The *FLC*-dependent vernalization pathway in *Sinapis alba*

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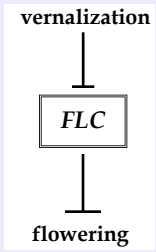
In many plants, flowering is promoted by a long exposure to cold, a process known as 'vernalization'. In *Arabidopsis*, the vernalization pathway was shown to promote flowering *via* the repression of the *FLOWERING LOCUS C* (*FLC*) gene, which encodes a repressor of flowering (1).
The genetical control of flowering seems to be well conserved among Brassicaceae, and it was reported elsewhere cloning of flowering time genes of the photoperiodic pathway in *Sinapis alba*, based on sequence similarity with *Arabidopsis* (2). However, little is known about vernalization in *Sinapis*. We therefore undertook a physiological and molecular study of this process.



Flowering in *Sinapis* is quantitavely accelerated by cold

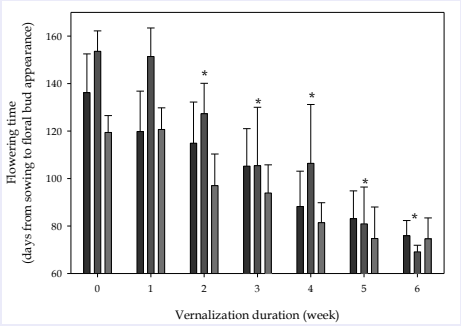


Figure I. Vernalization effect on flowering time in *Sinapis*.
* Significantly different from non-vernalized plants.

Plants were grown in 8-h short days (SD), 20°C, for 2 weeks, then exposed to cold (7°C) for 1 to 6 weeks before being returned to 20°C. Flowering was recorded as 'days from sowing to floral bud appearance' in three independent experiments.
Figure I shows that, without vernalization, flowering of *Sinapis* in SD occurred between 3 and 5 months after sowing. Vernalization was found to accelerate flowering, provided that its duration was more than 1 week. Thereafter, the longer the vernalization, the shorter the time to floral bud appearance.

Cloning of an *FLC* homolog

By screening a cDNA library we have obtained a fragment of an *FLC* homolog: *SaFLC*. The complete sequence cloned by RT-PCR shares 95% identity with the *Brassica napus FLC1* (*BnFLC1*) and 85% identity with *AtFLC*. We have found one *FLC* copy in the genome of *Sinapis* (data not shown). Based on phylogenetic analyses with the deduced amino acid sequence, SaFLC falls into the *Brassica* FLC1 clade, wich is the most closely related to AtFLC (Figure II). Thus *SaFLC* gene could be orthologous to *AtFLC*; complementation experiments are on the way to further check this hypothesis.

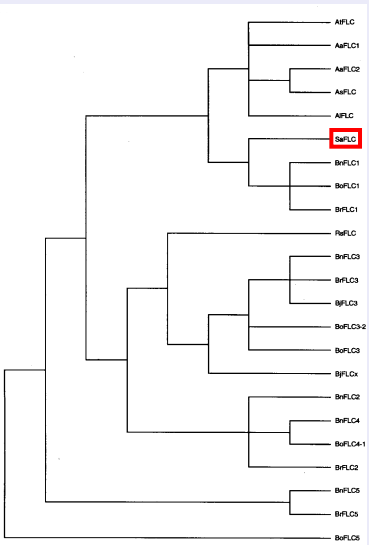
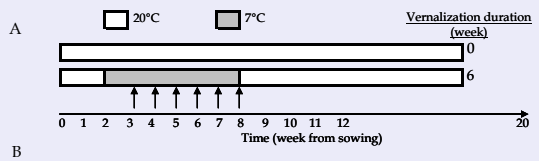


Figure II. Phylogenetic analysis of Brassicaceae FLC proteins done by maximum-parsimony.

SaFLC is quantitavely repressed by cold ... but not durably after one week of vernalization



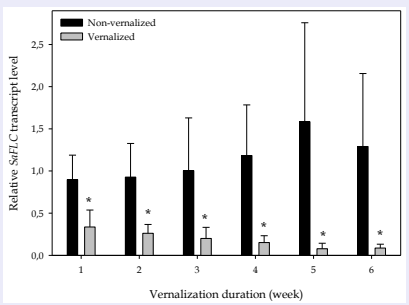


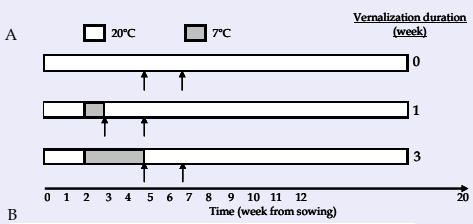
Figure III.

(A) Experimental design showing sampling times.

(B) *SaFLC* transcript level in shoot apices of *Sinapis* measured by real-time RT-PCR on total RNA. The expression of *SaTUBULINE* has been used for data normalization.

* Significantly different from non- vernalized plants.

SaFLC sequence was used to design primers and perform expression analyses by real-time RT-PCR on total RNA.
Vernalization was found to decrease strongly the transcript level of *SaFLC* in shoot apices: a sharp reduction occurred during the 1st week at 7°C and a minimum level was reached after 5 weeks (Figure III). Trying to correlate this kinetics with the flowering response to vernalization (Figure I) raised the question of why *SaFLC* repression obtained after one week of cold was not sufficient to accelerate flowering.



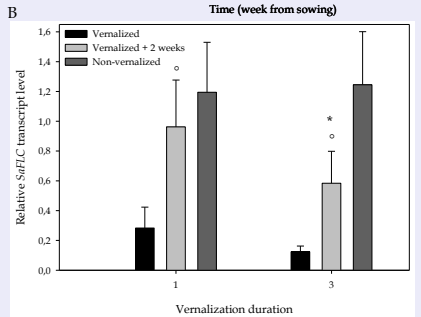


Figure IV.

(A) Experimental design showing sampling times.

(B) *SaFLC* transcript level in shoot apices of *Sinapis* measured by real-time RT-PCR on total RNA. The expression of *SaTUBULINE* has been used for data normalization.

° significantly different from vernalized plants.
* Significantly different from non-vernalized plants.

We then quantified *SaFLC* transcripts after the vernalization treatment and observed that it increased to the non-vernalized level when the cold treatment had been one-week long, but remained significantly lower after longer cold exposure (Figure IV).
Hence, *SaFLC* repression needs to be maintained in *Sinapis* – as in *Arabidopsis* – to accelerate flowering.

Physiological and molecular aspects of vernalization are well conserved in *Sinapis*.

References:
(1) Michaels S.D. and Amasino R.M. (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *The Plant Cell* **11**, 949-956.
(2) Bonhomme F., Kurz B., Melzer S., Bernier G. and Jacqmard A. (2000) Cytokinin and gibberellin activate SaMADSA, a gene apparently involved in the regulation of the floral trasition in *Sinapis alba*. *The Plant Journal*, **24**, 103-111.
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