The FLOWERING LOCUS T LIKE 2-1 gene of Chenopodium triggers precocious flowering in Arabidopsis seedlings

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Abstract

The *FLOWERING LOCUS T* (*FT)* gene is the essential integrator of flowering regulatory pathways in angiosperms. The paralogs of the *FT* gene may perform antagonistic functions, as exemplified by *BvFT1*, that suppresses flowering in *Beta vulgaris*, unlike the paralogous activator *BvFT2*. The roles of *FT* genes in other amaranths were less investigated. Here, we transformed *Arabidopsis thaliana* with the *FLOWERING LOCUS T like* (*FTL*) genes of *Chenopodium ficifolium* and found, that both *CfFTL1* and *CfFTL2-1* accelerated flowering, despite having been the homologs of the *Beta vulgaris* floral promoter and suppressor, respectively. The floral promotive effect of *CfFTL2-1* was so strong that it caused lethality when overexpressed under the *35S* promoter. *CfFTL2-1* placed in inducible cassette accelerated flowering after the induction with methoxyphenozide. The spontaneous induction of *CfFTL2-1* led to precocious flowering in some primary transformants even without chemical induction. The *CqFT2-1* homolog from *Chenopodium quinoa* had the same impact on viability and flowering as *CfFTL2-1*, when transferred to *A. thaliana*. After the *FTL* gene duplication in Amaranthaceae, the *FTL1* copy maintained the role of floral activator. The second copy *FTL2* underwent subsequent duplication and functional diversification, which enabled to control the onset of flowering in amaranths to adapt to variable environments.

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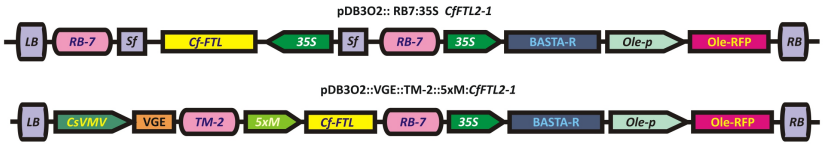

Figure 1. Schematic representation of T-DNA constructs used for the transformation of Arabidopsis. *LB*, *RB* – left and right T-DNA borders respectively; *RB-7*, *TM-2* –matrix attachment regions from tobacco; Sf- short stuffer fragment 35 bp, *Cf-FTL – C. ficifolium FTL* ORF, , *35S* – Cauliflower mosaic virus 35S promotor; BASTA-R phosphinothricin N-acetyltransferase gene conferring tolerance to Basta herbicide; *Ole-p* - oleosin promotor from Arabidopsis, *Ole-RFP* – gene for RFP reporter protein fused to Arabidopsis oleosin; *CsVMV* promotor from Cassava vein mosaic virus; VGE - chimeric transcription factor VGE reactive to methoxyfenozide, 5xM – minimal 35S promoter fused with 5 copies of Gal4 binding domain. Not drawn to scale.

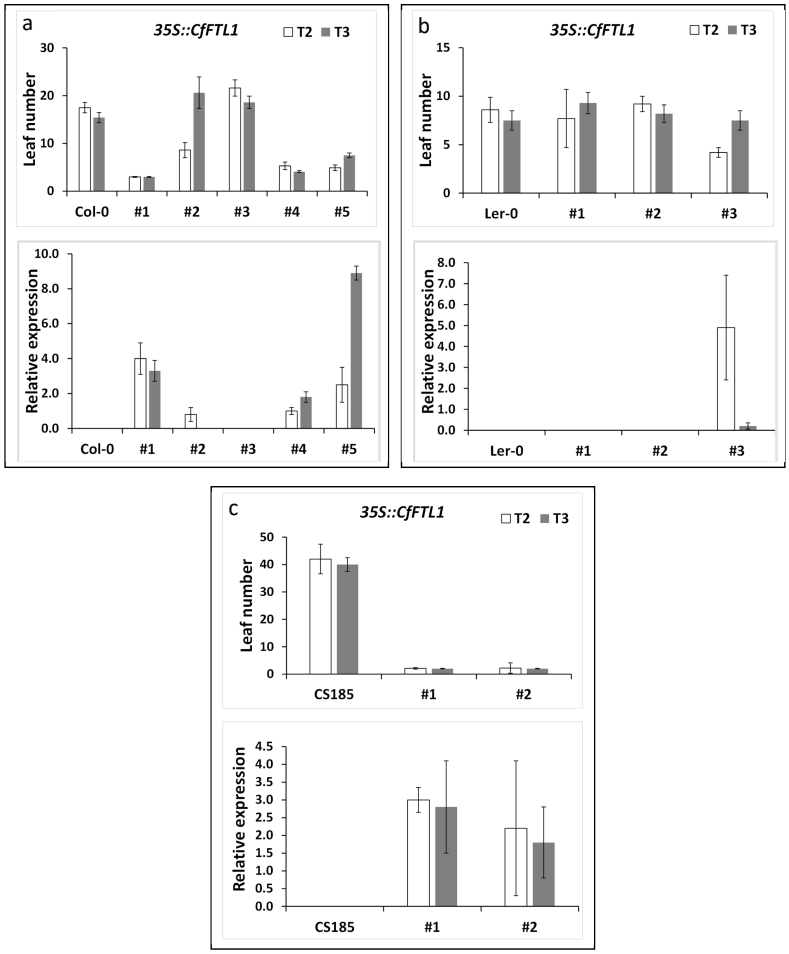

Figure 2. The number of rosette leaves and relative transgene expression at flowering time in Arabidopsis transformed with the CfFTL1 gene under the 35S promoter in the T2 and T3 generations. a. The CfFTL1 transformants in the Col-0, b. Ler, and c. CS185 (ft-3) backgrounds. The averages and standard deviations were calculated from 20 to 35 plants of the respective independent lineages, which are labeled by the numbers on the x axis. Asterisks represent honestly significant difference (HSD) estimated by Tukey test.

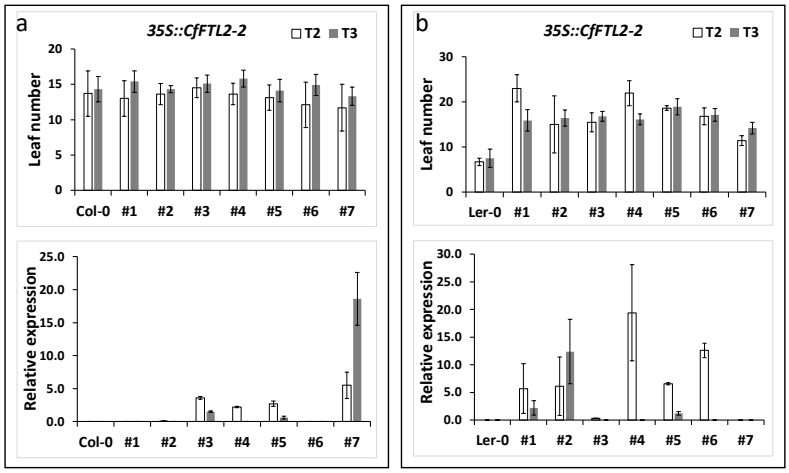

Figure 3. The number of rosette leaves and relative transgene expression at flowering time in Arabidopsis transformed with the CfFTL2-2 gene under the 35S promoter in the T2 and T3 generations.a. The CfFTL2-2 transformants in the Col-0, and b. Ler backgrounds. The averages and standard deviations were calculated from 20 to 35 plants of the respective independent lineages, which are labeled by the numbers on the x axis. Asterisks represent honestly significant difference (HSD) estimated by Tukey test.

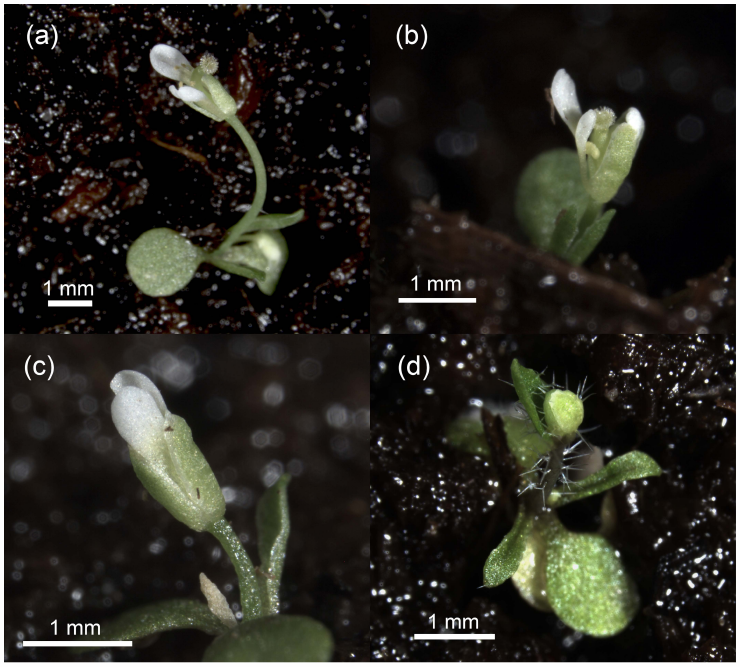

Figure 4. Phenotypes of primary transformants of Arabidopsis Col-0 carrying CfFTL2-1 under the complex metoxyfenozide-inducible promoter (VGE::TM-2::5xM:CfFTL2-1), which flowered without chemical induction. Plants started to bolt immediately after germination. Some of them formed minuscule flowers (a, b, c), others produced tiny flower buds with long trichomes (d). All the plantlets died without generating viable seed. Photo: Lukáš Synek.

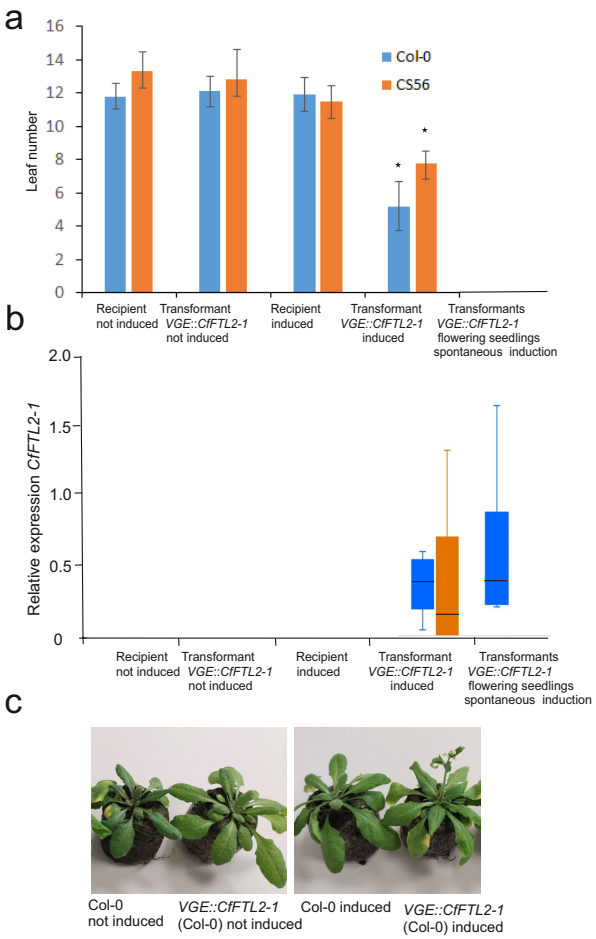

Figure 5. The acceleration of flowering and the CfFTL2-1 gene expression in Arabidopsis carrying the CfFTL2-1 transgene. a. The number of rosette leaves formed since the time of metoxyfenozide treatment till flowering in Col-0 and CS56 (Ler ft-1) backgrounds, calculated as the average with standard deviation from 20 -30 plants of the same homozygous transgenic line. Asterisks represent honestly significant difference (HSD) estimated by Tukey test. The seedlings (6 primary transformants in Col-0 background) with the spontaneously induced transgene flowered without forming rosette leaves. b. The CfFTL2-1 gene expression relative to the reference AtUBQ10 in induced and not induced plants and in spontaneously induced transformants (6 individuals) at flowering time. Median, the first and third quartile, maximum and minimum values are shown. c. The pictures of Col-0 and transgenic Arabidopsis plants taken 9 days after the metoxyfenozide treatment.

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