The promotion of flowering in response to cold: from molecular bases to manipulation in *Brassicaceae*

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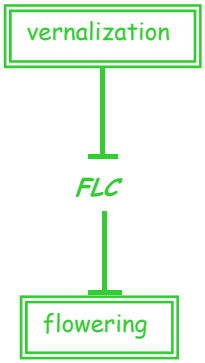
In many plant species, flowering is promoted by a long exposure to low temperature, a process known as 'vernalization'. Some plants even have an absolute requirement for winter cold before being able to flower the next summer, hence behave as biennials or winter crops. A recent breakthrough in the understanding of the molecular bases of vernalization has been the cloning of the *FLOWERING LOCUS C* (*FLC*) gene in *Arabidopsis thaliana* (**1**). *FLC* encodes a repressor of flowering and is downregulated by vernalization. So vernalization relieves the inhibitory role that *FLC* plays on downstream genes involved in the floral transition of the shoot apical meristem (SAM).

In the present work, we have investigated the key role of *FLC* by two different strategies.



Although vernalization has been shown, by physiological studies, to be sensed by the SAM, molecular evidence is missing. Such analyses are impaired in *Arabidopsis* by the small size of the plant and the rosette growth habit. We therefore used a caulescent relative *Brassicaceae* : mustard (*Sinapis alba* L.).

1) Characterization of vernalization effect on flowering time in *Sinapis*. Plants were grown in 8-h short days, 20°C for two weeks, then were exposed to cold (7°C) for 1, 2, 3, 4, 5 or 6 weeks before being returned to 20°C. Flowering time was recorded as 'days to floral bud appearance' (Figure 1).

2) Isolation of *FLC* homologues. A cDNA library made from *Sinapis* leaf mRNA (**2**) was screened with a PCR probe from *Arabidopsis FLC*.

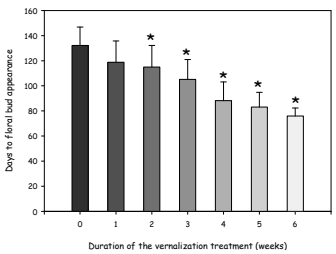


Figure 1. Vernalization effect on flowering time of *Sinapis alba*.

*statistically significant

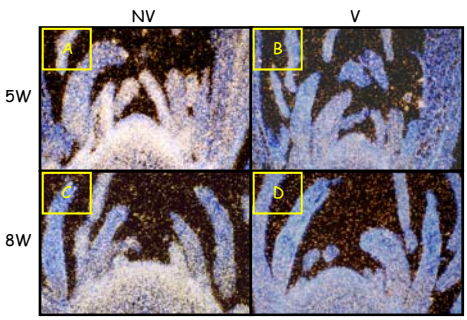


Figure 2. *FLC* expression in the SAM of 5-week (A and B) and 8-week (C and D) old plants, non vernalized (NV) or vernalized (V) from the age of 2-week.

Results

1) Figure 1 shows that a two-week duration of vernalization is sufficient to accelerate flowering. The longer the vernalization period, the shorter the time to floral bud appearance.

2) We isolated two *FLC* homologues. One of them, *SaFLC1*, was used for *in situ* hybridizations on SAM sections and it was observed that *FLC* transcript level decreased with vernalization (Figure 2).

Because of its agronomical importance, we have also tried to manipulate the vernalization process in crops. By using a transgenic approach, we have attempted to bypass the strict vernalization requirement of winter colza (*Brassica napus* L.), the third most important source of vegetable oil in the world (**3**).

We have over-expressed a gene which acts as a positive regulator of flowering and is repressed by *FLC* in non-vernalized wild type plants. This gene, cloned from *Sinapis,* is called *SaMADS A* (**2**).

Table 1. Effect of *SaMADS A* over-expression on flowering time of winter colza. Unvernalized plants were grown in 16-h long days, 20°C.

		Days to floral bud appearance
WT		∞
SaMADS A over-expressor	Line 1	122,5 ± 36,3
	Line 2	114,5 ± 18,3
	Line 3	105,2 ± 10,5
	Line 4	102,8 ± 24,1

Figure 3. Phenotype of wild type (A) and of a transgenic line over-expressing *SaMADS A* (B).

Results

We observed that transgenic lines did flower without vernalization while the WT did not (table 1 and figure 3). Thus the strict vernalization requirement of winter colza can be bypassed by constitutive expression of *SaMADS A*.

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.

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(**3**) **Sovero M.** (1993) Rapeseed, a new oilseed crop for the United States. In *New crops* (eds J. Janick and J.E. Simon), Wiley, New York, pp 302-307.

Acknowledgements

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