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Short communication

# Analysis of promoter activity reveals that *GmFTL2* expression differs from that of the known *Flowering Locus T* genes in soybean☆

*Limin Liu*[a,1], *Xiaomei Zhang*[b,1], *Fulu Chen*[b], *Asia Adam Elzamzami Mahi*[a], *Xiaoxia Wu*[a,*], *Qingshan Chen*[a,*], *Yong-Fu Fu*[b,*]

[a]*College of Agriculture, Northeast Agricultural University, Harbin 150030, China*
[b]*MOA Key Lab of Soybean Biology (Beijing), National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China*

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ABSTRACT

Regulation of flowering is one of the key issues in crop yield. The *Flowering Locus T* (*FT*) gene is a well-known florigen, which integrates various signals from multiple flowering-regulation pathways to initiate flowering. We previously reported that there are at least six *FT* genes (*GmFTL1–6*) in soybean displaying flowering activity. However, the individual functions of genes *GmFTL1–6* remain to be identified. In this study, we cloned the *GmFTL2* promoter (*GmFTL*$^{pro}$) from soybean (*Glycine max*) cultivar Tianlong 1 and analyzed its motifs bioinformatically and its expression patterns using both a transgenic approach and quantitative RT-PCR (qRT-PCR). In *GmFTL*$^{pro}$::*GUS* transgenic lines, GUS signals were enriched in cotyledons, hypocotyledons, pollen, embryos, and root tips in a photoperiod-independent manner. qRT-PCR confirmed the GUS reporter results. Our results suggest that *GmFTL2* expression is regulated by developmental and tissue-specific clues and plays roles in seedling establishment and the development of microgametophytes, embryos, and roots.



## 1. Introduction

In a long evolutionary process, the plant genome has expanded by genomic duplication, tandem duplication, and other events [1]. As a result, daughter genes have been forced to evolve by neofunctionalization, subfunctionalization, or functional redundancy, for survival by natural selection [1,2]. For this reason, homologs in a modern species may have totally different functions from their ancestors, and a gene displays reduced pleiotropic effects compared to its ancestral gene [3–6].

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* *Corresponding authors.*
E-mail addresses: Xxwu2012@163.com (X. Wu), qschen@neau.edu.cn (Q. Chen), fuyongfu@caas.cn (Y.-F. Fu).

[1] These authors contributed equally to this work.

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*FLOWERING LOCUS T* (*FT*) is a key florigen that is produced in leaves and then moves to meristems [7,8] to promote flowering in all plant species studied [9,10]. However, its homologs may play distinct roles depending on environmental condition or organ/tissue [11]. In *Arabidopsis*, *FT* induces the transition of meristems from vegetative to reproductive growth [7], but controls opening of stomata in the leaf epidermis [12]. In rice (*Oryza sativa* L.), one *FT* homolog, *Hd3a*, enhances flowering under long-day conditions, whereas another *FT* homolog *RFT* promotes flowering under short-day conditions [8,13,14]. Of two *FT*-like genes in poplar (*Populus deltoides* L.), one induces flower formation and the other is associated with bud dormancy [15,16]. Potato (*Solanum tuberosum* L.) *FT* homolog genes also have distinct functions: *StSP3D* has high flowering activity and *StSP6A* promotes tuber formation [17].

*AtFT* is expressed in nearly all organs during the *Arabidopsis* life cycle, from seed germination to silique maturation. *AtFT* is photoperiodically [7,18] and developmentally [19] regulated in leaves for flowering regulation. After flowering, *AtFT* is expressed at much higher levels in cauline leaves, sepals, petals, and developing siliques [20]. In these organs, *AtFT* expression is independent of photoperiod [21] and regulated by distal putative enhancers in the *AtFT* promoter [22].

In the soybean genome there are at least six *FT*-like genes (*GmFTL1–6*), whose overexpression results in early-flowering phenotypes in *Arabidopsis* [23,24]. However only two *FT*-like genes (*GmFTL3* and *GmFTL5*) show potential *FT* functions of flowering regulation [25,26], whereas *E10* (*GmFT4*) shows inhibition of flowering in soybean (*Glycine max*) [27,28]. The function of other *FT*-like genes in soybean remains unknown. In this study, we cloned the *GmFTL2* promoter, of around 10 kb in length, from the soybean genome and analyzed its activity in *Arabidopsis* using β-glucuronidase (GUS) as a reporter. Our results showed that the activity of the *GmFTL2* promoter was regulated by developmental clues as well as tissue/organ-specific signals and independent of photoperiods. GUS signals in transgenic plants were observed in young seedlings, stamens (pollen), pistils (embryos), and root tips. Thus, *GmFTL2* has a different expression pattern from that of *GmFTL3* and *GmFTL5*.

## 2. Materials and methods

### 2.1. *GmFTL2* promoter cloning and expression vector construction

According to the Phytozome database (https://phytozome.jgi.doe.gov/pz/portal.html), primers were designed (Table 1) to amplify the *GmFTL2* promoter (*GmFTL2$^{pro}$*) sequence. DNA was extracted from Tianlong 1 soybean using a DNA extraction kit (TransGen Biotech). PCR was performed according to a standard protocol. PCR products were recovered from 1.0% agarose gel (Fig. 1) and cloned into the Fu76 entry vector [29]. The ligated products were transformed into *Escherichia coli* DH5α and positive clones were screened out by PCR and sequenced. The right entry vector (Fu76-*GmFTL2$^{pro}$*) combined with another entry vector, Fu79-GUS, and the binary vector Fu39-2 [29] were subjected to an LR reaction (Invitrogen) to prepare the Fu39-2-*GmFTL2$^{pro}$*::*GUS* expression vector, which was used to transform *Arabidopsis* by floral dipping with *Agrobacterium* EHA105 [30].

### 2.2. Bioinformatic analysis of *GmFTL2$^{Pro}$* sequence

PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to identify potential motifs in the *GmFTL2$^{Pro}$* and *AtFT$^{pro}$* sequences.

### 2.3. GUS histochemical staining analysis

The Fu39–2-*GmFTL2$^{pro}$*::*GUS* expression vector was transformed into *Arabidopsis* wild type Col-0, yielding 32 T1 transgenic lines, of which five were selected for screening of homozygous lines. The homozygous transgenic seeds were sown on plates containing MS medium (Sigma) and grown under either long-day (16 h/8 h, light/dark) or short-day (8 h/16 h, light/dark) conditions, with 100 μmol $m^{-2}$ $s^{-1}$ light. GUS activity was detected following Xiao et al. [31]: samples were fixed in 90% acetone for 5 min, placed on ice for 20 min, washed in distilled water for 5 min, and then stained in X-Gluc (5-bromo-4-chloro-3-indolyl-beta-D-glucuronide) reaction liquid at 37 °C overnight. Samples were decolorized in

**Table 1 – Primers used in this study.**

| Primer | Sequence (5′–3′) | Annotation |
|---|---|---|
| pGmFTL2-1-F | GAGAGGTCGACCATGTAAAACCTTCATTGTACTTCT | Promoter cloning |
| pGmFTL2-1-R | AAGCCGCCAACGTTATAT | Promoter cloning |
| pGmFTL2-2-F | ACAATGCATCAATATATATAACGTT | Promoter cloning |
| pGmFTL2-2-R | TCTAGAAATAAAAATGAAAAAAATA | Promoter cloning |
| pGmFTL2-3-F | TAAGGGAGCCAAAATCCAAACAATA | Promoter cloning |
| pGmFTL2-3-R | TTTGTAAGTCCACCTGAACCTCCAC | Promoter cloning |
| pGmFTL2-4-F | TCAGCTAGTGCCCGTTACAG | Promoter cloning |
| pGmFTL2-4-R | CTAGTGTTTTCTCCCTTC | Promoter cloning |
| pGmFTL2-5-F | GTCTAAGTCGATCACCT | Promoter cloning |
| pGmFTL2-5-R | TCTATGCGCAAACTAACTCACACACTTC | Promoter cloning |
| qGmFTL2-U5F | ATCTCTTCTGTTAATGTACCAAAAGTG | qRT-PCR |
| qGmFTL2-U110R | GTGACCCATGAGTGTTATTATATGTAG | qRT-PCR |
| ACT11-F | ATCTTGACTGAGCGTGGTTATTCC | qRT-PCR |
| ACT11-R | GCTGGTCCTGGCTGTCTCC | qRT-PCR |

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