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GATA25, a novel regulator, accelerates the flowering time of *Arabidopsis thaliana*

Kihwan Kim[1], Jooeun Lee[1], Byeonggyu Kim[2], Juhyung Shin[2], Tae-An Kang[1] and Won-Chan Kim[1,2*]

Abstract

Intrinsic and exogenous signals in conjunction precisely regulate the initiation of flowering. Both signals influence flowering time, which is an integral part of plant reproduction. The signals converge through different pathways, and their coordinated action leads to the onset of flowering. Genetic pathways related to the regulation of flowering time are well-known from research into the molecular genetics of *Arabidopsis thaliana*. Specifically, crucial components of the photoperiodic pathway and floral integrators play a critically significant role in flowering. In this study, we found that GATA25 is a novel transcription factor that accelerates flowering time under long days. GATA25 encodes $C\text{-}X_2\text{-}C\text{-}X_{20}\text{-}C\text{-}X_2\text{-}C$ conserved cysteine residues of the zinc-finger domain and CCT domain which process photoperiodic flowering and regulate circadian rhythms. Flowering was accelerated by overexpression of GATA25 throughout the *Arabidopsis thaliana*. In contrast, GATA25 fused to SRDX (SUPERMAN repressive domain X)-motif plants showed delayed flowering. We also demonstrated that GATA25 induced the expression of floral integrator genes and photoperiodic pathway-related genes. Together, these results suggest that GATA25 might act to accelerate flowering time.

Keywords: *Arabidopsis thaliana*, Flowering, Floral integrators, GATA25, Photoperiodic pathway

Introduction

Terrestrial plants have adapted their flowering and growth to environmental changes over evolutionary time, and as a result, precisely regulate flowering patterns in different growing environments. Controlling the flowering patterns of plants can be a solution that maximizes crop yields in a limited space to overcome rapid environmental changes and reductions in crop yields caused by global warming. Molecular genetic analysis of flowering regulation in *Arabidopsis thaliana* to elucidate the flowering timing regulation mechanism confirms that flowering is regulated by various distinct pathways [1, 2]. As a result, regulatory proteins and genetic pathways involved in the regulation of flowering time have been identified. Typically, the control of flowering time can be attributed to both intrinsic and exogenous signals for flowering. The intrinsic signals associated with flowering include gibberellic acid (GA), autonomous signals, and aging signals whereas the exogenous signals are light intensity and day length (photoperiod). These distinct signals together converge to control activation of flowering initiation [3]. Especially for exogenous signals, it regulates the flowering pathway in a complex and sophisticated manner through circadian regulation of post-transcriptional regulation of protein stability and gene transcription by photoperiod [4, 5]. Therefore, the study of the photoperiodic pathway regulation mechanism for exogenous signals is the key to studying plant flowering patterns. The photoperiodic pathway involved in the exogenous signals regulates key components GIGANTEA (GI) and CONSTANS (CO). The CO transcription factor, which enables sensing of long days (LDs), is known to be regulated by physical interact with FLOWERING HTH1 (FHTH1) [6]. Also, The CO transcription factor is expressed in phloem companion cells and positively regulates FLOWERING LOCUS T (FT) expression under LDs photoperiods only [7, 8]. The FT protein, induced through the sequential activation of *CO*, interacts with FD to form the FT-FD

*Correspondence: kwc@knu.ac.kr
[1] Department of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea
Full list of author information is available at the end of the article

complex [9]. The FT-FD complex activates the expression of a *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) [10, 11]. The *SOC1* gene, a type of floral integrator, is generated in the meristem during floral transition and is regulated by the phytohormone GA [12, 13]. Therefore, it can be inferred that it is an essential component for floral induction. The GI transcription factor regulates *CO* transcription through binding FLAVIN BINDING KELCH REPEAT F-BOX 1 (FKF1). The GI-FKF1 complex promotes the degradation of CYCLING DOF FACTOR 1 (CDF1) [14, 15]. Here, we studied the *Arabidopsis* gene GATA25, also known as ZIM [16, 17]. GATA25 is a member of subfamily III containing the C-X_2-C-X_{20}-C-X_2-C conserved cysteine residues of the zinc-finger domain and a CONSTANS, CO-like, and TOC1 (CCT) domain [18]. The transcription factors containing the CCT domain are known to regulate flowering time and circadian rhythm [19, 20]. Therefore, we expected that the GATA25 transcription factor, including the CCT domain, would act as a regulator of flowering time. We observed that flowering initiation was accelerated when the GATA25 gene was overexpressed from the 2X CaMV 35S promoter. In contrast, flowering initiation was delayed when GATA25 fused to an SRDX repression motif was overexpressed from the 2X CaMV 35S promoter. Moreover, we demonstrated that GATA25 can control the expression of GI and CO and subsequent FT and SOC1 involved in the photoperiodic flowering response. Our results suggest that GATA25 acts as a positive regulator of flowering by regulating key floral integrators and photoperiodic pathways.

Material and methods

Plant materials and growth conditions

All of the *Arabidopsis thaliana* plants used in this study came from the *Columbia* ecotype. Plants were grown in soil in a plant growth room, at 23 ℃ with a controlled light/dark cycle (16 h light/8 h dark). Light intensity was approximately 90 μmol/ m^2/ s.

Construction of vectors and generation of transgenic plants

To generate the overexpression of GATA25 (At4g24470) transgenic plants, full-length GATA25 CDS was amplified from leaf cDNA of *Arabidopsis* by PCR using primers linked to *Spe*I and *Xma*I sites, respectively. The PCR product was cloned into a binary pTK-BMLC vector which replaced the pCB302-3 vector with a CaMV 35S promoter to a 2X CaMV 35S promoter, and multiple cloning sites were *Xba*I, *Spe*I, *Bam*HI, *Xma*I [21]. To generate the inducible expression of GATA25 transgenic plants, a GATA25 fragment was introduced at the *Spe*I site behind the GAL4 upstream activation sequence, positioned at *Spe*I in the pTA7002-BMLC vector, in which a CaMV 35S promoter replaced the 2X CaMV 35S promoter in the pTA7002 vector [22]. To generate the *GATA25-SRDX* chimeric repressor transgenic plant, full-length GATA25 CDS without the stop codon was amplified by PCR using primers tagged with the sequence encoding the SRDX motif (5′-LDLELRLGFA-3′) [23] and inserted into the pTK-BMLC vector. All binary vectors were verified by DNA sequencing.

Each binary vector was transformed into the *Agrobacterium tumefaciens* strain GV3101 used for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana* (Col-0) [24, 25] and transgenic plants were screened using a 0.1% Basta solution as a selection marker.

RNA extraction and gene expression analysis

Total RNA was extracted from leaves using Trizol reagent and then was treated with RNase-free DNaseI. As previously described, the first-strand cDNA was synthesized using Superscript III reverse transcriptase [26, 27].

For semi-quantitative RT-PCR analysis, we performed PCR reactions to measure gene expression profiles using Emerald Amp GT PCR Master Mix. Amplified PCR products were separated on 1% agarose gel and stained with ethidium bromide. To compare gene transcription levels, each cDNA reaction product was normalized to *PP2A* (At1g13320) [28, 29].

For quantitative real-time PCR (qRT-PCR) analysis, gene expression levels were calculated using SYBR green to monitor double-strand DNA synthesis. To compare data from each cDNA reaction product, cycle threshold (C_T) values for specific-target genes were normalized to the C_T value of *PP2A*. All experiments were performed with three independent biological replicates. Details of the primers used in this study are provided in Additional file 1: Table S1.

Dexamethasone-inducible transcription activation system

The GATA25/pTA7002-BMLC transgenic plants were grown in soil for 2 weeks, and groups were divided according to their treatment with or without dexamethasone (DEX). To activate GATA25, DEX was applied by spraying at 10 μM with 0.02% silwet surfactant. The control groups were sprayed with water containing 0.01% ethanol and 0.02% silwet surfactant. After treatment, young rosette leaves were harvested at the indicated time for expression profile analysis [22].

Isolation of protoplasts and transcriptional activation analysis

Protoplasts were prepared from *Arabidopsis* leaves, and polyethylene glycol-mediated transformation of effector and reporter constructs was conducted as previously

described [30]. For transcriptional activation analysis, protoplasts transfected with effector and reporter were lysed after incubation for 12 h, and GUS activity was calculated using the soluble extract.

To activate GATA25, which fused to the N-terminus of the glucocorticoid receptor (GR), the protoplasts were treated with 10 μM DEX. To inhibit new protein synthesis, cycloheximide was treated with 2 μM 30 min before DEX was added.

Statistics

The data are presented as mean of at least three biological replications ± standard deviation (SD). A difference was considered to be statistically significant when the P-value was less than 0.05 using a pairwise Student's *t*-test.

Results

GATA25 accelerates the flowering time under LDs

GATA25, also known as ZIM, is a transcription factor that has transcriptional activation and is localized in the nucleus [16, 17]. Transcription factors bind to a DNA promoter sequence, such as a regulatory sequence, and regulate transcription of the downstream genes [31–33]. To investigate the characteristics of GATA25, a transcriptional regulatory factor, we isolated twelve independent overexpression *GATA25* lines and six independent chimeric repressor *GATA25-SRDX* lines in which there was fusion of the SRDX motif to the dominant repression of target genes. A SUPERMAN repressor domain X (SRDX), LDLELRLGFA sequence was recruited as a co-repressor to build a repressive complex on specific targeted promoters [34]. We analyzed the flowering phenotype of WT and transgenic plants under LD photoperiods, which efficiently induced flowering [10]. When strongly expressed transcription levels occurred in plants with *GATA25*, the transgenic plants were early flowering at the same times as WT plants. Interestingly, in contrast, *GATA25-SRDX* lines had significantly delayed bolting (Fig. 1A). When we counted the total number of rosette leaves to measure the flowering time [35, 36], we found that the number of rosette leaves flowering under LDs was associated with decreased overexpression of *GATA25* lines as compared to WT, whereas *GATA25-SRDX* lines increased the number of rosette leaves at flowering (Fig. 1B). To reduce variation in the growth of plants and growth conditions within the experiment, WT

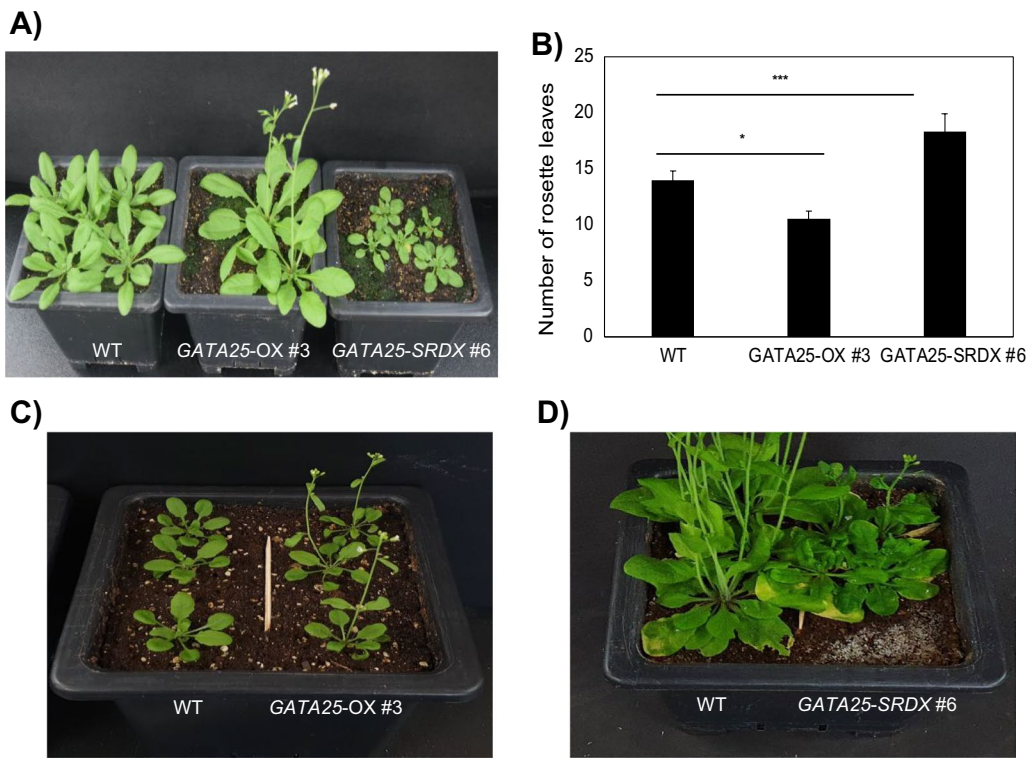

Fig. 1 The early-flowering phenotype in *GATA25* over-expressing plants. **A** Phenotypes of representative plants from wild-type (WT), *GATA25-OX*, and *GATA25-SRDX* plants. **B** Plants were scored for flowering time by measuring the number of rosette leaves at bolting. The mean ± SD were obtained from 6 plants. Phenotypes of *GATA25-OX* (**C**) and *GATA25-SRDX* (**D**) in a single pot with WT plants. Asterisks indicated statistically significant differences compared to WT as determined by Student's *t*-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

and each transgenic plant were grown in the same pot (Fig. 1C, D). The results clearly showed that overexpression of *GATA25* lines were early flowering and *GATA25-SRDX* lines were delayed in bolting compared to WT.

Inducible expression of GATA25 affects the flowering development in *Arabidopsis*

Various pathways controlling flowering time regulate the floral pathway integrators in *Arabidopsis* [1, 2]. Floral promotive genes can serve as a molecular signal to identify the affected flowering pathways. To understand the molecular mechanism of GATA25 in the early flowering phenotype, we generated transgenic plants using transient induction of GATA25 transcription levels under the chemical control of transcription using the GVG system [37]. The DEX-inducible expression of GATA25 transgenic plants was accomplished by inserting the full-length cDNA of GATA25 into the pTA7002-BMLC vector containing a chimeric transcription factor that was driven by a 2X CaMV 35S promoter (Fig. 2A). It is known that key floral integrators, *SOC1* and *FT,* converge to regulate various pathways [3, 12] and overexpression of these genes accelerate flowering [38]. To examine the expression of gene profiling, which is known to affect flowering time, transgenic plants of different lines in which GATA25 transcription was induced with DEX were collected at 0, 1, 3, and 6 h after the initial DEX treatment (Fig. 2B). When induction of GATA25 transcription with DEX led to the accumulation of floral pathway integrator transcripts, *SOC1* and *FT*. Moreover, *GI* and *CO* involving photoperiodic pathways also accumulated with the expression of GATA25 transcription. Interestingly, the GI and CO were assigned to the photoperiodic pathway, which promoted flowering in response to long photoperiods [39–41] and were highly correlated with GATA25 in response to circadian rhythm [42]. Thus, our results suggest that increased transcription levels of GATA25 alter the circadian rhythm, which causes accelerated flowering.

GATA25 is a flowering time genes-regulated transcriptional activator

The transient gene expression system using *Arabidopsis* mesophyll protoplasts was used to analyze the functions of cellular regulatory pieces of machinery by manipulating macromolecules. Many studies extensively apply transient analysis of promoter activity using transient transcriptional activation [43–45]. We used a transient transcriptional activation analysis to test the hypothesis that GATA25 activates transcription of *GI*, *CO*, *FT,* and *SOC1* (Fig. 3). As previously described, construction of an effector and reporter in the experiments was performed [46]. A 2X CaMV 35S promoter-driven GATA25 expression construct (effector) and 1 kb ~ 2 kb promoter-driven *GUS* expression construct (reporter) were co-transfected in *Arabidopsis* mesophyll protoplasts (Fig. 3A). As

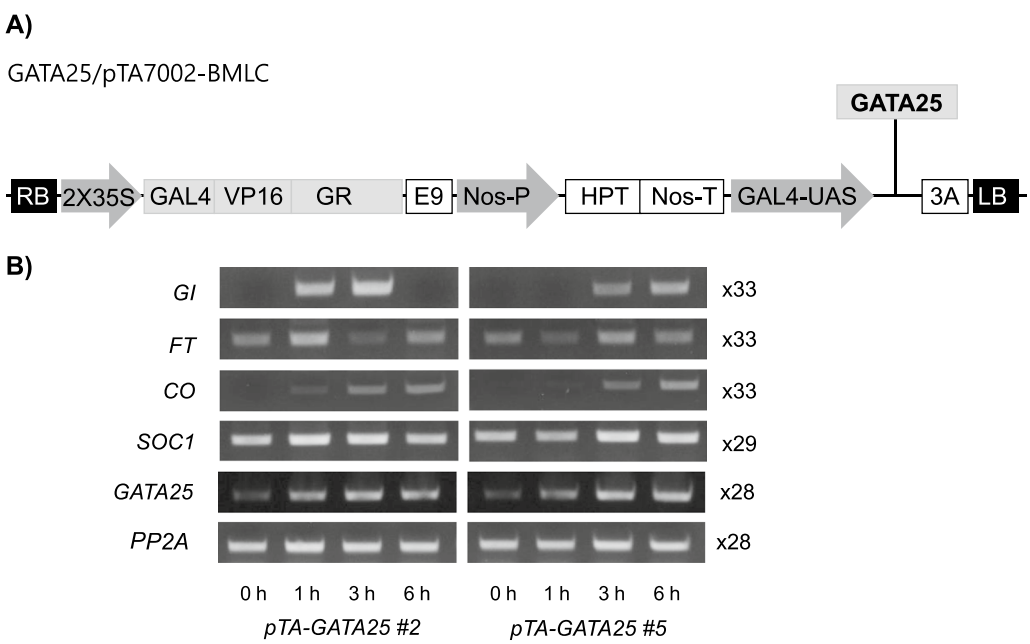

Fig. 2 Over-expression of *GATA25* shown accelerated flowering and induced expression levels of *GI*, *FT*, *CO*, and *SOC1*. **A** Schematic diagram of the vector used for DEX-inducible expression of *GATA25* in pTA7002-BMLC. **B** Expression patterns according to DEX treatment time of several flowering response-related genes of DEX-inducible *GATA25* independent lines. Semi-quantitative RT-PCR was performed using the *PP2A* gene as control

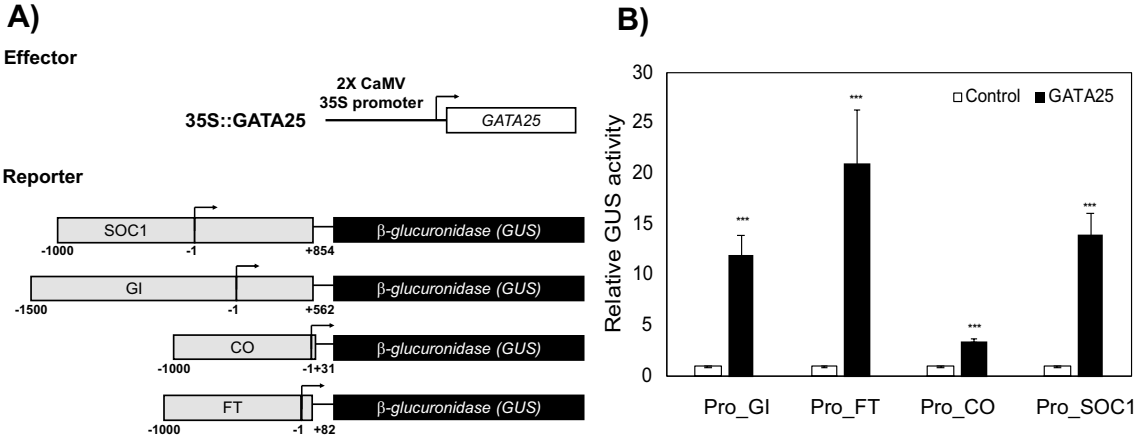

Fig. 3 GATA25 activates the expression of flowering response-related genes. **A** Schematic diagram of the effector and reporter constructs used in the transcriptional activation analysis. **B** Transcriptional activation analysis showing that the promoters of *GI*, *FT*, *CO*, and *SOC1* was activated by expressing *GATA25*. The activity of the GUS in the reporter construct transfected protoplasts with no effector was used as a control. Error bars represent the standard deviation (SD) of three biological replicates. Asterisks indicated statistically significant differences compared to Control as determined by Student's *t*-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

expected, when expressed effector was combined with 2X CaMV 35S promoter-driven GATA25, GUS activities of the reporters with the *GUS* gene driven by the selected promoters were significantly increased compared to control with transfection of the only reporter in protoplasts (Fig. 3B).

Transcription of flowering time genes is directly activated by GATA25

To further investigate whether GATA25 directly activates the expression of *GI*, *CO*, *FT,* and *SOC1*, we used a more powerful method, the steroid receptor-based inducible activation system [47, 48]. This system uses the glucocorticoid receptor hormone-binding domain (GR) fused transcription factor that binds the heat shock protein 90 (HSP90) in the cytoplasm and allows the fusion protein to enter the nucleus by addition of DEX. Simultaneous treatment with DEX and an inhibitor of protein biosynthesis, cycloheximide (CHX), prevents the accumulation of additional protein that subsequently affects gene expression [49–52]. First, the effector was constructed such that the GATA25 gene fused to the GR (GATA25-GR) was driven by the 2X CaMV 35S promoter (Fig. 4A). The inducible system was tested by co-transfection of the reporters and effectors described above in *Arabidopsis* mesophyll protoplasts. GUS activities of the reporters in which the *GUS* gene was driven by the selected promoters was highly induced by the addition of DEX. However, using simultaneous treatment with CHX and DEX, GUS activities of the reporters were not induced (Fig. 4B). We found that CHX was sufficient to inhibit additional protein synthesis, and DEX could activate the inducible system. Under this condition, GATA25-GR, which was treated simultaneously with CHX and DEX, resulted in an induction of the expression of target genes over time compared to only CHX (Fig. 4C). These results suggest that GATA25 regulates transcription of downstream target genes *GI*, *CO*, *FT*, and *SOC1* by activating each promoter [32, 53].

Discussion

We found that *Arabidopsis* GATA25 is a novel regulator of the floral transition. This novel function of the GATA25 transcription factor was revealed when our study showed that transgenic plants with increased GATA25 transcription levels were early flowering, whereas the GATA25-*SRDX* chimeric repressor transgenic plants were late flowering (Fig. 1). This result is consistent with the fact that early flowering occurs when *XTH33*, known as a downstream target gene of GATA25, is overexpressed [17, 54].

Various genes have been identified that play a vital role in regulating flowering through the combination of and cooperative actions of different pathways [55–59]. To determine whether GATA25 affects the expression of genes involved in the flowering promotive pathway, we used the GVG system, which controls transcription of the gene of interest by DEX treatment. We confirmed that the GATA25 transcription level induced by DEX treatment increased the transcription levels of *SOC1* and *FT*, known as key floral integrators (Fig. 2). Specifically, gibberellin (GA) signaling positively regulates the expression of floral pathway integrators [13, 60]. However, Shikata et al. (2004) showed that GATA25 leads to petiole

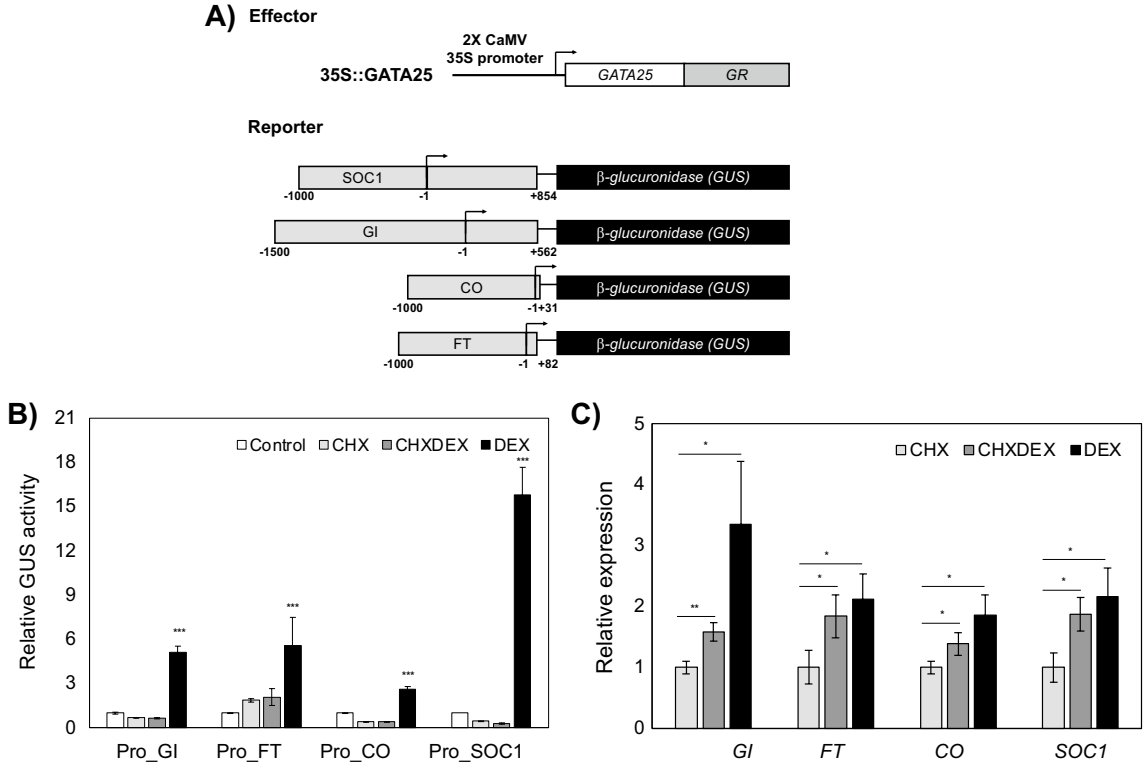

Fig. 4 Direct activation of flowering response-related genes by GATA25. **A** Schematic diagram of the GATA25-GR (effector) and reporter constructs used in the transcriptional activation analysis. GATA25 was fused to the glucocorticoid receptor (GR) under the 2XCaMV 35S promoter. **B** GATA25 activates the promoters of *GI*, *FT*, *CO*, and *SOC1* in the presence of DEX. Still, adding of the protein synthesis inhibitor cycloheximide at 2 μM completely abolished the DEX-induced GUS activity, indicating that the CHX treatment completely inhibited new protein synthesis. The activity of the GUS in the reporter construct transfected protoplasts with no effector was used as a control. **C** GATA25 directly induces the expression of flowering response-related genes by DEX activation of GATA25 occurred in the presence of CHX by performing the qRT-PCR analysis. The expression of flowering response-related genes in protoplasts, co-transfected with effector and reporter, treated with CHX was used as a control. Error bars represent the standard deviation (SD) of three biological replicates. Asterisks indicated statistically significant differences compared to Control as determined by Student's *t*-test ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

elongation in a GA-independent pathway. This indicates that GATA25 regulates the floral pathway integrators in a GA-independent pathway. In addition, we showed that the GATA25 transcription level increased the transcription levels of *GI* and *CO* involved in the photoperiodic pathway. In the photoperiodic pathway, circadian clock control of gene transcription by light can provide control of the flowering promotive pathway [4]. We confirmed that the expression of GATA25 is regulated in response to light [42]. This suggests that GATA25 is closely related to circadian-regulated gene expression and the photoperiodic pathway.

We have demonstrated that GATA25 is a transcriptional activator of floral pathway integrators and photoperiodic pathways (Fig. 3). Also, direct target analysis using the steroid receptor-based inducible activation system reveals that GATA25 directly activates the expression of floral pathway integrators and photoperiodic pathways (Fig. 4). In addition, we confirmed that each promoter of the genes activated by GATA25 has a putative GATA binding sequence (TGATAA and AGATAA) [54] and reverse orientation putative GATA binding sequence (TTATCA and TTATCT), which has the same transcription activation as in the forward orientation [61]. Therefore, these results indicate that GATA25 binds to the promoter of the selected target genes and activates transcription levels.

In this study, we analyzed the crucial role of GATA25, clustered as members of subfamily III within the *Arabidopsis* GATA gene family [42]. Our results demonstrate that GATA25 upregulates the photoperiodic flowering response-related genes together with their downstream target genes. Interestingly, a loss-of-function mutation in four *CDF* genes (*CDF1*, *CDF2*, *CDF3*, and *CDF5*) resulted in an early flowering phenotype but still showed photoperiodic response and circadian rhythms of *CO* expression [62]. This result suggests that there is a novel regulator in the regulation of *CO* expression

and photoperiodic response. Therefore, it indicates that GATA25 is a novel regulator that leads to an early flowering phenotype.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-022-00698-7.

Additional file 1: Table S1. List of primers used in this study

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Author contributions
KK and W-CK conceived and designed the experiments, prepared the figures, wrote the manuscript, and analyzed the data. KK, JL, JS, BK, and T-AK performed the experiments. All authors read and approved the final manuscript.

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Declarations

Competing interests
All of the authors declare no conflicts of interests.

Author details
[1]Department of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea. [2]Department of Integrative Biology, Kyungpook National University, Daegu 41566, Republic of Korea.

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