Supplemental Data

Short Title: Virus-induced Flowering (VIF) Assay

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Primary Research Area: Flowering and Reproduction/Breakthrough Technologies

2nd Research Area: Genes and Development

Article Title: # A Virus-induced Flowering Assay for Functional Dissection and Analysis of Monocot and Dicot *FT* genes[1]

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[2]These authors contributed equally to this work.

Author Contributions: C.Q. and W.C. designed and performed experiments; J-J.S., L.C., F.A., K.Z., C.Y., C.L. and P.Z. performed research; C.Q., N.S., Q.C., Y.L. and S.J.

were involved in the analysis of data and helped writing the paper. S.J. was also involved in designing experiments. Y.H. conceived and initiated the project, designed experiments, analysed data and wrote the paper.

One-sentence Summary: A PVX-based VIF assay was designed in order to identify amino acids in the FT protein that are essential for flowering, and to examine floral induction by mono- and dicotyledonous *FT* genes.

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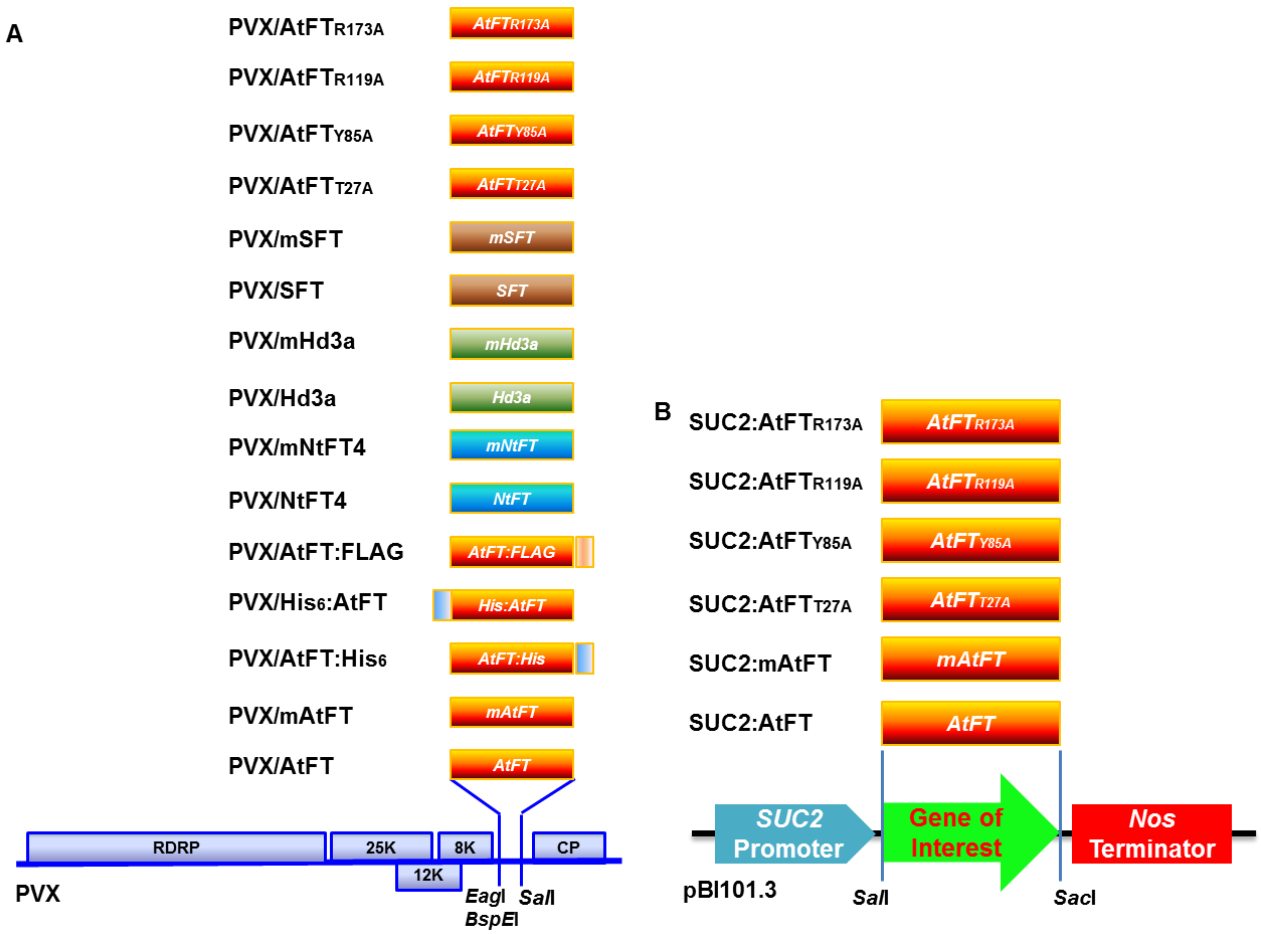
Supplemental Figure



Supplemental Figure S1. Constructs used in this study. A, Diagrammatic of Potato virus X (PVX)-based ViFA vectors. Wild-type, mutated non-translatable (m) and single amino acid substituted Arabidopsis *AtFT* coding sequences were cloned into the PVX vector to produce each of the ViFA vector as indicated. B, Plant transformation vector for wild-type and mutant AtFT alleles as indicated. The genome organization of PVX (A) and the outline of the binary vector pBI101.3 (B) are indicated. Restriction enzyme sites used to clone relevant genes into the two vectors are also indicated.

Supplemental Tables

Supplemental Table S1. Oligonucleotide primers used in this study.

Primer	Sequences (5' – 3')	Genes and origin*
RC0061	TCAAGA**TCCGGA**ATGTCTATAAATATAAGAGAC	*AtFT*, *Bsp*EI
RC0062	GAAGAA**GTCGAC**CTAAAGTCTTCTTCCTCCGCA	*AtFT*, *Sal*I
RC0066	TCAAGA**TCCGGA**TAGTCTTAAAATATAAGAGAC	*AtFT*, *Bsp*EI
RC0536	TCAAGA**CGGCCG**ATGGCCGGAAGTGGCAGGGACAG	Rice *Hd3a*, *Eag*I
RC0537	GAAGAA**GTCGAC**CTAGGGGTAGACCCTCCTGCCGCC	Rice *Hd3a*, *Sal*I
RC0538	TCAAGA**CGGCCG**TAGGCCGGAAGTGGCAGGGACAG	Rice *Hd3a*, *Eag*I
RC0541	TCAAGA**CGGCCG**ATGCCTAGAGAACGTGATCCTCTT	Tomato *SFT*, *Eag*I
RC0542	GAAGAA**GTCGAC**TCAATCAGCAGATCTTCTACGTCC	Tomato *SFT*, *Sal*I
RC0543	TCAAGA**CGGCCG**TAGCCTAGAGAACGTGATCCTCTT	Tomato *SFT*, *Eag*I
RC0779	TCAAGA**CGGCCG**ATGCCAAGAATAGATCCTTTGATA	MM *NtFT4*, *Eag*I
RC0780	GAAGAA**GTCGAC**TTAATATGCGCGGCGGC	MM *NtFT4*, *Sal*I
RC1639	TCAAGA**CGGCCG**TAGCCAAGAATAGATCCTTTGATA	MM *NtFT4*, *Eag*I
RC0693	ATAAGTAACCTTTAGAGCGATTGATCTATTAAA	*AtFT*$_{T27A}$
RC0694	TTTAATAGATCAATCGCTCTAAAGGTTACTTAT	*AtFT*$_{T27A}$
RC0695	CACCAACCAATGGAGAGCTTCTCGGAGGTGAGG	*AtFT*$_{Y85A}$
RC0696	CCTCACCTCCGAGAAGCTCTCCATTGGTTGGTG	*AtFT*$_{Y85A}$
RC0699	CAATATAAACACGACAGCATGAATTCCTGCAGT	*AtFT*$_{R119A}$
RC0700	ACTGCAGGAATTCATGCTGTCGTGTTTATATTG	*AtFT*$_{R119A}$
RC0701	GAAGAA**GTCGAC**CTAAAGTCTTGCTCCTCCGCA	*AtFT*$_{R173A}$, *Sal*I
RC0783	TCAAGA**GTCGAC**ATGTCTATAAATATAAGAGACCCT	*Sal*I
RC0784	GAAGAA**GAGCTC**CTAAAGTCTTCTTCCTCCGCAGCC	*Sac*I
RC0798	TCAAGA**GTCGAC**TAGTCTATAAATATAAGAGACCCT	*Sal*I
RC0799	GAAGAA**GAGCTC**CTAAAGTCTTGCTCCTCCGCAGCC	*Sac*I
RC1305	AAGCAACCCAAACCTGAGGGAGTATCTG	qRT-PCR *NtFT1*
RC1306	GCAGCAACAGGCGAATTGAGATTATGAAATCTC	qRT-PCR *NtFT1*

RC1307	AGATATCCCTGCAACCACAGAAGCAAC	qRT-PCR *NtFT2*
RC1308	AAACAGCGGCAACAGGCAAATTGAGAC	qRT-PCR *NtFT2*
RC1309	AATTGTCCACCAACCTAGGGTTGACGTG	qRT-PCR *NtFT3*
RC1310	CATTCACAACATCTCGAGTCAATTGTCGAAACAG	qRT-PCR *NtFT3*
RC1311	GATATCCCAGCAACTACAGATACAAG	qRT-PCR *NtFT4*
RC1312	GAAACGGGCAAACCAAGATTGTAAAC	qRT-PCR *NtFT4*
RC1313	TGAGATGCACCACGAAGCTC	qRT-PCR *NtEF1α*
RC1314	CCAACATTGTCACCAGGAAGTG	qRT-PCR *NtEF1α*
RC1317	AGGCCTTCTCAGGTTCAAAACAAGC	qRT-PCR *AtFT*
RC1318	TGCCAAAGGTTGTTCCAGTTGTAGC	qRT-PCR *AtFT*
PP82	CAGTGTTGGCTTGCAAACTAG	PVX
PP356	AGGAAGAA**GTCGAC**TAAAGTCTTCTTCCTCCGCAG	*AtFT*, *Sal*I
His-FTF	GGC**CGGCCG**ATGCATCATCACCATCACCACTCTATAAATATAAGA	*AtFT*, *Eag*I
His-FTR	GGC**GTCGAC**CTAAAGTCTTCTTCCTCCGCAG	*AtFT*, *Sal*I
FT-HisR	GCG**GTCGAC**CTAGTGGTGATGGTGATGATGAAGTCTTCTTCCTCCGC	*AtFT*, *Sal*I
FT-HisF	GGC**CGGCCG**ATGTCTAT AAATATAAGA	*AtFT*, *Eag*I
FT-FLAGR	GCG**GTCGAC**CTACTTGTCATCGTCATCCTTGTAGTCAAGTCTTCTTCCTCCGCAG	*AtFT*, *Sal*I
EFNBF	CTCCAAGGCTAGGTATGATG	RT-PCR *NtEF1α*
EFNBR	CTTCGTGGTTGCATCTCAAC	RT-PCR *NtEF1α*

[*]Each introduced restriction endonuclease site is highlighted in bold. Sense or non-sense mutated codons are underlined.

Supplemental Table S2. Flowering time in *Arabidopsis*.

Genotype	Average number of rosette leaves when flowering (n: plants)	Average number of cauline leaves when flowering (n: plants	Phenotype
Col (wild-type)	11.1 ±1.4 (n = 20)	2.9 ±1.2 (n = 20)	Normal flowering
ft-10 (*FT* mutant)	42.3 ±9.8 (n = 20)	7.8 ±1.9 (n = 20)	Late flowering
ft-10/SUC2:AtFT	5.3 ±0.8 (n = 19)	1.7 ±0.8 (n = 19)	Early flowering
ft-10/SUC2:mAtFT	40.2 ±5.5 (n = 20)	7.4 ±2.1 (n = 20)	Late flowering
ft-10/SUC2:FT$_{Y85A}$	30.0 ±9.3 (n = 18)	9.4 ±3.1 (n = 18)	Late flowering
ft-10/SUC2:FT$_{R119A}$	43.6 ±1.5 (n = 16)	6.6 ±0.6 (n = 16)	Late flowering
ft-10/SUC2:FT $_{R173A}$	35.0 ±2.5 (n = 20)	7.8 ±2.5 (n = 20)	Late flowering
ft-10/SUC2:FT$_{T27A}$	5.4 ±2.0 (n = 19)	3.7 ±2.5 (n = 19)	Early flowering