Cloning of *CONSTANS* and *FLOWERING LOCUS T* in *Sinapis alba*

Tamseddak, K*., D'Aloia, M.*, and Périlleux, C.

Laboratory of Plant Physiology, Department of Life Sciences, University of Liège, B22 Sart Tilman, B-4000 Liège, Belgium
corresponding author : K.Tamseddak@student.ulg.ac.be

Studies in *Arabidopsis* have disclosed a genetical cascade controlling the induction of flowering by long days (LD) (Fig. 1). CONSTANS (*CO*) integrates circadian and light inputs in the leaves and, in inductive LD, promotes the expression of its target gene *FLOWERING LOCUS T* (*FT*). The transcript and/or the protein encoded by *FT* then moves towards the shoot apical meristem (SAM) where downstream genes are activated at floral transition : *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*), *APETALA 1* (*AP1*) and *LEAFY* (*LFY*) (1).

At the physiological level, systemic signalling from leaves to SAM during the transition to flowering has been shown to involve nutritional and hormonal components. In order to get a comprehensive view of the flowering process, we analysed the genetical cascade in a species where physiological signals and timing of floral transition were previously analysed in great detail: *Sinapis alba*, which can be induced to flower by a single LD (2). We report here cloning of *CO* and *FT* homologs, and show the sequential timing of their activation in the leaves during a 22-h LD and the expression in the SAM of *SOC1*, *AP1* and *LFY*.

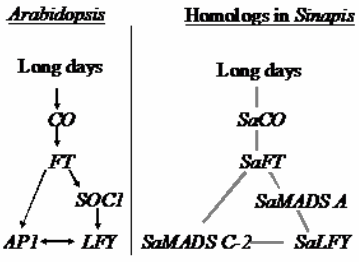

Fig. 1. Interactions between genes involved in the photoperiodic pathway controlling flowering in *Arabidopsis* and in *Sinapis*.

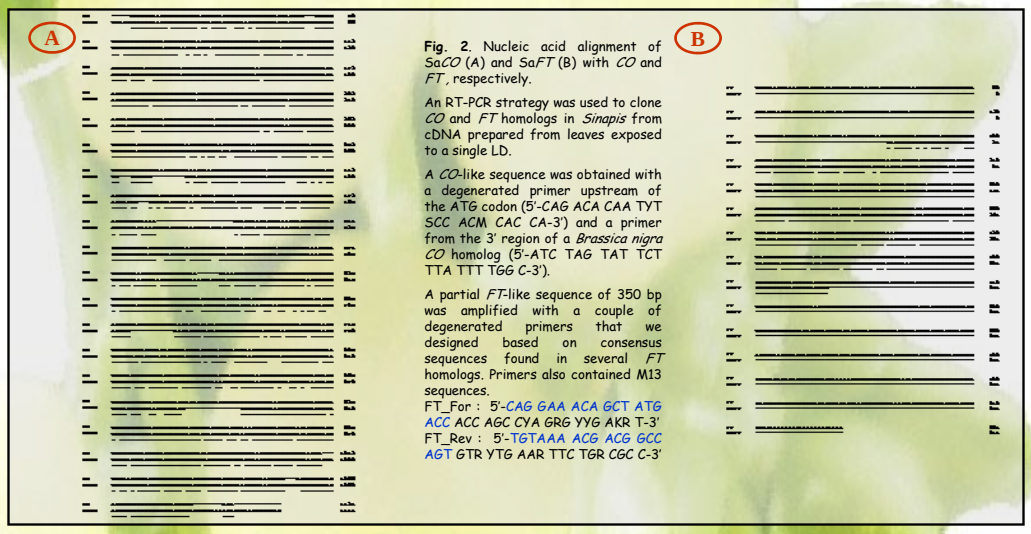

Fig. 2. Nucleic acid alignment of Sa*CO* (A) and Sa*FT* (B) with *CO* and *FT*, respectively.

An RT-PCR strategy was used to clone *CO* and *FT* homologs in *Sinapis* from cDNA prepared from leaves exposed to a single LD.

A *CO*-like sequence was obtained with a degenerated primer upstream of the ATG codon (5'-CAG ACA CAA TYT SCC ACM CAC CA-3') and a primer from the 3' region of a *Brassica nigra CO* homolog (5'-ATC TAG TAT TCT TTA TTT TGG C-3').

A partial *FT*-like sequence of 350 bp was amplified with a couple of degenerated primers that we designed based on consensus sequences found in several *FT* homologs. Primers also contained M13 sequences.
FT_For : 5'-CAG GAA ACA GCT ATG ACC ACC AGC CYA GRG YYG AKR T-3'
FT_Rev : 5'-TGTAAA ACG ACG GCC AGT GTR YTG AAR TTC TGR CGC C-3'

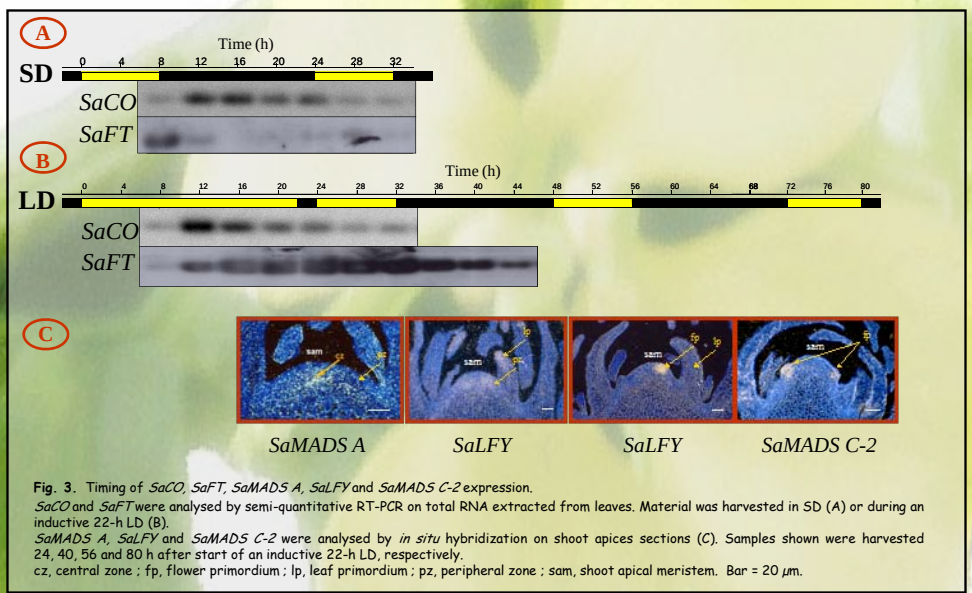

Fig. 3. Timing of *SaCO*, *SaFT*, *SaMADS A*, *SaLFY* and *SaMADS C-2* expression.
SaCO and *SaFT* were analysed by semi-quantitative RT-PCR on total RNA extracted from leaves. Material was harvested in SD (A) or during an inductive 22-h LD (B).
SaMADS A, *SaLFY* and *SaMADS C-2* were analysed by *in situ* hybridization on shoot apices sections (C). Samples shown were harvested 24, 40, 56 and 80 h after start of an inductive 22-h LD, respectively.
cz, central zone ; fp, flower primordium ; lp, leaf primordium ; pz, peripheral zone ; sam, shoot apical meristem. Bar = 20 µm.

We have cloned two sequences – hereafter called *SaCO* and *SaFT* – showing 79% and 91% identity with *CO* and *FT* from *Arabidopsis*, respectively (Fig. 2). Cloning of full length *SaFT* and complementation experiments are still needed to confirm that the isolated sequences are orthologous to *CO* and *FT*, but we performed expression analyses that gave results consistent with timing and functions described in *Arabidopsis*. Time course analyses were performed by semi-quantitative RT-PCR on total RNA extracted from leaves of *Sinapis* harvested every 4 h during the inductive LD, or in control short day (SD).

In SD, a peak in *CO* transcript level was found at h 12-16, i.e. during the night, while expression of *SaFT* was hardly detectable (Fig. 3A).

In LD, *CO* expression was not much different than in SD (Fig. 3B) but, as in *Arabidopsis*, the highest level was found during the light period of the inductive cycle. It was followed by a strong increase in *SaFT* expression, from h 24 to h 40 at least. Interestingly, the expression of *SaMADS A*, which is orthologous to *SOC1*, was previously reported to start in the corpus of the SAM at the same time (Fig. 3C) (3). *SaLFY* was found to be activated in two successive waves : a first pattern was observed from h 24 – 32 that consisted in expression in the peripheral zone of the SAM and in the leaf primordia, while the second pattern was typically limited to flower primordia, and started at h 56 (D. Bonhomme, unpublished). Expression of *SaMADS C-2*, orthologous to *AP1*, was also detected in the flower primordia, but later (F. Bonhomme, unpublished). Thus the sequential activation of *SaCO* and *SaFT* in the leaves fits well with the activation of *SOC1*, *LFY* and *AP1* homologs in the SAM. Our goal will be to correlate this cascade with the physiological signals involved in floral transition (4).

References :
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