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Yu Kinoshita<sup>4</sup>

Ko Motoki<sup>2</sup>,  
Munetaka Hosokawa<sup>3</sup>

1 Kyoto University,  
Kizugawa, Japan,

2 Okayama University,  
Okayama, Japan

3 Kindai University, Nara,  
Japan

## Changes in the spatiotemporal expression patterns of the tandem duplicated FLC homologs associated with the non-flowering trait in *Brassica oleracea*

### Background and Objective:

Flowering time is an important trait directly related to yield in Brassica crops. We discovered the non-flowering natural cabbage (*Brassica oleracea*, CC genome) mutant 'nfc' from the breeding line 'T15' with normal flowering characteristics in 1978. However, the molecular basis underlying the non-flowering mechanism of 'nfc' has not been elucidated. Therefore, this study aimed to identify the candidate causal genes responsible for the non-flowering trait of 'nfc' and to analyse the expression pattern of the candidate causal genes.

### Methods:

To use the genetically based QTL mapping, nfc was artificially induced to flower using the grafted floral induction method with FLOWERING LOCUS T-high expressing radish lines, and three F2 segregating populations were created. The flowering dates of these F2 populations were investigated and QTL-seq and QTL analyses were performed. Next, RNA-seq analysis in leaves and shoot apices of 'nfc' and 'T15' to identify the differentially expressed genes. Additionally, the temporal expression patterns of the identified candidate causal genes were examined by quantitative RT-PCR in the upper and maximum leaves of field-grown plants.

### Results:

QTL-seq and QTL analyses identified the QTL at 50.2–51.5 Mb on chromosome 9 in the *B. oleracea* reference genome TO1000. This QTL region encompassed 241 genes, of which 4 genes were homologs of flowering time-related genes. RNA-seq analysis showed that two of the four genes were significantly up-regulated in 'nfc'. Thus, these two genes were identified as the candidate genes responsible for the non-flowering trait of 'nfc'. They were tandem duplicated BoFLC1 genes (BoFLC1a and BoFLC1b), homologs of the floral repressor FLOWERING LOCUS C (FLC), which were located close to each other (17.5 kb apart) in the TO1000 reference genome. The expression levels of BoFLC1a and BoFLC1b in 'T15' gradually decreased upon exposure to low temperatures during winter in both upper and maximum leaves. On the other hand, the expression levels of BoFLC1a and BoFLC1b in the 'nfc' maximum leaves decreased, but significantly higher than in 'T15'. Furthermore, their expression levels in 'nfc' upper leaves hardly decreased, and especially for BoFLC1b rather increased.

### Conclusions:

This study showed that changes in the spatiotemporal expression pattern of tandem duplicated BoFLC1 homologs have a significant effect on flowering time in *Brassica oleracea* through the analysis of the non-flowering cabbage mutant. *B. oleracea* (CC genome) is the diploid ancestor of allotetraploid *B. napus* (AACC genome), and *B. napus* also has genes corresponding to BoFLC1a and BoFLC1b. The results of this study provide useful insights into the control of flowering time in Brassica crops.

### References:

Kinoshita, *et al.* (2023). Upregulation of tandem duplicated BoFLC1 genes is associated with the non-flowering trait in *Brassica oleracea* var. *capitata*. *Theor. Appl. Genet.* 136: 41

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