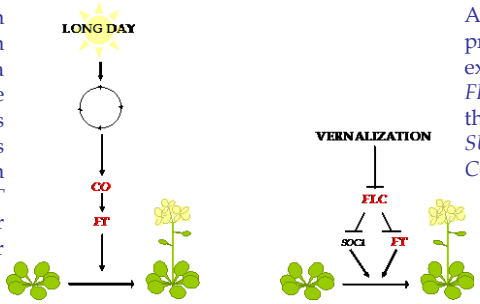
# Vernalization enhances flowering response to photoperiod by changing the timing of *CONSTANS*

**D'Aloia M. & Périlleux C.**
Laboratoire de Physiologie Végétale, Université de Liège
maria.daloia@ulg.ac.be

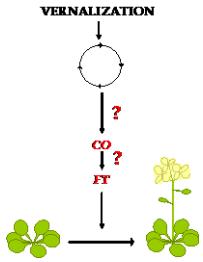
Flowering is controlled by different environmental factors such as photoperiod (day length) or cold.
Signalling pathways are deeply studied in the mustard *Arabidopsis thaliana* and key genes have been identified:

Photoperiod is measured by the cooperative function of photoreceptors and the circadian clock. Integration occurs at the level of *CONSTANS* (*CO*), encoding a transcription factor: the coincidence between the circadian time of *CO* expression and light – that is required for stabilization of the protein - conditions activation of its targets (1). In favourable daylength (long days), CO activates *FLOWERING LOCUS T* (*FT*). FT is as a systemic signal that acts – as a major limiting factor - in the shoot meristem to trigger floral transition.



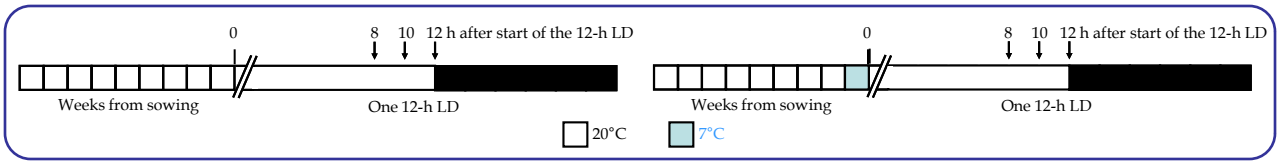

A long exposure to cold (vernalization) promotes flowering by inhibiting the expression of a strong repressor: *FLOWERING LOCUS C* (*FLC*). This relieves the activity of promotive genes *FT* and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) and so induces flowering (2).

We and other have observed that flowering response to photoperiod is enhanced by previous vernalization (3). Since vernalization has been shown recently to shorten circadian period (4), we have analyzed the effect of vernalization on the molecular sensor of photoperiod: *CO* and *FT* expression. This study was performed on another mustard: *Sinapis alba*, which can be induced to flower by exposure to a single long day (LD).




First, we have cloned two sequences – hereafter called *SaCO* and *SaFT*- showing a high identity level with *CO* and *FT* from *Arabidopsis*. Expression analyses were performed in different physiological experiments, the results of which are fully consistent with timing and functions of these genes in *Arabidopsis*. These results, together with the sequential activation of downstream genes in the shoot apical meristem of *Sinapis* during the inductive LD will be correlated with the physiological signals involved in floral transition (D'Aloia *et al.*, in preparation).

We have then analysed the vernalization effect on *SaCO* and *SaFT* expression patterns. For this experiment, 9-week old plants of *Sinapis alba* grown in non-inductive 8-h SD, 20°C, were exposed to a single 12-h LD, which is suboptimal for flowering. One half of the plants had been previously exposured to 7°C for one week. This cold treatment did reduce significantly *SaFLC* expression in leaves (Fig. 1) and also affected *SaCO* and *SaFT* (Fig. 2).



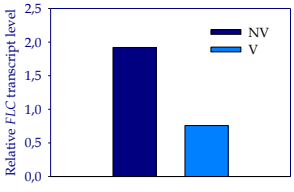




Figure 1. Expression analysis of *SaFLC* in the leaves of non vernalized (NV) and vernalized (V) plants.

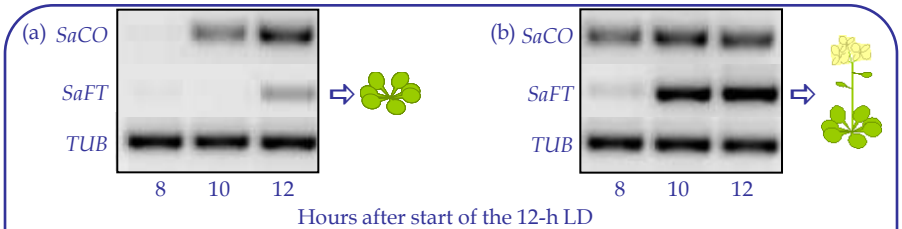



Figure 2. Expression analysis of *SaCO*, *SaFT* and *TUB* in the leaves collected 8, 10 and 12 hours after the start of the LD in non vernalized (a) and in vernalized (b) plants.

In non vernalized plants (Fig. 2a), expression of *SaCO* during the 12-h LD started at h10 and weak expresion of *SaFT* was detected at h12.
⇨ no plants flowered.
In vernalized plants (Fig. 2b), expression of *SaCO* during the 12-h LD started at h8 and strong expresion of *SaFT* was detected from h10.
⇨ 40% of the plants flowered.

**In conclusion:** Timing of *SaCO* and *SaFT* expression was advanced by vernalization. This change correlated with an enhanced flowering response to a suboptimal LD. This result suggests that vernalization affects circadian processes involved in photoperiod measurement.

References:
(1) **Imaizumi T. and Kay S. A.** (2006). Photoperiodic control of flowering: not only by coincidence. *TRENDS in Plant Science*, 11, 550-558.
(2) **Lee J.H., Hong S. M., Yoo S. J., Park O. K., Lee J.S. and Ahn J.H.** (2006). Integration of floral inductive signals by *FLOWERING LOCUS T* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*. *Physiologia Plantarum*, 126, 475-483.
(3) **D'Aloia M., Tocquin P. and Périlleux C.** (2008) Vernalization-induced repression of *FLOWERING LOCUS C* stimulates flowering in *Sinapis alba* and enhances plant responsiveness to photoperiod. *New Phytologist*, 178, 755-765.
(4) **Salathia N., Davis S.J., Lynn J.R., Michaels S.D., Amasino R.M. and Millar A.J.** (2006) *FLOWERING LOCUS C* -dependent and -independent regulation of the circadian clock by the autonomous and vernalization pathways. *BMC Plant Biology*, 6:10.

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