Title

CsFTL3, a chrysanthemum *FLOWERING LOCUS T-like* gene, is a key regulator of photoperiodic flowering in chrysanthemums

Running title

CsFTL3 induces flowering in chrysanthemums

Authors

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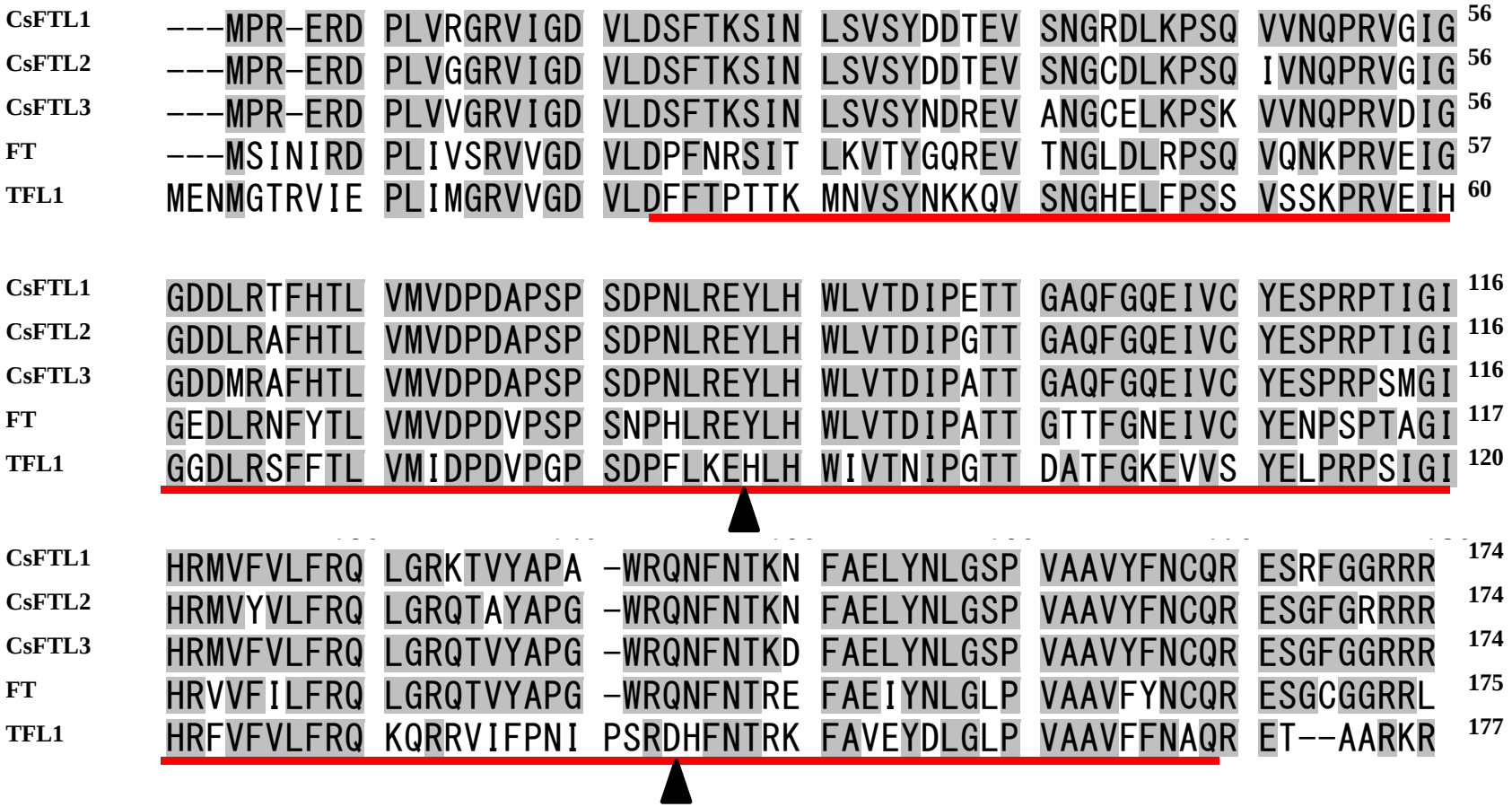


Figure S1. Comparison of deduced amino acid sequences of CsFTL1, CsFTL2, CsFTL3, FT, and TFL1. Identical residues of at least three residues are framed. The PEBP domain boundaries are marked by underlines. Arrow heads indicate the key amino acid residue contributing to FT and TFL functioning (Hanzawa et al., 2005).

Oda et al., Fig. S1

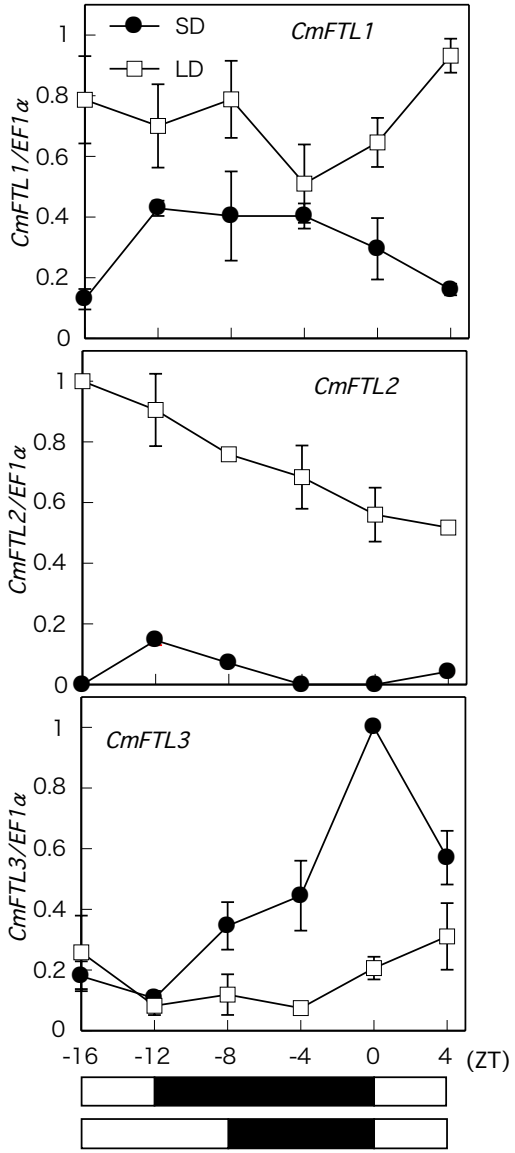
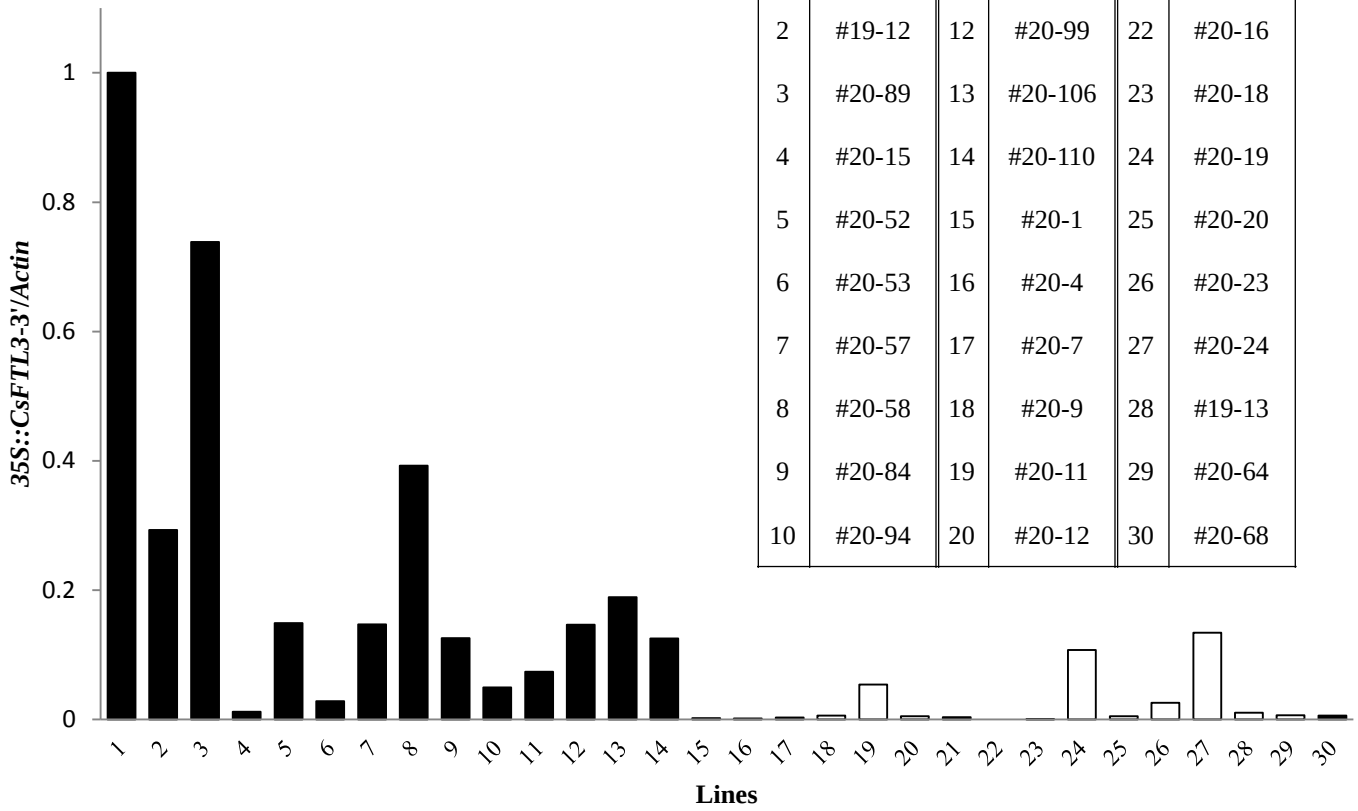


Figure S2. Expression of *CsFTL* family genes in leaves of chrysanthemum Reagan under SD and LD conditions.

The plants were grown under LD (16-h) conditions and transferred to SD (12-h; closed circle) conditions or kept in LD (opened circle) conditions. A week after transfer, leaves were harvested every 4 h and subjected to QRT-PCR analysis.



No.	Line name	No.	Line name	No.	Line name
1	#20-66	11	#20-98	21	#20-13
2	#19-12	12	#20-99	22	#20-16
3	#20-89	13	#20-106	23	#20-18
4	#20-15	14	#20-110	24	#20-19
5	#20-52	15	#20-1	25	#20-20
6	#20-53	16	#20-4	26	#20-23
7	#20-57	17	#20-7	27	#20-24
8	#20-58	18	#20-9	28	#19-13
9	#20-84	19	#20-11	29	#20-64
10	#20-94	20	#20-12	30	#20-68

Figure S3. Relative expression levels of exogenous *CsFTL3* mRNA in *in vitro* transgenic (35S::*CsFTL3*) chrysanthemum Jimba plants.

The plants of 14 transgenic lines had flower buds (black), whereas the plants of the other 16 lines (white) had no flower buds under *in vitro* conditions.

Oda et al., Fig. S3

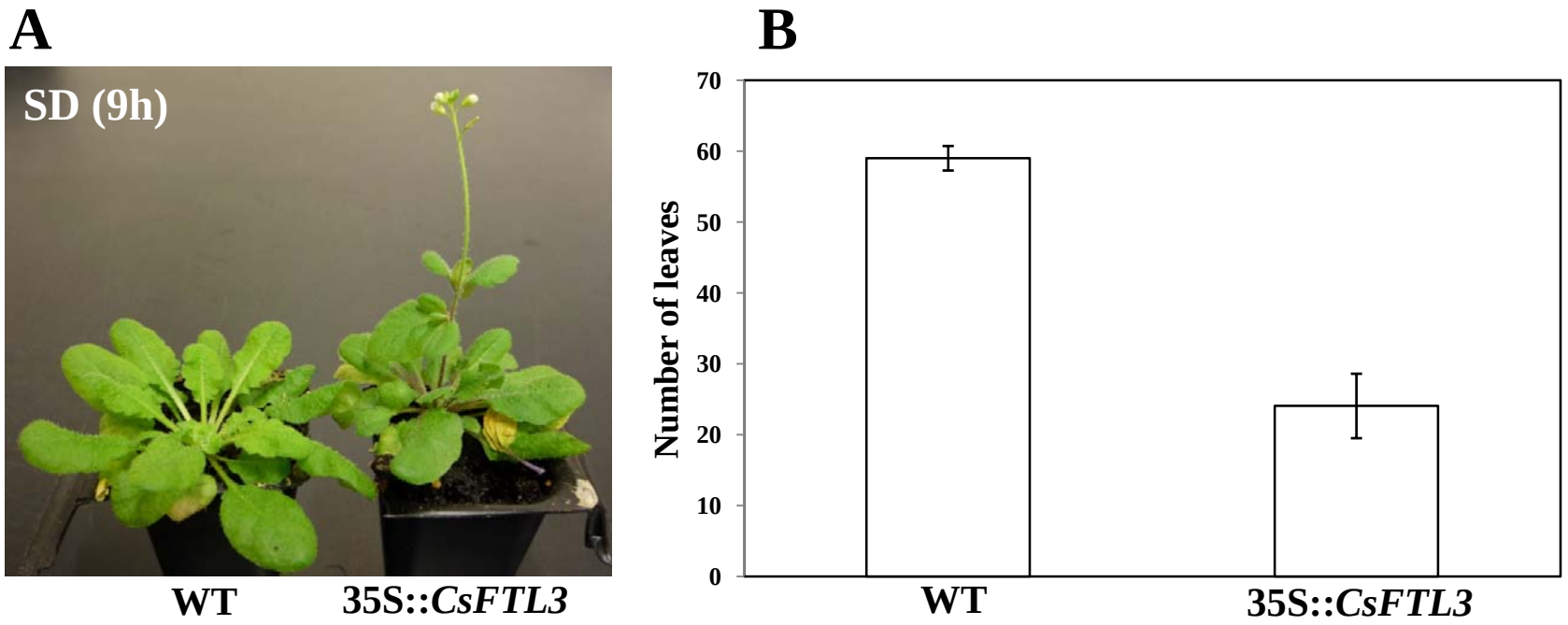


Figure S4 The effect of *CsFTL3* overexpression on flowering in *Arabidopsis* under SD conditions.
(A) Early flowering phenotype of *CsFTL3-ox Arabidopsis* plants under SD conditions.
(B) Total number of leaves at flowering in the T_3 population.

Oda et al., Figure S4

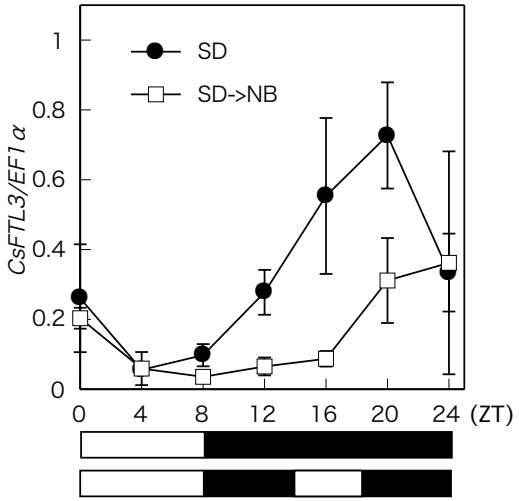


Figure S5. Transfer from SD to NB conditions down-regulates the expression of *CsFTL3* in *C. seticuspe*.

The plants exposed to four 8-h SD cycles were moved to NB conditions (SD plus 4-h NB; opened circle) or kept under SD (closed circle). Transcription levels were monitored from the beginning of second NB cycles over the subsequent 24 h. Leaves were harvested every 4 h and subjected to QRT-PCR analysis. The values were normalised by *CsEF1±*.

Table S1. Primers sequences and PCR conditions used in the study

Gene	Forward primer	Reverse primer	Detection temperature (℃)	PCR conditions
CsFTL1	AATCGTGTGCTATGAGAGCC	GCTTGTAACGTCCTCTTCATGC	82	A
CsFTL2	GAAAGCACGCATATCATAC	CATCGATCAAACACGTACAGTA	72	B
CsFTL3	GGGAAAGTGGATTTGGTGGACG	GTCTTACAATTTGGTACTGTCG	77	C
FTL3	TCCAAGTCCTAGTGACCCTAAC	TATCGGTAACCAACCAGTGT	72	B
CsFTL3 transgene 3'	GGGAAAGTGGATTTGGTGGACG	AATTCGAGCTCTAAGCGCTG	72	B
CsFTL3 transgene 5'	GTACAAAAAAGCAGGCTCC	CTAGGACTTGGAGCATCAGG	72	B
CsAFL1	CAAGCTCAACCATCAATAGTC	TGCAGCACATGAACGAGTAG	72	B
CsFL	CATTGATGCCATATTTAACTC	ACACGGATCATTCATTGTATA	72	B
CsM111	GGTCTCAAGAATATTCGCAC	TCATTAGTCATCCCATCAGC	72	B
CsACTIN	GATGACGCAGATCATGTTCG	AGCATGTGGAAGTGCATACC	72	B
CsEF1α	CTTGTTGCTTGATGACTGTGG	ACCATTCAAGCGACAGACTC	72	B

A) PCR was performed with an initial denaturing step at 95 ℃ for 20 s, followed by 40 cycles at 95 ℃ for 5 s, 60 ℃ for 20 s, 72 ℃ for 15 s, and 82 ℃ for 2 s. Fluorescence was quantified after the incubation at 82℃.

B) PCR was performed with an initial denaturing step at 95 ℃ for 20 s, followed by 40 cycles at 95 ℃ for 5 s, 60 ℃ for 20 s, 72 ℃ for 15 s. Fluorescence was quantified after the incubation at 72℃.

C) PCR was performed with an initial denaturing step at 95 ℃ for 20 s, followed by 40 cycles at 95 ℃ for 5 s, 60 ℃ for 20 s, 72 ℃ for 15 s, and 77 ℃ for 2 s. Fluorescence was quantified after the incubation at 77℃.

Table S2. The effect of de-foliation on the transition from vegetative to reproductive phase in grafting plants between wild type and 35S::*CsFTL3* transgenic plants

	De-foliation		No	Yes
	Stock	Scion		
Expt.1	35S::*CsFTL*	WT	1/3	3/3
	WT	WT	0/4	0/5
Expt.2	35S::*CsFTL*	WT	-	3/3
	WT	WT	-	0/3

The values indicate 'number of plants with phase transition/total number of plants tested'.
Wild-type Jimba plants (for experiment 1) and Nagano-queen plants (for experiment 2) were used as scion, respectively.