Supplementary Information

## Molecular characterization of a soybean FT homologue, GmFT7

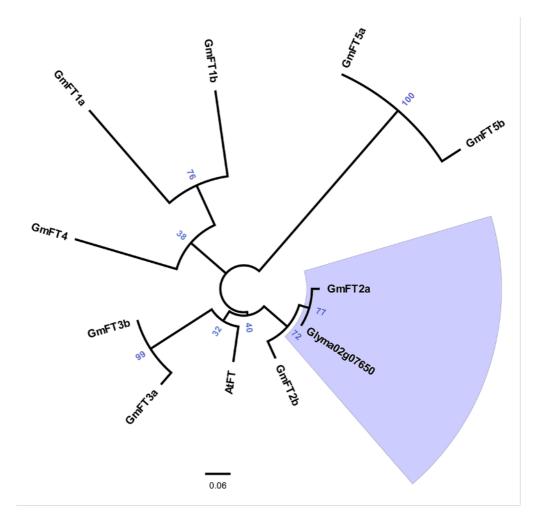
Senhao Zhang<sup>1</sup>, Mohan B. Singh<sup>1</sup> and Prem L. Bhalla<sup>1\*</sup>

 Plant Molecular Biology and Biotechnology Laboratory, School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria 3010, Australia.
\*Corresponding author: Prem L. Bhalla (premlb@unimelb.edu.au)

GmFT7	1		0
GsFT2c	1	MPSGSRDPLVVGRVIGDVLDPFECSIPMRVSYNNRDVSNGCEFKPSQVVN	50
GmFT7	1		0
GsFT2c	51	QPRINIGGDDLRNFYTLIAVDPDAPSPSDPNLREYLHWLVTDIPATTGPS	100
GmFT7	1	MMGIHRLVFVLFRQLGRETVYAPGWRQNFNTREFAEL	37
GsFT2c	101	FGHEVVTYESPRPMMGIHRLVFVLFRQLGRETVYAPGWRQNFNTREFAEL	150
GmFT7	38	YNLGLPVAAVYFNIQRESGSGGRRLYH 64	
GsFT2c	151	YNLGLPVAAVYFNIQRESGSGGRRLYH 177	

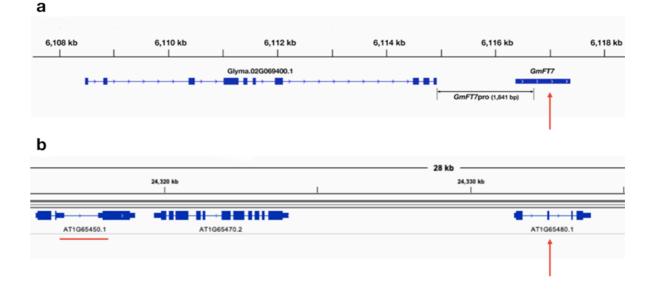
### Supplementary Figure 1. Pairwise protein sequence alignment of GmFT7 and GsFT2c.

Protein sequences of soybean (*Glycine max*) GmFT7 (Glyma02g07650) and wild soybean (*Glycine soja*) GsFT2c (XP\_028198760) were retrieved from Phytozome (v12; https://phytozome.jgi.doe.gov/pz/portal.html#) and NCBI, respectively. Pairwise protein sequence alignment was performed using Needle (https://www.ebi.ac.uk/Tools/psa/emboss\_needle/) with default parameters.



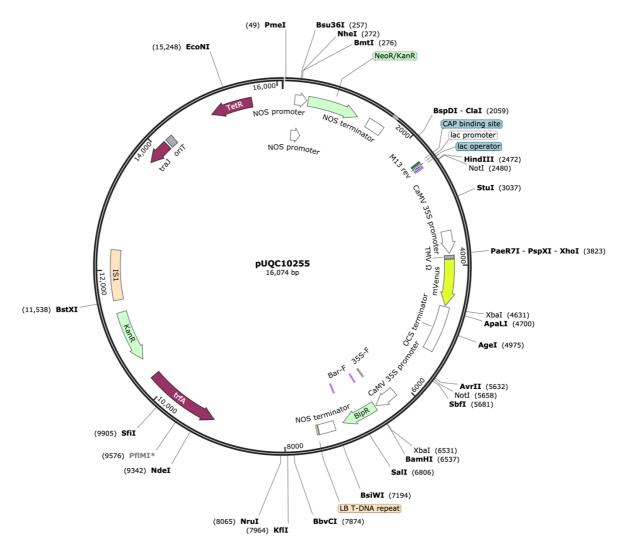
## Supplementary Figure 2. Phylogenetic tree of FT homologues.

Protein sequences of ten GmFT homologues and Arabidopsis FT were retrieved from Phytozome (v12; https://phytozome.jgi.doe.gov/pz/portal.html#). Maximum Likelihood tree was constructed using MEGA7.



# Supplementary Figure 3. Locations showing distances with the upstream genes of soybean GmFT7 (a) and Arabidopsis FT (b) on their corresponding chromosomes.

1.8 kb promoter for *GmFT7* was chosen because there is another gene (*Glyma.02G069400.1*) located upstream of *GmFT7* (a). From the end of *Glyma.02G069400.1* to the ATG of *GmFT7*, the length is 1841 bp. Hence, the promoter of *GmFT7* was cloned as 1841 bp. On the other hand, Arabidopsis, *AtFT* (*AT1G65480*, + strand) is located downstream of gene *AT1G65450* (+strand). The distance between *AtFT* and its closest upstream gene *AT1G65450* is more than 10 kb (b). Locations on chromosomes were displayed using Integrative Genomics Viewer (IGV) (http://software.broadinstitute.org/software/igv/home).



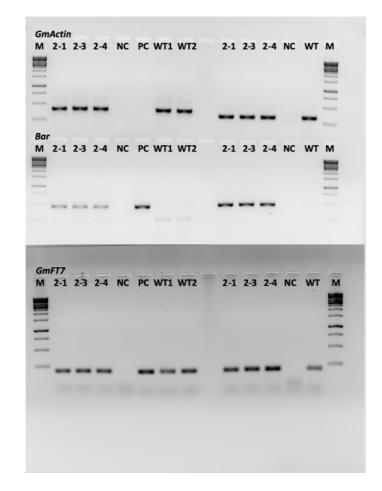
#### Supplementary Figure 4. Map of the expression vector pUQC10255 used in this study.

The map of the expression vector pUQC10255 is displayed using SnapGene Viewer 5.2.3 (https://www.snapgene.com/snapgene-viewer/). Main features of pUQC10255 are labelled accordingly.

Leaf-SD0 GmAct M 0 4 8 12 16 20	GmFT7. M 0 4 8 12 16 20 M	Leaf-SD2 GmAct GmFT7 M 0 4 8 12 16 20 M 0 4 8 12 16 20
=		
Leaf-SD1 GmAct M 0 4 8 12 16 20	GmFT7. M 0 4 8 12 16 20 N	Leaf-SD3 GmAct GmFT7 M 0 4 8 12 16 20 M 0 4 8 12 16 20
Leaf-SD4 GmAct M 0 4 8 12 16 20	GmFT7.	SA-SD1 GmAct GmFT7 M 0 4 8 12 16 20 M 0 4 8 12 16 20
	GmFT7. M 0 4 8 12 16 20	SA-SD2 GmAct GmFT7 M 0 4 8 12 16 20 M 0 4 8 12 16 20
SA-SD3 GmAct	 GmFT7	
M 0 4 8 12 16 20		
SA-SD4 GmAct M 0 4 8 12 16 20	GmFT7 M 0 4 8 12 16 20	

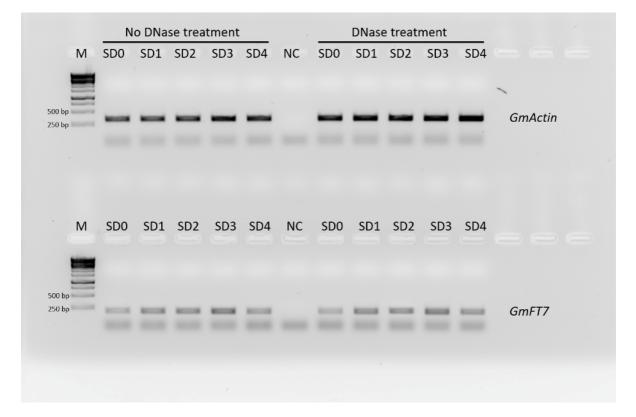
## Supplementary Figure 5. Original gel electrophoresis pictures for Fig. 5.

M, GeneRuler 1kb DNA Ladder (Thermo Fisher, Cat. Number: SM0311). SA, shoot apex. SD, short day. *GmAct*, soybean reference gene *Actin*.



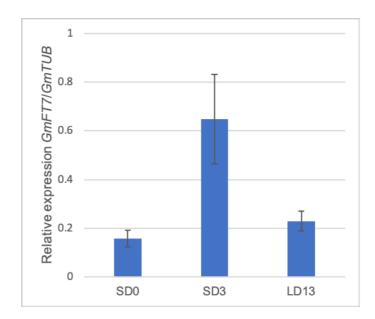
# Supplementary Figure 6. Genomic PCR (a) and RT-PCR (b) to confirm transgenic status of soybean plants.

This is the original gel picture for Fig. 8a/b in the main manuscript. Transgenic soybean plants over-expressing *GmFT7* in soybean cv. Bragg were used.Genomic PCR (upper panel) confirmed the presence of *Bar* gene while RT-PCR (lower panel) analysis showed the expression of the *Bar* and *GmFT7* transcripts. Soybean reference gene *GmActin*, herbicide resistance *Bar* gene, and *GmFT7* CDS region. 2-1, 2-3 and 2-4, three individual T2 transgenic soybean plants. WT1, WT2 and WT, wild type soybean plants. NC, negative control (water was used as template for PCR). PC, plasmid control (*35S:GmFT7:polyA* cassette in vector pUQC10255) as a positive control. M, GeneRuler 1kb DNA Ladder (Thermo Fisher, Cat. Number: SM0311).



#### Supplementary Figure 7. Expression of *GmFT7* using PCR.

Total RNA was extracted using TRIzol and followed by "without DNase treatment" to compare the PCR results with previous PCR experiments we performed. M, GeneRuler 1kb DNA ladder (Thermo Fisher); SD0-SD4, 0 to 4-SD treatment. *GmActin*, reference gene for PCR. NC, negative control. DNase treatment, total RNA was treated using DNase to remove potential genomic DNA contamination (previous RT-PCR on *GmFT7* we performed); No DNase treatment, total RNA was not treated with DNase for potential genomic DNA removal.



#### Supplementary Figure 8. Relative expression of *GmFT7* under SD0, SD3 and LD13.

qPCR was performed to check *GmFT7* expression on SD3 and LD13. Bar chart was shown as mean and standard deviation with three replicates. *GmTUB* was used as a reference gene for normalization. Student's T-test was performed in R (https://www.r-project.org/) and it showed that the expression of *GmFT7* was higher under SD3 compared to LD13, however the difference was not significant (*p*-value = 0.2142). While expression of *GmFT7* was significantly (p < 0.05) higher under SD3 compared to SD0.

Name	Sequence (5' -> 3')	Length (nt)	Note	
GmFT7mRNA-F	I7mRNA-F CCGTCGTTAAAAGCACTTAATAACCG		GmFT7 full-length mRNA	
GmFT7mRNA-R	CCCCATAATTTAACTTGTGCTCAAG	25		
FT7-cloF	AATGCTCGAGATGATGGGGGATTCATCGTTTA		Cloning of GmFT7 CDS	
FT7-cloR	CGCGGATCCTCAATGGTATAACCTTCTTC	29		
GmFT7-1F	ATGATGGGGATTCATCGTTTAGTG	24	Amplification of <i>GmFT7</i> CDS; qPCR fpr <i>GmFT7</i>	
GmFT7-1R	TCAATGGTATAACCTTCTTCCACC	24		
GmActin-F	ATCATGTTTGAGACCTTCAATGTG	24	Reference gene GmActin	
GmActin-F	CTCGAGTTCTTGCTCATAATCTAGG	-		
BAR-1F	GTACCGGCAGGCTGAAGTCC	20	Bar gene	
BAR-1R	CGGTCTGCACCATCGTCAAC	20	-	
AT4G34270-F	GTGAAAACTGTTGGAGAGAAGCAA	24	qPCR reference gene for	
AT4G34270-R	TCAACTGGATACCCTTTCGCA	21	Arabidopsis	
GmTUB-q-F	GAGAAGAGTATCCGGATAGG	20	qPCR reference gene for	
GmTUB-q-R	GAGCTTGAGTGTTCGGAAAC	20	Soybean	
GmFT2a-q-F	GGATTGCCAGTTGCTGCTGT	ATTGCCAGTTGCTGCTGT 20 qP		
GmFT2a-q-R	GAGTGTGGGAGATTGCCAAT	20	-	
GmFT5a-q-F	GCCTTACTCCAGCTTATACT 20		qPCR	
GmFT5a-q-R	GGCATGCTCTAGCATTGCAA	20	-	
GmAP1-q-F	TGAACATGGGTGGCAATTAC	20	qPCR	
GmAP1-q-R	TGTCAAATGCCATACCAAAG	20	-	
GmSOC1b-q-F	AAGAAGCCCAACTGCAATGT	20	qPCR	
GmSOC1b-q-R	GGGCTTCAGAAATGAGGAAAGG	22	1	
GmLFY2-q-F	TGACGAAGGAAACATTAACACTGG 24		qPCR	
GmLFY2-q-R	GCCTGAACCTGCATCAAGAA	20	1	

## Supplementary Table 1. Primers used in this study.

Primers for *GmFT7* in genomic PCR and RT-PCR (Fig. 8) were GmFT7-1F/ GmFT7-1R to amplify *GmFT7* CDS.