in *Arabidopsis*. Furthermore, because transgenic lines overexpressing *PehFT* in a wild type background flowered earlier than the control under both LD and SD conditions, *PehFT* is likely to function in the photoperiodic pathway. In *Arabidopsis*, *FT* induces flowering by promoting the expression of downstream flowering inducers such as *APETALA 1* (*AP1*) and *FRUITFUL* (*FUL*) (Abe et al. 2005, Wigge et al. 2005). Because PehFT protein was found to have similar functional domains as AtFT, PehFT was most likely to have been recognized as FT proteins inside the *Arabidopsis* cells, and like FT, PehFT was most likely to have bound with the FD protein and accelerated flowering by promoting expression of flowering genes such as *AP1* and *FUL*.

PehFT had a conserved PEBP domain. In PEBP domain, TY-85 and Gln-140 of FT protein in Arabidopsis could distinguish FT protein from TFL protein that is a floral repressor (Ahn et al. 2006; Hanzawa et al. 2005). PehFT protein has both TY-84 and Gln-139 corresponding to those amino acids in FT protein in *Arabidopsis*. Ho and Weigel (2014) suggested that two

aromatic residues in Tyr-134, Trp-138 and Gln-140 found only in FT protein from *Arabidopsis* and those residues could isolate it from TFL1 protein by increasing the selectivity to co-activator of FT protein to promote floral transition. As show in figure 2, PehFT protein has also three important residues Tyr-133, Trp-137 and Gln-139 in the position corresponding to Tyr-134, Trp-138 and Gln-140 in *Arabidopsis*. In our experiment, as overexpression of *PehFT* promoted the flowering timing in *Arabidopsis*, because PehFT protein has a similar structure and function in *Arabidopsis*. From those results, it was shown that PehFT protein has a similar structure to FT protein of *Arabidopsis* and have a function to promote the floral initiation in *Arabidopsis*.

PehFT expression pattern in petunia plants

The expression of *FT* in *Arabidopsis* starts in early developmental stages, 4 days after sowing (Kobayashi et al. 1999). The level of expression continues to increase as the plant grow, and reaches the highest expression right before flowering. In this study, the expression of *PehFT* in petunia grown under white fluorescent light was the highest at the five leaves stage, decreased significantly after the 7th day and kept low expression levels until flowering. Thus, the expression pattern of *PehFT* was similar to *AtFT* in the way that it starts expressing in early developmental stages, but the pattern was different from *AtFT* in later in stages.

In *Arabidopsis* grown under LD conditions, the expression of *FT* peaks at the end of the daytime, and decreases during the nighttime (Searle and Coupland 2004). This is mainly because the direct inducer of *FT*, CO protein, which is regulated by the clock genes such as *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*) and *TIMING OF CAB EXPRESSION 1* (*TOC1*), accumulates the most at the end of daytime, and decreases during the night (Searle and Coupland 2004). In this study, the expression level of *PehFT* was also found to increase during daytime and decrease during nighttime. Although the change of expression levels under continuous light and darkness must still be checked, there is a possibility that *PehFT* is receiving signals from clock genes, just like *FT* which receives signals from clock genes such as *CCA1*, *LHY*, and *TOC1*. A *FT* orthologue in rice, *Hd3a*, and *FT* orthologues in *Pharbitis*, *PnFT1* and *PnFT2*, are also known to be expressed in a clock-controlled manner (Hayama et al. 2007; Kojima et al. 2002). In our study, the variability of the expression levels at the peaks were quite high, and this was probably due to the fact that *PehFT* is a relatively low expressed gene, and as shown in our experiments, *PehFT* expression changes significantly with developmental stage.

As Ni (2005) reported, the integration of light signaling regulates flowering in *Arabidopsis*. Signals from phytochrome B and cryptochrome are intersected in PIF4 and HRB which affect *FT* expression. *PehFT* were affected by different light qualities, with white and red light inducing the high expression level during daytime compared to blue light condition. Although high *PehFT* expression under blue LED might have been expected due to the extra early flowering timing in petunia grown under blue LED (Fukuda et al. 2016), this was not the case. In the case of *Fragaria vesca*, light qualities of end of day (EOD) treatment, affected to the expression level of *FvFT1* and *FvSOC1* (Rantanen et al. 2014). Blue light could induce the *FvFT1* and red light inhibited it. Our results in petunia showed an antagonistic response of *PehFT* expression inhibited under blue light. However, previously, we could show the elevated expression of *FBP28*, *SOC1 like gene* in petunia under blue light (Fukuda et al. 2009). From those results, we should consider some possibilities such as the other *FT* homologue genes has a main role to induce the early flowering under blue light.

Overexpression of PehFT did not rescue the delayed flowering of petunia grown under red LEDs

In this study, we tested the hypothesis that delayed and induced flowering in petunia under red and blue LEDs, respectively, is due to suppression and induction of *PehFT* expression level under these respective light qualities. When expression levels were measured every 4 h throughout the day, higher *PehFT* expression was detected in petunia grown under red compared to blue LEDs. In *Arabidopsis*, higher *FT* expression under blue LEDs compared to plants grown under red LEDs has been reported (Fukuda et al. 2011). Thus, although *PehFT* expression in this study was influenced by light quality, opposite the expression pattern compared to *AtFT* was observed.

Though one out of the two petunia transformants overexpressing *PehFT* flowered earlier than the wild type under white fluorescent light, the flowering function of the gene in petunia could not be completely verified. Also, because the delayed flowering phenotype of petunia grown under red LEDs was not rescued in the two transformants, our hypothesis was also disproved. The discovery of the genes such as *UNS*, *ALF*, *SOC1* and *PFG* that are homologous to flowering genes in *Arabidopsis* suggest that genes that are similar to flowering genes in *Arabidopsis* also exist in petunia (Ferrario et al. 2004; Immink et al. 1999; Preston et al. 2014; Souer et al. 2008). However, there is a possibility that the functions of the genes and number of homologues may have changed through the course of evolution. The result of the Southern blot analysis suggest that indeed there are about 2 to 3 homologues of *PehFT* in petunia, and isolation and analysis of the functions of these genes is

required to understand the genetic control of flowering of petunia. However, in our preliminary experiments, when we have tried to isolate *FT*-like genes from whole young plant grown under blue light condition sampled during different timings at day and night, but we have failed to find out those. In petunia, the activities of *FT*-like genes would not be so high under such blue light condition to induce early floral bud initiation.

In our study, although *PehFT* had a high sequence similarity with *FT*, we could not completely verify the flowering function of the *PehFT* in petunia. However, other studies show that in flowering genes, sequence similarity does not always suggest functional similarity. Rather, in many cases, flowering is controlled by sets of genes that are similar in sequence but has opposite functions. For instance, in *Arabidopsis*, flowering is controlled by the flowering inducer *FT*, and flowering repressor *TFL1*, a gene that has high sequence similarity with *FT* (Hanano and Goto 2011). Flowering in tomato is controlled by the ratio of *SFT*, a *FT* homologue, and *SP*, a flowering repressor which also belongs in the same gene family as *SFT* (Lifschiz et al. 2014). *TFL1* is a strong repressor of floral induction (Koskela et al. 2012). In short day type *F. vesca*, EOD of far-red and blue light induced not only the expression of *FvFT1*, but also the expression of *FvTFL1* that prevented the floral induction and reversed the responsibility to light qualities. For an explanation on the early flowering of petunia under blue light condition, we should suppose the other factors, such as *TFL1* could be inhibited by blue light, or other *FT* homologue genes shown in figure 10 is the main control factor corresponded to the light quality condition. In addition, as a hypothesis, it can be discussed that the function of *PehFT* would induce the expressions of floral repressors such as *TFL1* as shown in short day type *F. vesca*, because the excess expression of *PehFT* in petunia strongly inhibited floral induction under white light. In the *F. vesca*, SOC1 over expression could repress flower initiation under short day condition by activation of *Fv TFL1* (Mouhu et al. 2013). *SP*, *TFL1* and *FT* genes share the homology to REBP genes, and those genes family can change the plant architecture (McGarry and Ayre 2012). In our experiment, the strong expression line #2 showed different plant shape with non-flower compared to WT and line #1 with relatively low expression of *PehFT*. Strong expression of *PehFT* could change the morphogenesis of petunia as the REBP gene family.

In conclusion, the gene isolated in this study, *PehFT*, is a gene which expression is influenced by photoperiod and light quality. However, since the gene expression was not suppressed under red LEDs, and the transformants overexpressing *PehFT* did not rescue the delayed flowering of petunia grown under red LEDs, this gene is unlikely to be the main gene that controls the light quality-flowering. To gain a better understanding of the flowering control system in petunia and its relation to light quality, the homologues of *PehFT* and other flowering genes of petunia must be isolated and analyzed.

Acknowledgements

Thanks are due to Dr. Anna Holefors, Norwegian University of Life Sciences, for technical assistance with a first trial to clone the *FT* like gene in petunia. Thanks are due to Dr. Takashi Akihiro, Shimane University, for the technical suggestions on our project. The MEXT/JSPS KAKENHI Grant Number 22580028 is acknowledged for financial support and the Norwegian University of Life Sciences (guest researcher scholarship to Dr. N. Fukuda) and is acknowledged for financial support.

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