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

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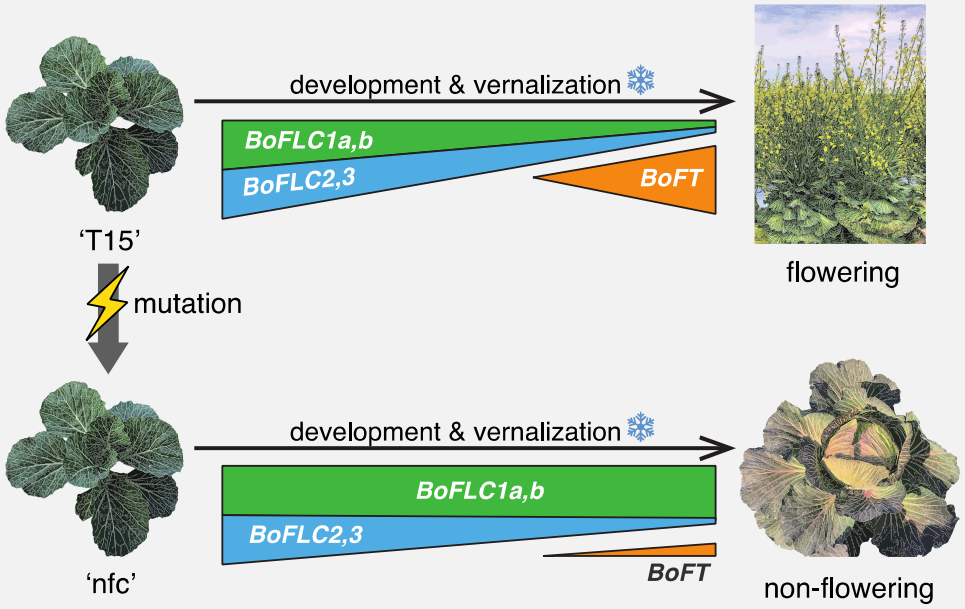
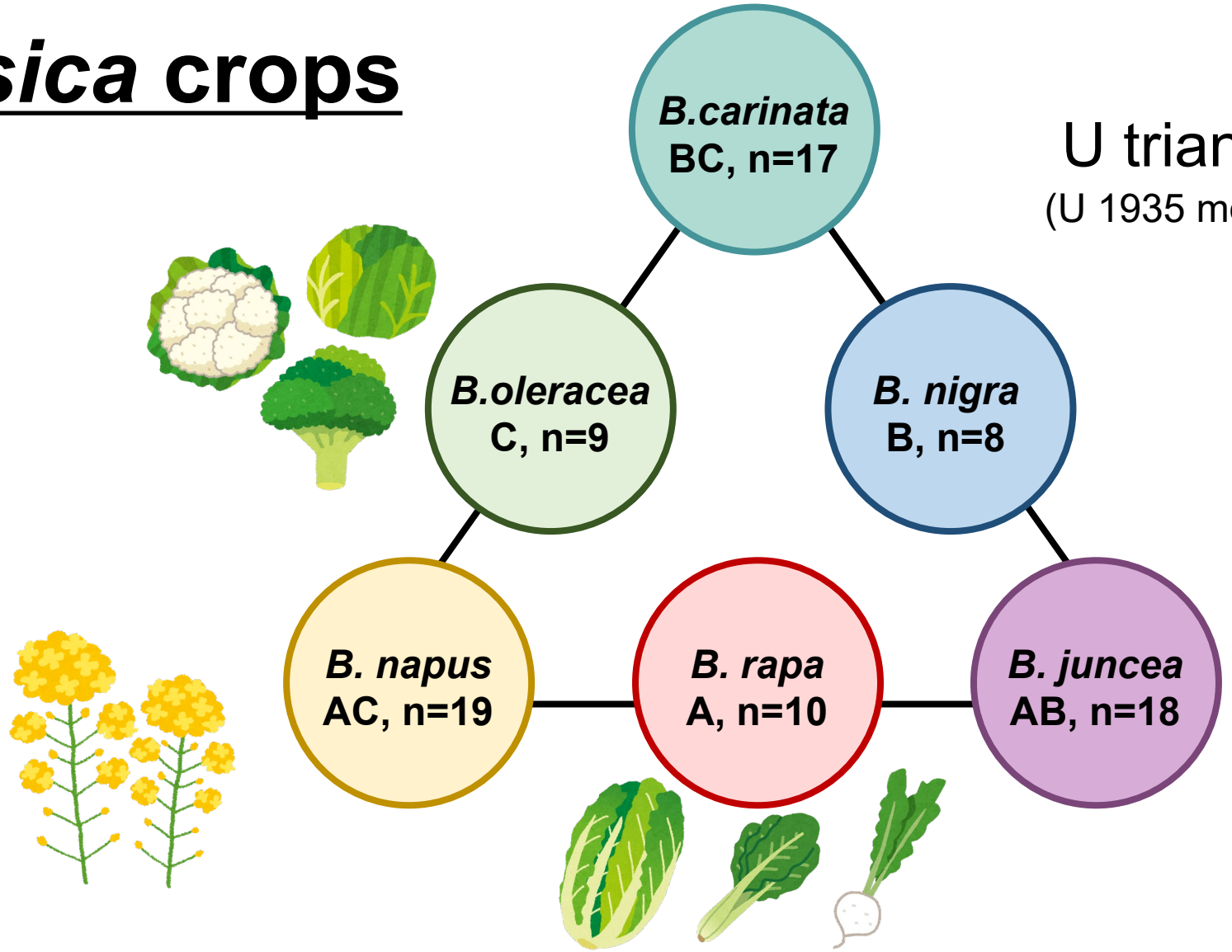


***Brassica* crops**



U triangle
(U 1935 modified)

- Flowering time is important for production and breeding
- *B. oleracea* is the ancestor of the *B. napus*

Cabbage (*Brassica oleracea*)

- Leafy, head-forming vegetable
- Flowering is induced by low temperatures (vernalization)
- Cabbage normally flowers in the spring after over wintering

winter

Head forming

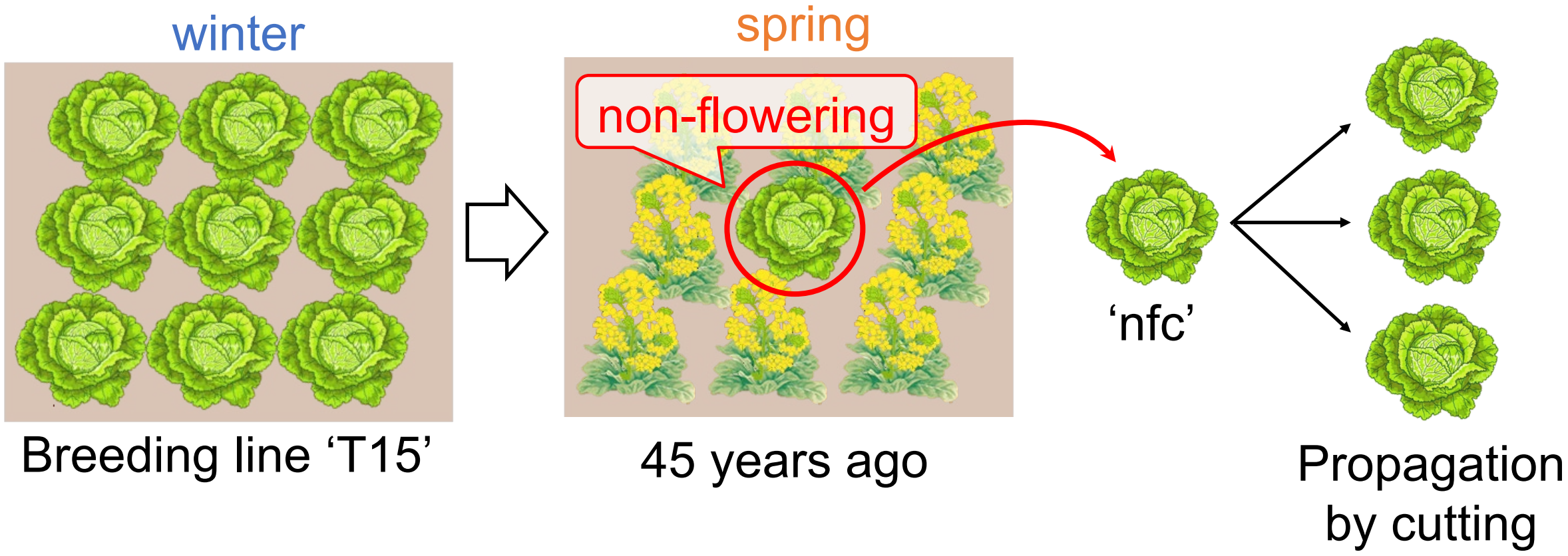
early-spring

Floral induction

mid-spring

Flowering

‘nfc’ : non-flowering cabbage mutant



- ‘nfc’ was discovered from breeding line ‘T15’ 45 years ago
- ‘nfc’ has been clonally propagated by cutting

(Kinoshita et al. 2021)

‘nfc’ : non-flowering cabbage mutant

‘nfc’ (non-flowering)

Commercial cultivar (flowering)

Aim of this study:

To identify the causal gene responsible for the non-flowering trait of ‘nfc’

Flowering state of fall-planted cabbage in May

‘nfc’ cultivated for 4 years

(Kinoshita et al. 2021)

1. QTL-seq – creation of F_2 population

×

cross

‘nfc’
(scion)

Radish
(rootstock)

Grafting floral induction
(Motoki et al. 2019, 2022)

→ F_1 → self

F_2 population

[・phenotypic segregation
・random genome distribution]

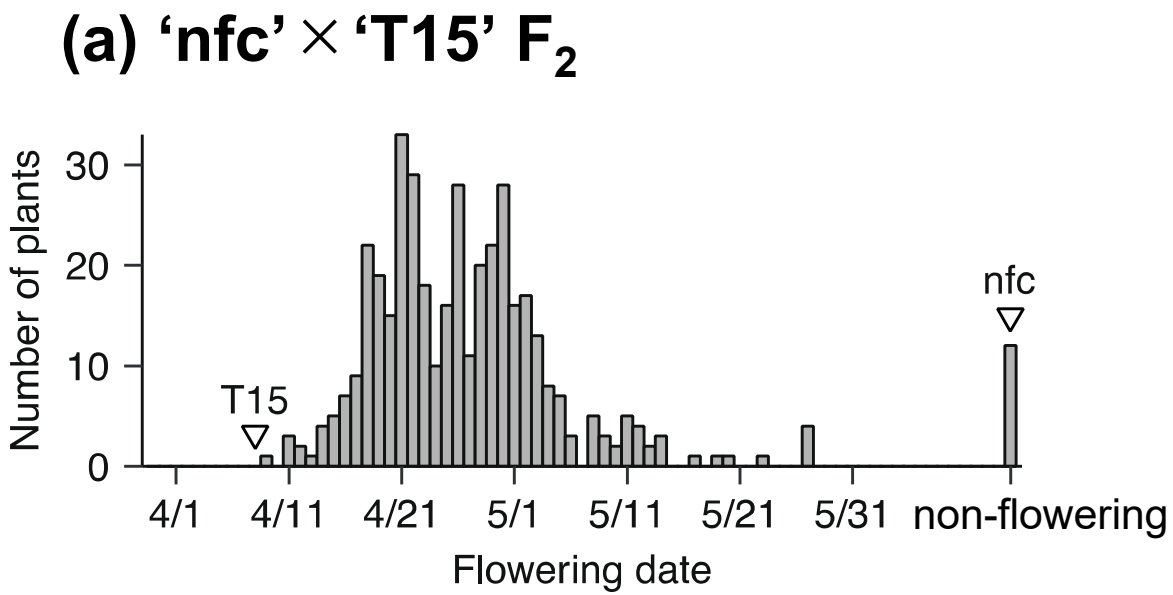
‘T15’
(wild type of ‘nfc’)

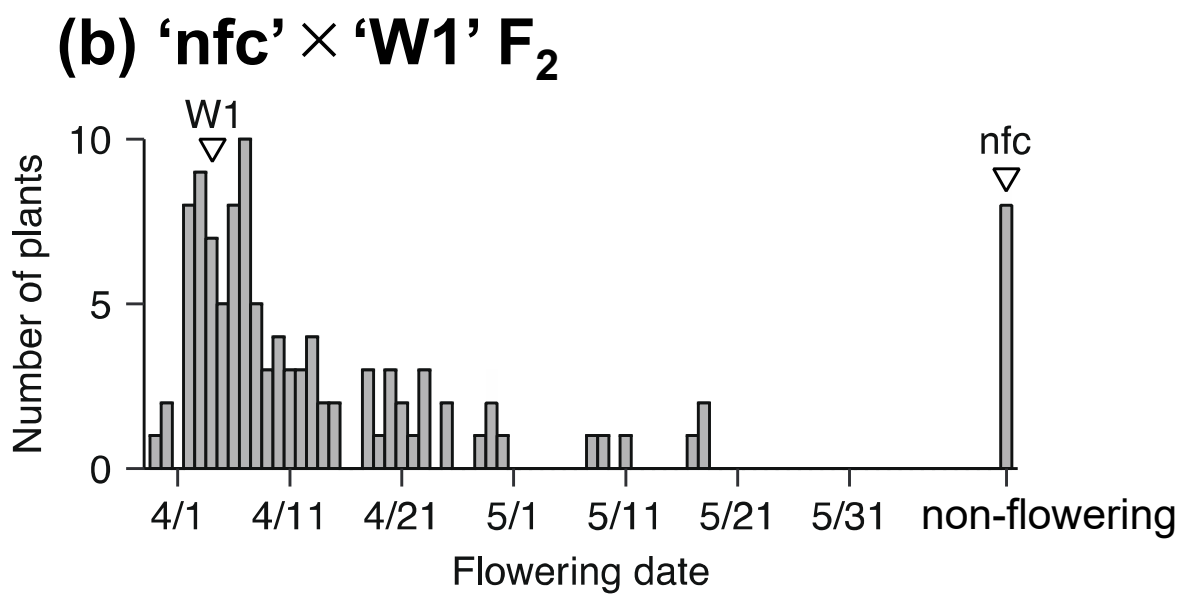
‘W1’
(commercial cultivar)

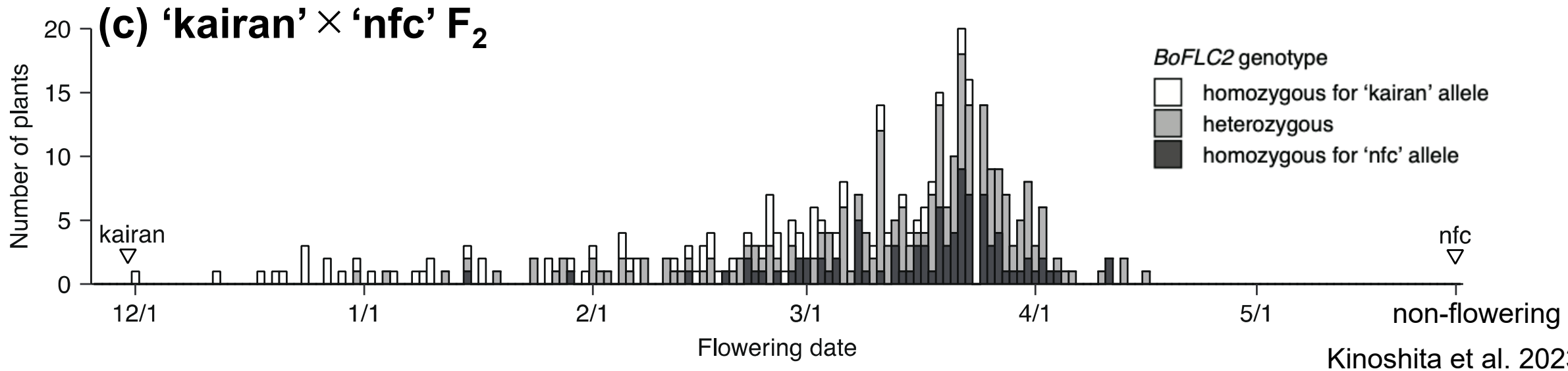
‘kairan’
(*B. oleracea* var. *alboglabra*)

Relatedness to ‘nfc’

1. QTL-seq – phenotype (flowering date)







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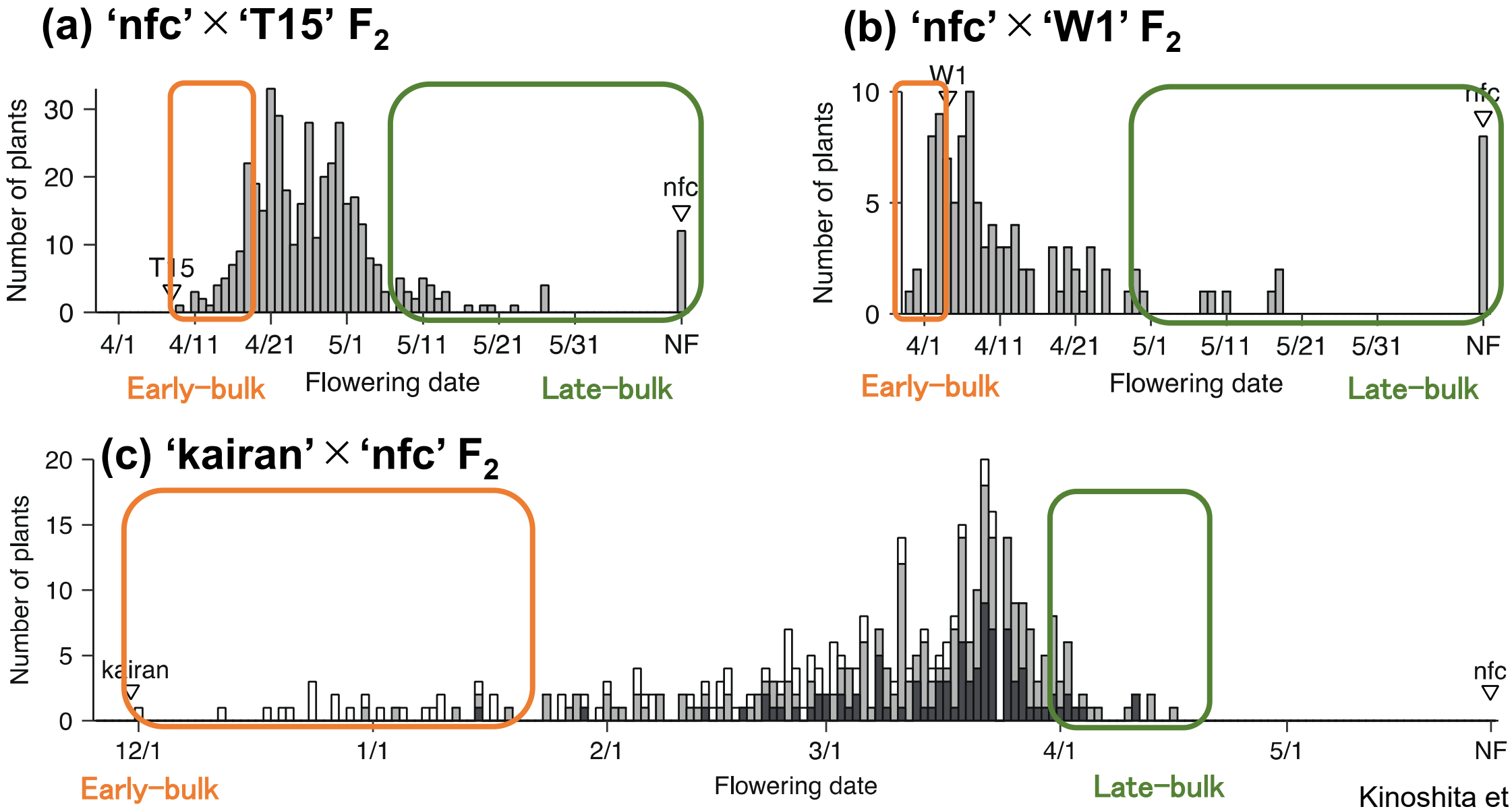
1. QTL-seq – phenotype (flowering date)



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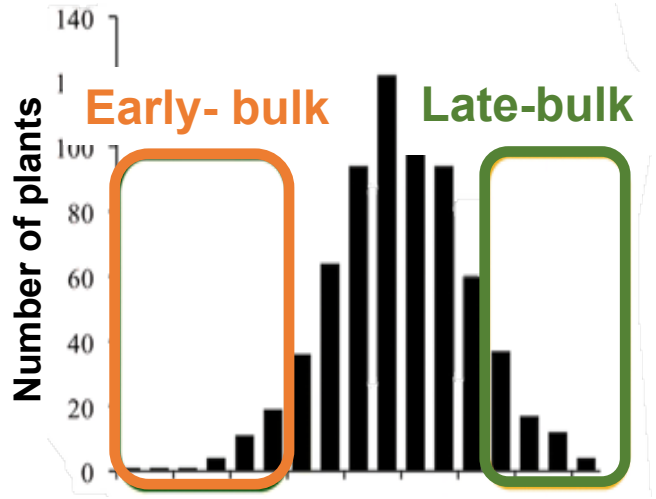
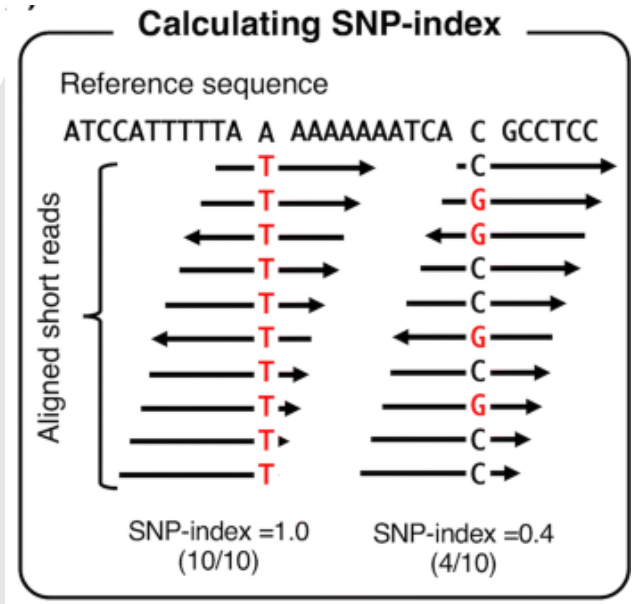
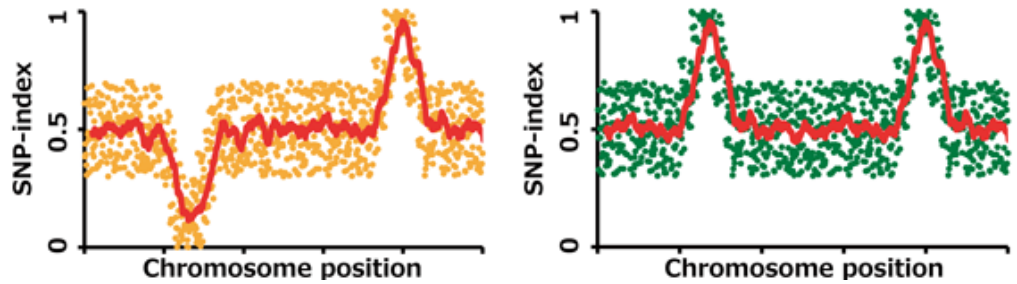
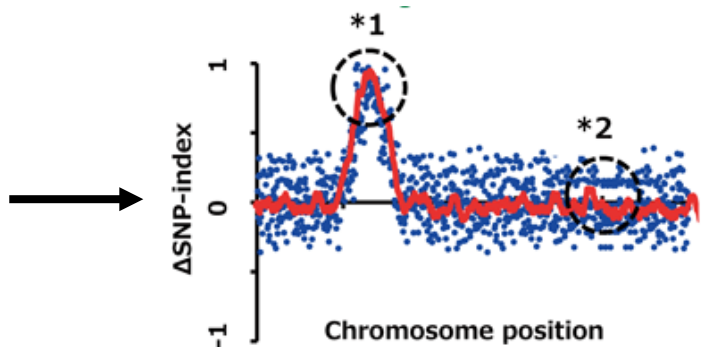
1. QTL-seq – methodology







Calculation of SNP-index

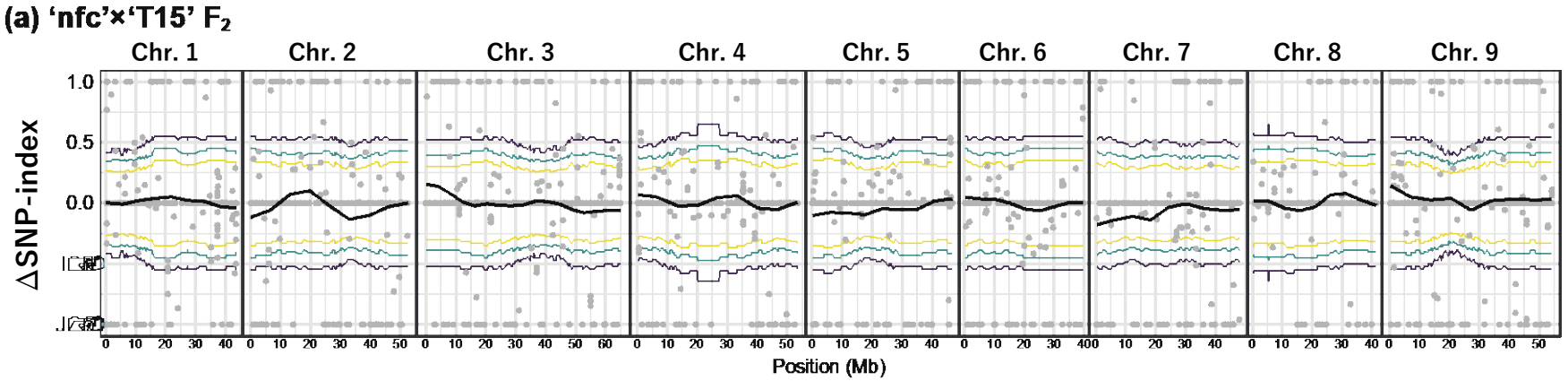


ΔSNP-index=Late-bulk—Early-bulk

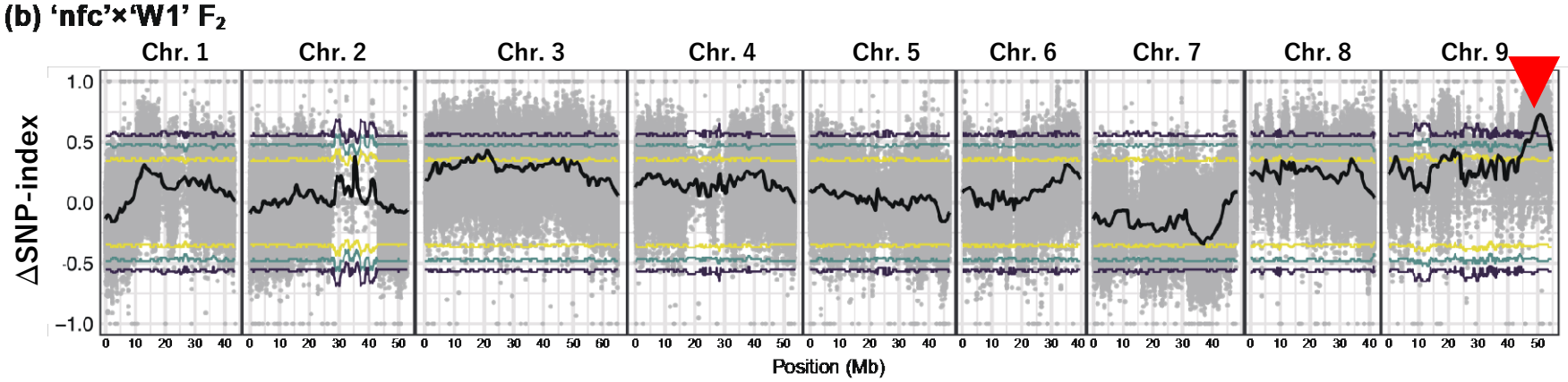
(Takagi et al. 2013; Itoh et al. 2019)

1. QTL-seq – result

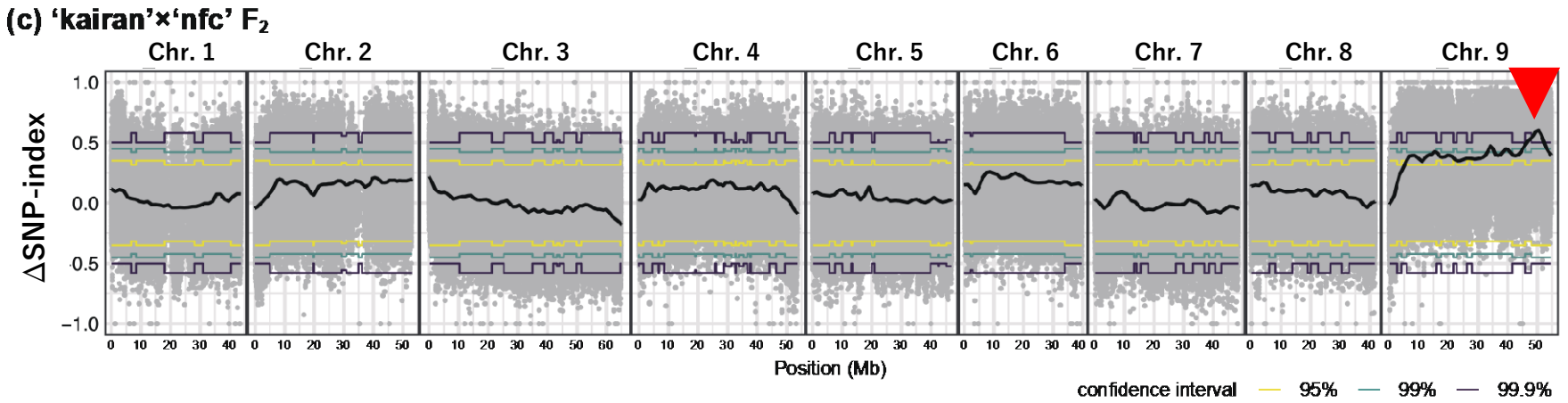
‘nfc’ allele frequency =



‘nfc’ allele frequency =



‘nfc’ allele frequency =



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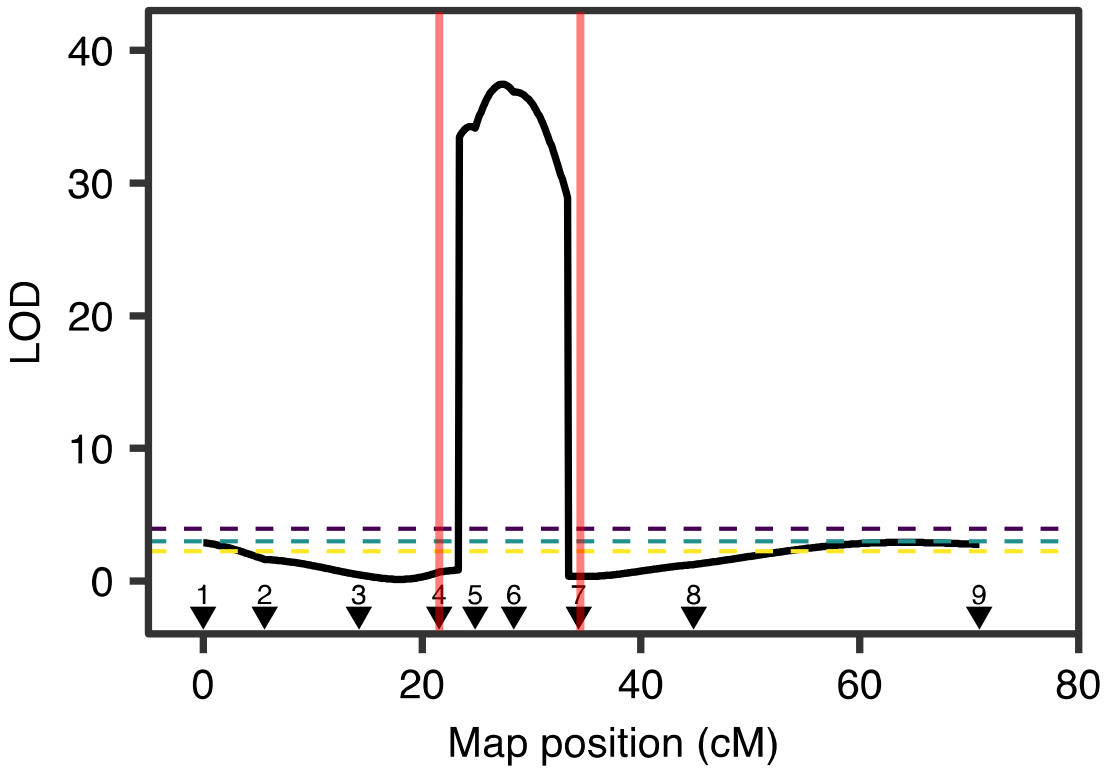
2. QTL analysis – fine mapping



- ✓ Classical QTL analysis using recombinant plants of ‘kairan’ × ‘nfc’ F_2 (n = 432)
- ✓ Genotyping using 9 DNA markers
- ✓ Phenotype：Days to flowering

QTL region was narrowed down to the region corresponding to the physical interval of **about 1.3 Mb (from 50.2Mb to 51.5Mb)** on chromosome9 in the reference genome ‘TO1000’

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3. BLAST analysis – identification of flowering-related genes

- the QTL region covered 241 genes in the reference genome ‘TO1000’
- BLAST analysis for these 241 genes against the flowering-related genes in *Arabidopsis thaliana* identified the following four homologs

Gene ID	Gene Name	
LOC106318968	**CDKC;2**	CYCLIN-DEPENDENT KINASE C;2
LOC106315498	**HAM1/2**	HISTONE ACETYLTRANSFERASE OF THE MYST FAMILY 1/2
LOC106318712	**FLC**	FLOWERING LOCUS C
LOC106318713	**FLC**	FLOWERING LOCUS C

⇩

the coding sequences were identical between ‘T15’ and ‘nfc’

⇩

RNA-seq analysis to compare gene expression levels

4. RNA-seq – ‘T15’ vs. ‘nfc’

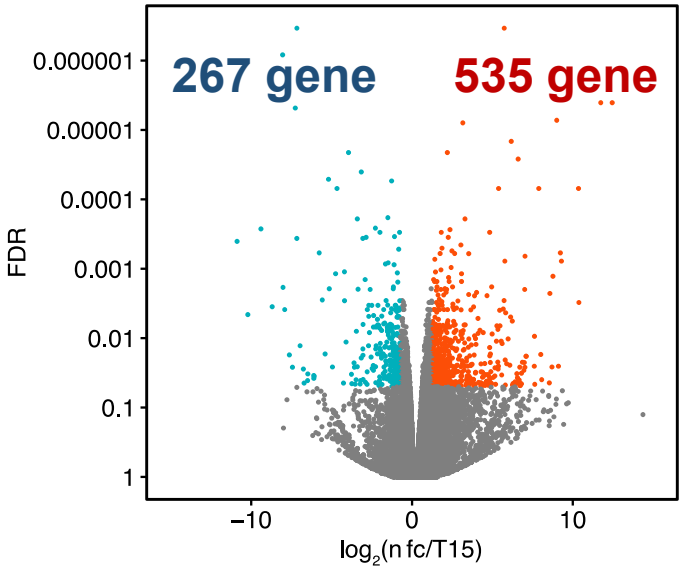
○ Upper leaves

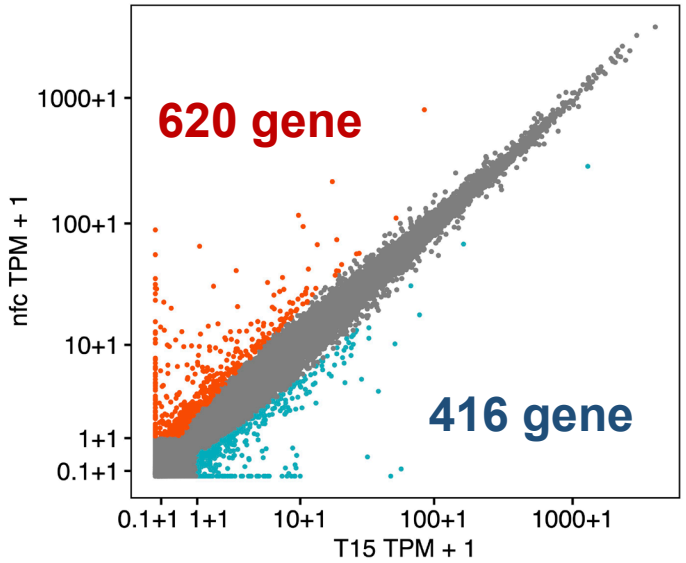
- plants cultivated in the field
- sampled in November
- n = 3

○ Shoot apex

- plants cultivated at 22℃
- shoot apical meristems containing 2–3 leaf primordia
- pooling of 10 samples (n = 1)

RNA extraction → Sequencing using NovaSeq 6000 → Differential gene expression analysis





Differentially expressed genes (DEGs)：$\log_2$Fold-Change > 1 or $\log_2$Fold-Change < -1

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4. RNA-seq – ‘T15’ vs. ‘nfc’

Upper leaves (19 gene)

Gene ID	Gene name	log_2 T15.TPM	log_2 nfc.TPM	FDR	log_2FC (nfc/T15)
LOC106318713	***FLC***	5.0	6.5	0.001	1.5
LOC106335903	*LHY*	0.2	2.5	0.002	2.4
LOC106294303	*BRC1*	-6.6	0.9	0.002	7.5
LOC106301238	*GLK1*	2.9	4.5	0.008	1.6
LOC106307179	*TEM2*	4.2	5.2	0.011	1.0
LOC106318712	***FLC***	1.4	3.6	0.012	2.2
LOC106343287	*TEM1*	0.6	4.2	0.017	3.6
LOC106328407	*TEM1*	0.6	4.1	0.024	3.5
LOC106332186	*BRC1*	-3.1	1.0	0.037	4.2
LOC106312145	*GLK1*	1.9	3.2	0.043	1.3
LOC106317875	*NF-YB2*	2.8	4.0	0.003	1.3
LOC106304211	*ULT1*	3.0	1.0	0.006	-2.0
LOC106322748	*TPS1*	1.5	0.3	0.009	-1.1
LOC106325069	*XAL2*	-0.6	2.9	0.013	3.5
LOC106305004	*FPF1*	2.3	-1.2	0.019	-3.5
LOC106313686	*MYB30*	-0.9	1.3	0.025	2.2
LOC106337599	*SOC1*	-2.9	0.4	0.025	3.3
LOC106302148	*AGL19*	1.9	0.0	0.035	-1.9
LOC106322776	*MYB30*	1.0	2.3	0.043	1.3

Shoot apex (15 gene)

Gene ID	Gene name	log_2 T15.TPM	log_2 nfc.TPM	log_2FC (nfc/T15)
LOC106318713	***FLC***	6.4	9.7	3.2
LOC106318712	***FLC***	4.3	6.2	2.0
LOC106292870	*ATHB16*	0.3	2.1	1.8
LOC106299005	*AGL18*	0.2	1.5	1.3
LOC106316090	*UBC2*	1.6	2.6	1.0
LOC106317055	*TFL1*	0.6	-0.4	-1.0
LOC106322389	*UBP12*	3.7	1.5	-2.2
LOC106300247	*SPL15*	2.4	-0.6	-3.0
LOC106316732	*XAL2, AGL14*	1.4	-1.1	-2.6
LOC106307983	*STO*	1.1	-1.2	-2.2
LOC106316836	*CO*	1.8	-0.2	-2.0
LOC106310692	*IDD8, NUC*	2.1	1.0	-1.1
LOC106298783	*GID1B*	2.0	1.0	-1.0
LOC106325361	*GA20ox1*	2.6	1.5	-1.0
LOC106312330	*PIF5*	-0.9	0.3	1.2

* Blue and red colors indicate genes with negative and positive effects on flowering time, respectively.

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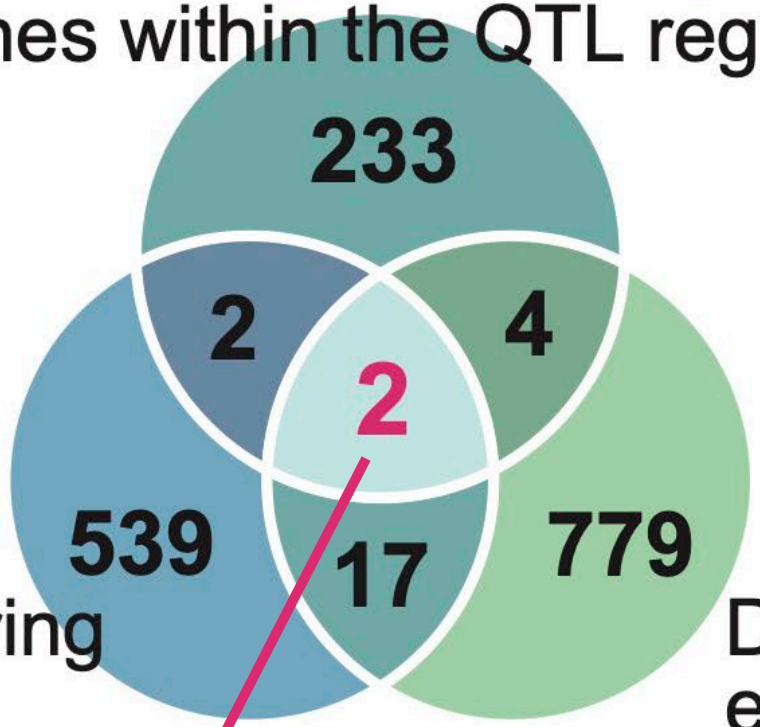
Two *FLC* homologs (floral repressor) within the QTL were highly expressed in ‘nfc’

Summary so far

F_2 generation using grafting floral induction

1. QTL-seq, QTL analysis
Genes within the QTL region



Homologs of flowering time-related genes
2. BLAST analysis

Differentially expressed genes
3. RNA-seq analysis

Two *FLC* homologs (LOC106318712, LOC106318713) were considered to be the candidate genes responsible for the non-flowering trait of 'nfc'

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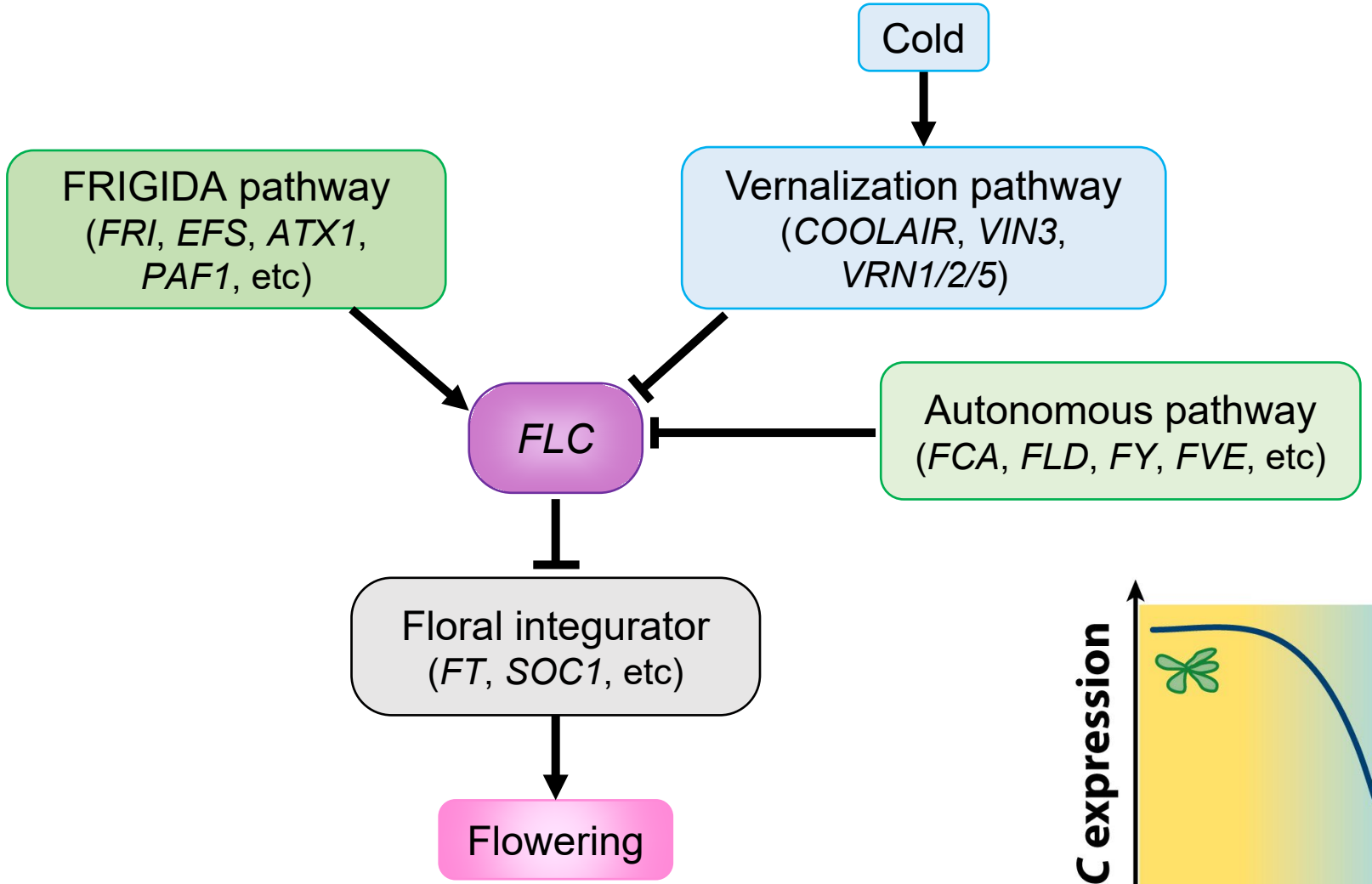
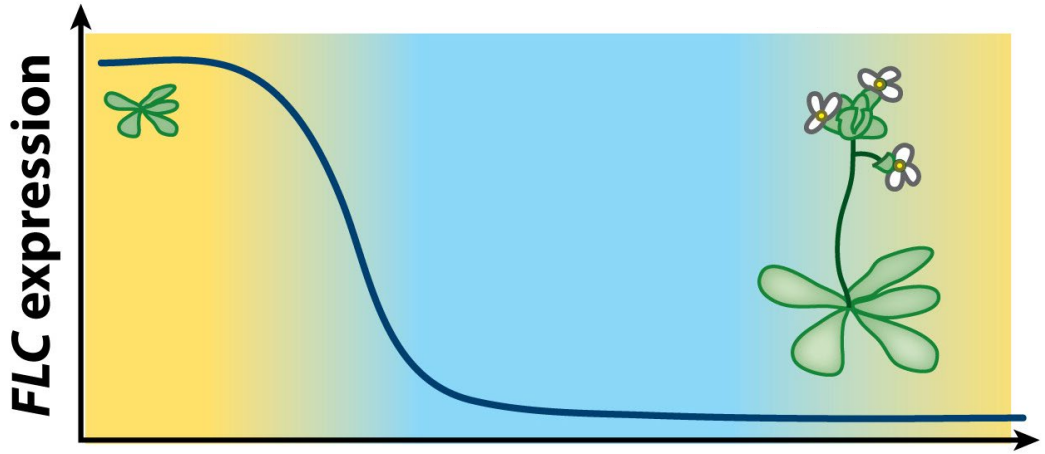
FLOWERING LOCUS C (FLC)

・・・ MADS-box transcription factor

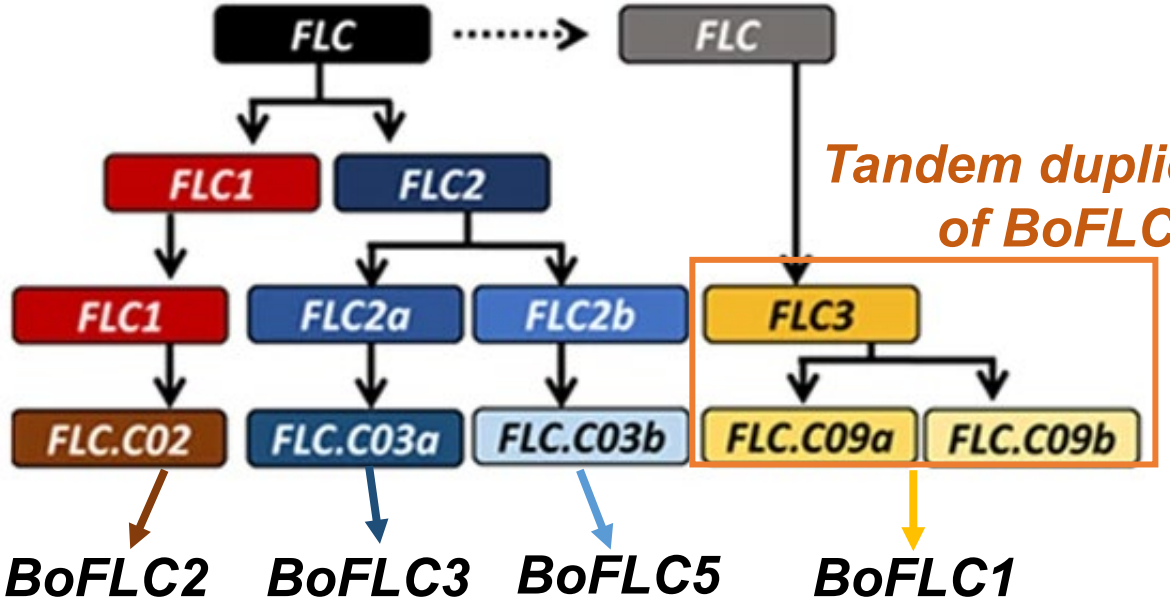
Systemic expression





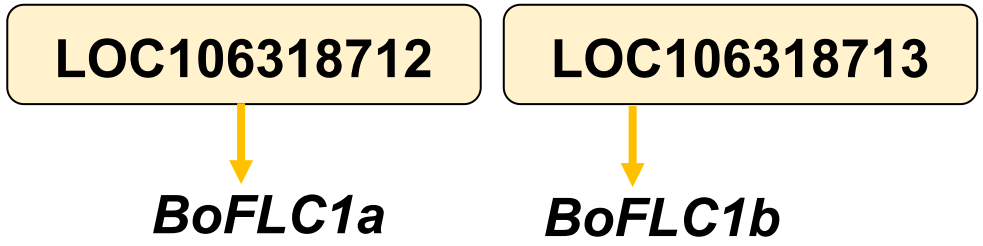
(Whittaker・Dean, 2017)

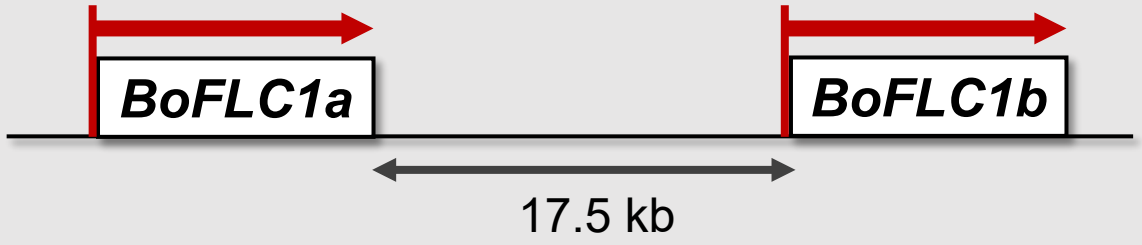
Brassica oleracea FLC homologs



(Schiessl et al. 2019)

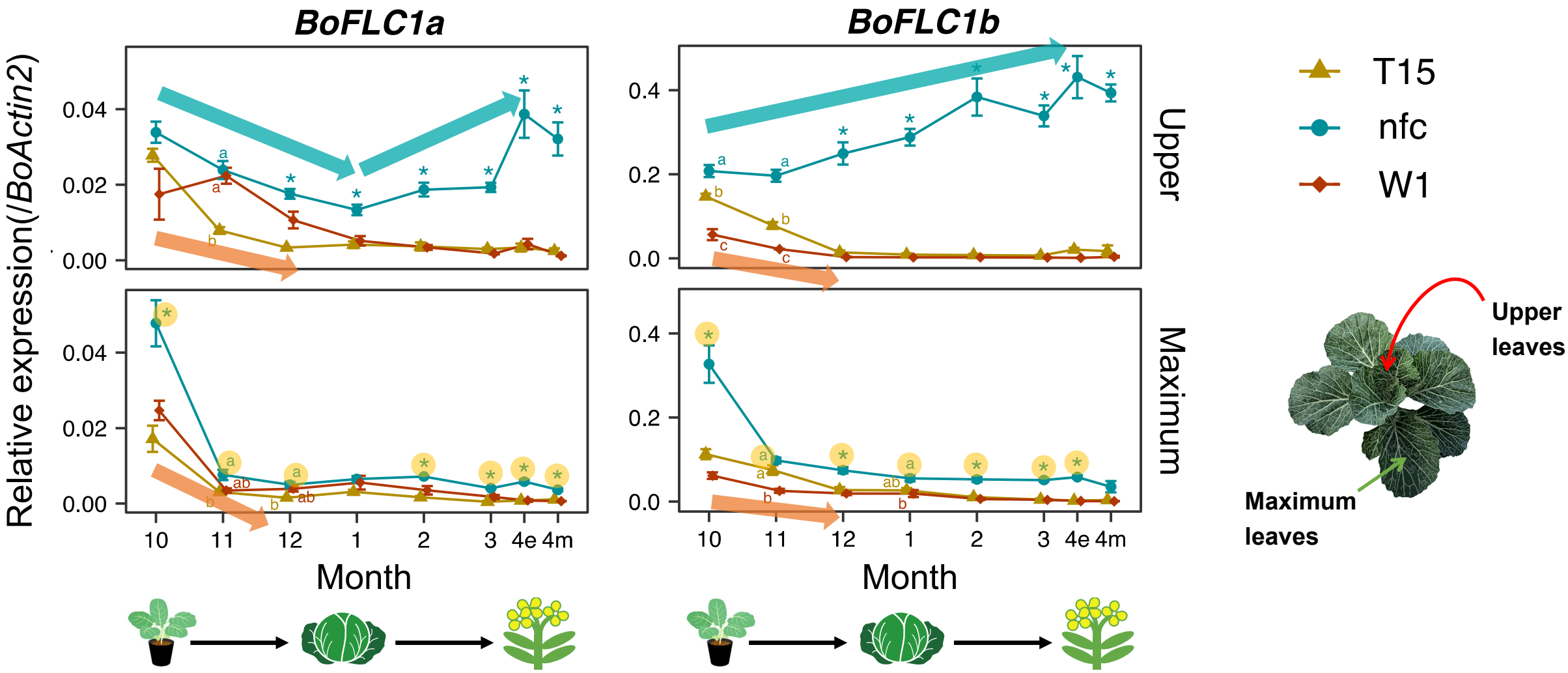
The two *FLC* homologs identified as candidate genes





- They are located only about 17.5 kb apart
- They have high sequence similarity in reference genome TO1000

5. Gene expression analysis – qPCR



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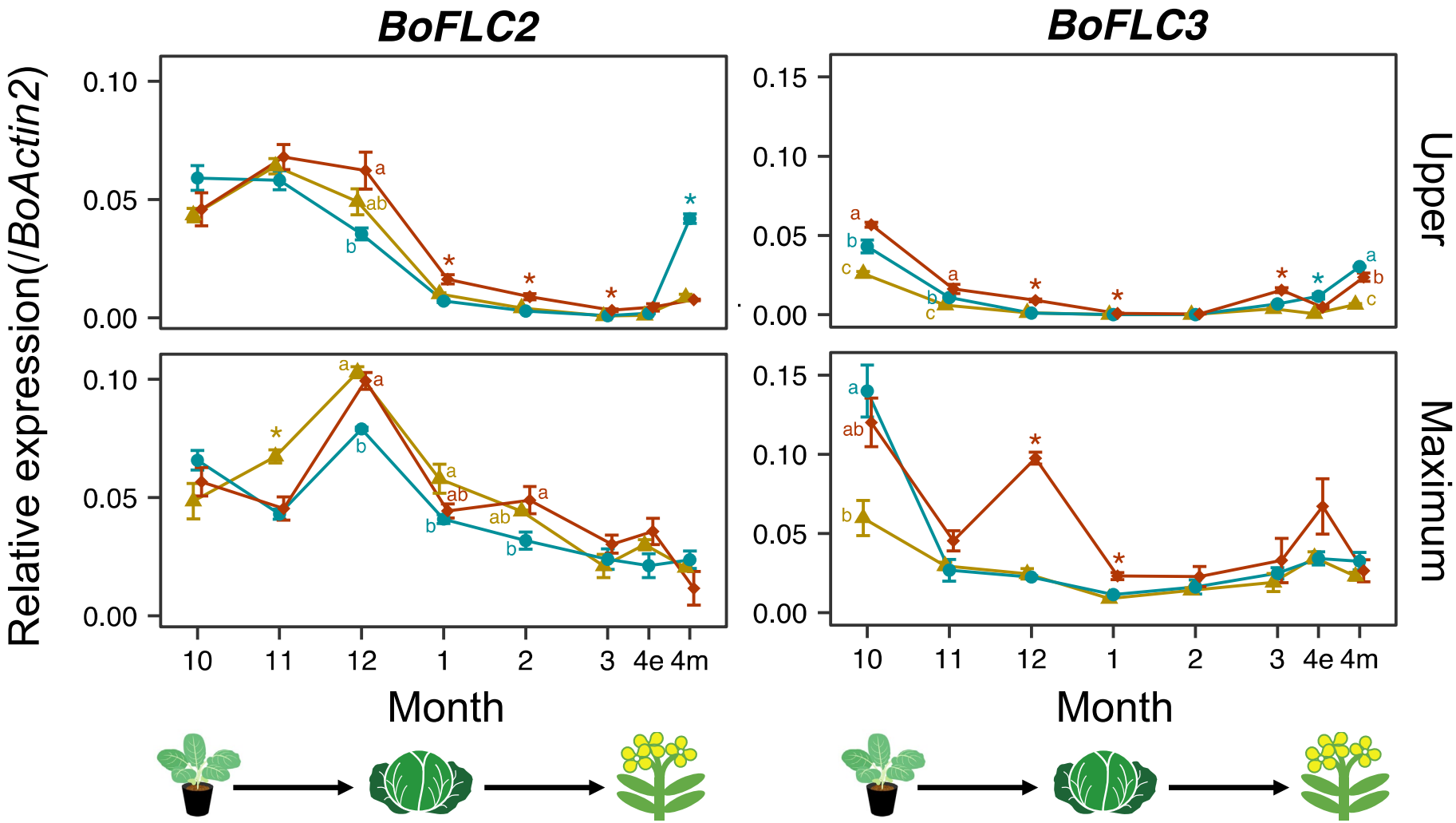
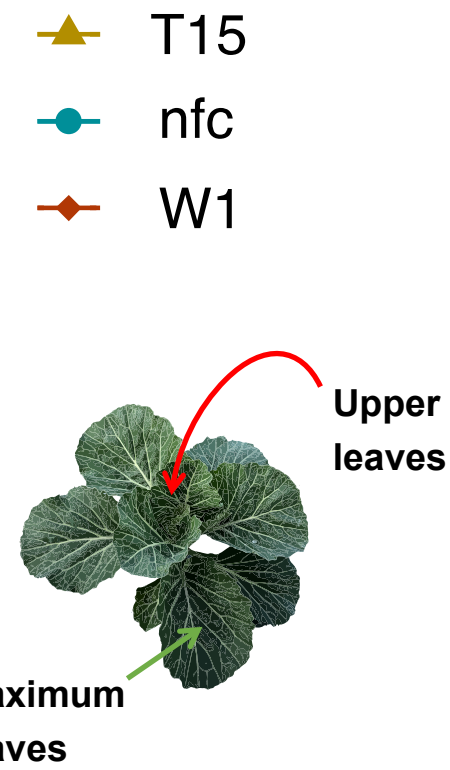
5. Gene expression analysis – qPCR





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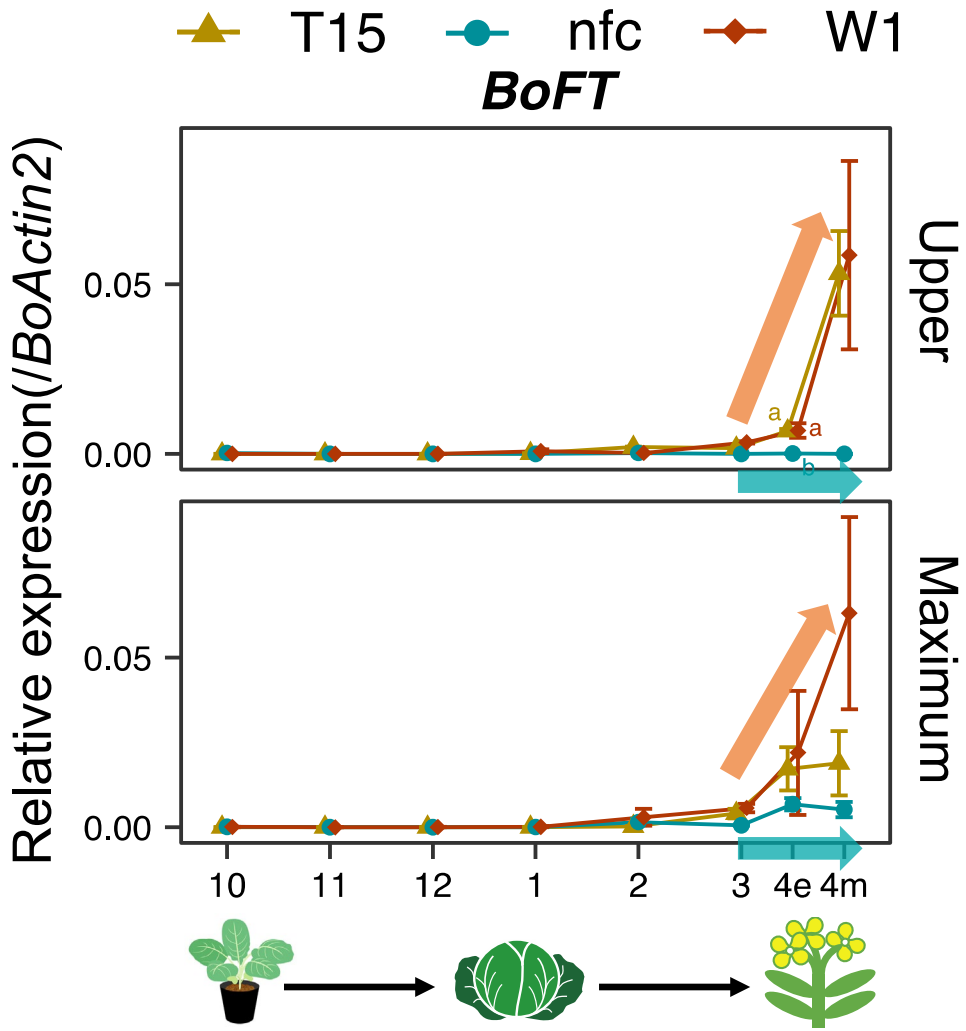
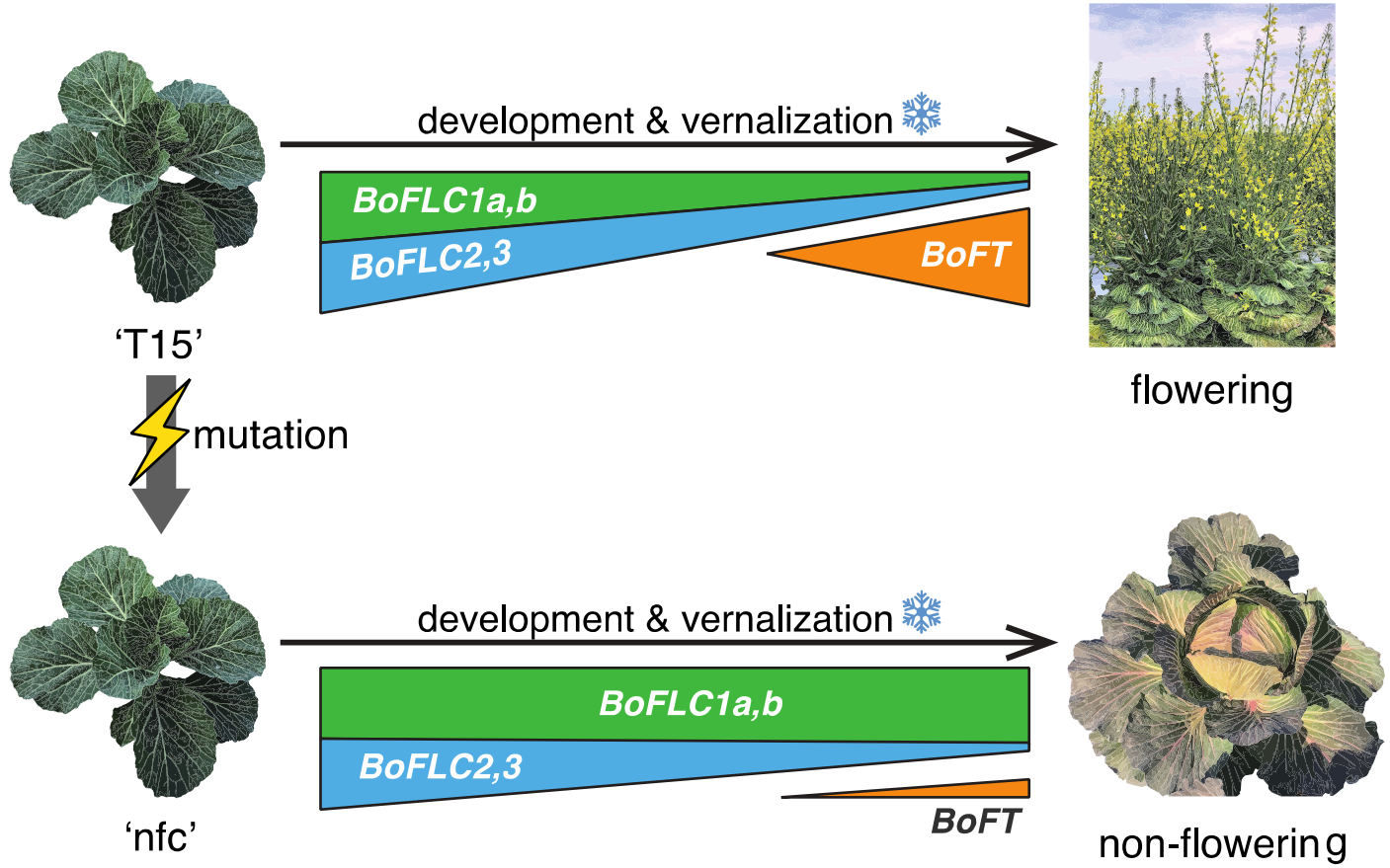
5. Gene expression analysis – qPCR





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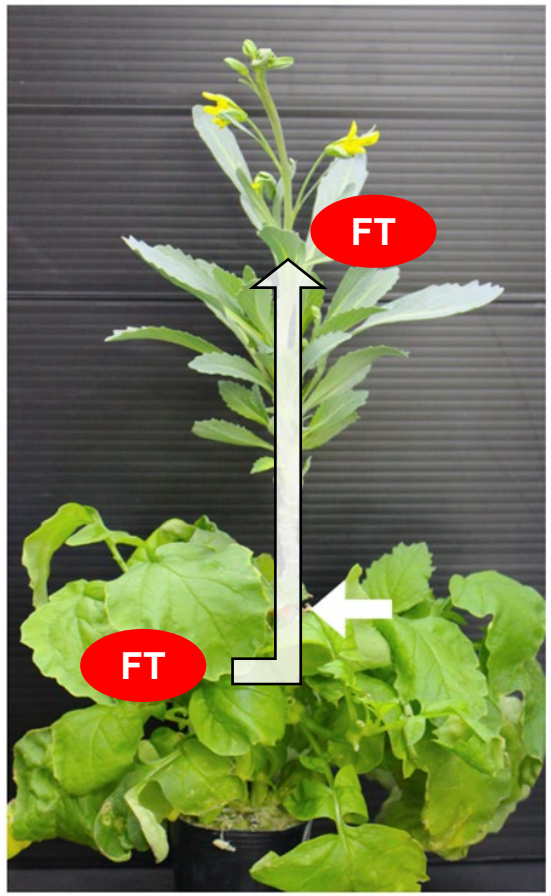
The constitutive high expression of both *BoFLC1a* and *BoFLC1b*

▶ repression of *BoFT* expression ▶ the non-flowering trait of 'nfc'

Flowering of ‘nfc’ by grafting floral induction



‘nfc’
(scion)

Radish
(rootstock)

‘T15’

development & vernalization

BoFLC1a,b

BoFLC2,3

BoFT

flowering

mutation

‘nfc’

development & vernalization

BoFLC1a,b

BoFLC2,3

BoFT

non-flowering

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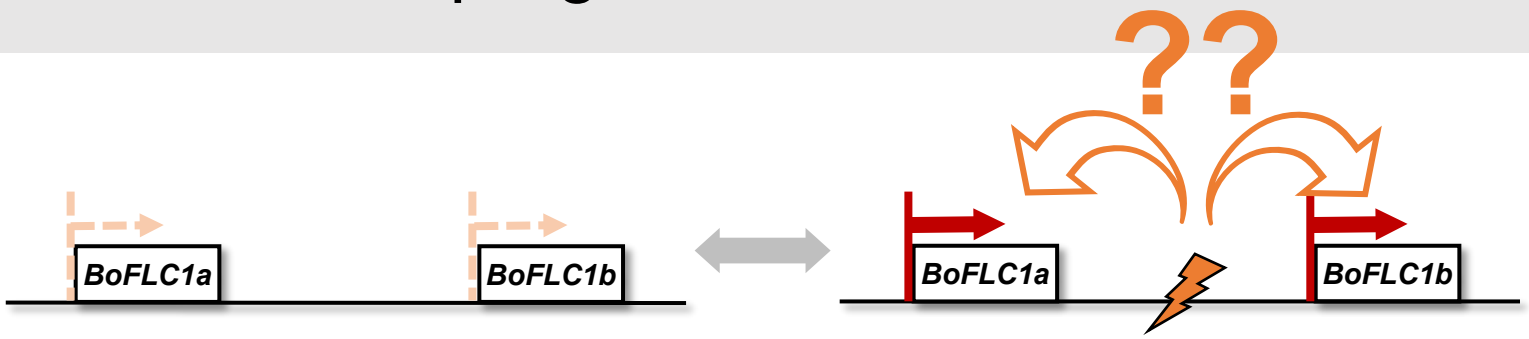
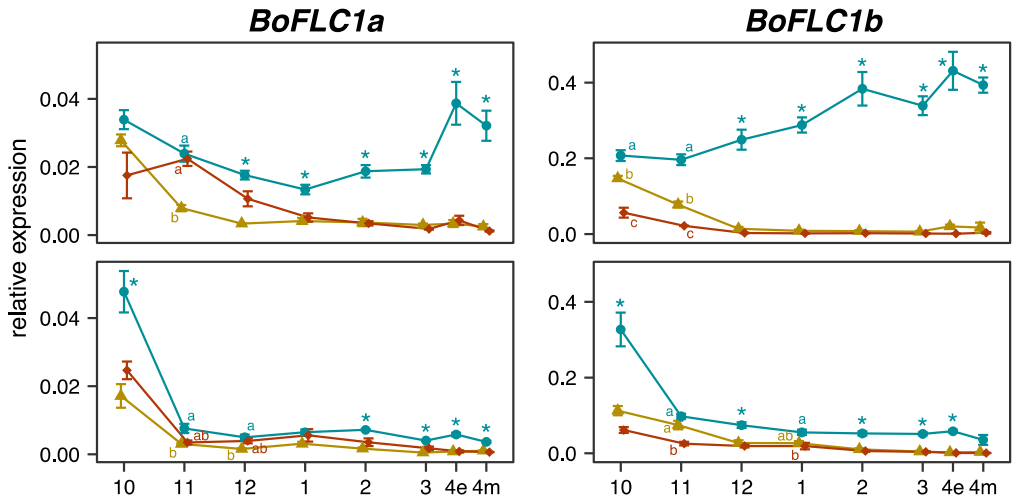
FT protein supplied from the radish rootstock may
bypass and overcome the floral repression by *BoFLC1a* and *BoFLC1b*

A cis-regulatory mutation in the vicinity of *BoFLC1a* and *BoFLC1b* may contribute to their upregulation

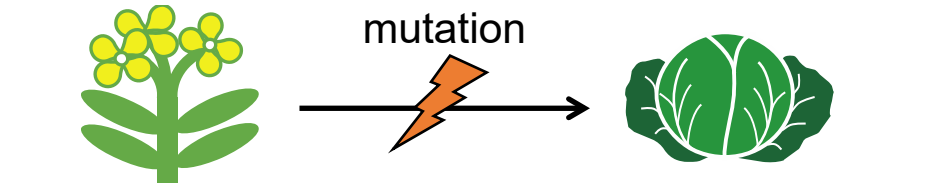


'T15' (flowering) **'nfc' (non-flowering)**

1. *BoFLC1a* and *BoFLC1b* are tandemly arranged

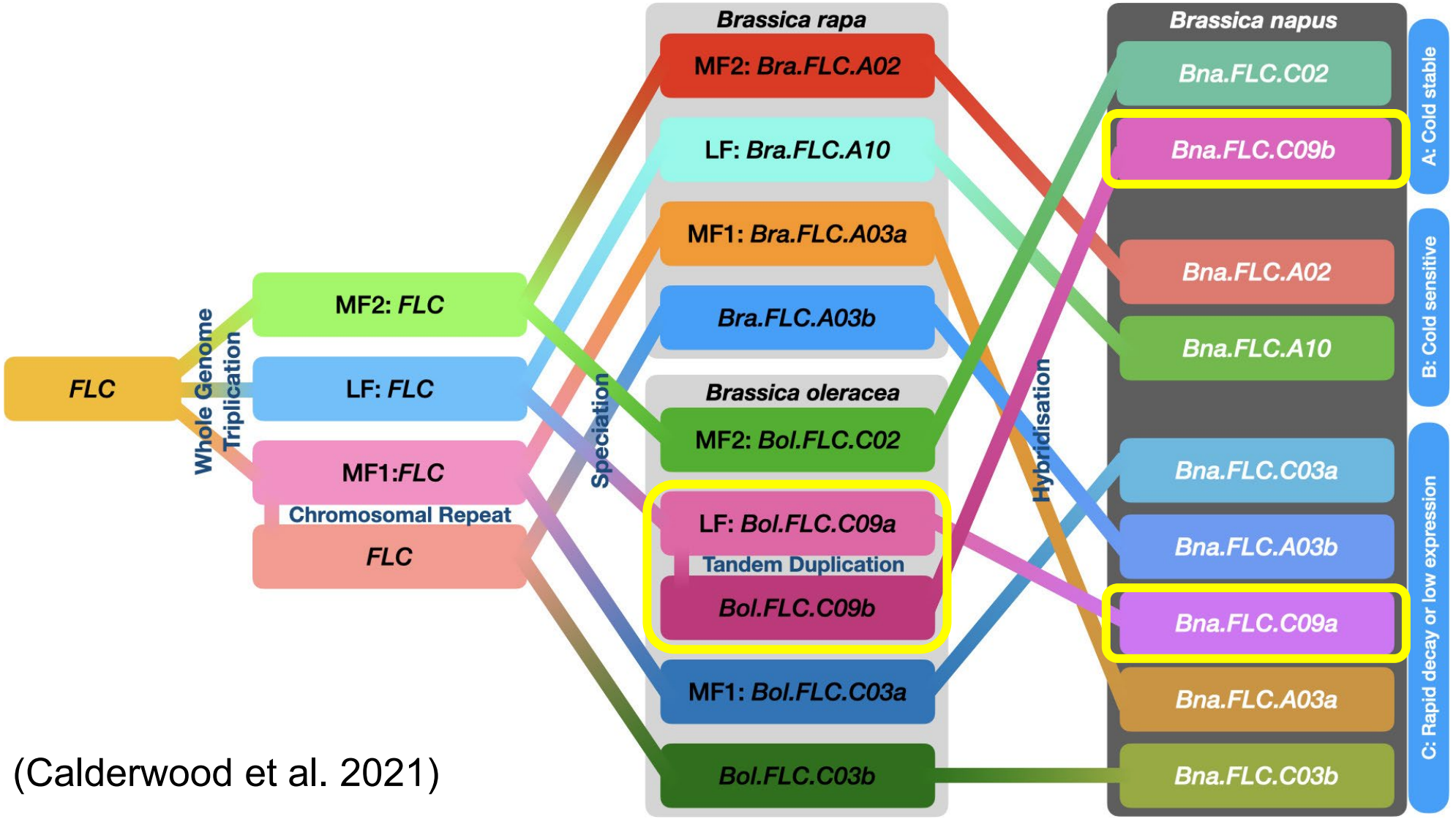


2. The expression pattern is generally similar



'T15' (flowering) **'nfc' (non-flowering)**

3. 'nfc' is a natural mutant that appeared from 'T15'



(Calderwood et al. 2021)

Elucidation of the regulatory mechanism of *FLC* expression in *Brassica oleracea* would provide a useful insight into that in *Brassica napus*

Acknowledgment

Providing ‘nfc’ and information

Prof. Susumu Yazawa (Professor Emeritus, Kyoto University)

Providing plant materials

‘W1’ seeds were kindly provided by the Genebank Project, NARO, Japan.

Support

Members of the Laboratory of Vegetable and Ornamental Horticulture, Kyoto University

Funding

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Thank you for your attention

Reference

1) Kinoshita Y, Motoki K, Hosokawa M (2021) Characterization of a non-flowering cabbage mutant discovered 42 years ago. *Hort J* 90: 374–381. (https://doi-org.kyoto-u.idm.oclc.org/10.2503/hortj.UTD-277)
2) Kinoshita Y, Motoki K, Hosokawa M (2023) Upregulation of tandem duplicated *BoFLC1* genes is associated with the non-flowering trait in *Brassica oleracea* var. *capitata. Theor Appl Genet* 136: 41. (https://doi.org/10.1007/s00122-023-04311-3)