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Induction of flowering in Arabidopsis through functional peptide-mediated *FT* mRNA delivery

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Flowering is a key developmental event for plants, marking the transition from the vegetative phase to the reproductive phase. Artificial induction of flowering holds great impact on agriculture by accelerating fruit and seed setting. However, to date, only a limited number of externally applicable methods have been established for flowering induction. In this study, we focused on *FLOWERING LOCUS T* (*FT*), a pivotal flowering inducer conserved in many flowering plants. We developed a non-transgenic flowering induction system in the model plant Arabidopsis, employing the exogenous introduction of *FT* mRNA through a functional peptide-mediated delivery. Utilizing the functional peptide BP100-KH_9 as a nanocarrier for nucleic acid delivery into plant cells, we confirmed the production of FT protein from the introduced *FT* mRNA, which was fused with a reporter gene Citrine. The introduction of *FT* mRNA was also evidenced by the induction of expression of floral genes in treated seedlings, leading to the optimization of the timing for mRNA delivery. Measurement of flowering demonstrated accelerated flowering resulting from the introduction of *FT* mRNA as indicated by a shortened flowering time and a reduced number of leaves at flowering. This proof-of-concept study proposes an innovative non-transgenic flowering induction method using mRNA delivery.

Keywords Flowering, FT, RNA delivery, Peptide, *Arabidopsis thaliana*

Flowering is a crucial developmental stage for plants, signifying the shift from vegetative growth to reproductive growth, which enables the production of the next generations and introduces genetic diversity. Flowering is regulated precisely at the molecular level, with studies in Arabidopsis significantly advancing our understanding of its mechanisms. The process integrates information about temperature and day length through various pathways: the thermosensory and vernalization/autonomous pathways, and the photoperiod pathway[1]. *FLOWERING LOCUS T* (*FT*), which encodes a small globular protein similar to the mammalian Raf kinase inhibitor protein[2] is known as a florigen and plays a critical role in the photoperiodic pathway in the long-day plant *Arabidopsis thaliana*[2,3]. *FT* expression is induced by the zinc-finger protein *CONSTANS* (*CO*), a transcription factor that acts under long day condition[4,5], in coordination with the developmental growth of plants. *FT* is normally expressed in the phloem companion cells of leaves[6] and its protein is transported to the shoot apical meristem (SAM) via phloem sieve elements[7–9]. In the SAM, FT binds to the transcription factor FD to directly or indirectly induce floral genes such as *APETALA1* (*AP1*), LEAFY (LFY), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*), *SEPALLATA3* (*SEP3*)[10–16].

Inducing flowering has significant potential for applications in breeding and agriculture by promoting faster seed and fruit setting. Genetic engineering approaches, such as overexpressing *FT* or other flowering-promoting genes, can effectively induce flowering by either enhancing these genes or suppressing their regulators. For example, overexpression of *FT* under a strong constitutive promoter significantly promotes early flowering and reduces the number of rosette leaves in Arabidopsis Col-0 [17]. Flowering induction by *FT* overexpression has also been observed in other plant species, including the annual monocot rice[18] and the perennial dicot tree poplar[19]. Other than genetic engineering, limited number of phytohormones and compounds are known to

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induce flowering. Phytohormones such as salicylic acid and cytokinin have been reported to induce flowering in Arabidopsis through external application[20,21]. While endogenous molecules like reactive oxygen species (ROS) and sugars are involved in flowering induction, their external application has not yet been demonstrated to induce flowering in Arabidopsis[22].

Recent advances in molecular-level understanding of plant mechanisms have stimulated their application for plant modification. Genetic engineering is the major and forceful method for the modification and has been utilized for many years. Agrobacterium-mediated transformation[23] and biolistic transformation[24] are well-established genetic engineering techniques used for plant modification. In addition to these traditional methods, emerging methods utilizing nanomaterials (such as peptides, carbon materials, and metals) are now being employed for plant modification[25]. Functional peptides are short peptides (~ 50 amino acids) with specific in vivo functions. Cell-penetrating peptides (CPPs) are a subset of functional peptides that can traverse cell membranes[26,27]. BP100 is a widely used CPP for various plant species, except for rice leaf[28]. When combined with a tandemly fused polycationic peptide KH_9, which efficiently binds to nucleic acids through electrostatic interactions, this fusion peptide can deliver a range of biomacromolecules, including DNA and mRNA, to produce proteins in plant cells[29–31]. Thus, this peptide-mediated method allows for non-transgenic protein production in plants via mRNA delivery. In this study, we applied the peptide-mediated mRNA delivery method to accelerate flowering in Arabidopsis as a model system. We optimized the conditions for delivering *FT* mRNA into Arabidopsis seedlings using the fusion peptide BP100-KH_9. This delivery method led to upregulation of floral genes and earlier flowering in the treated plants.

Results and discussion

Formation of *FT* mRNA-peptide complexes

We first prepared *A. thaliana FT* mRNA by in vitro transcription and obtained high quality *FT* mRNA without any prominent by-product (Fig. S1). To analyze the complexation of the *FT* mRNA and the peptide BP100-KH_9, which is critical for delivering nucleic acids into cells through nanocarrier-mediated delivery methods, we performed gel shift and hydrodynamic diameter analyses at various N/P ratios (the ratio of the number of positive charges in peptides to the number of negative charge in nucleic acids). The gel shift analysis showed formation of RNA-peptide complexes, evidenced by decreased RNA band intensity and retarded mobility in the gel (Fig. 1A). Complexation between *FT* mRNA and peptide was observed at every N/P ratio, with binding efficiency reaching 100% at an N/P ratio 2 or higher. Notably, the mobility of the complexes was highly retarded at N/P ratios 2 and 4, with some complexes retained in the well (Fig. 1A), suggesting the formation of higher-order mRNA-peptide complexes at higher N/P ratios. Next, we evaluated the in vitro protection of mRNA against RNase through complex formation. Following RNase digestion of the complexes formed between *FT* mRNA and BP100-KH_9 at various N/P ratios, we observed reduced mRNA degradation at all tested N/P ratios (Fig. 1B). This protection of *FT* mRNA by binding with the peptides via the cationic KH_9 region supports the notion that the peptide acts not only as a carrier but also provides protection from RNase during delivery into plant cells.

The hydrodynamic diameter of *FT* mRNA without peptide showed a broad size distribution with a peak at around 500 nm. The size distribution of the complexed molecules shifted with the addition of the peptide; an additional peak with smaller size around 50 nm at N/P ratio of 0.25 and peaks with larger sizes at other N/P ratios. The size distribution of the complexes at an N/P ratio 2 was bimodal, with a main peak larger than 1000 nm, supporting the formation of higher order complex at this N/P ratios (Fig. S1). Despite the higher order complex formation at an N/P ratio 4 as confirmed by the gel shift assay, the complexes at this ratio exhibited a main peak around 200 nm in hydrodynamic diameter. This may be because the predicted larger complex were removed by filtration before the analysis. These properties of *FT* mRNA-peptide complexes collectively suggest the formation of smaller complex at lower N/P ratios, with larger complexes forming at higher N/P ratios. Considering that the maximum size of molecules to pass through the cell wall is approximately 50 nm[32], the physicochemical properties of the *FT* mRNA-peptide complexes suggest that mRNA delivery should be performed at lower N/P ratios. Additionally, it is advisable to use the lowest possible peptide concentration (N/P ratio), as higher concentration of CPPs may cause stress to plants due to cytotoxicity[28].

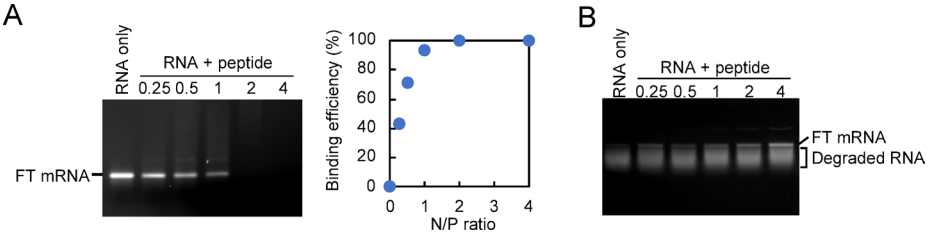

Fig. 1. Complexation of ***FT*** mRNA with the peptide BP100-KH_9. (**A**) Gel shift assay for mRNA-peptide complexes. *FT* mRNA complexed with the peptide BP100-KH_9 at N/P ratio = 0.25, 0.5, 1, 2, and 4 were analyzed by agarose gel electrophoresis. Binding efficiency of the RNA at each N/P ratio was calculated from the gel image. (**B**) Protection of mRNA from RNase by peptides. *FT* mRNA was complexed with BP100-KH_9 at N/P ratio = 0.25, 0.5, 1, 2, and 4, and then treated with RNase A. After dissociation from BP100-KH_9, *FT* mRNA was analyzed by agarose gel electrophoresis. Original gel images are shown in Fig. S6.

Production of FT-Citrine from the delivered mRNA

To obtain evidence of protein production from exogenously delivered mRNA, we tested *FT*-Citrine mRNA delivery mediated by the carrier peptide BP100-KH_9. The *FT*-Citrine mRNA, synthesized by in vitro transcription, was complexed with BP100-KH_9 and introduced into Arabidopsis leaves via infiltration using a needleless syringe[33]. Confocal laser-scanning microscopy (CLSM) analysis of the infiltrated leaves showed Citrine fluorescence, with FT-Citrine distributed in both the cytoplasm and nucleus of the leaf cells (Fig. 2A). The fluorescence was observed in stomata, likely through the permeation pathway used by non-viral carriers for nucleic acid cargo[30] and this biased incorporation complicates the accurate measurement of *FT*-Citrine mRNA delivery efficiency in leaves. To validate the results, *FT*-Citrine was expressed under the constitutive promoter CaMV 35 S from exogenously introduced DNA using biolistics. The produced FT-Citrine was localized to both cytoplasm and nucleus, consistent with the localization observed from the introduced mRNA (Fig. 2B). While the fluorescence patterns resembled the characteristic autofluorescence typically observed in the cytosol and the chloroplasts of guard cells[34], the presence of fluorescence within the nuclei suggests that the signal was derived from FT-Citrine. Furthermore, these observations align with the localization of FT-GFP in both the nucleus and cytoplasm when expressed endogenously in Arabidopsis shoot apex and leaf cells[11]. Collectively, the observed fluorescence and intracellular localization of FT-Citrine validate the production and correct localization of FT-Citrine protein from the exogenously introduced mRNA in Arabidopsis leaf cells. However, FT-Citrine protein was not detected by Western blotting, likely due to its low abundance. Since FT protein fused to GFP is reported to exhibit reduced activity in Arabidopsis[8], we employed FT without any reporter protein in subsequent analyses.

Induction of floral gene expression by the introduction of *FT* mRNA

Since *FT* transcript levels dramatically increase during seedling development in Arabidopsis under long day conditions[17], seedling stage is thought to be more effective for flowering induction by *FT* mRNA delivery. Peptide-mediated mRNA delivery has only been applied to mature leaves so far[31]. Thus, we first tested the delivery of *FT* mRNA into Arabidopsis seedlings using the peptide-mediated method and analyzed the retention of the delivered *FT* mRNA. As shown in Fig. S2, the relative amount of *FT* mRNA in the seedlings treated with *FT* mRNA and peptide showed a marked increase one day after the introduction (DAI) compared to the controls. The *FT* mRNA level in the treated seedlings was then decreased to almost basal level by 3 DAI. These results suggest that the externally introduced *FT* mRNA persists in Arabidopsis seedlings for at least two days after introduction. However, the assessment does not reflect the amount of functional *FT* mRNA, as the assay quantifies total *FT* mRNA in the seedlings, including that in intercellular spaces.

In Arabidopsis, *FT* expression remains in a low level until 9 days after germination (DAG), then abruptly increases around 11 DAG[35]. We therefore assessed the effect of *FT* mRNA delivery at various growth stages up to 11 DAG in Arabidopsis seedling by analyzing expression of floral genes, which should be upregulated in response to the internalization of functional *FT* mRNA into plant cells. This analysis aim to determine the optimal timing for *FT* mRNA delivery for effective flowering induction and to demonstrate the functionality of externally introduced *FT* mRNA in Arabidopsis seedlings.

Using the peptide BP100-KH_9, we introduced *FT* mRNA into Arabidopsis seedlings at 7, 9, and 11 DAG and analyzed the expression of the floral genes *AP1*, *LFY*, *SEP3*, and *SOC1*, which are directly or indirectly induced by FT (Fig. 3A), two days after the introduction. Expression analysis showed a stepwise increase in all the tested floral gene transcripts according to the growth of seedlings treated with water or peptide only (Fig. 3B), suggesting a minimal effect of the delivery method on seedling growth. The introduction of *FT* mRNA mediated by the peptide significantly induced the expression of the floral genes, with the most pronounced induction observed at 7 DAG and 9 DAG, compared to the controls (Fig. 3B). While the addition of only peptide

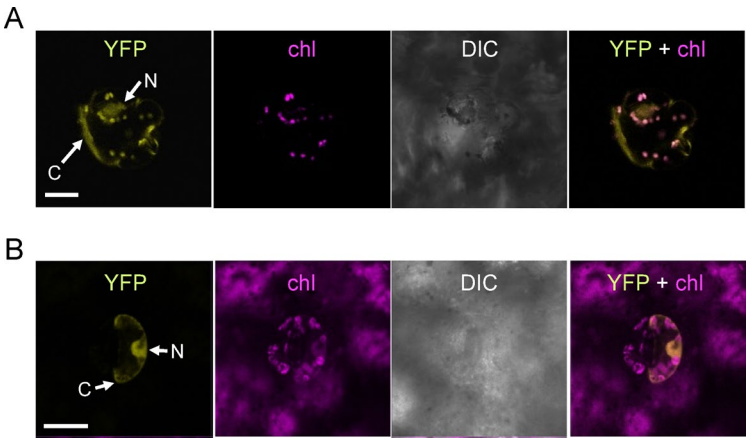

Fig. 2. Production and localization of FT-Citrine protein in Arabidopsis leaf cells. (**A**) Production of FT-Citrine protein from *FT*-Citrine mRNA introduced by the peptide-mediated method. Chlorophyll fluorescence shows leakage to YFP fluorescence. (**B**) Production of FT-Citrine protein from plasmid DNA introduced by biolistic delivery method. Confocal laser-scanning microscopy (CLSM) of the cells represents fluorescence of Citrine (YFP) and chlorophyll (chl). N and C denote nucleus and cytosol, respectively. Bars, 20 mm.

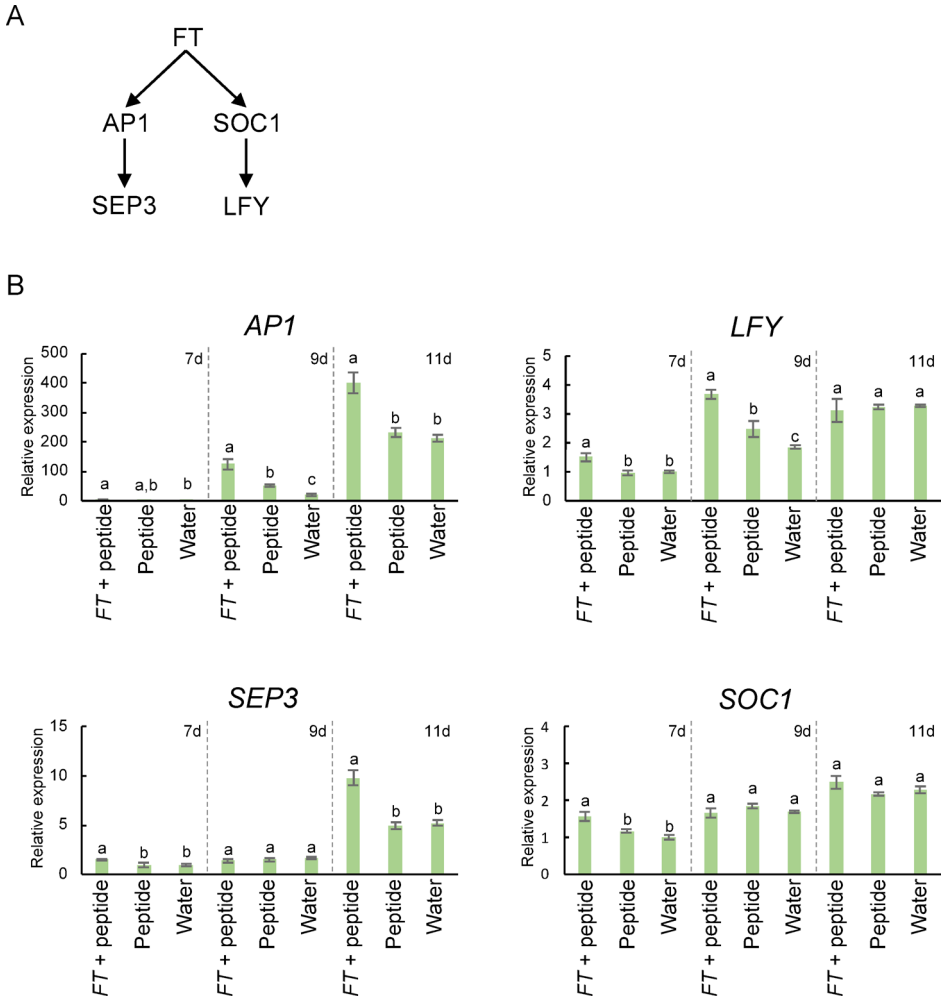

Fig. 3. Expression analysis of floral genes after peptide-mediated delivery of *FT* mRNA in Arabidopsis seedlings. (**A**) Transcriptional regulation of floral genes by *FT*. FT protein associated with FD induces transcription of floral genes (*AP1*, *LFY*, *SEP3*, *SOC1*) directly or indirectly. (**B**) Transcript abundance of floral genes was assessed by quantitative reverse transcription PCR and normalized by that of an internal control *PEX4*. *FT* mRNA was delivered into 7, 9, or 11 days after germination Arabidopsis seedlings by the peptide BP100-KH_9, and total RNA was extracted from the shoot apex of the seedlings two days after the introduction. Seedlings were treated only with the peptide BP100-KH_9 or water as controls. Data are shown as means with standard deviation of three biological replicates ($n = 3$). The lowercase letters indicate statistically significant differences (Tukey's multiple comparison test, $P < 0.05$).

occasionally induced the expression especially in *AP1*, likely be due to stress-induced flowering[36] caused by cytotoxicity of CPPs[28], the induction caused by the introduction of *FT* mRNA with peptide exceeded that induced by peptide, demonstrating that the induction of floral gene expression is primarily attributed to *FT* mRNA delivery. Furthermore, these results suggest that our *FT* mRNA delivery system is more effective when introduced into 7 to 9 DAG seedlings for inducing floral genes. This effectiveness can be explained by two reasons. First, the ratio of delivered *FT* mRNA to endogenous *FT* mRNA was higher in younger seedlings due to the abrupt increase in endogenous *FT* transcript levels after 9 DAG. Second, the cell wall network becomes denser with the increase of cell wall stiffness as seedlings grow[37], which impedes the mRNA-peptide nanocomplex from passing through cell wall pores. Either or both of these factors likely contribute to the effective introduction of *FT* mRNA into 7–9 DAG Arabidopsis seedlings. Collectively, the induction of floral genes by *FT* mRNA delivery suggests that FT protein was produced and functioned to induce flowering in younger seedlings at 7 to 9 DAG. These results, on the other hand, raise the question of whether delivering *FT* mRNA into seedlings younger than 7 DAG or using multiple introductions would be more effective for flowering induction. However, delivery to very young seedlings or multiple infiltrations would be unsuitable, since the delivery method utilizes pressure and compression of seedlings in the mRNA/peptide solution, which substantially delays growth.

Acceleration of flowering by *FT* mRNA delivery

Using the optimized conditions for the *FT* mRNA to peptide ratio and the timing for delivery, we delivered *FT* mRNA into Arabidopsis seedlings to test the acceleration of flowering. 8 DAG seedlings were subjected to the

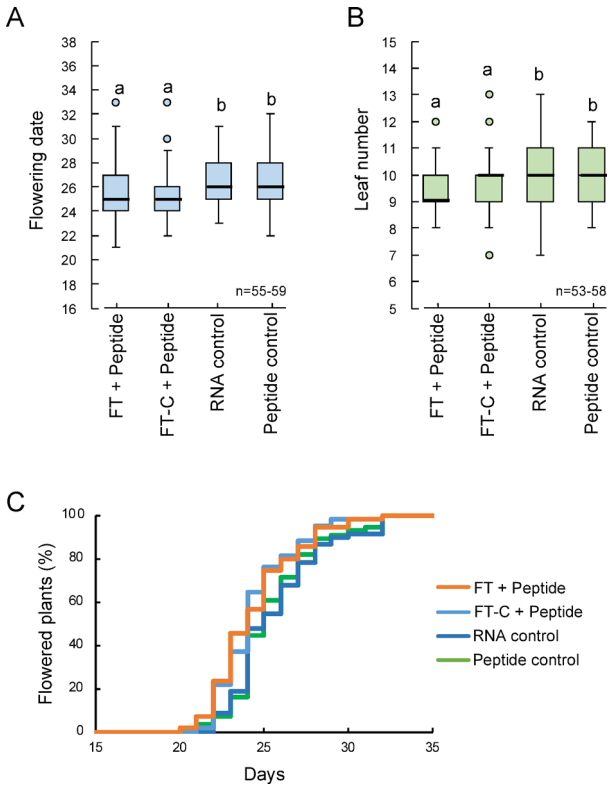

Fig. 4. Flowering induction of Arabidopsis plants by *FT* mRNA delivery. (**A**) and (**B**) Box plot showing flowering date (**A**) and leaf number at flowering (**B**) of plants treated with peptide-mediated delivery of *FT* mRNA, *FT* mRNA with 5'Cap and polyA (FT-C), and the controls treated with *FT* mRNA (RNA control) or peptide only (Peptide control). The asterisks indicate statistically significant differences ($P < 0.05$, Tukey's multiple comparison test, excluding outliers). (**C**) Flowering rate of plants treated with *FT* mRNA and/or peptides shown by days after germination.

peptide-mediated *FT* mRNA delivery and then cultivated under long-day condition, along with *FT* mRNA only and peptide only controls. Since flowering is affected by light intensity and temperature, we measured flowering time by cultivating plants on an agar medium, ensuring a homogenous condition for all plants being compared. As a result of measurement of the flowering date, plants treated with either *FT* mRNA or peptide flowered at a median of 26 DAG (Fig. 4A and C), which nearly agrees with that of Arabidopsis Col-0 in a pot[38], confirming that plants flowered normally under these conditions. In comparison, plants treated with the *FT* mRNA-peptide complex flowered at a median of 25 DAG, which is statistically earlier than any of the controls (Fig. 4A). We also investigated the effect of *FT* mRNA delivery on the leaf number at bolting, a common metric for assessing the impact on flowering[2]. As shown in Fig. 4B, the results largely agreed with the flowering time of the plants; plants treated with the *FT* mRNA-peptide complex flowered with significantly fewer leaves compared to any of the controls. We validated the flowering induction with *FT* mRNA delivery by testing peptide-mediated mRNA delivery of FT-Citrine, which has limited ability to induce flowering[39], and observed a significant reduction in flowering acceleration with *FT*-Citrine mRNA delivery (Fig. S4). These findings collectively suggest that the introduction of *FT* mRNA mediated by the peptide BP100-KH_9 accelerates flowering in the Arabidopsis wild-type plants, even under the long-day condition.

Since both 5' cap and 3' polyA modifications of mRNA enhance expression of externally introduced mRNA in plant cells by increasing stability and translation efficiency[40], we tested these modifications on *FT* mRNA. The modified *FT* mRNA formed a complex with the peptide BP100-KH_9 similarly to the unmodified *FT* mRNA (Fig. S3). As shown in Fig. 4, plants treated with the modified *FT* mRNA flowered earlier than the controls, with statistical significance in both flowering date and leaf number. However, there was no significant difference in the flowering status between plants treated with modified or unmodified *FT* mRNA (Fig. 4), suggesting that the flowering acceleration by the delivery of modified *FT* mRNA was comparable to that of the unmodified version. Since our carrier peptide binds to mRNA to compact the complex and thus protect it from RNase (Fig. 1B), this likely reduced the effect of mRNA modification on stability. Moreover, the complexation of mRNA with the peptide by N/P ratio results in the introduction of a reduced number of mRNA copies into cells for the modified mRNA due to its higher molecular weight. Considering these factors, the results suggest a minor effect of mRNA modification on our peptide-mediated *FT* mRNA delivery for flowering acceleration.

Given that the uptake of nucleic acids mediated by the BP100-KH_9 peptide is mainly via leaf hydathodes in Arabidopsis leaves[30], FT protein, which is thought to be the predominant mobile form[8] translated from the exogenously delivered *FT* mRNA in leaves, would move to the SAM via the phloem and subsequently induce

the floral genes in our system. Additionally, the delivered *FT* mRNA might be directly transported to the SAM, leveraging its potential mobility to induce floral genes[41], though induction of flowering via direct transport of *FT* mRNA to the SAM is still debated[42]. Direct delivery of *FT* mRNA to the SAM should accelerate flowering more efficiently. However, the SAM region is likely protected against externally introduced biomolecules, as demonstrated against the viruses[43]. Thus, we believe that our system demonstrated in this study offers the only feasible method for flowering acceleration utilizing *FT* mRNA delivery to date.

Conclusion

In this study, we established a method to accelerate flowering in a model Arabidopsis by utilizing *FT* mRNA delivery mediated by the carrier peptide BP100-KH_9. The Arabidopsis ecotype used in the study was the early flowering ecotype Col-0[44], and the conditions were long-day, which typically make flowering acceleration challenging. Despite these conditions, we observed a shortened flowering time, confirmed both by the flowering time and the number of leaves at flowering, validating the effectiveness of the method. Considering the short half-life of the delivered *FT* mRNA, the acceleration of flowering is likely due to the advanced progression of the flowering stage triggered by a transient increase in FT protein levels, rather than a sustained elevation. We chose *FT* mRNA for external flowering induction, because *FT* is functionally conserved in many crop plants[45]. Indeed, genetic engineering with *FT* overexpression has been shown to induce flowering in various plant species[18,19,46–48], suggesting the applicability of the method established in this study. The method also offers promise for biosafety when used for crop plants, as it employs a biocompatible peptide carrier, and mRNA delivery is already utilized for vaccination in human[49]. The method established in this study serves as a proof of concept for external *FT* mRNA delivery for flowering acceleration and mRNA delivery for plant modification.

Materials and methods

Plant materials and cultivation

Arabidopsis thaliana ecotype Col-0 was used in the study. Arabidopsis seeds were sterilized with 70% ethanol for 1 min and subsequent 2.5% hypochlorite for 5 min. After vernalization at 4 °C storage for 3 days, the sterilized seeds were cultivated on a Murashige-Skoog (MS) medium (Sigma-Aldrich, US) solidified with 0.25% gellan-gum at 22 °C under a 16 h light condition in a growth chamber. For infiltration using a needle-less syringe, Arabidopsis Col-0 plants were cultivated in soil (Promix, Rivièredu-Loup, Canada) supplemented with vermiculite at a ratio of 2:1 at 22 °C under a 8 h light condition.

Synthesis of mRNA

Template DNA for in vitro transcription was amplified by PCR using Arabidopsis 1 st strand cDNA with p1 and p2 for *FT* (Sequences of primers are listed in Table S1). For *FT*-Citrine, template DNA was amplified by PCR with p3 and p4, and p5 and p6 for *FT* and Citrine, respectively, and subsequently the PCR products were mixed and then fused by overlapping PCR with p3 and p6. mRNA was synthesized by in vitro transcription using the template DNA (Fig. S5) and in vitro Transcription Kit (TaKaRa, Japan) or HiScribe T7 ARCA mRNA Kit (with Tailing) (New England BioLabs, US). The synthesized mRNA was purified by a column. Purity of mRNA was analyzed by Bioanalyzer 2100 (Agilent, US).

Complexation of mRNA with peptides and physicochemical evaluation of mRNA-peptide complex

BP100-KH_9 [KKLFKKILKYLKHKHKHKHKHKHKHKH: 3809.8 Da] was mixed with mRNA at various N/P ratios, and then left them for 20 min at room temperature. For gel shift assay, the complexes were analyzed by electrophoresis in a 1% agarose gel with 0.5 x TBE. The hydrodynamic diameter of the complex was measured by Dynamic Light Scattering using Zeta Sizer Nano ZS instrument (Malvern Instruments Ltd., UK) after filtering the complex solution.

mRNA protection assay

FT mRNA (200 ng) was mixed with BP100-KH_9 at various N/P ratios, and then incubated at room temperature for 20 min. Following addition of 0.16 ng RNaseA (QIAGEN, Germany) to the mRNA-peptide complex solution, the mixture was incubated at room temperature for 20 min. After adding a loading buffer containing SDS to the solution to stop RNase activity and dissociate the peptide from the mRNA, the RNA was analyzed by agarose gel electrophoresis.

Observation of FT-Citrine in leaves

For delivery of *FT*-Citrine mRNA into leaf cells, 1 µg of *FT*-Citrine mRNA was complexed with 0.125 µg of BP100-KH_9 in 100 µl solution, and then infiltrated into 2 months-old Arabidopsis leaf by infiltration using needleless syringe as described previously[33]. For biolistic delivery of *FT* gene, *FT*-Citrine used for the template for in vitro transcription was placed downstream of a constitutive P35S promoter in pUC19. The resulting plasmid was introduced into 2 months-old Arabidopsis leaves by biolistic particle delivery system PDS-1000 (Bio-Rad, US)[50]. The fluorescence of the FT-Citrine in the infiltrated and bombarded leaves was observed by a confocal laser scanning microscopy LSM700 (Zeiss, Germany) with chlorophyll autofluorescence.

Peptide-mediated delivery of mRNA into Arabidopsis seedlings

For delivery of *FT* mRNA into Arabidopsis seedlings, 10 µg of *FT* mRNA was mixed with 1.25 µg of BP100-KH_9 in 1 ml solution, and the complex was then incubated at room temperature for 20 min. Arabidopsis seedlings cultivated for eight days on a solidified medium were dipped in 0.02% (v/v) Silwet L-77 (PhytoTechnology

Laboratories, Shawnee Mission, USA) and then submerged in the mRNA-peptide complex solution (~ 40 seedlings in the 1 ml complex solution). The seedlings were subjected for vacuum at −0.08 MPa for 1 min and compression at + 0.08 MPa for 1 min for infiltration of the complex. After 1 h incubation in the solution, the seedlings were washed with water, and then cultivated on a solidified medium. For the analysis of abundance of the internalized *FT* mRNA in Arabidopsis seedlings, the seedlings were washed three times 1, 2 and 3 days after introduction, and then total RNA was extracted by RNeasy Plant Mini kit (QIAGEN, Germany).

Expression analysis of *FT* and floral genes

For expression analysis of the floral genes, *FT* mRNA was introduced into 7, 9, or 11 DAG Arabidopsis seedlings, and 2 days after the introduction, shoot apex of the seedlings were collected. Total RNA was extracted from the shoot apex by RNeasy Plant Mini kit (QIAGEN, Germany), and first strand cDNA was synthesized with the total RNA and ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO, Japan). Quantitative real-time PCR analyses of floral genes were performed by using Applied Biosystems StepOnePlus Real-Time PCR Systems (Thermo Fisher, US), the cDNA and primers p7 and p8 for *AP1*, p9 and p10 for *LFY*, p11 and p12 for *SEP3*, and p13 and p14 for SOC1, and p15 and p16 for an internal control gene *PEX4*. For analysis of *FT*, primers p17 and p18 were used, and the amount of *FT* mRNA was normalized with that of *PEX4*. Quantification was performed with standard curves for each target gene.

Measurement of flowering time

For measurement of flowering time, *FT* mRNA was introduced into 8 DAG seedlings as described above, and after 1 day cultivation on a solidified medium for recovery, the seedlings were transferred to a MS solidified medium. For precise comparison of flowering time, seedlings to be compared were cultivated on a same solidified medium, and such plates were replicated for statistical analysis. The plants were cultivated at 22 °C under a 16 h light condition in a growth chamber (Biotron, NK System, Japan). The light intensity was 100 μmol m^{-2} s^{-1}. The number of leaves was counted without cotyledons after bolting. The data was statistically evaluated by the Tukey's multiple comparison test, excluding outliers identified by Smirnov-Grubbs test. During the measurements, dwarf and dark green individuals were occasionally observed, likely as a result of the vacuum–pressure infiltration treatment. Because these plants were abnormal, exhibited delayed flowering, and were identified as outliers that could confound accurate measurement of flowering time, they were excluded from the analyses.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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MO, SK and KN conceived the research, MO and MM performed the experiments, MO, SI, SK and KN analyzed the data, and MO and KN wrote the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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