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RESEARCH PAPER

Generation and analysis of a complete mutant set for the *Arabidopsis FT/TFL1* family shows specific effects on thermo-sensitive flowering regulation

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Abstract

The FLOWERING LOCUS T (FT)/TERMINAL FLOWER 1 (TFL1) family proteins play an important role in the regulation of flowering time. In the *Arabidopsis thaliana* genome, there are six genes in the *FT/TFL1* family. To determine how these *FT/TFL1* family genes contribute to the regulation of flowering time, this study generated a comprehensive set of mutants (sixty-three multiple mutants in all combinations) of the *FT/TFL1* family genes and analysed their flowering times at 23 and 16°C under long-day conditions. The analysis confirmed that *FT* and *TFL1* are major determinants of flowering time under long-day conditions. At 23 °C, *ft-10 tsf-1 mft-2* showed the latest flowering, whereas *tfl1-20 atc-2 bft-2* showed the earliest flowering. Flowering occurred in the sextuple mutants. Introduction of *tsf-1* led to reduced sensitivity to ambient temperature change. Introduction of *tfl1-20* caused a stronger effect in accelerating flowering time at 16 °C than at 23 °C. Overexpression of miR156 did not block flowering of sextuple mutants, suggesting that there is a pathway to induce flowering independent of the *FT/TFL1* pathway and miR156 pathway. This study proposes that this mutant population will be useful in further investigation of the functions of the *FT/TFL1* family genes in plant development.

Key words: *Arabidopsis thaliana*, *ATC*, *BFT*, flowering time, *FT*, *MFT*, miR156, *TFL1*, *TSF*.

Introduction

The *Arabidopsis* life cycle is divided into vegetative and reproductive growth phases. Extensive molecular genetic analysis in *Arabidopsis* has provided considerable information on how plants integrate environmental and endogenous signals to transition from the vegetative phase to the reproductive phase (Srikanth and Schmid, 2011). Multiple, interdependent genetic pathways control the developmental transition to the flowering phase (Lee *et al*., 2006; Michaels, 2009); these pathways include the photoperiod, autonomous, vernalization, gibberellic acid, and thermosensory pathways. Under long-day conditions, genes that act within the photoperiod pathway play a major role in controlling flowering.

FLOWERING LOCUS T (FT) and TERMINAL FLOWER 1 (TFL1) belong to a small group of proteins that show structural similarities to mammalian phosphatidylethanolamine-binding protein (Kardailsky *et al*., 1999; Kobayashi *et al*., 1999; Ahn *et al*., 2006). In addition to *FT* and *TFL1*, four highly similar genes are present in the *Arabidopsis thaliana* genome, namely *TWIN SISTER OF FT* (*TSF*) (Yamaguchi *et al*., 2005), *MOTHER OF FT AND TFL1* (*MFT*) (Yoo *et al*., 2004), *BROTHER OF FT AND TFL1* (*BFT*) (Yoo *et al*., 2010), and *ARABIDOPSIS THALIANA CENTRORADIALIS HOMOLOGUES* (*ATC*) (Mimida *et al*., 2001). These six genes are found in many species and

are commonly referred to as the *FT*/*TFL1* family (Chardon and Damerval, 2005; Ahn *et al*., 2006; Karlgren *et al*., 2011; Harig *et al*., 2012).

A major function of *FT*/*TFL1* family genes is the regulation of photoperiodic flowering. *FT* encodes a floral activator that integrates signal inputs from various pathways that regulate flowering time (Wigge, 2011; Pin and Nilsson, 2012). *FT* is a major target of CONSTANS in the photoperiod pathway (Valverde *et al*., 2004) and mediates signalling from the vernalization and autonomous pathways by the direct interaction with FLOWERING LOCUS C (Helliwell *et al*., 2006). Interestingly, despite its sequence similarities to the floral activator FT, TFL1 acts as a floral inhibitor, an opposite role to FT (Ratcliffe *et al*., 1998). In addition, TFL1 controls plant architecture by regulating the expression of *LEAFY* and *APETALA1* (*AP1*) in the shoot apical meristem (Bradley *et al*., 1997; Ferrandiz *et al*., 2000). The opposite functions of FT and TFL1 proteins map to a single amino acid in the second exon (Hanzawa *et al*., 2005) and a small external loop domain in the 4th exon (Ahn *et al*., 2006). *TSF* is most similar to *FT* within the *FT*/*TFL1* family. The *tsf* mutation on its own did not show any clear alteration of flowering time under long-day conditions, but it had an additive effect when combined with *ft* (Michaels *et al*., 2005; Yamaguchi *et al*., 2005). This indicated that *TSF* plays a redundant role with *FT*. However, the effect of *tsf* loss-of-function is apparent under short-day conditions, suggesting that *TSF* makes a major contribution to flowering under short-day conditions. Based on overexpression studies, it was suggested that *MFT* and *ATC* have weak *FT*- and *TFL1*-like activity, respectively (Mimida *et al*., 2001; Yoo *et al*., 2004). *ATC* was also shown to be a short-day-induced floral inhibitor (Huang *et al*., 2012). Finally, *bft* mutation produced more secondary inflorescences when combined with *tfl1*, suggesting that *BFT* has a *TFL1*-like activity and functions redundantly with *TFL1* in inflorescence meristem development (Yoo *et al*., 2010). It was recently demonstrated that *FT* regulates stomatal opening (Kinoshita *et al*. 2011) and *MFT* regulates abscisic acid- and gibberellic acid-mediated seed germination (Xi *et al*., 2010), raising the possibility that *FT*/*TFL1* family genes function in diverse aspects of plant development.

Flowering is also significantly affected by changes in the ambient temperature (Fitter and Fitter, 2002; Lee *et al*., 2008). Among flowering time mutants, a subset of mutants showed flowering that was insensitive to ambient temperature (23 and 16 °C), indicating that these genes mediate ambient temperature-responsive flowering; later, these genes were proposed to act within the thermosensory pathway (or ambient temperature pathway) (Blazquez *et al*., 2003; Fornara *et al*., 2010). A group of genes [*FCA*, *FVE*, *HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1* (*HOS1*), *PHYTOCHROME INTERACTING FACTOR4* (*PIF4*), *SHORT VEGETATIVE PHASE* (*SVP*), *EARLY FLOWERING3* (*ELF3*), and *TFL1*] (Blazquez *et al*., 2003; Lee *et al*., 2007; Strasser *et al*., 2009; Kumar *et al*., 2012; Lee *et al*., 2012b) and ambient temperature-responsive miRNAs including miR156, miR172, and miR399 (Lee *et al*., 2010; Kim *et al*., 2011; Kim *et al*., 2012) are involved in this pathway. Increasing evidence points to a complex interplay of components within the thermosensory pathway. For instance, miR172 is subjected to multiple layers of regulation (at both transcriptional and biogenesis levels) (Cho *et al*., 2012; Jung *et al*., 2012b), which may allow plants to fine-tune their responses to changes in ambient temperature. In addition, the ambient temperature transcriptome is regulated by H2A.Z-containing nucleosomes (Kumar and Wigge, 2010). Although there are many components that affect ambient temperature signalling, the ambient temperature response is likely mediated by *FT* and *TFL1* (Lee *et al*., 2007; Strasser *et al*., 2009; Kumar *et al*., 2012; Lee *et al*., 2012a).

Based on phenotypic analyses of single or double mutants of the *FT*/*TFL1* family members, it was suggested that *FT, TFL1*, and *TSF* are the major players in the control of flowering time. However, the combinatorial effect of mutations of the *FT*/*TFL1* family is unknown, due to the absence of a comprehensive set of mutants of the *FT*/*TFL1* family. To determine how *FT*/*TFL1* family genes contribute to the regulation of flowering time, this study generated a comprehensive set of mutants (63 multiple mutants in all combinations) of the *FT*/*TFL1* family and analysed their genetic interactions. In addition, this study tested the hypothesis that ablation of *FT*/*TFL1* family genes blocks flowering, since the *FT*/*TFL1* family is suggested to play an important role in flowering. This study also tested whether miR156 overexpression in the sextuple mutant background inhibits flowering.

The analysis confirmed that *FT* and *TFL1* are major determinants of flowering time under long-day conditions. A sextuple mutant, in which all the *FT*/*TFL1* family genes are impaired, still flowered, indicating that the *FT*/*TFL1* family genes are not essential to induce flowering. It was also found that *tsf-1* caused reduced sensitivity to ambient temperature changes. Overexpression of miR156 delayed flowering of sextuple mutants, suggesting the possibility that there is an alternative pathway to induce flowering independent of the *FT*/*TFL1* and miR156 pathways. This study proposes that this mutant population will be useful for further investigation of the functions of the *FT*/*TFL1* family genes in plant development.

Materials and methods

Plant materials and growth conditions

All of the mutants used in this study were in the *A. thaliana* Columbia (Col) background. Single mutants of the *FT*/*TFL1* family used to generate multiple mutants were described elsewhere (*ft-10*: Yoo *et al*., 2005; *tsf-1*: Yamaguchi *et al*., 2005; *mft-2*: Xi *et al*., 2010; *tfl1-20*: Yoo *et al*., 2010; *atc-2*: Huang *et al*., 2012; and *bft-2*: Yoo *et al*., 2010). The plants were grown in soil or MS medium at 23 °C or 16 °C in long-day conditions (16/8 h light/dark cycle) at a light intensity of 120 μmol m^{-2} s^{-1}.

PCR genotyping

The genomic DNA was extracted from fresh young leaves, which were homogenized in a tissue disrupter (Automill, Tokken, Japan) using metal beads. To increase accuracy of genotyping to isolate multiple mutants, two independent PCR reactions were used to detect mutant and wild-type alleles, instead of multiplex PCR. To amplify

the mutant allele, a primer set (T-DNA primer and a gene-specific primer) was used. To amplify the wild-type allele, two gene-specific primers that hybridize adjacent to a T-DNA insertion site were used. The primers used for genotyping are described in Supplementary Table S1 (available at *JXB* online).

Measurement of flowering time

Flowering time was measured by scoring total leaf number (at least 10 plants) under long-day conditions (16 and 23 °C). The total leaf number was recorded when the primary inflorescence had reached a height of 5 cm. The effect of each mutation is expressed as the difference in leaf numbers between two mutant combinations that contained or did not contain the mutation. The leaf number ratio (16 °C/23 °C, LNR) under long-day conditions was used as an indicator of ambient temperature-responsive flowering (Blazquez *et al*., 2003; Lee *et al*., 2007). A hypothetical ambient temperature-insensitive plant produces an identical total number of leaves at both 23 and 16°C; thus, its LNR is 1.0.

RT-qPCR and small RNA northern hybridization

For RNA extraction, whole seedlings were harvested at zeitgeber time (ZT) 16, at which point FT expression levels were high (Corbesier *et al*., 2007). Total RNA was extracted using Plant RNA Purification Reagent (Invitrogen), according to the manufacturer's instructions. For real-time quantitative PCR (RT-qPCR), 1 µg of total RNA was treated with DNaseI (New England Biolabs) and used for cDNA synthesis with First-Strand cDNA Synthesis Kit (Roche).

Expression levels were analysed by RT-qPCR as described by Udvardi *et al*. (2008). RT-qPCR was performed in a 384-well plate with a LightCycler 480 using LightCycler 480 SYBR Green I Master Mix (Roche). For quantification, two stably expressed genes (At1G13320 and At2G28390) were used as reference genes (Hong *et al*., 2010). The threshold cycle (Ct) and PCR efficiency of the primers used were calculated using LinRegPCR (Ramakers *et al*., 2003). Oligonucleotide sequences used for RT-qPCR are given in Supplementary Table S2. All RT-qPCR experiments were performed in biological triplicate, and technical triplicates for each, with similar results. The results from a biological triplicate are shown.

For small RNA Northern blots, 10 µg of total RNA was separated on a denaturing 17% (w/v) polyacrylamide gel (8 M urea) in TBE buffer and transferred to an N+ Hybond membrane (Amersham). Hybridization was carried out at 42°C using PerfectHyb Plus hybridization buffer (Sigma). DNA oligonucleotide probes specific to miR156 (Lee *et al*., 2010; Kim *et al*., 2012) were end labelled with γ^{32}P-ATP using Optikinase (USB). U6 RNA was used to show an equal amount of loading in small RNA hybridization analyses.

Results

Generation of a comprehensive FT/TFL1 family mutant set

T-DNA insertion alleles of *FT* (*ft-10*) (Yoo *et al*., 2005), *TSF* (*tsf-1*) (Yamaguchi *et al*., 2005), *MFT* (*mft-2*) (Xi *et al*., 2010), *TFL1* (*tfl1-20*) (Yoo *et al*., 2010), *ATC* (*atc-2*) (Huang *et al*., 2012), and *BFT* (*bft-2*) (Yoo *et al*., 2010) in the Columbia background were used to generate multiple mutants. All alleles were reported to be strong loss-of-function mutants with T-DNA insertions in the introns or exons: a single T-DNA was inserted in the first intron for *ft-10* and *mft-2*, in the second intron for *tsf-1* and *tfl1-20*, in the first exon for *atc-2*, and in the third exon for *bft-2* (Fig. 1A).

The *FT*/*TFL1* family consists of six homologous genes in the *Arabidopsis* genome; thus, there are 63 possible combinations (6 single, 15 double, 20 triple, 15 quadruple, 6 quintuple, and 1 sextuple) in a comprehensive mutant set. All six genes are located on different chromosomes or far apart on the same chromosome in the *Arabidopsis* genome and therefore are unlikely to be linked. *FT*, *MFT*, *ATC*, *TSF*, *TFL1*, and *BFT* are located on chromsome I, I, II, IV, V, and V, respectively. The genes on the same chromosome, *FT* and *MFT* on chromosome I (18.1 Mb apart) and *TFL1* and *BFT* on chromosome V (23.9 Mb apart) are located in different arms,

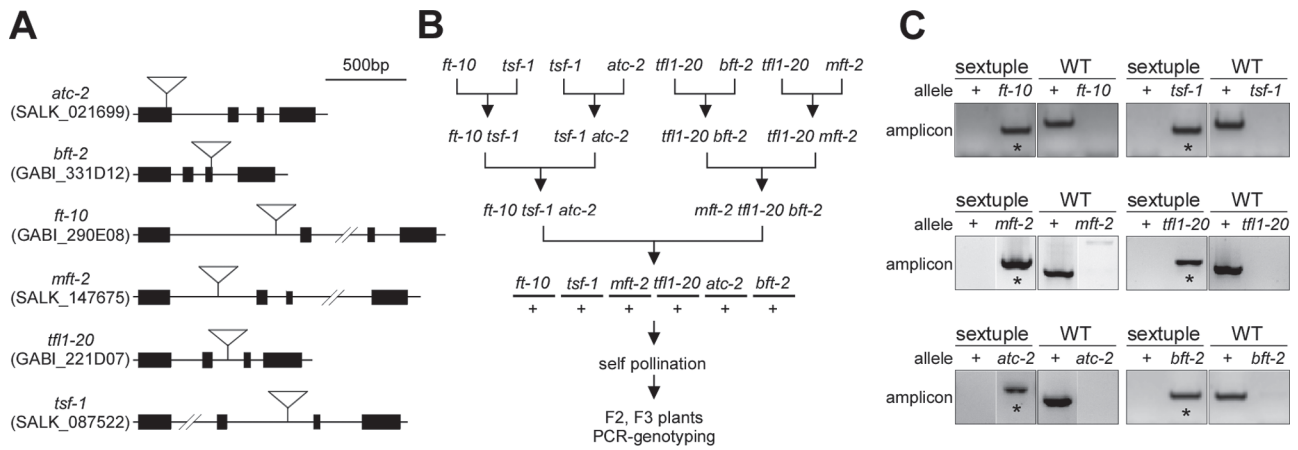

Fig. 1. Map of T-DNA insertions of mutants used in this study and strategy for generating the mutant population. (A) T-DNA insertions in the *FT/TFL1* family mutants used in this work. Closed boxes indicate exons; solid lines indicate introns; inverted triangles indicate T-DNA insertion. Both the allele name and its public T-DNA library identifier (Alonso *et al*., 2003; Rosso *et al*., 2003) are presented. (B) The strategy for generating a comprehensive set of mutants of the *FT/TFL1* family genes. (C) Confirmation of the genotype of sextuple (*ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2*) mutants by PCR. For example, *ft-10* genotyping produced a single band of 926 bp in size from the homozygous *ft-10* allele (*), whereas the wild-type allele (WT, +) produced a single band of 1392 bp in size. Genotyping primer information and the sizes of the expected amplicon of each mutant allele are provided in Table S1.

according to the *Arabidopsis* Information Resource (TAIR, version 10). Therefore, linkage should not affect generation of mutant combinations.

Since introducing each mutation by repetitive crossing would be a time-consuming way to generate a complete mutant set, this study first generated two triple mutants that were complementary to each other (*ft-10 tsf-1 atc-2* and *mft-2 tfl1-20 bft-2*) (Fig. 1B). These triple mutants were generated by introducing *atc-2* and *mft-2* mutations into *ft-10 tsf-1* and *tfl1-20 bft-2* double mutants, respectively, which have been previously reported (Yoo *et al.*, 2010). The *ft-10 tsf-1 atc-2* and *mft-2 tfl1-20 bft-2* triple mutants were then crossed to generate a line heterozygous for all *FT*/*TFL1* family genes (*ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2*). This line was self-pollinated and the resulting F2 and F3 plants were subjected to PCR genotyping to isolate individual triple, quadruple, quintuple, and sextuple homozygous mutants. For example, in the PCR genotyping of sextuple mutants (*ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2*), an amplicon corresponding to each mutant allele was detected (asterisks in Fig. 1C), but amplicons corresponding to wild-type alleles were not. This confirmed the successful isolation of a sextuple mutant.

Absence of cross-regulation among the FT/TFL1 family genes

To determine whether the *FT*/*TFL1* genes regulate each other, mRNA expression of the *FT*/*TFL1* family genes was examined in each single mutant. There was no apparent reduction or increase (>2-fold) of mRNA levels of other *FT*/*TFL1* family genes in any single mutant (Fig. 2). For instance, *FT* expression levels were unaltered in *tsf-1*, *mft-2*, *tfl1-20*, *atc-2*, and *bft-2* mutants (Fig. 2A). This was also true for the other genes. These results indicated that a single mutation in a *FT*/*TFL1* family gene did not affect the mRNA level of other members, excluding a possibility that transcriptional cross-regulation occurs among the *FT*/*TFL1* family, which may have interfered with data obtained from this study of genetic interactions.

Flowering time analysis at 23 °C under long-day conditions

The flowering time of all 63 mutants under long-day conditions at 23 °C (Table 1) was measured by counting the number of leaves at flowering. Among the six single mutants, only *ft-10* and *tfl1-20* plants showed late (*ft-10*, 36.1 ± 2.6 leaves) and early (*tfl1-20*, 9.6 ± 0.7 leaves) flowering, respectively, compared to wild-type plants (13.8 ± 0.8 leaves). However, the flowering time of *tsf-1* (14.3 ± 0.6 leaves), *mft-2* (15.3 ± 0.5 leaves), *bft-2* (13.8 ± 1.1 leaves), and *atc-2* (13.6 ± 1.4 leaves) mutants was not significantly different from that of wild-type plants, consistent with previous observations (Mimida *et al.*, 2001; Yoo *et al.*, 2004; Yamaguchi *et al.*, 2005; Yoo *et al.*, 2010).

The effect of introducing each mutation to another mutation on flowering time was analysed by measuring the difference in leaf numbers at flowering between mutants with or without the mutation: for instance, the effect of the introduction of *ft-10* into *tfl1-20* was calculated thus: *ft-10 tfl1-20* (32.9 leaves) – *tfl1-20* (9.6 leaves) = 23.3 leaves. Introducing *ft-10* generally delayed flowering time regardless of the genotype (Fig. 3A); however, a group of mutants apparently exhibited even more delayed flowering (red arrows in Fig. 3A). For instance, among double mutants, *ft-10 tsf-1* mutants (56.2 ± 4.4 leaves) flowered significantly later than other double mutants (*ft-10 mft-2*, 37.1 ± 4.5 leaves; *ft-10 tfl1-20*, 32.9 ± 2.9 leaves; *ft-10 atc-2*, 36.5 ± 3.8 leaves; and *ft-10 bft-2*, 33.5 ± 4.3 leaves). Among triple mutants, *ft-10 tsf-1 mft-2* (59.6 ± 3.3 leaves), *ft-10 tsf-1 bft-2* (52.1 ± 2.2 leaves), *ft-10 tsf-1 tfl1-20* (48.3 ± 3.5 leaves), and *ft-10 tsf-1 atc-2* (50.5 ± 6.4 leaves) mutants flowered significantly later than other triple mutants. The same is also true for quadruple and quintuple mutants. These results demonstrated that *ft-10* caused a severe delay when combined with *tsf-1*, which strongly supports the observation that an additive delay was seen in *ft-1 tsf-1* double mutants (Yamaguchi *et al.*, 2005). Interestingly, the increase in the number of leaves at flowering caused by the introduction of *ft-10* is similar in all mutant combinations. The introduction of *ft-10* into any genotype with *tsf-1* or without *tsf-1* caused a flowering time delay of 36.7 ± 4.3 and 21.2 ± 2.5 leaves, respectively (Fig. 3B).

Introducing *tsf-1* generally showed no effect or only weak effect (black arrows in Fig. 3C). However, the introduction of *tsf-1* into a genotype that already contained *ft-10* dramatically delayed flowering (red arrows in Fig. 3C). As already

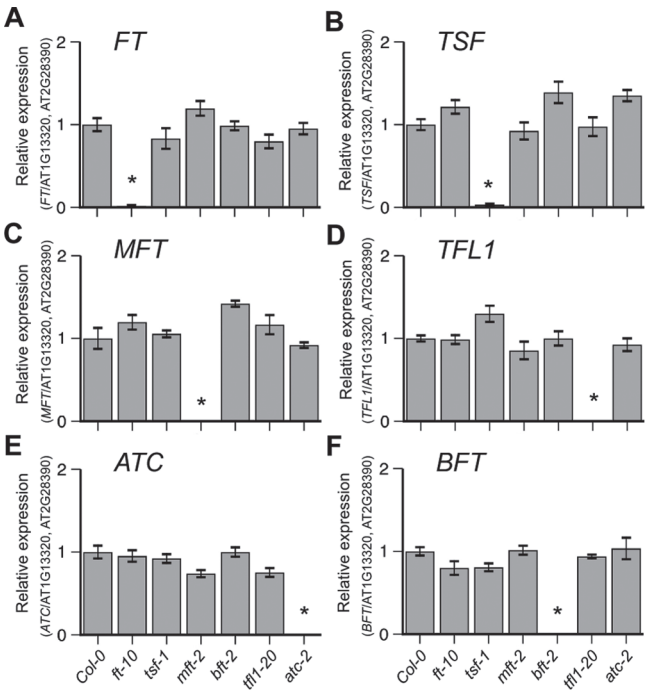

Fig. 2. Expression levels of *FT/TFL1* family genes in each single mutant determined via RT-qPCR: (A) *FT*, (B) *TSF*, (C) *MFT*, (D) *TFL1*, (E) *ATC*, and (F) *BFT*. Expression levels were normalized to At1G13320 and At2G28390 (Hong *et al.*, 2010). Asterisks indicate near absence of transcript levels of the gene in the corresponding mutants.

Table 1. Flowering time of the *FT/TFL1* family mutants under long-day conditions. CL, cauline leaves; ND, not determined; RL, rosette leaves; TL, total number of leaves.

Genotype	23 °C			16 °C			Leaf number ratio (16 °C/23 °C)
	RL	CL	TL	RL	CL	TL	
Col-0	11.0±0.8	2.8±0.4	13.8±0.8	23.8±1.9	3.3±0.7	27.2±1.8	2.0
ft-10	27.8±2.4	8.2±0.7	36.1±2.6	42.7±3.3	7.7±0.5	50.5±3.5	1.4
tsf-1	11.4±0.7	2.8±0.6	14.3±0.6	21.7±1.2	5.8±0.7	27.4±1.6	1.9
mft-2	12.3±0.5	3.0±0.0	15.3±0.5	24.1±2.7	7.5±1.2	31.6±3.9	2.1
tfl1-20	8.6±0.7	1.0±0.0	9.6±0.7	11.7±1.3	0.6±0.5	12.4±1.1	1.3
atc-2	11.1±1.1	2.5±0.5	13.6±1.4	20.3±1.4	5.1±0.9	25.5±2.1	1.9
bft-2	11.2±0.8	2.6±0.7	13.8±1.0	19.3±1.9	6.3±1.2	25.6±2.6	1.9
ft-10 tsf-1	43.2±3.5	13.0±2.3	56.2±4.4	47.7±2.2	11.7±2.5	59.5±3.2	1.1
ft-10 mft-2	28.6±2.8	8.5±1.7	37.1±4.5	37.8±3.0	7.6±1.1	45.5±3.8	1.2
ft-10 tfl1-20	25.4±2.2	7.5±1.0	32.9±2.9	29.0±1.7	6.7±0.9	35.7±2.0	1.1
ft-10 atc-2	28.8±3.0	7.7±1.3	36.5±3.8	34.0±1.6	7.1±1.4	41.1±2.4	1.1
ft-10 bft-2	24.8±3.3	8.7±1.6	33.5±4.3	33.4±2.6	8.6±1.5	42.0±3.9	1.3
tsf-1 mft-2	13.6±1.7	4.1±0.8	17.7±2.4	23.6±3.4	6.7±0.5	30.3±3.9	1.7
tsf-1 tfl1-20	9.5±0.7	1.3±0.7	10.8±0.9	13.5±1.6	0.3±0.5	13.8±1.7	1.3
tsf-1 atc-2	11.4±0.7	2.8±0.6	14.2±0.6	21.3±1.8	5.1±0.6	26.4±1.9	1.9
tsf-1 bft-2	11.4±0.7	2.7±0.6	14.2±1.2	20.1±1.2	5.4±0.8	25.6±1.6	1.8
mft-2 tfl1-20	8.6±0.9	1.3±0.6	10.0±1.5	15.3±1.4	0.5±0.5	15.8±1.3	1.6
mft-2 atc-2	11.9±1.0	2.6±0.5	14.5±1.3	28.8±1.7	8.4±1.4	37.3±2.7	2.6
mft-2 bft-2	11.5±0.7	2.2±0.4	13.7±1.0	23.5±1.2	6.9±0.5	30.4±1.2	2.2
tfl1-20 atc-2	9.1±0.6	1.4±0.5	10.5±0.8	12.8±0.8	1.3±0.7	14.1±0.9	1.3
tfl1-20 bft-2	8.3±0.6	1.0±0.4	9.3±0.6	12.5±1.1	0.7±0.8	13.3±1.1	1.4
atc-2 bft-2	10.9±0.6	2.6±0.7	13.5±0.5	23.0±1.3	5.9±0.7	28.8±1.7	2.1
ft-10 tsf-1 mft-2	46.1±2.8	13.5±1.5	59.6±3.3	56.7±2.1	11.3±0.5	68.0±2.6	1.1
ft-10 tsf-1 tfl1-20	37.6±2.6	10.7±2.8	48.3±3.5	39.8±2.6	7.1±0.6	47.0±2.8	1.0
ft-10 tsf-1 atc-2	42.5±5.4	8.0±1.8	50.5±6.4	51.7±4.0	9.5±1.0	61.2±4.8	1.2
ft-10 tsf-1 bft-2	40.1±1.6	12.0±1.3	52.1±2.2	40.8±1.3	11.5±1.5	52.4±1.6	1.0
ft-10 mft-2 tfl1-20	28.5±0.6	7.8±1.0	36.3±2.3	29.8±0.7	7.0±1.1	36.8±1.2	1.0
ft-10 mft-2 atc-2	26.4±1.7	9.0±1.0	35.4±2.4	39.8±2.6	9.2±0.9	49.1±3.0	1.4
ft-10 mft-2 bft-2	26.6±0.6	7.9±1.2	34.5±3.4	47.0±1.0	7.0±0.0	54.0±1.0	1.6
ft-10 tfl1-20 atc-2	21.0±2.2	6.6±1.0	27.6±2.8	34.5±2.5	7.0±1.1	41.5±1.9	1.5
ft-10 tfl1-20 bft-2	23.2±2.2	6.2±0.7	29.4±2.4	29.1±2.0	4.7±2.0	33.9±2.7	1.1
ft-10 atc-2 bft-2	26.0±1.7	8.7±0.6	34.7±2.3	33.7±1.5	8.7±1.2	42.4±2.2	1.2
tsf-1 mft-2 tfl1-20	9.1±1.2	0.9±0.3	10.0±1.1	13.1±1.7	1.9±0.7	15.0±2.3	1.5
tsf-1 mft-2 atc-2	12.5±1.2	2.9±0.3	15.3±1.3	23.8±2.2	6.2±0.4	30.0±2.3	1.9
tsf-1 mft-2 bft-2	12.6±1.1	2.9±0.7	15.6±1.5	21.8±2.1	5.6±0.8	27.5±2.9	1.8
tsf-1 tfl1-20 atc-2	9.8±1.0	0.7±0.5	10.5±1.2	10.9±0.7	0.7±0.8	11.6±0.8	1.1
tsf-1 tfl1-20 bft-2	9.5±3.3	1.3±0.6	10.8±3.2	10.0±1.2	0.1±0.3	10.1±1.3	0.9
tsf-1 atc-2 bft-2	12.2±1.2	3.1±0.8	15.3±1.8	21.3±0.9	4.8±0.6	26.1±0.8	1.7
mft-2 tfl1-20 atc-2	8.2±1.4	0.9±0.3	9.1±1.7	14.1±0.9	1.7±0.5	15.8±1.1	1.7
mft-2 tfl1-20 bft-2	8.4±0.7	1.1±0.6	9.5±1.0		ND		ND
mft-2 atc-2 bft-2	14.0±1.4	3.4±0.9	17.4±1.9	25.2±1.5	7.3±0.9	32.4±2.2	1.9
tfl1-20 atc-2 bft-2	7.8±1.0	0.7±0.5	8.5±1.5	11.3±0.9	1.3±0.5	12.6±1.1	1.5
ft-10 tsf-1 mft-2 tfl1-20	39.3±2.5	5.4±0.6	44.7±2.4		ND		ND
ft-10 tsf-1 mft-2 atc-2	40.1±2.3	12.4±1.9	52.5±4.2	48.1±1.4	12.7±0.8	60.8±1.3	1.2
ft-10 tsf-1 mft-2 bft-2	40.1±2.6	11.7±2.3	51.8±4.9	47.1±1.6	11.2±0.7	58.3±1.5	1.1
ft-10 tsf-1 tfl1-20 atc-2	37.8±2.9	11.0±2.8	48.8±5.7	39.3±1.8	9.7±0.7	49.0±1.7	1.0
ft-10 tsf-1 tfl1-20 bft-2	41.8±1.6	9.4±0.8	51.2±2.4	41.8±1.6	9.4±0.8	51.3±2.1	1.0
ft-10 tsf-1 atc-2 bft-2	35.6±4.7	11.6±1.7	47.2±5.4	34.5±4.4	6.1±1.2	40.7±5.2	0.9
ft-10 mft-2 tfl1-20 atc-2	21.7±1.6	6.5±0.8	28.2±2.3	30.0±3.6	6.2±0.9	36.2±4.1	1.3
ft-10 mft-2 tfl1-20 bft-2	23.2±1.4	6.9±1.0	30.2±2.1	27.5±1.7	5.7±1.6	33.2±2.7	1.1
ft-10 mft-2 atc-2 bft-2	28.6±4.1	5.5±1.9	34.1±4.5		ND		ND
ft-10 tfl1-20 atc-2 bft-2	20.0±1.8	6.5±0.8	26.5±2.3	29.3±1.7	8.0±0.6	37.3±2.1	1.4
tsf-1 mft-2 tfl1-20 atc-2	9.2±0.6	1.3±0.5	10.5±0.8	14.1±1.3	1.4±0.5	15.5±1.1	1.5
tsf-1 mft-2 tfl1-20 bft-2	8.9±0.8	1.0±0.0	9.9±0.8	14.2±1.5	1.4±0.5	15.6±1.4	1.6
tsf-1 mft-2 atc-2 bft-2	15.5±1.0	4.2±1.3	19.7±1.0	23.2±2.7	5.0±1.6	28.2±4.0	1.4
tsf-1 tfl1-20 atc-2 bft-2	8.9±0.6	1.0±0.0	9.9±0.6	12.7±1.4	1.3±0.5	14.0±1.6	1.4

Table 1. Continued.

Genotype	23 °C			16 °C			Leaf number ratio (16 °C/23 °C)
	RL	CL	TL	RL	CL	TL	
mft-2 tfl1-20 atc-2 bft-2	8.1±1.1	1.0±0.4	9.1±1.0	12.9±1.6	1.4±0.7	14.2±1.5	1.6
ft-10 tsf-1 mft-2 tfl1-20 atc-2	40.8±4.3	10.8±1.5	51.6±5.8	39.2±2.8	7.6±0.9	46.8±2.9	0.9
ft-10 tsf-1 mft-2 tfl1-20 bft-2	34.7±4.5	10.0±3.4	44.7±7.4	41.2±2.3	9.8±1.0	51.1±2.7	1.1
ft-10 tsf-1 mft-2 atc-2 bft-2	33.1±2.6	11.4±2.1	44.5±3.3	47.1±1.4	9.5±1.9	56.7±1.6	1.3
ft-10 tsf-1 tfl1-20 atc-2 bft-2	31.0±1.9	7.4±1.1	38.4±2.4	39.1±1.1	8.0±1.0	47.1±1.3	1.2
ft-10 mft-2 tfl1-20 atc-2 bft-2	21.5±1.8	6.9±1.1	28.3±2.2	35.8±2.7	7.5±1.1	43.3±2.8	1.5
tsf-1 mft-2 tfl1-20 atc-2 bft-2	8.1±1.1	0.6±0.7	8.7±1.1	11.0±1.4	1.0±0.7	12.0±1.4	1.4
ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2	39.3±4.5	9.4±1.9	48.7±6.5	38.0±1.1	7.4±2.0	45.1±2.4	1.0

mentioned, increase in the number of leaves caused by the introduction of *tsf-1* into a genotype containing *ft-10* was similar. The introduction of *tsf-1* into any genotype with or without *ft-10* caused a flowering time delay of 16.1±3.2 and 1.0±0.9 leaves, respectively (Fig. 3D).

The introduction of *mft-2* did not induce a dramatic alternation in flowering time. Although *MFT* is suggested to act as a flowering activator based on an overexpression study (Yoo *et al.*, 2004), a slight increase in leaf number caused by introduction of *mft-2* was only observed in *tsf-1 mft-2*, *mft-2 atc-2 bft-2*, *ft-10 tsf-1 mft-2 bft-2*, and *tsf-1 mft-2 atc-2 bft-2* mutants (red arrows in Fig. 3E). The significant decrease of leaf number by *mft-2* was observed only in *ft-10 mft-2 tfl1-20 atc-2* mutants (blue arrows in Fig. 3E). The introduction of *mft-2* in some mutants containing *tfl1-20* appeared to have a weak effect; however, an analysis of leaf number changes by *mft-2* revealed an insignificant difference between mutants that did or did not contain *tfl1-20* (Fig. 3F).

Introduction of *tfl1-20* caused a general decrease in leaf number (red arrows in Fig. 3G). For instance, introducing *tfl1-20* into *tsf-1 mft-2 bft-2* mutants caused slightly earlier flowering (from 15.6 to 9.2 leaves). Interestingly, introduction of *tfl1-20* into the *ft-10 tsf-1 atc-2* mutants failed to accelerate flowering (black arrows in Fig. 3E). Flowering time of *ft-10 tsf-1 atc-2* and *ft-10 tsf-1 tfl1-20 atc-2* mutants was similar (50.5 versus 48.8 leaves). The introduction of *tfl1-20* into any genotype without *ft-10 tsf-1 atc-2* reduced flowering time by 5.7±2.8 leaves, whereas the introduction of *tfl1-20* into any genotype with *ft-10 tsf-1 atc-2* produced no significant change in leaf number (Fig. 3H).

The effect of the introduction of *atc-2* and *bft-2* was similar (Fig. 3I, K) and generally caused weak acceleration of flowering only in the mutants containing *ft-10*. In mutants without *ft-10*, the introduction of *atc-2* and *bft-2* had only a minor acceleration of flowering. The introduction of *atc-2* into the genotypes with *ft-10* caused a slight decrease in leaf number (2.3±3.8 leaves) (Fig. 3J). Similarly, the introduction of *bft-2* into the genotypes with *ft-10* caused a slight decrease in leaf number (3.7±2.7 leaves) (Fig. 3L). In contrast, the introduction of *atc-2* or *bft-2* into the genotypes without *ft-10* did not cause an apparent alteration in flowering time.

Under long-day conditions at 23 °C, *ft-10 tsf-1 mft-2* mutants flowered the latest (59.6±3.3 leaves), and *tfl1-20 atc-2 bft-2* mutants flowered the earliest (8.5±1.5 leaves) (Table 1).

Flowering time analysis at 16 °C under long-day conditions

The flowering time also measured at 16 °C under long-day conditions. Among single mutants, altered flowering time was only seen in *ft-10* (50.5±3.5 leaves) and *tfl1-20* plants (12.4±1.1 leaves), compared to wild-type plants (27.2±1.8 leaves) (Table 1). Flowering time of *tsf-1*, *mft-2*, *atc-2*, and *bft-2* single mutants was similar to that of wild-type plants, which was similar to that seen at 23 °C (Fig. 3).

The effect of introducing each mutation on flowering time at 16 °C was analysed by calculating the difference of leaf numbers between mutants with or without the mutation at flowering (Fig. 4). Introducing *ft-10* generally delayed flowering time regardless of genotype. Introduction of *ft-10* into mutants containing *tsf-1* generally led to very delayed flowering at 16 °C (red arrows in Fig. 4A), similar to flowering at 23 °C; however, its effect at 16 °C was not as distinct as at 23 °C. In spite of the absence of *tsf-1*, some mutants (e.g. *ft-10 mft-2 atc-2*) flowered as late as mutants carrying both *ft-10* and *tsf-1*. Among double mutants, *ft-10 tsf-1* mutants (59.5±3.2 leaves) flowered significantly later than other double mutants (*ft-10 mft-2*, 45.4±3.8 leaves; *ft-10 tfl1-20*, 35.7±2 leaves; *ft-10 atc-2*, 41.1±2.4 leaves; and *ft-10 bft-2*, 42±3.9 leaves). Among triple mutants, *ft-10 tsf-1 mft-2* (68.0±2.6 leaves), *ft-10 tsf-1 bft-2* (52.4±1.6 leaves), *ft-10 tsf-1 atc-2* (61.2±4.8 leaves), and *ft-10 mft-2 bft-2* (54.0±1.0 leaves) mutants flowered significantly later than other triple mutants.

The introduction of *ft-10* into the mutants with *tsf-1* caused a delay in flowering time (29.0±6.4 leaves), whereas the introduction of *ft-10* into genotypes without *tsf-1* caused a smaller delay in flowering time (15.5±7.0 leaves) (Fig. 4B). Considering that the introduction of *ft-10* into any genotype led to a clearer difference depending on the presence of the *tsf-1* mutation at 23 °C (Fig. 3B), this indicated that the additive effect of *ft-10* and *tsf-1* is diminished at 16 °C.

Introducing *tsf-1* into a genotype that contained *ft-10* delayed flowering with wide variation (red arrows in Fig. 4C). However, introducing *tsf-1* into the genotypes without *ft-10* showed slight acceleration of flowering or no clear effect

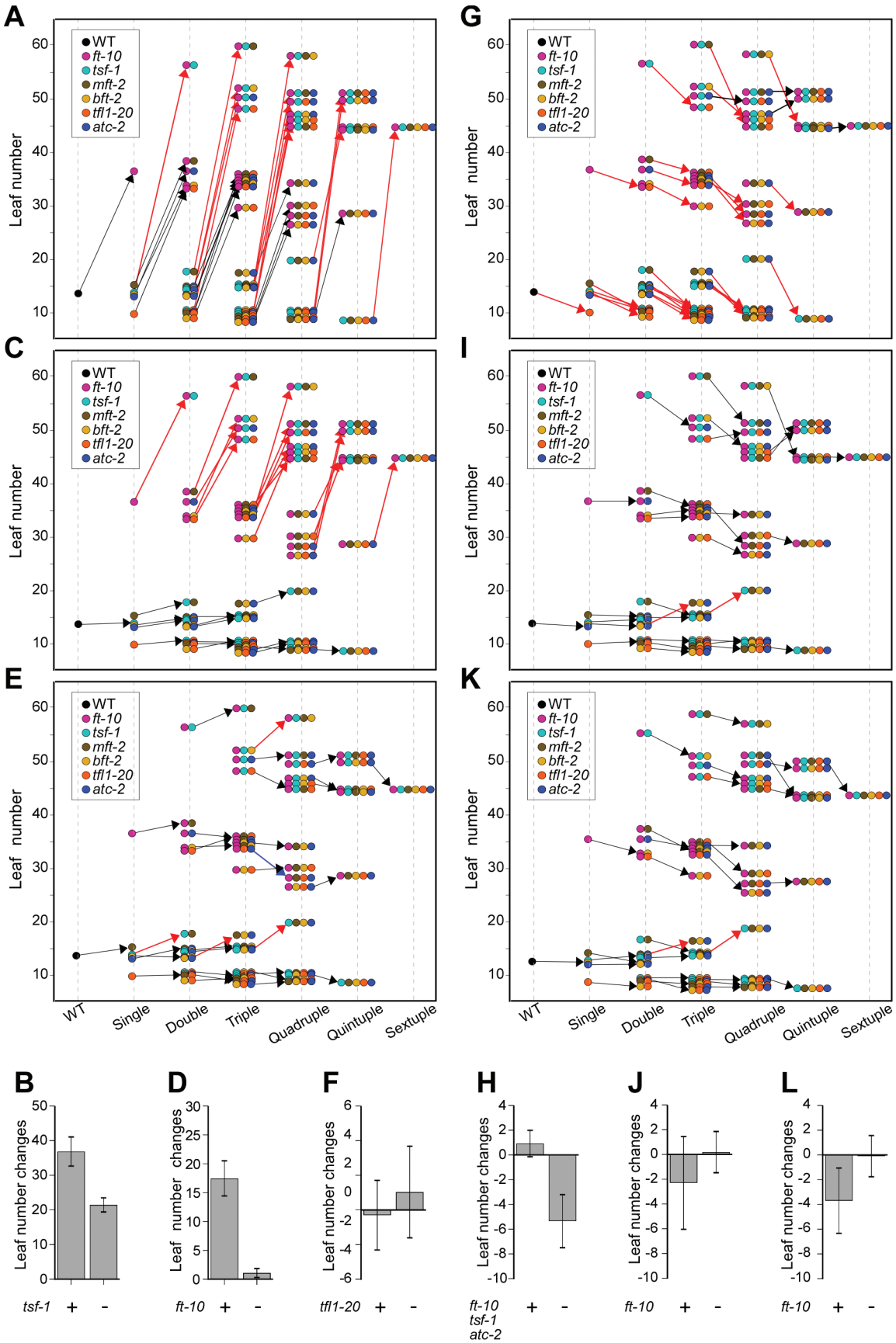

Fig. 3. Leaf number changes caused by the introduction of each mutation at 23 °C under long-day conditions. (A, C, E, G, I, and K) The effect of introducing *ft-10* (A), *tsf-1* (C), *mft-2* (E), *tfl1-20* (G), *atc-2* (I), and *bft-2* (K); the arrows indicate leaf number changes after addition of a certain mutation to a genotype. (B, D, F, H, J, and L) Leaf number changes caused by the introduction of a single mutation in mutants

(black arrows in Fig. 4C). The introduction of *tsf-1* into a genotype with or without *ft-10* caused a delay in flowering time (9.2 ± 8.0 leaves) and no clear effect (–1.6 ± 2.3 leaves), respectively (Fig. 4D). This result indicated that the additive effect of *ft-10* and *tsf-1* at 16 °C was not as clear as at 23 °C.

The introduction of *mft-2* did not cause a dramatic change in flowering time at 16 °C (Fig. 4E). A significant delay in flowering caused by *mft-2* was observed only in *mft-2 atc-2*, *ft-10 mft-2 bft-2*, *ft-10 mft-2 atc-2*, and *ft-10 tsf-1 mft-2 atc-2 bft-2* mutants (red arrows in Fig. 4E), which showed no change from addition of *mft-2* at 23 °C. Conversely, *ft-10 mft-2 tfl1-20 atc-2* mutants, which showed a significant decrease in leaf number by addition of *mft-2* at 23 °C, did not show an apparent alternation in flowering at 16 °C.

The introduction of *tfl1-20* caused a dramatic decrease in leaf number (red arrows in Fig. 4F), except for the introduction of *tfl1-20* into *ft-10 atc*-2, *ft-10 tsf-1 bft-2*, *ft-10 tsf-1 atc-2 bft-2*, and *ft-10 mft-2 atc-2 bft-2* mutants (black arrows in Fig. 4F). Although introducing *tfl1-20* into *ft-10* mutants caused only slightly early flowering (from 36.1 to 32.9 leaves) at 23 °C, more apparent acceleration in flowering by the introduction of *tfl1-20* was observed in *ft-10 tfl1-20* double mutants (from 50.5 to 35.7 leaves) at 16 °C. The same is also true for *ft-10 tsf-1 tfl1-20*, *ft-10 mft-2 tfl1-20 bft-2*, and *ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* mutants. This suggested that the effect of *tfl1-20* on the control of flowering time is ambient temperature dependent.

The introduction of *atc-2* and *bft-2* had little effect on flowering time at 16 °C. The acceleration of flowering time by introduction of *atc-2* was seen only in some mutants (Fig. 4G). For instance, the introduction of *atc-2* into the *ft-10*, *ft-10 tsf-1 bft-2*, and *ft-10 mft-2 bft-2* backgrounds decreased leaf number at flowering by 9.4, 11.7, and 22.5 leaves, respectively. In contrast, introducing *atc-2* into some mutants such as *mft-2*, *ft-10 tfl1-20*, and *ft-10 mft-2 tfl1-20 bft-2* rather increased leaf number. The remaining mutants did not show a clear alteration (0.4 ± 2.4 leaves). The introduction of *bft-2* had only a minor effect in accelerating flowering at 16 °C. In spite of the suggested role of *BFT* as a flowering repressor, only some mutants, such as *ft-10 mft-2 bft-2* (+15.7 leaves) and *ft-10 mft-2 tfl1-20 atc-2 bft-2* (+7.1 leaves), showed a delay in flowering that was increased by addition of *bft-2* (red arrows in Fig. 4H).

tfl1-20 strongly accelerated flowering at 16 °C

The change in leaf numbers in response to the introduction of each single mutation was next compared at 23 and 16°C to examine the effect at different ambient temperatures (Fig. 5).

containing (+) or not containing (–) another mutation: (B) introduction of *ft-10* into mutants with or without *tsf-1*; (D) introduction of *tsf-1* into mutants with or without *ft-10*; (F) introduction of *mft-2* into mutants with or without *tfl1-20*; (H) introduction of *tfl1-20* into mutants with or without *ft-10 tsf-1 atc-2*; (J) introduction of *atc-2* into mutants with or without *ft-10*; (L) introduction of *bft-2* into mutants with or without *ft-10*.

The introduction of *ft-10* caused a severe delay in flowering time (ranging from 18.1 to 41.9 leaves) at 23 °C. The flowering response was clearly divided into two categories, namely with (open box in Fig. 5) or without *tsf-1*. However, at 16 °C, the delay in flowering by *ft-10* mutation was attenuated and the additive delay by combination of *ft-10* and *tsf-1* was less distinct. The introduction of *tsf-1* caused no delay on its own but significant flowering time delay when combined with *ft-10* (grey box in Fig. 5) at both 23 and 16°C. *mft-2* did not induce an apparent effect at either temperature, although there were a few mutants that showed a strong effect of *mft-2* at 16 °C. The introduction of *tfl1-20* caused a weak acceleration in flowering time at 23 °C (–4.7 leaves in average). A particularly interesting observation was that *tfl1-20* had a stronger effect at 16 °C (–11.6 leaves in average) (horizontal bar in Fig. 5). Most mutants containing *atc-2* or *bft-2* did not show a clear alteration in flowering time at both temperatures. This comparison revealed that *FT*, *TSF*, and *TFL1* play an important role in ambient temperature-responsive flowering and that the effect of *tfl1-20* was stronger at 16 °C.

tsf-1 caused reduced sensitivity to ambient temperature-responsive flowering

The effect of introducing each mutation on the response to ambient temperature was analysed by measuring the leaf number ratio (LNR, 16 °C/23 °C). *FT* is suggested to be an important mediator of flowering time in the response to ambient temperature, since the LNR of *ft-10* single mutants was reduced comparing to that of wild-type plants (Lee *et al.*, 2007). Introducing *ft-10* generally decreased LNR. The LNR of all mutants containing *ft-10* was lower than that of wild-type plants (Fig. 6A). The average LNR of all mutant combinations containing *ft-10* was 1.2. Noticeably, the LNR of *ft-10 tsf-1*, *ft-10 tsf-1 bft-2*, *ft-10 tsf-1 tfl1-20*, *ft-10 mft-2 tfl1-20*, *ft-10 tsf-1 tfl-20 atc-2*, and *ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* mutants was near 1.0, suggesting that they showed a flowering phenotype insensitive to ambient temperature changes. The hypersensitive flowering response to ambient temperature changes of *mft-2 atc-2* mutants (LNR 2.6) was also significantly suppressed by the introduction of *ft-10* (*ft-10 mft-2 atc-2* LNR 1.4). This analysis demonstrated that *ft-10* has a strong effect on reducing ambient temperature sensitivity.

Interestingly, the introduction of *tsf-1* into other mutants caused a general decrease in LNR (Fig. 6B), independent of the presence of *ft-10*, although *tsf-1* on its own failed to change ambient temperature sensitivity (LNR 1.9). Introducing *tsf-1* in some genotypes induced a dramatic decrease in LNR (more than –0.5). These include *tsf-1 mft-2 bft-2* (change in LNR –0.5), *ft-10 tsf-1 mft-2 bft-2* (–0.7), and *ft-10 tsf-1 mft-2 tfl1-20 atc-20* (–0.5). The general reduction in LNR by *tsf-1* suggested that *TSF* plays a role in the regulation of ambient temperature-responsive flowering.

Introducing *mft-2* caused a slight increase in LNR (Fig. 6C). For instance, the increase in LNR of *mft-2 atc-2* and *ft-10 mft-2 bft-2* mutants by introduction of *mft-2* was 0.7 and 0.5, respectively. An interesting observation was that introducing *mft-2* even increased the LNR of mutants containing *tfl1-20*.

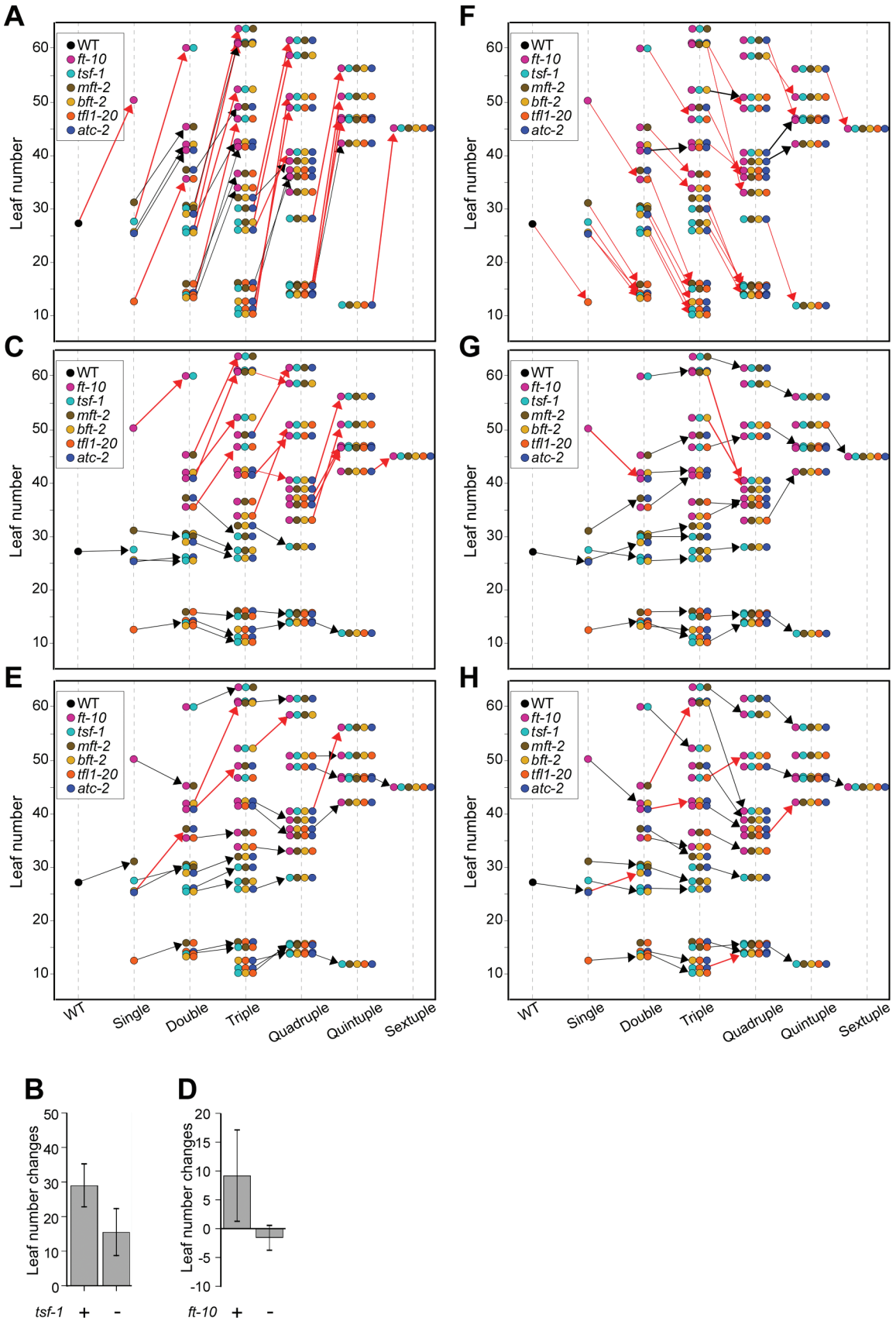

Fig. 4. Leaf number changes caused by the introduction of each mutation at 16 °C under long-day conditions. (A, C, E, F, G, and H) The effect of introducing *ft-10* (A), *tsf-1* (C), *mft-2* (E), *tfl1-20* (F), *atc-2* (G), and *bft-2* (H); the arrows indicate leaf number changes after addition of a certain mutation to a genotype. (B and D) Leaf number changes caused by the introduction of a single mutation in mutants containing (+) or not containing (–) another mutation: (B) introduction of *ft-10* into mutants with or without *tsf-1*; (D) introduction of *tsf-1* into *ft-10*.

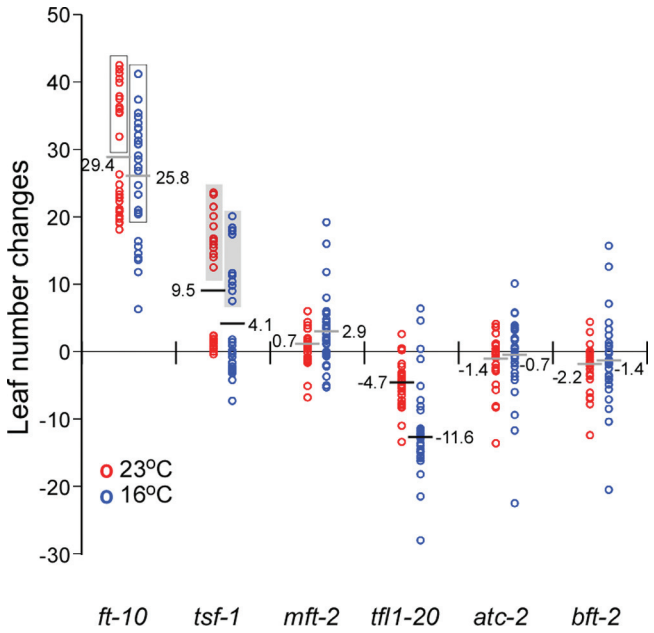

Fig. 5. Plotting of leaf number changes in all mutant combinations caused by introduction of each single mutation at 23 and 16°C. Open boxes indicate leaf number changes by *ft-10* in the presence of *tsf-1* (except *ft-10 mft-2 bft-2* mutants at 16 °C). Grey boxes indicate leaf number changes caused by *tsf-1* in the presence of *ft-10* (except *ft-10 tsf-1 mft-2 bft-2*, *ft-10 tsf-1 atc-2 bft-2*, and *ft-10 tsf-1 mft-2 tfl1-20* mutants at 16 °C). Horizontal bars indicate the average leaf number changes caused by the introduction of each mutation: note that *tfl1-20* and *tsf-1* caused a stronger effect at 16 °C than at 23 °C (black bars, $P < 0.05$), and that *ft-10*, *mft-2*, *atc-2*, and *bft-2* did not show a clear effect (grey bars).

The increase in LNR of *mft-2 tfl1-20*, *mft-2 tfl1-20 atc-2*, *tsf-1 mft-2 tfl1-20 bft-2*, and *ft-10 tsf-1 mft-2 atc-2 bft-2* was 0.3, 0.4, 0.8, and 0.4, respectively. Although *tfl1-20* is known to induce ambient temperature-insensitive flowering (Strasser *et al*., 2009), *mft-2* weakly suppressed the effect of *tfl1-20* in ambient temperature-responsive flowering.

Introducing *tfl1-20* caused a general decrease in LNR (Fig. 6D). For instance, the decrease in LNR in *tsf-1 tfl1-20*, *tsf-1 bft-2 tfl1-20*, and *tsf-1 mft-2 tfl1-20 atc-2* mutants by introduction of *tfl1-20* was 0.6, 0.9, and 0.5, respectively. However, the introduction of *tfl1-20* into a genotype that already contained *ft-10 tsf-1* had a weak effect. The decrease in LNR in *ft-10 tsf-1 tfl1-20* and *ft-10 tsf-1 tfl1-20 bft-2* mutants by introduction of *tfl1-20* was 0.1 and 0.1, respectively. This suggested that ambient temperature-insensitive flowering of mutants containing *ft-10* and *tsf-1* was not strongly enhanced by *tfl1-20*.

Introducing *atc-2* and *bft-2* did not produce a clear pattern in response to ambient temperature changes (Fig. 6E and F). Some mutants showed an increase in LNR caused by the introduction of *atc-2* (*mft-2 atc-2*, LNR 0.5; *tsf-1 mft-2 atc-2*, LNR 0.3; and *tsf-1 tfl1-20 atc-2 bft-2*, LNR 0.5) (Fig. 6E). In contrast, some mutants showed a decrease in LNR caused by the introduction of *atc-2* (*ft-10 mft-2*, LNR –0.3; *mft-2 atc-2 bft-2*, LNR –0.4; and *tsf-1 mft-2 atc-2 bft-2*, LNR –0.3). No significant change was observed by introducing *bft-2* in any double mutants. But among triple mutants and other higher-order mutants, the effect of introducing *bft-2* appeared to be dependent on *ft-10* and *tfl1-20*, which caused weak temperature insensitivity. The introduction of *bft-2* into a genotype containing *ft-10* or *tfl1-20* caused an increase in LNR (Fig. 6F). The increase of LNR in *ft-10 mft-20 bft-2* and *ft-10 mft-2 atc-2 bft-2* mutants by introduction of *bft-2* was 0.6 and 0.3, respectively. However, the introduction of *bft-2* into the other genotypes that were sensitive to ambient temperatures produced a decrease in LNR.

The LNR of *ft-10* single mutants was 1.4, but the LNRs of the double mutants were even lower than that of *ft-10* single mutants. The LNRs of *ft-10 bft-2*, *ft-10 mft-2*, *ft-10 atc-2*, *ft-10 tfl1-20*, and *ft-10 tsf-1* double mutants were 1.3, 1.2, 1.1, 1.1, and 1.1, respectively (Table 1). This indicated that the addition of a mutation in any *FT*/*TFL1* family member into the *ft-10* mutant background reduced sensitivity to ambient temperature-responsive flowering. However, such an additive effect was not seen in other single-mutant backgrounds. This suggested that *FT* plays a redundant role with other *FT*/*TFL1* family genes in ambient temperature-responsive flowering.

Taken together, these results show that, for ambient temperature-responsive flowering, the introduction of *ft-10*, *tfl1-20*, and *tsf-1* caused flowering to be less sensitive to ambient temperatures. However, the introduction of *mft-2*, *bft-2*, and *atc-2* did not show a clear general pattern.

miR156 overexpression in ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2 sextuple mutants

These genetic analyses revealed that *Arabidopsis* plants flowered in the absence of all *FT*/*TFL1* family genes, suggesting that *FT*/*TFL1* family genes are not essential for flowering. This further suggested a possibility that there is an *FT*/*TFL1*-independent pathway to induce flowering. One such pathway may include miR156, which delays flowering time by negatively regulating *SPL* genes (Wang *et al*., 2009; Jung *et al*., 2012a). Thus, this study tested whether the introduction of miR156 into our *ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* sextuple mutants blocks flowering. For this experiment, the *35S::miR156a* construct was introduced into the sextuple mutants and wild-type plants by *Agrobacterium*-mediated transformation. The introduction of *35S::miR156* into wild-type plants and sextuple mutants caused a general delay in flowering. The distribution of flowering times of *35S::miR156a* and *35S::miR156a ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* plants in the T1 generation is shown (Supplementary Fig. S1). This study selected a line that showed strong late flowering and confirmed miR156 overexpression in transgenic plants (approximately 5-fold) (Fig. 7A).

To exclude the possibility that the *FT*/*TFL1* family genes regulate miR156 expression, small RNA blot analysis was performed for each single mutant. The results indicated that miR156 expression was not altered in any single mutant (Fig. 7B). However, the miR156 level in the sextuple mutants was slightly higher than that of wild-type plants (Fig. 7A),

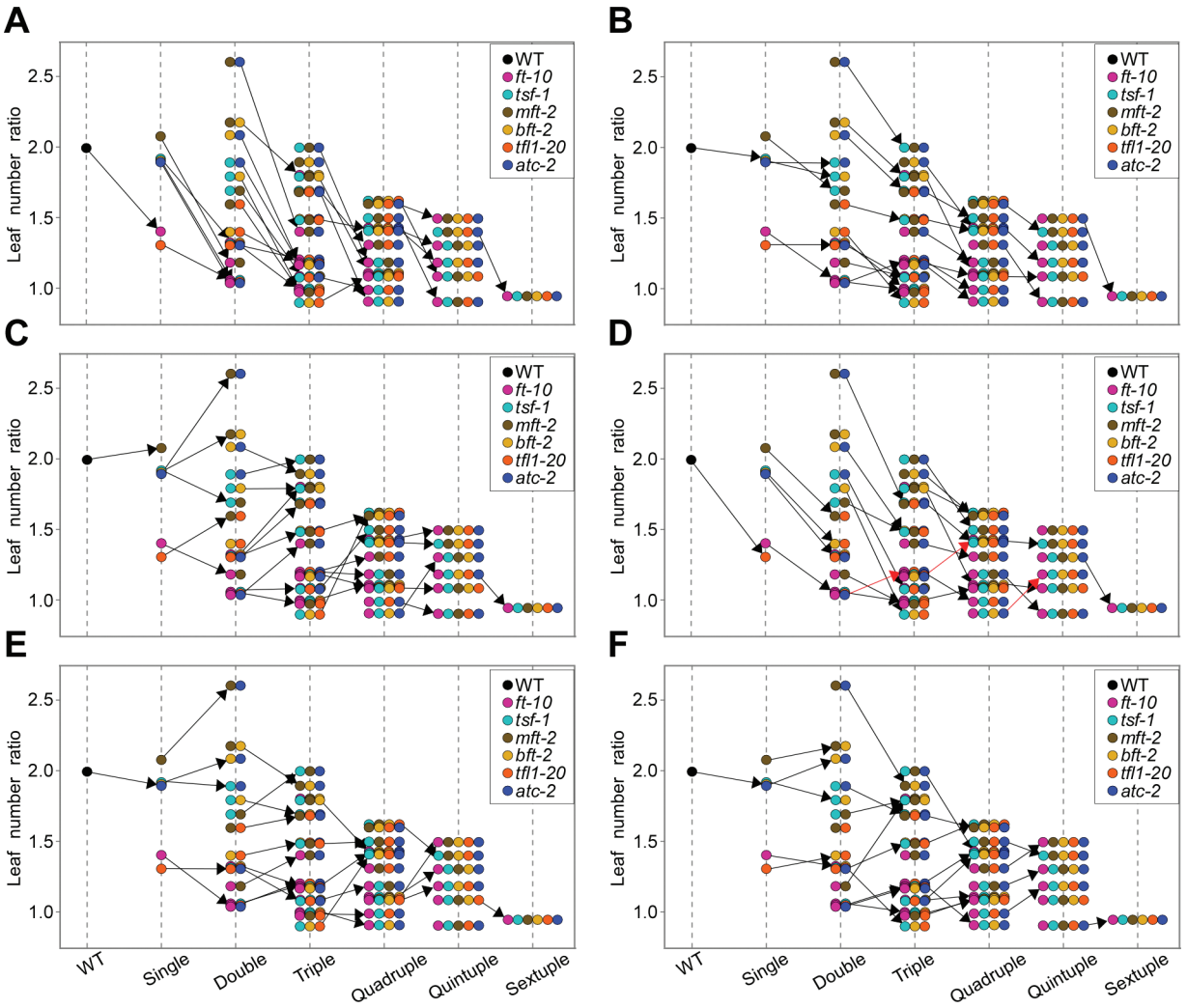

Fig. 6. Changes in leaf number ratios (16 °C/23 °C) by the introduction of mutations *ft-10* (A), *tsf-1* (B), *mft-2* (C), *tfl1-20* (D), *atc-2* (E), and *bft-2*(F). Arrows indicate the changes in leaf number ratios after addition of a certain mutation into a genotype.

suggesting a possibility that miR156 expression is negatively affected by combined mutations in the *FT*/*TFL1* family.

Interestingly, *35S::miR156a ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* plants still flowered, but later than that of *ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* and *35S::miR156a* plants. *35S::miR156a ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* plants flowered with 58.1 leaves under long-day conditions, whereas *35S::miR156a* control plants flowered with 24.7 leaves under the same conditions (Fig. 7C). Such late flowering was comparable to that seen in *ft-10 tsf-1 mft-2* mutants (Table 1). This study also observed that *35S::miR156a ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* plants frequently generated multiple rosettes with many secondary leaves (Fig. 7D), although such phenotype was absent in the sextuple mutants.

Discussion

The *FT*/*TFL1* family encodes six important regulators (*FT*, *TSF*, *MFT*, *TFL1*, *ATC*, and *BFT*) that control flower development in *Arabidopsis*. This study constructed a comprehensive mutant set for this family, including the sextuple mutant, and measured the flowering time of each mutant. Lesions in all six *FT*/*TFL1* family genes and ectopic miR156 expression in the sextuple mutants did not inhibit flowering under long-day conditions. Also, *tsf-1* reduced sensitivity to ambient temperature changes and *tfl1-20* had a stronger effect at 16 °C than at 23 °C.

Flowering time studies of a subset of double and triple mutants have been reported (Hanzawa *et al.*, 2005; Yamaguchi *et al.*, 2005; Jang *et al.*, 2009; Ahn *et al.*, 2006; Yoo *et al.*, 2010). The previous analyses using overexpression lines and a handful of mutants suggested that *FT*, *TSF*, and *MFT* are floral activators (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999; Yoo *et al.*, 2004) and that *TFL1*, *ATC*, and *BFT* are floral repressors (Bradley *et al.*, 1997; Mimida *et al.*, 2001; Yoo *et al.*, 2010), and this study's analysis of flowering time provided conclusive evidence to support the notion. Among them, *FT* and *TFL1* exert a strong effect, whereas the contribution from other genes was minor. An excellent example was the flowering time of sextuple mutants. The *ft-10 tsf-1 mft-2* mutants flowered latest (59.6 ± 3.3 leaves) and the *tfl1-20 atc-2 bft-2* mutants flowered earliest (8.5 ± 1.5 leaves). The

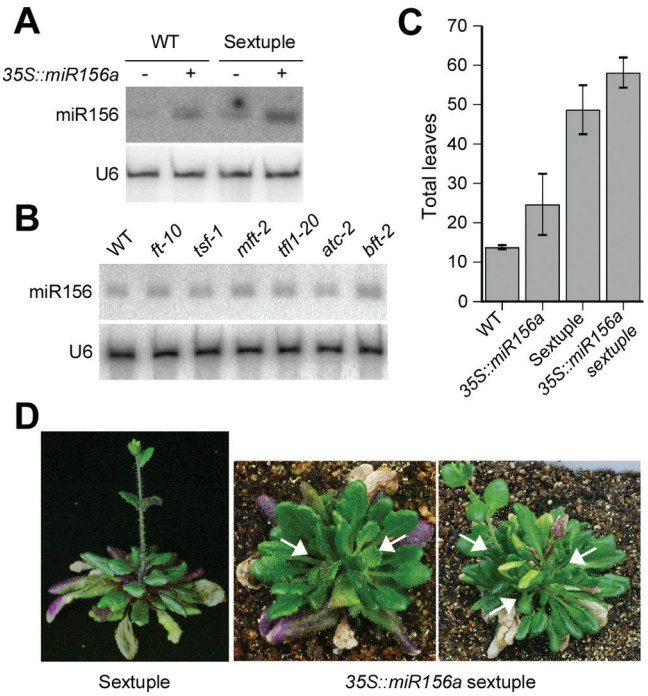

Fig. 7. Flowering phenotype of *35S::miR156a ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* mutants under long-day conditions. (A and B) Small RNA blots showing expression levels of miR156 in transgenic plants generated in this study (*35S::miR156a* plants and *35S::miR156a ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* mutants) (A) and in single mutants (B); U6 RNA served as a loading control (Lee *et al.*, 2010). (C and D) Flowering time (C) and morphology (D) of *35S::miR156a ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* mutants under long-day conditions. Note multiple rosettes generated from *35S::miR156a ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* mutants (arrows). Total leaf number of *ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* mutants in (C) came from Table 1.

flowering time of *ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* mutants (48.7 ± 6.5 leaves) was intermediate (Table 1).

An important question is which gene activates flowering in the sextuple mutants, because the sextuple mutants still flower and *ft-10 tsf-1 mft-2* mutants flowered later than *tfl1-20 atc-2 bft-2* mutants. This study measured expression levels of *SOC1*, *SPL3*, *FUL*, and *AP1* in the wild type, *ft-10 tsf-1 mft-2*, *tfl1-20 atc-2 bft-2*, and the sextuple mutants. Although *SOC1* and *FUL* mRNA levels were lower in *ft-10 tsf-1 mft-2* mutants, their mRNA levels in *tfl1-20 atc-2 bft-2* mutants were not significantly higher than those of wild-type plants (Supplementary Fig. S2). This suggested the possibility that *SOC1* and *FUL* did not activate flowering. *SPL3* mRNA levels seemed to be unaffected by these mutations. However, *AP1* transcript levels were significantly lower in *ft-10 tsf-1 mft-2* mutants, but higher in *tfl1-20 atc-2 bft-2* plants than in wild-type plants. These results suggested that increased *AP1* expression is responsible for the early flowering of *tfl1-20 atc-2 bft-2* plants. However, since this study tested a subset of flowering time genes, a genome-wide analysis would be necessary to identify the gene responsible for the flowering phenotype of the sextuple mutants.

One notable finding is that *tsf-1* reduces sensitivity to ambient temperature changes. It was previously suggested that under long-day conditions, *TSF* on its own did not play a role in regulating flowering time (Michaels *et al.*, 2005; Yamaguchi *et al.*, 2005). However, the current data revealed that *tsf-1* reduced the temperature response even without *ft-10* (Fig. 6B). This finding is consistent with this study group's previous proposal that the ambient temperature response is mediated by both *FT* and *TSF*, based on the weak effect of *ft-10* single mutation in ambient temperature-responsive flowering (Lee *et al.*, 2007). Indeed, the leaf number ratios of *ft-10 tsf-1* double mutants and higher-order mutants containing both *ft-10* and *tsf-1* were close to 1.0. Thus, it seems likely that *FT* and *TSF* act downstream of the thermosensory pathway (Lee *et al.*, 2007; Lee *et al.*, 2010; McClung and Davis, 2010; Kumar *et al.*, 2012). *TSF* plays a redundant role with *FT* under long-day conditions, mainly acts under short-day conditions (Yamaguchi *et al.*, 2005) and acts in response to cytokinin treatment (D'Aloia *et al.*, 2011). This group's studies suggest an additional role for *TSF* under long-day conditions.

The observation that *tfl1-20* had a stronger effect at 16 °C than at 23 °C further supports Cerdán group's finding that *TFL1* may be a positive regulator of the response to low temperature (Strasser *et al.*, 2009). They performed a modified gene set enrichment analysis and found that *TFL1* plays more general roles in the plant response to ambient temperature. How TFL1 regulates ambient temperature response is largely unknown, but its localization at the endomembrane compartment (Sohn *et al.*, 2007) may provide a clue to its precise function. Membrane homeostasis including membrane thickness (Cybulski *et al.*, 2010) and membrane integrity (Mansilla *et al.*, 2004) is suggested to be a general cue for sensing temperature. Thus, the changes in biochemical properties of cell membrane lipids in response to ambient temperature changes may lead to alterations in the activity of a signalling molecule that regulates ambient temperature response. Considering that TFL1 is highly similar to an animal PEBP protein that encodes a Raf kinase inhibitor (Yeung *et al.*, 1999), it is tempting to speculate that TFL1 may be involved in relay of temperature-responsive sensor kinase signalling (Mansilla *et al.*, 2004), which is associated with thermal control of membrane lipid homeostasis.

Another interesting observation was that *tfl1-20* did not accelerate flowering in the *ft-10 tsf-1 atc-2* background, although *tfl1-20* generally reduced the leaf number at flowering in almost all mutant combinations. This suggests that *FT*, *TSF*, and *ATC* are required for *TFL1* function in the regulation of flowering time. *ATC* was recently described as a short-day-induced floral inhibitor that is graft transmissible (Huang *et al.*, 2012). FT and TSF proteins interact with FD (Abe *et al.*, 2005; Wigge *et al.*, 2005; Jang *et al.*, 2009), probably at the shoot apex. TFL1 protein also interacts with FD to transcriptionally repress flowering time genes that are induced by *FT* (Hanano and Goto, 2011). Thus, a possible scenario to explain the absence of the effect of *tfl1-20* mutation is that recruitment of a coactivator or corepressor to the FD protein complex is inhibited in the *ft-10 tsf-1*

atc-2 background, as previously suggested (Ahn *et al*., 2006). Failure to recruit such cofactors may then render FD inactive or nearly inactive, explaining the absence of an effect of the addition of *tfl1-20*.

In summary, this study constructed a comprehensive set of mutants of the six *A. thaliana FT*/*TFL1* family genes and analysed their genetic interactions in the regulation of flowering time. This mutant population will be useful to further define the role of the *FT*/*TFL1* family genes in broad aspects of plant development. Further analyses using this population will provide strong genetic evidence of the functional roles and importance of the *FT*/*TFL1* family.

Supplementary material

Supplementary data can be found at *JXB* online.

Supplementary Fig. S1. Distribution of flowering time of wild type, *35S::miR156a* and *35S::miR156a ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* plants at 23°C in the T_1 generation.

Supplementary Fig. S2. Relative expression of *SPL3*, *FUL*, *SOC1*, and *AP1* in WT, *tfl1-20 atc-2 bft-2, ft-10 tsf-1 mft-2 tfl1-20 atc-2 bft-2* and *ft-10 tsf-1 mft-2* plants.

Supplementary Table S1. Oligonucleotide sequences used for genotyping.

Supplementary Table S2. Oligonucleotide sequences used for RT-qPCR.

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References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T. 2005. FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* **309,** 1052–1056.

Ahn JH, Miller D, Winter VJ, Banfield MJ, Lee JH, Yoo SY, Henz SR, Brady RL, Weigel D. 2006. A divergent external loop confers antagonistic activity on floral regulators FT and TFL1. *The EMBO Journal* **25,** 605–614.

Alonso JM, Stepanova AN, Leisse TJ, *et al.* 2003. Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. *Science* **301,** 653–657.

Blazquez MA, Ahn JH, Weigel D. 2003. A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. *Nature Genetics* **33,** 168–171.

Bradley D, Ratcliffe O, Vincent C, Carpenter R, Coen E. 1997. Inflorescence commitment and architecture in *Arabidopsis*. *Science* **275,** 80–83.

Chardon F, Damerval C. 2005. Phylogenomic analysis of the PEBP gene family in cereals. *Journal of Molecular Evolution* **61,** 579–590.

Cho HJ, Kim JJ, Lee JH, Kim W, Jung JH, Park CM, Ahn JH. 2012. SHORT VEGETATIVE PHASE (SVP) protein negatively regulates miR172 transcription via direct binding to the pri-miR172a promoter in *Arabidopsis*. *FEBS Letters* **586,** 2332–2337.

Corbesier L, Vincent C, Jang S, *et al.* 2007. FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* **316,** 1030–1033.

Cybulski LE, Martin M, Mansilla MC, Fernandez A, de Mendoza D. 2010. Membrane thickness cue for cold sensing in a bacterium. *Current Biology* **20,** 1539–1544.

D'Aloia M, Bonhomme D, Bouche F, Tamseddak K, Ormenese S, Torti S, Coupland G, Perilleux C. 2011. Cytokinin promotes flowering of *Arabidopsis* via transcriptional activation of the *FT* paralogue *TSF*. *The Plant Journal* **65,** 972–979.

Ferrandiz C, Gu Q, Martienssen R, Yanofsky MF. 2000. Redundant regulation of meristem identity and plant architecture by *FRUITFULL*, *APETALA1* and *CAULIFLOWER*. *Development* **127,** 725–734.

Fitter AH, Fitter RS. 2002. Rapid changes in flowering time in British plants. *Science* **296,** 1689–1691.

Fornara F, de Montaigu A, Coupland G. 2010. SnapShot: control of flowering in *Arabidopsis*. *Cell* **141, 550,** 550 e551–552.

Hanano S, Goto K. 2011. *Arabidopsis* TERMINAL FLOWER1 is involved in the regulation of flowering time and inflorescence development through transcriptional repression. *The Plant Cell* **23,** 3172–3184.

Hanzawa Y, Money T, Bradley D. 2005. A single amino acid converts a repressor to an activator of flowering. *Proceedings of the National Academy of Sciences, USA* **102,** 7748–7753.

Harig L, Beinecke FA, Oltmanns J, *et al.* 2012. Proteins from the FLOWERING LOCUS T -like subclade of the PEBP family act antagonistically to regulate floral initiation in tobacco. *The Plant Journal* **72,** 908–921.

Helliwell CA, Wood CC, Robertson M, James Peacock W, Dennis ES. 2006. The *Arabidopsis* FLC protein interacts directly *in vivo* with *SOC1* and *FT* chromatin and is part of a high-molecular-weight protein complex. *The Plant Journal* **46,** 183–192.

Hong SM, Bahn SC, Lyu A, Jung HS, Ahn JH. 2010. Identification and testing of superior reference genes for a starting pool of transcript normalization in *Arabidopsis*. *Plant Cell Physiology* **51,** 1694–1706.

Huang NC, Jane WN, Chen J, Yu TS. 2012. *Arabidopsis thaliana CENTRORADIALIS homologue* (*ATC*) acts systemically to inhibit floral initiation in *Arabidopsis*. *The Plant Journal* **72,** 175–184.

Jang S, Torti S, Coupland G. 2009. Genetic and spatial interactions between *FT*, *TSF* and *SVP* during the early stages of floral induction in *Arabidopsis*. *The Plant Journal* **60,** 614–625.

Jung JH, Ju Y, Seo PJ, Lee JH, Park CM. 2012a. The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in *Arabidopsis*. *The Plant Journal* **69,** 577–588.

Jung JH, Seo PJ, Ahn JH, Park CM. 2012b. The *Arabidopsis* RNA-binding protein FCA regulates microRNA172 processing in thermosensory flowering. *Journal of Biological Chemistry* **287,** 16007–16016.

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D. 1999. Activation tagging of the floral inducer *FT*. *Science* **286,** 1962–1965.

Karlgren A, Gyllenstrand N, Kallman T, Sundstrom JF, Moore D, Lascoux M, Lagercrantz U. 2011. Evolution of the PEBP gene family in plants: functional diversification in seed plant evolution. *Plant Physiology* **156,** 1967–1977.

Kim JJ, Lee JH, Kim W, Jung HS, Huijser P, Ahn JH. 2012. The *microRNA156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3* module regulates ambient temperature-responsive flowering via *FLOWERING LOCUS T* in *Arabidopsis*. *Plant Physiology* **159,** 461–478.

Kim W, Ahn HJ, Chiou TJ, Ahn JH. 2011. The role of the miR399-*PHO2* module in the regulation of flowering time in response to different ambient temperatures in *Arabidopsis thaliana*. *Molecules and Cells* **32,** 83–88.

Kinoshita T, Ono N, Hayashi Y, *et al.* 2011. *FLOWERING LOCUS T* regulates stomatal opening. *Current Biology* **21,** 1232–1238.

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T. 1999. A pair of related genes with antagonistic roles in mediating flowering signals. *Science* **286,** 1960–1962.

Kumar SV, Lucyshyn D, Jaeger KE, Alos E, Alvey E, Harberd NP, Wigge PA. 2012. Transcription factor PIF4 controls the thermosensory activation of flowering. *Nature* **484,** 242–245.

Kumar SV, Wigge PA. 2010. H2A.Z-containing nucleosomes mediate the thermosensory response in *Arabidopsis*. *Cell* **140,** 136–147.

Lee H, Yoo SJ, Lee JH, Kim W, Yoo SK, Fitzgerald H, Carrington JC, Ahn JH. 2010. Genetic framework for flowering-time regulation by ambient temperature-responsive miRNAs in *Arabidopsis*. *Nucleic Acids Research* **38,** 3081–3093.

Lee JH, Hong SM, Yoo SJ, Park OK, Lee JS, Ahn JH. 2006. Integration of floral inductive signals by flowering locus T and suppressor of overexpression of Constans 1. *Physiologia Plantarum* **126,** 475–483.

Lee JH, Lee JS, Ahn JH. 2008. Ambient temperature signaling in plants: an emerging field in the regulation of flowering time. *Journal of Plant Biology* **51,** 321–326.

Lee JH, Kim JJ, Ahn JH. 2012a. Role of *SEPALLATA3* (*SEP3*) as a downstream gene of *miR156-SPL3-FT* circuitry in ambient temperature-responsive flowering. *Plant Signaling and Behavior* **7,** 1151–1154.

Lee JH, Kim JJ, Kim SH, Cho HJ, Kim J, Ahn JH. 2012b. The E3 ubiquitin ligase HOS1 regulates low ambient temperature-responsive flowering in *Arabidopsis thaliana*. *Plant and Cell Physiology* **53,** 1802–1814.

Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS, Ahn JH. 2007. Role of *SVP* in the control of flowering time by ambient temperature in *Arabidopsis*. *Genes and Development* **21,** 397–402.

Mansilla MC, Cybulski LE, Albanesi D, de Mendoza D. 2004. Control of membrane lipid fluidity by molecular thermosensors. *Journal of Bacteriology* **186,** 6681–6688.

McClung CR, Davis SJ. 2010. Ambient thermometers in plants: from physiological outputs towards mechanisms of thermal sensing. *Current Biology* **20,** R1086–R1092.

Michaels SD. 2009. Flowering time regulation produces much fruit. *Current Opinion in Plant Biology* **12,** 75–80.

Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM. 2005. Integration of flowering signals in winter-annual *Arabidopsis*. *Plant Physiology* **137,** 149–156.

Mimida N, Goto K, Kobayashi Y, Araki T, Ahn JH, Weigel D, Murata M, Motoyoshi F, Sakamoto W. 2001. Functional divergence of the *TFL1*-like gene family in *Arabidopsis* revealed by characterization of a novel homologue. *Genes to Cells* **6,** 327–336.

Pin PA, Nilsson O. 2012. The multifaceted roles of FLOWERING LOCUS T in plant development. *Plant, Cell and Environment* **35,** 1742–1755.

Ramakers C, Ruijter JM, Deprez RH, Moorman AF. 2003. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters* **339,** 62–66.

Ratcliffe OJ, Amaya I, Vincent CA, Rothstein S, Carpenter R, Coen ES, Bradley DJ. 1998. A common mechanism controls the life cycle and architecture of plants. *Development* **125,** 1609–1615.

Rosso MG, Li Y, Strizhov N, Reiss B, Dekker K, Weisshaar B. 2003. An *Arabidopsis thaliana* T-DNA mutagenized population (GABI-Kat) for flanking sequence tag-based reverse genetics. *Plant Molecular Biology* **53,** 247–259.

Sohn EJ, Rojas-Pierce M, Pan S, Carter C, Serrano-Mislata A, Madueno F, Rojo E, Surpin M, Raikhel NV. 2007. The shoot meristem identity gene TFL1 is involved in flower development and trafficking to the protein storage vacuole. *Proceedings of the National Academy of Sciences, USA* **104,** 18801–18806.

Srikanth A, Schmid M. 2011. Regulation of flowering time: all roads lead to Rome. *Cellular and Molecular Life Sciences* **68,** 2013–2037.

Strasser B, Alvarez MJ, Califano A, Cerdan PD. 2009. A complementary role for ELF3 and TFL1 in the regulation of flowering time by ambient temperature. *The Plant Journal* **58,** 629–640.

Udvardi MK, Czechowski T, Scheible WR. 2008. Eleven golden rules of quantitative RT-PCR. *The Plant Cell* **20,** 1736–1737.

Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G. 2004. Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science* **303,** 1003–1006.

Wang JW, Czech B, Weigel D. 2009. miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. *Cell* **138,** 738–749.

Wigge PA. 2011. FT, a mobile developmental signal in plants. *Current Biology* **21,** R374–R378.

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D. 2005. Integration of spatial and temporal information during floral induction in *Arabidopsis*. *Science* **309,** 1056–1059.

Xi W, Liu C, Hou X, Yu H. 2010. *MOTHER OF FT AND TFL1* regulates seed germination through a negative feedback loop modulating ABA signaling in *Arabidopsis*. *The Plant Cell* **22,** 1733–1748.

Yamaguchi A, Kobayashi Y, Goto K, Abe M, Araki T. 2005. *TWIN SISTER OF FT* (*TSF*) acts as a floral pathway integrator redundantly with *FT*. *Plant and Cell Physiology* **46,** 1175–1189.

Yeung K, Seitz T, Li S, *et al.* 1999. Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. *Nature* **401,** 173–177.

Yoo SJ, Chung KS, Jung SH, Yoo SY, Lee JS, Ahn JH. 2010. *BROTHER OF FT AND TFL1* (*BFT*) has *TFL1*-like activity and functions redundantly with *TFL1* in inflorescence meristem development in *Arabidopsis*. *The Plant Journal* **63,** 241–253.

Yoo SK, Chung KS, Kim J, Lee JH, Hong SM, Yoo SJ, Yoo SY, Lee JS, Ahn JH. 2005. *CONSTANS* activates *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* through *FLOWERING LOCUS T* to promote flowering in *Arabidopsis*. *Plant Physiology* **139,** 770–778.

Yoo SY, Kardailsky I, Lee JS, Weigel D, Ahn JH. 2004. Acceleration of flowering by overexpression of *MFT* (*MOTHER OF FT AND TFL1*). *Molecules and Cells* **17,** 95–101.