

Full Length Research Paper

# A new method for promoting lily flowering

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**FT is thought to be the florigen in plants. In this research, a new method for promoting lily flowering was introduced. The function of FT gene cloned from *Arabidopsis* on promoting lily flowering was analyzed. pET-30a-FT vector was constructed to indicate the expression of FT:eGFP fuse protein in prokaryotic cells. FT:eGFP was also constructed into plant virus vector-pGR106. *Agrobacterium GV3101* harboring pGR106-FT was injected into lily. The injected lily showed early flowering when compared with the control plants. The detection of eGFP and PCR analysis indicated that the virus harboring FT:eGFP was replicated and expressed in the host plants. The results showed that FT:eGFP fuse protein functioned in promoting lily flowering.**

**Key words:** FT, viral vector, lily.

## INTRODUCTION

The transition from vegetative phase to reproductive growth is controlled by day length in many plant species. It is believed that florigen triggers floral morphogenesis. Florigen is produced in the leaves under inductive day length conditions and transported to the shoot apex where it interacts with the other proteins and reprograms the SAM to form flowers. Recent studies from several laboratories have provided strong evidence that the FT protein and its corresponding proteins are an important part of the mobile signal-florigen. Studies on *Arabidopsis* and rice showed that FT protein and the rice Hd3a participated in long-distance signaling to induce flowering. Homologs of the FT genes have been isolated from many monocotyledonous and dicotyledonous species. The function of FT to promote flowering was conserved in all species tested until now. Presently, FT is the only known protein that serves as a long-range developmental signal in plants (Lin et al., 2007).

The presence of FT proteins in the phloem sap has been unambiguously demonstrated in the cucurbits (Lin et al., 2007). FT protein acts at the shoot apex of the plant in concert with a transcription factor FD to induce plant flowering (Abe et al., 2005; Wigge et al., 2005).

Transmission of FT:GFP protein via a graft junction from donor transgenic plants to the recipient plants promoted flowering in the recipient plants (Corbesier et al., 2007). Over-expression of FT homologs causes extreme early flowering in the dicotyledonous plants poplar, tomato and tobacco, as well as in the monocotyledonous plant rice (Takashi et al., 2009). In this research, the function of FT:eGFP fuse protein was studied in lily via virus vector injection, and its corresponding results were analyzed.

## MATERIALS AND METHODS

Seedlings of *Lilium × formolongi* were cultivated in medium containing peat : perlite : vermiculite (7:2:1) in a growth chamber at 25 to 28°C in the day and 15 to 17°C at night under 16/8 h light/dark photoperiod with light intensity of 3000 lux.

### Vectors construction

#### Construction of pET- 30a- FT vector

FT gene was cloned from *Arabidopsis*. FT:eGFP gene fragment with NcoI at the 5' end and XhoI at the 3' end was cloned from the binary vector pCR2.1 containing FT:eGFP. The primers were as the followings: forward primer, 5'-CACCATGGCTATGCGGGCCTCTATAAAT-3'; reverse primer, 5'-CACTCGAGGGTACCGGATCCCTTGT-3'.

PCR reactions were conducted with a pre-denaturation step at 94°C for 4 min, then a denaturation step at 94°C for 45 s,

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annealing step at 60°C for 60 s, a polymerization step at 72°C for 60 s (20 cycles), and finally an extension step at 72°C for 7 min. The PCR product digested with NcoI and XhoI was ligated to the pET-30a vector digested with NcoI and XhoI. The final vector pET-30a-FT was constructed.

### Construction of pGR106-FT vector

FT:eGFP DNA fragment was cloned via PCR amplification. The primers were as follows: forward primer, 5'-CCGATCGATATGCGGGCCTCTATAAT-3'; reverse primer, 5'-CAGTCGACCTAGGTACCGGATCCCTTGT-3'. The PCR procedure was the same with the earlier mentioned one.

The PCR product digested with ClaI and Sall was ligated to the pGR106 vector digested with ClaI and Sall. The final vector pGR106-FT was constructed.

### Expression of FT:eGFP in prokaryotic cells

pET-30a-FT was transformed into *Escherichia coli* DB2.1. A single colony was inoculated in LB liquid medium supplemented with 50 µgml<sup>-1</sup> kanamycin and cultured at 37°C, 220 rpm for 12 to 16 h until its OD<sub>600</sub> reached 0.6 to 1.0. IPTG was added with final concentration at 1 mM<sup>-1</sup>, and the bacteria were continued with the culture at 28°C and 180 rpm for 6 h.

### Injection of viral vector

pGR106-FT plasmid was transformed into *Agrobacterium GV3101*. *GV3101* harboring pGR106-FT was cultured at 28°C until its OD<sub>600</sub> reached 0.6 to 1.0. Then the agrobacterium was resuspended in liquid MS media, and 1 to 2 ml of the resuspended media was injected with sterile syringes (without needles) into the leaves. Sterile water was injected as control. This experiment was done with 3 repeats.

### PCR assay of injected lily

In order to detect FT:eGFP DNA fragment, genomic DNA was extracted from the injected lily leaves and control leaves, and primers were synthesized as follows: forward primer from FT gene, 5' TTCCCGTGGCCTCAGTTT3'; reverse primer from eGFP gene, 5' AGTTCACCTTGATGCCGTTTC-3'.

PCR reactions were conducted with a pre-denaturation step at 94°C for 4 min, then a denaturation step at 94°C for 45 s, annealing step at 53°C for 45 s, a polymerization step at 72°C for 60 s (35 cycles), and finally an extension step at 72°C for 7 min.

## RESULTS AND DISCUSSION

### The expression of FT:eGFP in prokaryotic cells

In order to prove the fluorescent character, the FT:eGFP fuse protein, the recombinant *E. coli* and also the purified fuse protein were detected under fluorescent microscope (Figure 1). Since eGFP can be observed under natural light, the liquid culture and pellet of recombinant *E. coli* harboring pET-30a-FT showed pale green color, while the culture and pellet of *E. coli* harboring pET-30a did not show pale green color (Figure 1A). Green fluorescence

was observed from the recombinant *E. coli* harboring pET-30a-FT (Figure 1B), but was not observed in the *E. coli* harboring pET-30a. FT:eGFP fuse protein was purified with His Trap Kit and green fluorescence was observed (Figure 1D); while nothing was observed with the control protein under fluorescent microscope (Figure 1C). These results indicated that FT:eGFP could be expressed in prokaryotic cells.

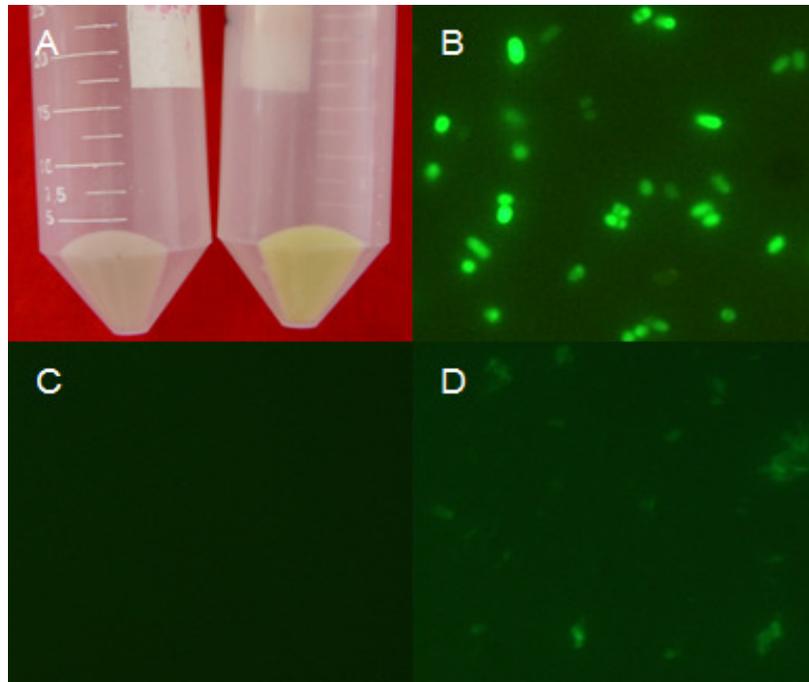
### FT:eGFP promoted lily flowering

*Agrobacterium GV3101* harboring pGR106-FT was injected into lily leaves. Two months later, the injected lily (marked with FT:eGFP) showed early flowering, while the control plants (marked with CK) and the plants injected with water (marked with H<sub>2</sub>O) showed no early flowering (Figure 2A) at the same time. The repeated experiments showed similar results. Only the flowering lily plants showed bolting but the lily plants without flowering did not bolt and had only basal leaves. The lily plants injected with *Agrobacterium GV3101* harboring pGR106-FT flowered over 30 days ahead of time when compared with the control plants. The leaves of the injected lily and control plants were observed under fluorescent microscope (Figure 2). Green fluorescence was observed in the injected lily (Figure 2B), and nothing was observed in the control plants (Figure 2C). Furthermore, leaves from different parts of the injected lily were also picked and observed under fluorescent microscope. The results showed that gradual decrease in expression of green fluorescence was detected from old leaves to young leaves.

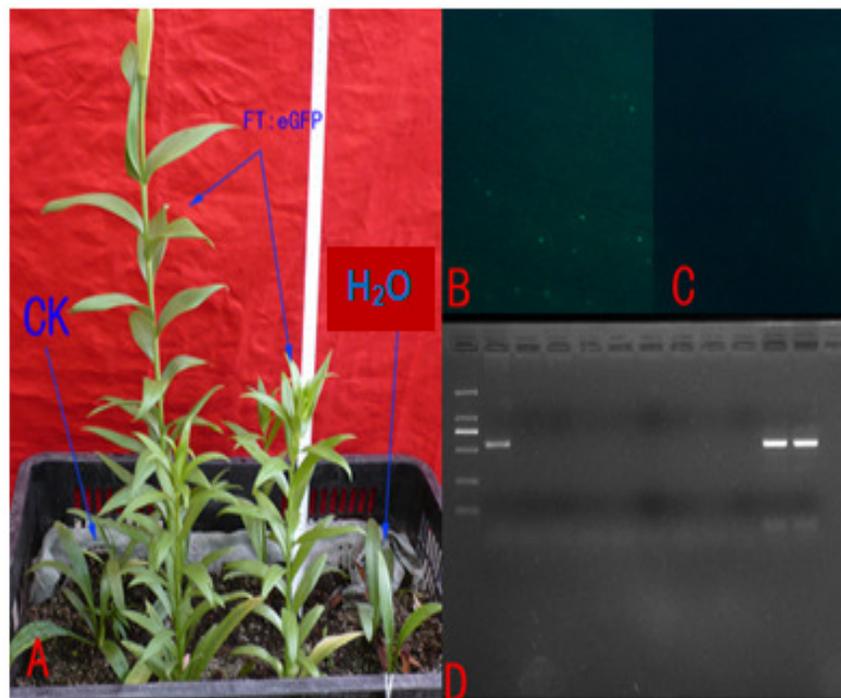
PCR analysis was conducted to detect FT:eGFP DNA fragment (Figure 2D). The expected size of 560 bp was only amplified with the positive control and injected lily. This indicated that virus harboring FT:eGFP was replicated in the host plants, and FT:eGFP DNA fragment was also replicated and expressed in the host plants.

Further detection of FT:eGFP fuse protein was carried out under confocal microscope (Figure 3). FT:eGFP fuse protein expression in stomatic cells in the injected lily leaves was obviously seen. This might be due to the movement of FT:eGFP fuse protein along the plant transport system.

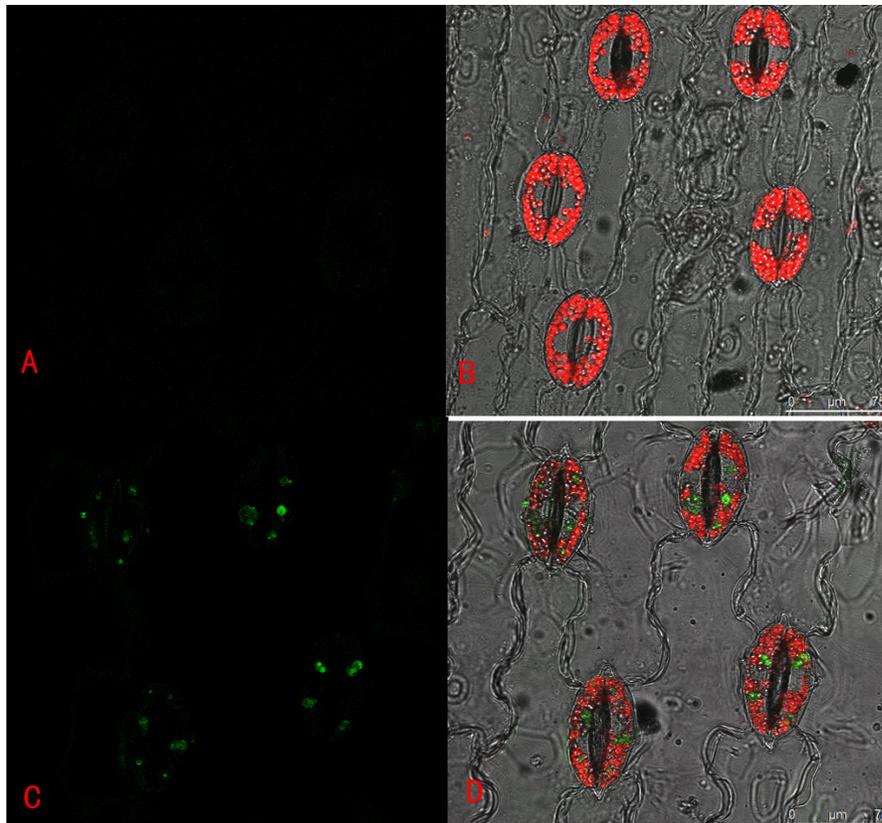
It has been reported that FT protein was expressed via viral vector and successfully promoted plant flowering in tobacco and cucurbits. In this work, similar research was also carried out to promote lily flowering. The pET-30a-FT vector was constructed and FT:eGFP fuse protein was purified. Purified fuse protein was also used in promoting flowering experiment via direct injection. However, the injected lily did not show early flowering. This might be due to the following reasons: (1) The dysfunction of purified fuse protein during the injection process and (2) the degradation of the purified protein injected into the lily plants. FT is a long distance mobile signal-florigen, and is



**Figure 1.** The observation of the recombinant *E. coli* and the fuse protein under the microscope. A, Under natural light, the collection of bacteria of the recombinant *E. coli* (right) and the negative control (left) after 6 h IPTG induction; B, the recombinant *E. coli* under fluorescent light after 6 h IPTG induction; C, the purified protein of negative control under fluorescent light; D, the purified FT:eGFP fuse protein under fluorescent light.



**Figure 2.** FT:eGFP promoted lily flowering via viral vector. A, FT:eGFP promoted injected lily flowering; B, leaves of injected lily under fluorescent microscope; C, leaves of control lily under fluorescent microscope; D, PCR analysis of the lilies.



**Figure 3.** FT:eGFP detection under confocal microscope. A and B, detection of FT:eGFP of control lily leaves; C and D: detection of FT:eGFP of injected lily leaves.

not only the injected leaves that showed green fluorescence, but also, the uninjected leaves from the injected lily plants. This indicated that FT:eGFP fuse protein can be moved from leaves to leaves. The reason is not too clear, because virus replication and immigration can also cause it. Further research should be done to resolve it.

Since the time for most lily varieties from seeds to flowering is long, this research might be helpful for lily breeders and producers.

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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 path-way integrator FT at the shoot apex. *Sci.*, 309: 1052-1056.

Avesani L, Falorni A, Tornielli GB, Marusic C, Porceddu A, Polverari A, Faleri C, Calcinaro F, Pezzotti M (2003). Improved in planta expression of human islet autoantigen glutamic acid decarboxylase (GAD65). *Transgenic Res.*, 12: 203-212.

Corbesier L, Vincent C, Jang S, Formara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, Coupland G (2007). FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science*, 316: 1030-1033.

Lin M, Belanger H, Lee Y, Varkonyi-Gasic E, Taoka K, Miura E, Xoonostle-Cazares B, Gendler K, Jorgensen RA, Phineey B, Lough TJ, Lucas WJ (2007). FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the cucurbits. *Plant Cell.*, 19: 1488-1506.

Takashi N, Yoshiko A, Yuko K, Akiko K, Norimoto S, Masahiro N (2009). Over-expression of *Arabidopsis* FT gene reduces juvenile phase and induces early flowering in ornamental gentian plants. *Euphytica*, 168: 113-119.

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.