The *splicing factor 1–FLOWERING LOCUS M* module spatially regulates temperature-dependent flowering by modulating *FLOWERING LOCUS T* and *LEAFY* expression

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Abstract

**Key message** The *AtSF1-FLM* module spatially controls temperature-dependent flowering by negatively regulating the expression of *FT* and *LFY* in the leaf and shoot apex, respectively.

**Abstract** Alternative splicing mediated by various splicing factors is important for the regulation of plant growth and development. Our recent reports have shown that a temperature-dependent interaction between *Arabidopsis thaliana* splicing factor 1 (AtSF1) and *FLOWERING LOCUS M* (*FLM*) pre-mRNA introns controls the differential production of *FLM*-β transcripts at different temperatures, eventually resulting in temperature-responsive flowering. However, the molecular and genetic interactions between the *AtSF1-FLM* module and floral activator genes remain unknown. Here, we aimed to identify the interactions among *AtSF1*, *FLM*, *FLOWERING LOCUS T* (*FT*), and *LEAFY* (*LFY*) by performing molecular and genetic analyses. *FT* and *TWIN SISTER OF FT* (*TSF*) expression in *atsf1-2* mutants significantly increased in the morning and middle of the night at 16 and 23 °C, respectively, under long-day conditions. In addition, *ft* mutation suppressed the early flowering of *atsf1-2* and *atsf1-2 flm-3* mutants and masked the temperature response of *atsf1-2 flm-3* mutants, suggesting that *FT* is a downstream target gene of the *AtSF1-FLM* module. *LFY* expression significantly increased in the diurnal samples of *atsf1-2* mutants and in the shoot apex regions of *atsf1-2 ft-10* mutants at different temperatures. The chromatin immunoprecipitation (ChIP) assay revealed that FLM directly binds to the genomic regions of *LFY* but not of *APETAL1* (*AP1*). Moreover, *lfy* mutation suppressed the early flowering of *flm-3* mutants, suggesting that *LFY* is another target of the *AtSF1-FLM* module. Our results reveal that the *AtSF1-FLM* module spatially modulates temperature-dependent flowering by regulating *FT* and *LFY* expressions.

**Keywords** Ambient temperature · *AtSF1* · *FLM* · *FT* · *LFY* · Temperature-dependent flowering

Introduction

In eukaryotes, splicing is the removal of non-coding intronic regions from between exons in primary transcripts (pre-mRNAs) to form mature messenger RNAs (mRNAs) using a large RNA–protein complex called the spliceosome, consisting of U1, U2, U4/U6, and U5 small nuclear ribonucleoproteins (snRNPs) as the main building blocks (Wahl et al. 2009; Will and Luhrmann 2011). During the splicing process, conserved *cis*-acting elements, such as the 5′-splicing site (5′-ss), the branch point sequence (BPS), the polypyrimidine tract, and the 3′-splice site (3′-ss) within the intron are required for the binding of various splicing factors. Pre-mRNA splicing begins with the recognition of 5′-ss by base pairing with U1 snRNA via U1 snRNP and the identification
of 3′-ss by the binding of splicing factor 1 (SF1) to the BPS followed by the recruitment of U2 snRNP auxiliary factor 65 (U2AF65) and U2AF35 to the polypyrimidine tract and 3′-ss with the help of U2 snRNP. During this process, the domain structures within SF1 and U2AF65 are also important for their binding to the conserved cis-acting elements in 3′-ss. The KH-QUA2 domain at the N-terminus of SF1 interacts with BPS and buries BPS-adenosine to facilitate the formation of the BPS-U2 snRNA helix (Arning et al. 1996; Liu et al. 2001). The U2AF ligand motif at the N-terminal region of SF1 is also required for its interaction with the U2AF homology motif of U2AF65 (Selenko et al. 2003; Wang et al. 2013; Zhang et al. 2013).

Diverse environmental cues, different developmental stages, and abiotic or biotic stresses affect 5′- or 3′-ss selection, thereby producing two or more mRNA isoforms from the same pre-mRNA in a process called alternative splicing (AS) (Chaudhary et al. 2019; Staiger and Brown 2013; Syed et al. 2012). AS is a widespread mechanism that has the potential to greatly increase the complexity of the transcriptome and proteome, with 60–70% of intron-containing genes in higher plants undergoing AS (Chamala et al. 2015; Zhang et al. 2019, 2017). AS generates non-productive transcript abundance (Kalyna et al. 2012; Kurihara et al. 2009). Moreover, AS leads to the formation of several protein variants with altered amino acid sequences, which control post-transcriptional regulation via protein–protein interactions (Marquez et al. 2015).

AS plays an important role in the regulation of flowering time in response to changes in ambient temperature, suggesting that the AS of pre-mRNAs serves as a ‘molecular thermometer’ (Capovilla et al. 2015; Deng and Cao 2017). One known example is the temperature-dependent AS of FLOWERING LOCUS M (FLM) pre-mRNA in temperature-responsive flowering. The alternative use of mutually exclusive second or third exons in this pre-mRNA produces two spliced isoforms, FLM-β and FLM-δ at low and high temperatures, respectively (Lee et al. 2013, 2014; Pose et al. 2013). The resulting proteins FLM-β and FLM-δ interact with SHORT VEGETATIVE PHASE (SVP) to form distinct protein complexes at different temperatures, thereby contributing to the repression and promotion of flowering, respectively. However, further studies have revealed that only the FLM-β protein is functional in the formation of a temperature-dependent repressor complex with SVP (Capovilla et al. 2017; Lutz et al. 2015, 2017). Furthermore, high ambient temperature causes AS coupled with nonsense-mediated mRNA decay of aberrant FLM transcripts containing premature termination codons (Sureshkumar et al. 2016). These results suggest that a subset of splicing factors affects the balance between functional and non-functional transcript isoforms of FLM to modulate the temperature-dependent flowering time. Several U2 snRNP-related factors, such as AtU2AF65A, AtU2AF65B, and AtSF1, regulate the AS of FLM pre-mRNA (Lee et al. 2020, 2017; Park et al. 2019). The U1 snRNP component RNA BINDING PROTEIN 45d (RBP45d) and the glycine-rich proteins AtGRP7 and AtGRP8 also modulate the temperature-dependent AS of FLM (Chang et al. 2021; Steffen et al. 2019).

We have previously shown that a lesion in Arabidopsis thaliana SF1 (AtSF1) leads to the misregulation of AS of FLM pre-mRNA, and a temperature-dependent interaction between AtSF1 and the introns of FLM pre-mRNA regulates temperature-responsive flowering via the differential production of FLM-β transcripts at varying temperatures (Lee et al. 2020). Although AtSF1 mutation leads to the reduced expression of floral repressors, such as FLM and SVP, explaining the early flowering phenotype of atsf1-2 mutants (Lee et al. 2017, 2020; Park et al. 2019), the molecular and genetic interactions between AtSF1 and floral activator genes involved in the ambient temperature pathway are poorly understood. In this study, we aimed to investigate the expression levels of the floral activator genes FLOWERING LOCUS T (FT), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), and TWIN SISTER OF FT (TSF) in atsf1-2 mutants as well as the genetic interactions among AtSF1, FLM, and FT. We hypothesised that the expression of LEAFY (LFY), which is specifically expressed in the shoot apex, will be increased in atsf1-2 mutants and FLM will be specifically bound to LFY genomic regions. Furthermore, we also expect to observe a genetic interaction between FLM and LFY.

Materials and methods

Plant materials and growth conditions

All mutants used in this study had a Columbia (Col-0) background, similar to that of wild-type plants. The processes of generating atsf1-2, flm-3, ft-10, and lfy-12 mutants have been previously described (Jang et al. 2014; Lee et al. 2013; Moon et al. 2005; Yoo et al. 2005). The lfy-12 and ft-10 mutants were provided by Dr. Lee (Seoul National University) and Dr. Ahn (Korea University), respectively. Wild-type and mutant plants were grown either in Sunshine Mix 5 (Sungro, Agawam, MA, USA) or in half-strength Murashige and Skoog (MS) medium at 16 and 23 °C under long-day (LD) (16 h light/8 h dark) conditions at a light intensity of 120 μmol m−2 s−1. Flowering time was measured by scoring the total number of rosette and/or cauline leaves, and the data were presented as box plots (Postma and Goedhart 2019). In the plots, the box indicates the interquartile range (IQR), the whiskers show the range of values within...
1.5 × IQR, and the horizontal line indicates the median. The number of plants counted is shown above each genotype in a box plot. To reveal the statistical differences in flowering time, the data were analysed using SPSS version 24 (IBM SPSS Statistics). Under LD conditions, the leaf number ratio (LNR; 16 °C/23 °C) was used as an indicator of temperature-responsive flowering (Lee et al. 2007, 2020).

**Generation of double mutants and genotyping**

Homozygosity of the double mutants (atsf1-2 flm-3, atsf1-2 ft-10, and atsf1-2 lfy-12) was verified by polymerase chain reaction (PCR) genotyping. The genomic DNA isolation was performed as previously described (Weigel and Glazebrook 2002). For genotyping of the atsf1-2 allele, 062177L and LBb1.3 primers were used (Jang et al. 2014). For genotyping of the flm-3 allele, JH4014 and mLBa1 primers were used (Lee et al. 2013). For the ft-10 allele, the JH2295, JH2296, and JH2297 primers were used (Yoo et al. 2005). For the lfy-12 allele, the PCR amplification was done using DW1228 and DW1019 primers, and the mutation was confirmed by sequencing (Moon et al. 2005). The oligonucleotide primers used for genotyping are listed in Table S1.

**RNA expression analyses**

For RNA expression analysis, total RNA was extracted from wild-type or mutant seedlings using TRIzol (Invitrogen, Carlsbad, CA, USA). For real-time quantitative polymerase chain reaction (RT-qPCR), the samples were harvested at the indicated Zeitgeber time (ZT) points, immediately frozen in liquid nitrogen, and stored at −80 °C until further use. RNA quality analysis and complementary DNA (cDNA) synthesis were performed as described previously (Lee et al. 2013). RT-qPCR analysis was carried out in 384-well plates using a LightCycler 480 (Roche Applied Science, Penzberg, Germany) or CFX real-time system (Bio-Rad, Hercules, CA, USA). A stably expressed gene (PP2AA3) was used as the reference gene. All RT-qPCR experiments were performed in three biological replicates (independently harvested samples), each with three technical replicates. The relative transcript abundance was determined using the geNorm algorithm and the PCR efficiency and Ct (threshold cycle) values (Lee et al. 2013). The oligonucleotide primers used for RT-qPCR are listed in Table S1.

**Chromatin immunoprecipitation (ChIP) analysis**

*pFLM:gFLM:GFP* seedlings (Lee et al. 2013; Pose et al. 2013) grown on MS medium at 16 and 23 °C under LD conditions (ZT16) were cross-linked in 1% formaldehyde on ice using vacuum infiltration. Nuclear extracts were isolated, and an immunoprecipitation assay was conducted as described by (Lee et al. 2013). After shearing chromatin via sonication, approximately 5 µg of rabbit anti-green fluorescent protein (GFP) polyclonal antibody (Santa Cruz Biotechnology, Dallas, TX, USA) was used to immunoprecipitate the genomic DNA fragments. The DNA recovered from immunoprecipitation or 10% input DNA was used for qPCR analysis. ChIP experiments were carried out in three biological replicates (samples independently harvested on different days) with three technical triplicates each (ChIP samples processed on the same day), and the results were presented as a percentage of input (% input) (Livak and Schmittgen 2001). Error bars indicate standard error of the mean (SEM) of three biological replicates. The oligonucleotide primers used for the ChIP analysis are listed in Table S1.

**Results**

**Effect of AtSF1 mutation on FT and TSF expression at different temperatures**

In a previous study, we found that atsf1-2 mutants exhibited early flowering phenotypes at different temperatures upon downregulation of the expression of SVP, FLM-β, and TEMPRANILLO 2 (Lee et al. 2020). As these important floral repressors affect the transcript levels of FT, SOC1, and TSF, all of which are involved in integrating temperature-dependent flowering signals within the ambient temperature pathway (Lee et al. 2013; Pose et al. 2013), we measured the expression of flowering-promoting genes over a 2-day diurnal time course in similar developmental atsf1-2 mutant and wild-type plants grown at 16 and 23 °C under LD conditions. Although the expression of FT and TSF was specifically increased in the middle of the night (ZT20) in 7- and 8-day-old atsf1-2 mutant seedlings grown at 23 °C, FT expression at 16 °C showed a statistically significant increase in the morning (ZT0 and 8, and ZT4 to 12 in 11- and 12-day-old seedlings, respectively), whereas TSF expression increased from night to morning (ZT8 and 20, and ZT0 to 8 in 11- and 12-day-old seedlings, respectively) (Fig. 1a, c). Therefore, phase shifts affected the expression patterns of these two genes. However, SOC1 expression in the atsf1-2 mutants decreased at 16 and 23 °C, when compared to the wild-type plants (Fig. 1b), indicating that SOC1 expression could not explain the early flowering phenotype in atsf1-2 mutants. These results suggest that atsf1 mutation leads to early flowering at different temperatures by partially regulating FT and TSF expression.

**Effect of genetic interactions among AtSF1, FLM, and FT on temperature-insensitive flowering of atsf1-2 mutants**

Because FT acts as a major floral integrator gene under LD conditions (Yoo et al. 2005), we tested the genetic effect of
AtSF1, FLM, and FT on flowering time. We first generated double and triple mutants with different combinations of \textit{atsf1-2}, \textit{flm-3}, and \textit{ft-10} single mutants and measured their flowering times at 16 and 23 °C under LD conditions. Our analysis showed that wild-type plants flowered with a mean total leaf number (TLN) of 27.8 and 14.1 at 16 and 23 °C, respectively. Moreover, the \textit{atsf1-2} and \textit{flm-3} mutants flowered significantly earlier than the wild-type plants, whereas the \textit{ft-10} mutants flowered later than other single mutants and wild-type plants (Fig. 2a), which was consistent with the previous reports (Lee et al. 2013, 2020). The \textit{atsf1-2 flm-3} mutants flowered with 10.3 and 9.1 leaves at 16 and 23 °C, respectively, which was comparable to \textit{flm-3} single mutants at 16 °C, but not at 23 °C (Fig. 2a). The \textit{atsf1-2 ft-10} mutants (TLN = 35.3 and 17.0 leaves at 16 and 23 °C, respectively) flowered earlier than \textit{ft-10} single mutants (TLN = 51.7 and 32.3 leaves) at different temperatures (Fig. 2a). However, \textit{flm-3 ft-10} mutants flowered with a TLN of 44.5 and 32.6 leaves at 16 and 23 °C, respectively, which was comparable to that of \textit{ft-10} mutants. Furthermore, the \textit{atsf1-2 flm-3 ft-10}
mutants flowered earlier than the flm-3 ft-10 mutants (40.1 and 28.2 leaves at 16 and 23 °C, respectively) (Fig. 2a). These results indicate that the flm or ft single mutation and flm ft double mutation did not completely suppress the flowering phenotype of atsf1-2 mutants at different temperatures.

To assess the temperature sensitivity of the various mutants, we calculated their LNRs using TLN values at 16 and 23 °C. An LNR close to 1.0 indicates that different temperatures have minor effects on flowering. The atsf1-2 or flm-3 single mutants and atsf1-2 flm-3 double mutants had LNR values of approximately 1.0, compared to those of wild-type plants (LNR = 2.0) (Fig. 2b). However, the LNRs of the atsf-2 ft-10, flm-3 ft-10, and atsf1-2 flm-3 ft-10 mutants were 2.1, 1.4, and 1.4, respectively, indicating that both FLM and FT acted downstream of AtSF1 during temperature response. These results suggest that AtSF1 regulates other flowering time genes, in addition to FLM and FT, to modulate flowering time at different temperatures.

**Effect of AtSF1 on LFY expression in the shoot apex regions**

Since atsf1-2 ft-10 mutants flowered earlier than ft-10 mutants (Fig. 2a) and our previous study have shown that LFY expression was increased at ZT16 in 8-day-old atsf1-2 mutants at 23 °C under LD conditions (Lee et al. 2017), we measured the transcript levels of floral activators LFY and APETALA1 (AP1) over an approximately 2-day diurnal time course in the atsf1-2 and wild-type plants grown to similar developmental stages at 16 and 23 °C under LD conditions. RT-qPCR analysis showed that LFY expression levels were increased throughout the time course in the whole seedlings of atsf1-2 mutants grown at 23 °C, but not in those grown at 16 °C (Fig. 3a). However, the expression of AP1 in atsf1-2 mutants decreased at most ZT points at 23 °C, but increased at 16 °C (Fig. 3b). These results indicate that lesions in AtSF1 affect LFY and AP1 expression significantly in all seedlings at 23 °C only.

It has been reported that AtSF1 is strongly expressed in shoot apex regions (Jang et al. 2014); thus, we also examined the expression levels of LFY and AP1 at ZT16 in the shoot apices of 8- and 12-day-old atsf1-2 mutant and wild-type plants grown at 23 and 16 °C under LD conditions, respectively. LFY expression was significantly increased in atsf1-2 and atsf1-2 ft-10 mutants at the different temperatures compared to that in the wild-type plants (Fig. 3c). In contrast, AP1 expression in atsf1-2 mutants was lower than that in the wild-type plants at 23 and 16 °C (Fig. 3d). Unlike diurnal expression levels of AP1 in the whole seedlings of atsf1-2 mutants, AP1 expression decreased in the shoot apex regions of the atsf1-2 mutants at different temperatures. Furthermore, LFY expression was almost similar in atsf1-2, flm-3, and atsf1-2 flm-3 mutants (Fig. 3e). These results suggest that AtSF1 influences flowering by negatively regulating LFY expression via FLM in the shoot apex.

**Direct binding of FLM to the LFY genomic region**

Since it has been established that AtSF1 regulates the AS of FLM pre-mRNA (Lee et al. 2020), we examined whether FLM protein is involved in directly regulating LFY expression. FLM is a type of MADS-box transcription factor, which are DNA-binding proteins that recognise the CArG or vCArG motifs in their target genes (Lee et al. 2013; Pose et al. 2013); thus, we performed ChIP experiments on 8-day-old pFLM:gFLM:GFP flm-3 mutant and wild-type plants at 16 and 23 °C under LD conditions (ZT16). In these experiments, two regions (upstream promoter region I and part of the first exon and intron region II) and one region (part of the first exon and intron region I) containing CAriG or CAreiG motifs of the LFY and AP1 loci, respectively, were explored (Fig. 4). Each region lacking the CAriG or CAreiG motifs of the LFY and AP1 loci was used as a negative control. The FLM-GFP protein was bound to two regions (I and II) in the LFY locus compared to that in the wild-type plants (Fig. 4a), which was consistent with the FLM ChIP-sequencing data (Pose et al. 2013). We observed that the FLM binding to one LFY region I was temperature-dependent, with an approximately 1.8-fold increase in binding at 16 °C compared to that at 23 °C (Fig. 4a). These observations indicate that FLM preferentially binds to the promoter region of LFY at low temperatures. In contrast, FLM binding occurred at both 16 and 23 °C in region II (Fig. 4a), suggesting that FLM can bind to the LFY genomic region in a temperature-independent manner. However, differential binding of FLM to the LFY region (NC), a negative control lacking conserved motifs, was not observed at different temperatures. Moreover, no significant FLM enrichment was observed in region I and negative control of the AP1 genomic region (Fig. 4b). Based on the increased LFY expression in atsf1-2 mutants (Fig. 3c) and the direct FLM binding to the LFY genomic locus (Fig. 4a), we conclude that LFY is an in vivo target of FLM and AtSF1 may influence LFY expression through FLM.

**Genetic interactions between FLM and LFY**

To test the genetic effects of FLM and LFY on flowering time, we measured the flowering times of flm-3, lfy-12, and flm-3 lfy-12 mutants at 16 and 23 °C under LD conditions. The flm-3 lfy-12 mutants flowered significantly later (18.8 and 13.4 leaves, respectively) than flm-3 single mutants at 16 and 23 °C (15.0 and 12.0 leaves, respectively) (Fig. 5a). This indicates that the lfy mutation
partially suppressed the early flowering phenotype of flm-3 mutants at different temperatures. The LNRs of flm-3 lfy-12 and flm-3 mutants were 1.4 and 1.3, respectively, compared to those of lfy-12 mutants (LNR = 2.3), indicating that the temperature insensitivity of flm-3 lfy-12 mutants was similar to that of flm-3 mutants (Fig. 5b).

In contrast, the LNR of the lfy-12 mutant was 2.3. This result indicates that the temperature sensitivity of lfy-12 mutants was completely suppressed by flm mutation. The results suggest that LFY is another target of the AtSF1-FLM module in addition to FT and that this module regulates flowering by modulating LFY expression.

Fig. 3 Expression patterns of LFY and AP1 in several mutants at different temperatures. a, b Diurnal expression patterns of LFY (a) and AP1 (b) in the atsf1-2 mutant at 16 and 23 °C under LD conditions, measured by RT-qPCR (Student’s t test, *P < 0.05; **P < 0.01). Expression levels in wild-type (Col-0) plants at ZT0 on day 7 or 11 at the indicated temperatures were defined as 1.0. Error bars indicate the SEM. c, d Expression levels of LFY (c) and AP1 (d) at ZT16 in the 12- and 8-day-old shoot apices of atsf1-2, ft-10, and atsf1-2 ft-10 mutants at 16 and 23 °C, respectively, under LD conditions. e LFY expression at ZT16 in the 12- and 8-day-old shoot apices of atsf1-2, flm-3, and atsf1-2 flm-3 mutants at 16 and 23 °C, respectively, under LD conditions. Error bars indicate the SEM of three biological replicates. Statistical analysis was performed as described in Fig. 2.
Discussion

Our previous reports have revealed that AS variants of FLM transcripts produced by temperature-dependent interactions between AtSF1 and FLM pre-mRNA control the flowering time in response to temperature fluctuations (Lee et al. 2020). However, the molecular and genetic interactions between the AtSF1-FLM module and the important floral integrator genes involved in temperature-dependent flowering remain unknown. In the present study, we showed that the AtSF1-FLM module spatially regulates FT and LFY, which play important roles in regulating temperature-dependent flowering.

We observed that the LNRs of ft-10 soc1-2 tsf-1 mutants was close to 1.0 compared to those of their single or double mutants (LNRs of ft-10, soc1-2, tsf-1, ft-10 soc1-2, and ft-10 tsf-1 = 1.4, 1.9, 1.7, 1.2, and 1.3, respectively), and that FT, TSF, and SOC1 are common downstream genes of SVP and FLM, two main ambient temperature pathway mediators, these factors are known to be the major outputs of this pathway (Lee et al. 2013; Pose et al. 2013). Since FT and
SOCI expression remains unaltered at ZT16 in 8-day-old atsf1-2 mutants grown at 23 °C (Lee et al. 2017) and atsf1 mutation greatly affects the AS of FLM pre-mRNA at different temperatures (Lee et al. 2020), it is important to determine whether the AtSF1-FLM module actually regulates FT, TSF, and SOCI. In this study, we showed that FT and TSF expression in atsf1-2 mutants was significantly increased in the morning and the middle of the night at 16 and 23 °C under LD conditions (Fig. 1). Increased expression levels of FT and TSF at specific ZT points under continuously low and high temperature conditions explain the early flowering phenotype of the atsf1-2 mutants. Moreover, several ambient temperature pathway mutants that show early flowering exhibit higher FT expression in the morning than in the afternoon under natural LD conditions (Song et al. 2018), and minor changes in FT expression are observed in syp mutants only at dawn relative to wild-type plants under day/night temperature regulation (Kinthmont-Schultz et al. 2016). Furthermore, our genetic interaction data showed that the ft mutation suppressed the early flowering of the atsf1-2 and atsf1-2 flm-3 mutants (Fig. 2). Results of the present study and previous reports indicate that FT is primarily expressed in leaves (Corbesier et al. 2007), making it a potential downstream target of the AtSF1-FLM module in leaves. As phytochrome A (phyA) and EARLY FLOWERING 3 (ELF3) regulate FT expression in the morning and CONSTANS (CO) protein is more stable in the morning under natural LD conditions (Song et al. 2018), further investigation is required to explain the role of AtSF1 in AS of phyA, ELF3, and CO at different temperatures.

Several reports have shown that LFY is a floral activator gene in the shoot apex (Klepikova et al. 2015; Michaels and Amasino 1999). In the present study, we identified the molecular and genetic interactions among the AtSF1-FLM module, FT, and LFY in response to ambient temperature. AtSF1 greatly affects the temperature-responsive AS of FLM pre-mRNA (Lee et al. 2020), and the AtSF1-FLM module spatially regulates FT and LFY expression to repress precocious flowering in a temperature-dependent manner.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00299-022-02881-y.

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Author contributions J-KK and JHL conceived and designed the research. KCL, HTL, HHJ, J-HP, and Y-CK conducted experiment. J-KK and JHL conceived and designed the experiment. KCL, HTL, HHJ, J-HP, and Y-CK analyzed data. JHL and J-KK wrote the manuscript. All authors read and approved the manuscript.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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