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Vernalization control in the Brassicaceae: a multidimensional translational landscape

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The Brassicaceae family represents an extraordinary case of both evolutionary diversification and agricultural success. Through centuries of classical selective breeding, this family has yielded a vast array of crop species—ranging from oilseeds to leafy vegetables—that exhibit remarkable adaptation to diverse climates and a wide range of morphological traits. Central to this success is the combination of diverse genome structures and an ingenious evolutionary acquisition, the process of vernalization. This review explores the current state of vernalization research across three critical dimensions of translational science. First, we examine the genomic translation from the model annual *Arabidopsis thaliana* to *Brassica* vegetable crops, which has provided the foundational molecular framework for the vernalization response. Second, we discuss epigenetic translation, shifting the focus from fixed genetic variation to the heritable but flexible chromatin states that govern transcriptional memory. By targeting the recruitment mechanisms—such as DNA elements and noncoding RNAs—of chromatin-modifying complexes, researchers can achieve precise, locus-specific control over crop performance using existing technologies. Third, we address environmental translation, moving from the highly controlled laboratory conditions to the fluctuating complexities of natural field environments, where plants must integrate noisy thermal signals to ensure timely reproduction. After outlining the core molecular and epigenetic mechanisms of the vernalization “double-negative” switch, we summarize and evaluate how specific biotechnological and omics-based approaches have advanced our understanding of three key species: *Brassica rapa*, *Brassica oleracea*, and *Raphanus sativus*.

KEYWORDS

Brassica rapa, *Brassica oleracea*, epigenetics, *FLOWERING LOCUS C*, *Raphanus sativus*, vernalization

1 Introduction

The Brassicaceae family, often colloquially known as the mustard family, stands as a uniquely fascinating and economically vital group within the plant kingdom. Its biological significance stems from three converging avenues of success that have driven extensive genetic and molecular research. First, the family demonstrates profound evolutionary success, having adapted to diverse ecological niches across the globe, a resilience likely rooted in genome complexity and flexible regulatory mechanisms. Second, the Brassicaceae family includes the origin of numerous high-value agricultural crops (Brassicaceae), including global staples such as oilseed rape, cabbage, broccoli, and turnip. Intensive selective breeding over centuries has resulted in an astonishing variety of morphological and biochemical traits, providing a rich natural laboratory for studying complex phenotypic variation (Cheng et al., 2014; Saban et al., 2023). Finally, the family hosts *Arabidopsis thaliana*, the premier model organism in plant science that has consistently pushed the frontiers of molecular genetics, providing a foundational blueprint for understanding gene function, signaling networks, and genome structure across the plant domain (Yaschenko et al., 2025).

Against the dual backdrop of agronomic importance and scientific accessibility, dissecting the mechanisms underlying trait variation in *Brassica* species is a central goal in breeding research. The morphological diversity of *Brassica* crops is largely shaped by the interaction between their complex genomes and environmental conditions, in particular, extended cold exposure and photoperiod, which precisely direct the shift from the vegetative phase to the reproductive phase (flowering), thereby initiating the development of a range of new morphological organs that become edible crop parts. A central gene for these developmental decisions is *FLOWERING LOCUS C (FLC)* (Kim, 2025). In this review, we summarize recent advances in understanding the genetic and molecular basis of flowering-time regulation in *Brassica* species, with particular emphasis on the roles of *FLC* and its regulatory network. We further explore how environmental cues such as vernalization and photoperiod interact with these molecular pathways and highlight the implications of these findings for breeding strategies aimed at optimizing flowering behavior and improving crop adaptability.

2 Brassica vegetable crops: genomic insights and the complexity of crop improvement

2.1 Genomic foundation for *Brassica* domestication

The genus *Brassica* comprises several agriculturally important species that are interconnected through the classical “U’s triangle”

model. This model describes three diploid progenitor species—*Brassica rapa* (AA genome, $2n = 2x = 20$), *Brassica nigra* (BB, $2n = 2x = 16$, genome B), and *Brassica oleracea* (CC, $2n = 2x = 18$)—and three derived amphidiploid species, *Brassica juncea* (AABB, $2n = 2x = 36$), *Brassica napus* (AACC, $2n = 2x = 38$), and *Brassica carinata* (BBCC, $2n = 2x = 34$) (U, 1935). Advances in genome sequencing technologies have enabled the assembly of high-quality whole-genome sequences for all six species, providing robust molecular evidence in support of the U’s triangle model (Chalhoub et al., 2014; Yang et al., 2016; Song et al., 2021). Furthermore, population genomic studies employing high-throughput sequencing have shed light on the domestication and diversification of *B. rapa* and *B. oleracea*. These analyses suggest that the earliest domestication of *B. rapa*, particularly the turnip type, occurred in Central Asia (McAlvay et al., 2021), whereas *B. oleracea* was first domesticated in the Eastern Mediterranean region (Mabry et al., 2021).

2.2 Economic importance, diversity, and nutritional value

The *Brassica* genus comprises economically important species that exhibit remarkable morphological diversity and provide a wide range of edible plant parts, including roots, stems, leaves, buds, flowers, and seeds. Vegetables belonging to this genus, such as cabbage, cauliflower, broccoli, Chinese cabbage, and turnip, represent valuable components of the human diet owing to their high nutritional value. They are rich sources of essential minerals, including potassium and calcium, as well as folic acid, vitamin C, and vitamin K (Zhang et al., 2025a). Several *Brassica* species also contain bioactive compounds with health-promoting properties; among these, glucosinolates are characteristic sulfur-containing secondary metabolites of the genus. Their hydrolysis products, particularly sulforaphane, which is abundant in broccoli and its sprouts, function as a potent antioxidant with well-documented benefits for human health (Cheng et al., 2014; Zhang et al., 2025a). This unique combination of morphological versatility and high nutritional density establishes the genus *Brassica* as an indispensable cornerstone of global food security and human health.

2.3 Breeding challenges and the molecular-environmental gap

The requirement for vernalization leads to distinct and critical breeding challenges across the diverse *Brassica* vegetable crop types. In leafy or root vegetables, such as cabbage and turnip, premature bolting before harvest severely reduces yield and market quality, making strong bolting resistance a highly desirable trait, particularly in temperate regions such as Europe and East Asia that experience cold winters and distinct seasonal changes. Conversely, flower (inflorescence) vegetables, such as broccoli and cauliflower, generally have a weaker vernalization requirement; however, precise control of the floral transition remains equally essential to ensure the development of commercially harvestable curds at the optimal time (Lin et al., 2005; Ridge et al., 2015). Furthermore, in F_1 hybrid breeding, synchronizing the flowering

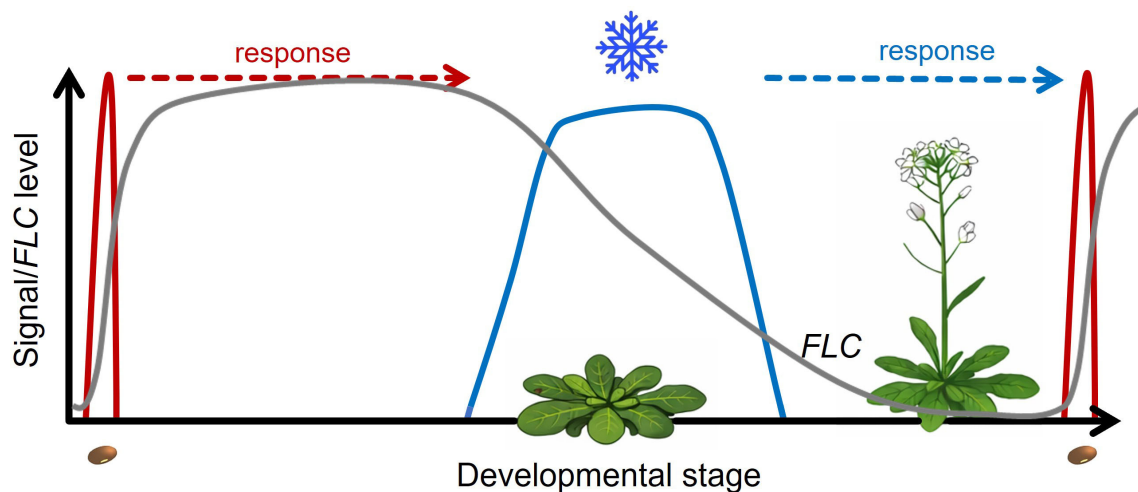


FIGURE 1

Schematic diagram of the dual-memory switch governing the vernalization checkpoint. Developmental memory (red curve, TF = transcription factor) shows the high *FLC* expression state established during embryogenesis and mitotically inherited to maintain vegetative growth. Environmental memory (blue curve) illustrates cold-induced *FLC* silencing, which acts as a timing mechanism to sense winter duration. Once silencing is complete, this repressed state is mitotically remembered, allowing the transition to the reproductive phase only after the winter signal is registered.

time of parental lines is paramount for efficient hybrid seed production (Fujimoto et al., 2018). Given these varied and stringent requirements, understanding the precise molecular mechanisms underlying flowering-time regulation is crucial for breeding programs in *Brassica* vegetables. Another significant challenge lies in translating basic research to field applications: the expression patterns of flowering-related genes can vary substantially between controlled experimental systems (where mechanisms are discovered and characterized) and field-grown plants (where food production takes place). This variation suggests that real-world environmental factors profoundly influence the complex regulatory network controlling floral transition. Consequently, to effectively translate molecular insights into predictable crop performance, integrative analyses combining genetic, molecular, and environmental data are indispensable to fully elucidate the mechanisms governing flowering in *Brassica* vegetable crops.

3 The vernalization checkpoint: molecular and epigenetic logic of *FLC* memory

In *A. thaliana*, the vernalization checkpoint operates as a classic double-negative switch, in which the transition to flowering is initiated only after *FLC*, the master inhibitor of floral transition, is silenced. This switch is versatile in distinguishing relevant signals from sporadic ones over long temporal intervals associated with seasonal changes. Importantly, it relies fundamentally on an information storage system that allows the plant to “remember” its developmental and environmental history. This information storage is currently best understood in the context of winter annual life history (Figure 1), where *FLC* regulation is framed as the sequential integration of developmental and environmental memories, each serving multiple roles.

3.1 Developmental memory and the active chromatin state

Developmental *FLC* memory is a form of active transcription that inhibits the transition to reproduction by maintaining high *FLC* levels throughout early growth. This high-expression state is established during a narrow embryonic window by specialized transcription factors and is mitotically inherited through all subsequent cell lineages, persisting from the embryo into the juvenile and adult phases (Tao et al., 2017). Physically, this state is maintained by the recruitment of activating chromatin machinery, primarily orchestrated by FRIGIDA (FRI) (Maple et al., 2024).

In eukaryotic nuclei, DNA is wrapped around histone octamers to form nucleosomes. The “on” state of *FLC* is characterized by the enrichment of H3K4me3 and H3K36me3—marks associated with active euchromatin (Fang et al., 2021). Trimethylation of histone H3 at lysine 4 (H3K4me3) is deposited at promoters by Trithorax-group (TrxG) complexes. In *A. thaliana*, the major writers include ARABIDOPSIS TRITHORAX1 (ATX1) and related SET-domain proteins, alongside the SET DOMAIN GROUP 2 (SDG2) methyltransferase (Ding et al., 2012; Binenbaum et al., 2025). These marks typically peak at the 5′ ends of genes, where they facilitate the recruitment of the general transcription machinery. ATX1, for instance, physically associates with the basal transcriptional machinery—including TATA-binding protein (TBP) and Pol II—and is essential for maintaining high levels of these components at promoters (Ding et al., 2012). This interaction promotes the formation of a robust pre-initiation complex, while the catalytic H3K4me3 mark itself is required for efficient transcriptional elongation; in its absence, Pol II tends to pause or stall more frequently, leading to reduced mRNA production. Active genes are further characterized by the presence of H3K36me3 throughout their transcribed regions—a pattern that supports open chromatin and

unobstructed Pol II passage (Roudier et al., 2011). In *A. thaliana*, the SDG8 enzyme catalyzes di- and tri-methylation of H3K36 on elongating genes. Mechanistically, SDG8 is directly coupled to transcription through its physical interaction with both the initiating and elongating forms of Pol II (Zhang et al., 2020). This coupling ensures that H3K36me3 is deposited in tandem with Pol II progression, thereby supporting transcriptional induction.

3.2 Environmental memory and the repressive machinery

Environmental *FLC* memory refers to the repressed state, serving as a timing mechanism during the cold and providing permanent repression afterwards. The gradual silencing of *FLC* allows the plant to “sense” the duration of winter, in which decreasing levels of the repressor serve as a cellular record of sufficient cold exposure. This transition is mediated by the accumulation of VERNALIZATION INSENSITIVE 3 (VIN3), which recruits the Polycomb Repressive Complex 2 (PRC2) to the locus.

The core repressive mark, H3K27me3, is deposited by PRC2. In *A. thaliana*, this complex comprises several functionally distinct subunits: the catalytic SET-domain proteins—including CURLY LEAF (CLF), SWINGER (SWN), and MEDEA (MEA); the SUZ12-like zinc-finger proteins such as EMBRYONIC FLOWER 2 (EMF2), VERNALIZATION 2 (VRN2), and FERTILIZATION-INDEPENDENT SEED 2 (FIS2)—which facilitate nucleosome binding; and the scaffolding proteins FERTILIZATION-INDEPENDENT ENDOSPERM (FIE) and MULTICOPY SUPPRESSOR OF IRA1 (MSI1) (Godwin and Farrona, 2022). Mechanistically, H3K27me3 promotes chromatin condensation, thereby restricting the accessibility of transcription factors and RNA Pol II. PRC2-mediated silencing can block the transition of RNA Pol II into productive elongation by inducing promoter-proximal pausing, or by physically compacting nucleosomes to exclude the transcriptional machinery entirely (Aranda et al., 2015).

Because PRC2 lacks inherent DNA-binding capabilities, its recruitment is guided by DNA-binding cofactors that recognize specific sequence motifs. For example, telobox DNA repeats recruit telomere-repeat-binding (TRB) proteins that physically interact with CLF and SWN to guide PRC2 to specific target genes (Deng et al., 2013; Zhou et al., 2018). Crucially, H3K36me3 and H3K27me3 tend to be mutually exclusive; active genes marked with H3K36me3 rarely acquire repressive Polycomb marks, and vice versa, thereby establishing a stable epigenetic boundary (Sequeira-Mendes et al., 2014).

3.3 Erasers and the regulatory tug-of-war

While the deposition of histone marks can propagate epigenetic states across cell divisions—creating stable memory of active/repressed states—these marks are ultimately reversible. The erasure of H3K27me3 is achieved by Jumonji demethylases such as RELATIVE OF EARLY FLOWERING 6 (REF6, JMJ12) and EARLY FLOWERING 6 (ELF6, JMJ13) (Noh et al., 2004). REF6 acts as a specific targeting factor, recognizing the DNA motif

CTCTGYTY via its C2H2 zinc-finger domains and directly binding to target loci (Qiu et al., 2019). Upon binding, REF6 recruits the SWI/SNF chromatin remodeler BRAHMA to open the chromatin structure and promote transcription, effectively switching silenced genes back to an active state. A critical aspect of this regulation is the selectivity of REF6; it binds only to motifs in relatively unmethylated, euchromatic regions and is actively repelled by H3K9me2-associated DNA methylation. This ensures that while developmental genes can be toggled between active and repressed states, constitutive heterochromatin remains stable. Similarly, histone H3K4 demethylases, such as JMJ14, act as molecular switches to remove activation marks and fine-tune gene expression levels (Lu et al., 2010; Searle et al., 2010). By balancing the activity of PRC2 writers against erasers such as REF6 and ELF6, the plant cell can precisely tune developmental gene expression and maintain a flexible yet heritable epigenetic memory.

The interplay between these opposing histone modifications establishes a robust yet reversible system of transcriptional control, exemplified most notably by the regulation of the floral repressor *FLC*. The *FLC* locus serves as a molecular battlefield where activating (H3K4me3/H3K36me3) and repressive (H3K27me3) complexes are recruited to specific, *cis*-regulatory elements to ultimately coordinate the timing of phase transitions (Buzas et al., 2012; Buzas, 2017). Notably, the same *cis*-acting elements can act as a core recruitment platform for both Polycomb and Trithorax complexes, having antagonistic activities. Ultimately, it is such tug-of-war that allows succession of the two *FLC* memory states: developmental and environmental (Figure 1).

3.4 Translating epigenetic knowledge into precision breeding

While traditional crop improvement focuses on fixed DNA sequences, the epigenome provides a flexible and dynamic layer of heritable information for translational research. Although histone modifications constitute the physical marks of memory, the actual frontier for breeding lies in manipulating the *cis*-regulatory logic—in case of *FLC*, including elements such as the first intron and noncoding RNAs—that directs chromatin-modifying complexes to specific genomic loci. The first intron of *FLC* is a particularly valuable target, as it contains a long DNA sequence peppered with *cis*-elements—such as the Vernalization Response Element (VRE) (Sung and Amasino, 2004) and the Cold Memory Element (CME) (Yuan et al., 2016)—that serve as recruitment hubs for epigenetic “writers” and “erasers” without compromising protein function (Buzas et al., 2011, 2012).

Beyond these DNA elements, the antisense transcript *COOLAIR* holds significant potential for engineering because both its promoter and its various exons—which are alternatively spliced into different isoforms (Marquardt et al., 2014)—are located outside the *FLC* coding region. Natural variations in *COOLAIR* sequences that modulate *FLC* regulation without altering the amino acid sequence of the *FLC* protein have already been identified in diverse *A. thaliana* accessions (Li et al., 2014) and *Brassica* crops (Ahn et al., 2024, see below). This natural diversity indicates that such noncoding regions are viable targets for crop development, providing a mechanism to

fine-tune environmental responses without pleiotropic effects on protein function. By using Clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) to target these specific recruitment and splicing mechanisms in crops such as *Brassica* and *Raphanus*, researchers can engineer how crops record and respond to their environment, leveraging conserved *A. thaliana* regulatory logic to achieve FLC-specific impacts on crop performance. Given the wealth of existing information on the function of natural variants in these noncoding regions, these sequences represent immediate, high-value targets for developing climate-resilient horticultural varieties.

4 From model to market: leveraging genomic paralog diversity and DNA elements for epigenetic regulation

The translation of findings from *A. thaliana* to related crops is complicated by their increased genomic complexity. Multiple *FLC* paralogs, arising from ancestral whole-genome triplication events, provide a genomic “control panel” in which different paralogs may contribute unequally to the flowering phenotype (i.e., sub-functionalization). Therefore, translational research requires the functional characterization of each paralog to determine which copies have retained dominance in controlling vernalization. While the protein-coding sequences are highly conserved, significant variation exists in the noncoding regions, particularly within the massive first introns characteristic of *Brassica* and *Raphanus* *FLC* genes. These intronic expansions often house the duplicated *cis*-regulatory elements that dictate how each paralog responds to environmental cues. By identifying which specific paralog—such as *BrFLC2* in certain Chinese cabbage varieties—exerts the strongest influence on bolting, breeders can move beyond broad phenotypic selection toward a “paralog-specific” breeding strategy.

4.1 Paralog diversification and epigenetic control of flowering time in *B. rapa*

4.1.1 Paralog diversity and function

The whole-genome triplication event in the genus *Brassica*, following its divergence from *A. thaliana*, resulted in multiple paralogs of genes that typically exist as single copies in *A. thaliana* (Cheng et al., 2013). Consequently, understanding flowering in *Brassica* requires accounting for both additive effects and functional divergence among these paralogs. Central regulators of flowering time exemplify this genomic complexity. For instance, *B. rapa* possesses two copies of *FRI*; while both retain the ancestral function of *AtFRI*, notable functional differences have emerged between them (Takada et al., 2019; Zheng et al., 2021). Similarly, all four *BrFLC* paralogs are repressed by cold, yet their varying expression levels prior to vernalization suggest divergence in regulatory control (Kawanabe et al., 2016; Takada et al., 2019). Among flowering integrators, two *BrFT* copies were identified in the ‘Chiifu-401–42’ reference genome (Del Olmo et al., 2019; Lee

et al., 2023), while 12 *FT*-related genes with distinct functional roles have been reported in turnip (Li et al., 2024). Furthermore, *BrSOC1* exists in three copies (*BrSOC1-1*, *-2*, and *-3*) with distinct expression patterns (Sri et al., 2020), while the two *BrVIN3* paralogs (*BrVIN3.1* and *BrVIN3.2*) are essentially the same, though there are a few minor differences in their regulatory control (Jung et al., 2021).

Notable functional studies have further dissected this complexity through several avenues. First, QTL mapping has been instrumental in identifying genes associated with flowering time. Studies using populations derived from early- and late-flowering accessions have identified loss-of-function alleles in major regulators such as *BrFT* and *BrFLC* (Shea et al., 2017; Akter et al., 2021; Kim et al., 2022). While promoter insertions that activate *BrFT* lead to early flowering (Wei et al., 2022; Nishikawa et al., 2023), the late-bolting trait is often more valuable in breeding leafy and root vegetables. Delayed flowering is associated with insertions in the first intron of *BrFLC2* and *BrFLC3*, which inhibit cold-induced suppression (Kitamoto et al., 2014). Additionally, variation in *BrFLC5*—which is non-functional in many lines but functional in others—correlates with flowering time, with functional alleles trending toward later flowering (Kakizaki et al., 2011; Xi et al., 2018; Akter et al., 2023). Association studies have linked polymorphisms in *BrFLC1* and *BrVIN3.1* to variation in flowering time (Su et al., 2018). Total *BrFLC* expression prior to vernalization is positively correlated with delayed bolting, suggesting that promoter sequence polymorphisms may drive divergent vernalization requirements (Takada et al., 2019). Conversely, reduced repression of *BrFLC* during cold treatment also contributes to late-bolting phenotypes, likely driven by sequence polymorphisms in critical repressive regions, such as the first intron (Kitamoto et al., 2014; Takada et al., 2019). In addition, genome editing has revealed critical dosage effects. In *F₁* plants created by crossing a *BrSOC1* loss-of-function mutant with the wild type, bolting was delayed relative to the wild type but was less severe than in the mutant. This dosage sensitivity highlights its potential as a tool for fine-tuning bolting time in hybrid breeding, though the effect is less pronounced than a complete loss-of-function (Jung et al., 2025). RNA-seq analyses pre- and post-vernalization have identified differential expression of core genes, including *BrFLC*, *BrVIN3*, and *BrSOC1* (Shea et al., 2019; Akter et al., 2020; Jung et al., 2021). Notably, late-flowering lines exhibit higher expression of nitric oxide signaling genes both before and after cold treatment (Jung et al., 2021). Among the *BrSOC1* paralogs, *BrSOC1-1* appears to play the most critical role; although loss of any paralog delays flowering, the effect is significantly more pronounced when *BrSOC1-1* is involved (Jung et al., 2021). Lastly, single-cell transcriptomics provides the clearest demonstration of spatiotemporal functional divergence. Profiling across five cell types revealed that mesophyll cells are highly responsive to vernalization. In contrast, vascular cells tend to maintain *BrFLC* expression longer during cold exposure, suggesting they may buffer against flowering induction during short-term cold exposure. Crucially, the patterns of specific paralogs vary significantly by cell type; while *BrFLC1* and *BrFLC2* expression decreases rapidly in mesophyll and epidermal cells in response to cold, vascular cells show delayed repression of the same genes. Furthermore, specific

paralogs, such as *BrFLC3*, show higher baseline stability in proliferating cells than in differentiated mesophyll, indicating that the triplicated genome allows for cell-type-specific “tuning” of the vernalization requirement (Zhang et al., 2025b).

4.1.2 Epigenetic marks and mechanisms

H3K27me3 is deployed genome-wide in *B. rapa* to silence developmental regulators, and its distribution is largely conserved among paralogs in the triplicated genome, underscoring its fundamental role in controlling developmental transitions (Akter et al., 2019; Payá-Milans et al., 2019; Nishimura et al., 2025). Prior to vernalization, *BrFLC* is actively transcribed and enriched in permissive histone marks, such as H3K4me3 and H3K36me3, across the gene body (Mehraj et al., 2021). During cold exposure, however, H3K27me3 gradually accumulates at the nucleation region near the transcription start site. Upon return to normal temperatures, this repressive mark spreads across the entire gene, thereby establishing a stably silenced state of *BrFLC* (Akter et al., 2019). Several loss-of-function mutants of PRC2 components have been identified in *B. rapa*. *BrCLF* is a single-copy gene and the *brclf* mutants exhibit early flowering (Huang et al., 2020; Tan et al., 2021; Nugroho et al., 2023; Poza-Viejo et al., 2024). Chromatin Immunoprecipitation (ChIP)-seq and RNA-seq analyses in *brclf* mutants have revealed reduced H3K27me3 levels, concomitant with the upregulation of floral integrator and homeotic genes, including *BrSOC1*, *BrFT*, and *BrSEP3* (Poza-Viejo et al., 2024). *BrSWN* is also present as a single-copy gene in *B. rapa*, and the *brswn* mutant displays delayed flowering accompanied by reduced vernalization sensitivity. After prolonged cold treatment, expression levels of *BrFLC2* and *BrFLC3* remained higher in the *brswn* mutant than in the wild type, whereas their H3K27me3 levels were lower, indicating that *BrSWN* is directly or indirectly required for efficient epigenetic silencing of *BrFLC* paralogs during vernalization (Zhao et al., 2025).

In addition to PRC2-mediated repression, histone methylation associated with transcriptional activation also contributes to flowering time regulation. *SDG8*, the ortholog of the *A. thaliana* histone H3K36 methyltransferase, was identified as the causal gene of another early-flowering mutant. In this mutant, expression levels of all four *BrFLC* paralogs were reduced compared to the wild type (Fu et al., 2020). Another TrxG-related H3K36 methyltransferase, *BrEFS* (the *B. rapa* homolog of *A. thaliana* *EFS*), was recently characterized in oilseed-type *B. rapa*. A loss-of-function *brefs* mutant carrying a premature stop codon in *BrEFS*, displays defects in floral organ development and shows strongly reduced H3K36me3 at key flower-development regulators, linking H3K36 methylation directly to reproductive development in *Brassica* (Moon et al., 2025). Together, these findings support a model in which TrxG enzymes lay down activating marks to sustain expression of floral regulators, thereby antagonizing the spread of PRC2-mediated H3K27me3.

Histone demethylases also modulate flowering through dynamic regulation of H3K27me3. *brelf6* and *brref6* mutants, each present as a single-copy gene, show contrasting phenotypes; *brelf6* flowers early, whereas *brref6* flowers late. The early-flowering

phenotype of *brelf6* is associated with increased H3K27me3 accumulation and repression of *BrFLC3*. In contrast, the late-flowering phenotype of *brref6* is not associated with *BrFLC3* expression or its H3K27me3 levels. Instead, genes involved in gibberellin (GA) biosynthesis exhibit elevated H3K27me3 and transcriptional repression in *brref6*, suggesting that impaired GA biosynthesis causes delayed flowering, a hypothesis supported by the observation that exogenous GA₃ application accelerates flowering in *brref6* but not in the wild type (Poza-Viejo et al., 2022). Functional diversification has also been observed among alleles of *BrJMJ18*, an H3K36me3/2 demethylase. Overexpression of the *BrJMJ18* allele from subsp. *chinensis* (Pak-Choi, PC) does not alter flowering time between normal and high temperature conditions. However, overexpression of the allele from subsp. *chinensis* var. *parachinensis* (*Par*) leads to delayed flowering at high temperatures but not under normal temperature conditions. This temperature-dependent delay is associated with increased H3K36me3 enrichment at the *BrFLC3* locus and elevated *BrFLC3* expression, indicating that the *BrJMJ18* allele from *Par* modulates thermal responsiveness of flowering through H3K36me3 dynamics (Xin et al., 2024).

Together, these findings highlight the diverse and interconnected roles of histone methylation and demethylation machinery in regulating *FLC* expression and flowering time in *B. rapa*. While *COOLAIR*-like transcripts have been identified for *BrFLC1* and *BrFLC2* and are induced by cold, they appear to be absent for *BrFLC3* and *BrFLC5* (Li et al., 2016; Shea et al., 2019; Dai et al., 2024). Consequently, although all four paralogs exhibit cold-induced H3K27me3 accumulation, the contribution of noncoding RNAs to PRC2 recruitment in *B. rapa* remains unclear. Instead, the RY element—a *cis*-regulatory sequence known to recruit PRC2 as part of the CME—has been identified in all four *BrFLC* paralogs, suggesting that it may play the primary role in vernalization-induced repression in *Brassica* (Moon et al., 2026).

4.2 Natural variation and morphotype-specific regulation of flowering in *B. oleracea*

4.2.1 Physiological and genetic divergence

While *B. oleracea* shares a requirement for prolonged cold exposure with *B. rapa*, their physiological responses are distinct. *B. rapa* is characterized by a seed-vernalization response, whereas *B. oleracea* exhibits a “green plant” vernalization type, requiring the plant to reach a specific vegetative size or age before becoming competent to perceive cold signals (Itabashi et al., 2019). Within the species, vernalization requirements vary drastically across morphotypes; leafy vegetables such as cabbage possess a high cold requirement, while flower-head crops like broccoli and cauliflower require significantly less (Ridge et al., 2015; Guo et al., 2024). Following the whole-genome triplication event shared across the *Brassica* genus, *B. oleracea* contains multiple paralogs of *A. thaliana* flowering-related genes (Cheng et al., 2013). For example, *FRI* is present in two copies, and the conserved *FRI-FLC* regulatory module remains a fundamental feature of *B. oleracea* flowering

(Irwin et al., 2012). Among the repressors, four *BoFLC* paralogs have been identified: *BoFLC5* is a pseudogene, while *BoFLC1* exists as a tandem duplication (*BoFLC1a* and *BoFLC1b*). As this tandem duplication is also observed in the C-subgenome of *B. napus*, the event likely occurred prior to the formation of *B. napus* approximately 7,500 years ago (Chalhoub et al., 2014).

4.2.2 The central role of natural variation in *BoFLCs*

Early QTL analyses established the genetic basis for flowering variation in *B. oleracea* (Camargo and Osborn, 1996; Guo et al., 2004). A major breakthrough identified *BoFLC2* as the causal gene for the largest flowering-time QTL; early-flowering genotypes often carry nonfunctional *BoFLC2* alleles due to frameshift mutations or deletions (Okazaki et al., 2007). Further modelling suggested that differences in cold sensitivity among genotypes are the primary drivers of flowering-time diversity (Uptmoor et al., 2012). Recent advances in next-generation sequencing technologies have deepened this understanding. QTL-seq identified the *Ej2.1* locus on chromosome C02, where *BoIGRF6* emerged as a strong candidate gene for early flowering (Shu et al., 2018). Similarly, GWAS and transcriptomic analyses across diverse accessions identified *miR172D* and *BoFLC2* as key candidate regulators for vernalization response (Woodhouse et al., 2021). While the functional roles of two *B. oleracea* copies of *miR172D* remain to be elucidated (Shivaraj et al., 2014), *BoFLC2* expression levels under non-vernalizing conditions show a strong correlation with the timing of visible bud formation and anthesis (Woodhouse et al., 2021). This relationship between pre-vernalization *FLC* expression levels and cold requirement mirrors findings in *B. rapa* and *B. napus* (Takada et al., 2019; Calderwood et al., 2021). Molecular evidence confirms that subtle variation in *BoFLC2* regulation facilitates climate adaptation. *Cis*-regulatory polymorphisms at this locus influence heading dates (Irwin et al., 2016), while a 215 bp insertion/deletion (indel) within the first intron reduces the efficiency of cold-induced silencing, thereby delaying flowering (Li et al., 2022). Furthermore, *BoFLC2* variants were likely selected during domestication to optimize flowering time across geographically distinct growing regions (Hou et al., 2025).

BoFLC1 has also been identified as a significant regulator of flowering time. A 67 bp insertion in the first intron of *BoFLC1* differentiates early- and late-flowering lines, serving as a reliable DNA marker for breeding (Abuyusuf et al., 2019). Notably, in the “natural non-flowering cabbage” (*nfc*) mutant, a tandem duplication and constitutive overexpression of *BoFLC1a* and *BoFLC1b* continuously suppress the floral integrator *BoFT*, thereby maintaining a vegetative state throughout winter (Kinoshita et al., 2023).

4.2.3 Spatiotemporal and morphotype-specific regulation

Analyses of cabbage at different developmental stages (e.g., four-leaf vs. fourteen-leaf) reveal distinct expression dynamics among *BoFLC* paralogs. While *BoFLC2* and *BoFLC3* are strongly

repressed following prolonged cold exposure regardless of age, *BoFLC1* expression remains relatively stable. These findings suggest that cabbage employs age-dependent and gene-specific responses to regulate flowering competence (Itabashi et al., 2019). Coordination between *BoFLC* paralogs and other regulators—such as *BoGI*, *BoCOOLAIR*, and *BoVIN3*—further modulates these responses (Ahn et al., 2024). Comprehensive omics approaches have expanded the regulatory network governing flowering. Transcriptomic comparisons between early- and late-flowering cabbage lines identified *BoSEP2-1* and *BoSEP2-2* as candidate genes (Wang et al., 2023). Furthermore, integrated metabolome and transcriptome analyses of bolting-tolerant lines revealed complex interactions among circadian-related genes (*BoCCA1*, *BoLHY*, *BoELF4*), flowering integrators (*BoSOC1*), and metabolic pathways such as ribosome biogenesis and raffinose metabolism (Yang et al., 2025). In specific morphotypes such as cauliflower, floral initiation requires a synergy between vernalization (optimally around 10 °C) and GA₁ activity to facilitate stalk elongation (Guo et al., 2004). The reduced vernalization requirement in cauliflower and broccoli is often linked to the loss of *BoFLC2* function (Ridge et al., 2015). Recent pangenome analyses revealed a large deletion of *BoFLC2* in Chinese kale and a widespread single-nucleotide deletion in the fourth exon of *BoFLC2*, found in over 90% of broccoli and cauliflower accessions (Guo et al., 2024).

In conclusion, while *BoFLC2* is clearly a dominant regulator of flowering in *B. oleracea*, evidence from *B. rapa* and *B. napus* suggests that the total expression of all *FLC* paralogs is critical. It remains to be seen if the current focus on *BoFLC2* reflects its high genetic diversity or if other paralogs exhibit more constrained natural variation. Moreover, the molecular mechanism underlying the juvenile-phase requirement for vernalization competence in *B. oleracea* remains a significant knowledge gap. Addressing these questions will be crucial for developing *Brassica* varieties that are adapted to a changing global climate.

4.3 Integrated control of flowering time in radish

4.3.1 Genomic background and conservation of flowering genes

Radish (*R. sativus* L.) is an important root vegetable crop belonging to the family Brassicaceae. Because an early floral transition results in significant yield loss, understanding the mechanisms regulating flowering time is essential for radish breeding. Radish is a diploid species ($2n = 2x = 18$), and comparative genomic studies have shown that *Raphanus* genomes originated from an ancestral genome that underwent whole-genome triplication, followed by extensive chromosomal rearrangements (Kitashiba et al., 2014; Mitsui et al., 2015; Jeong et al., 2016). Transcriptome analyses identified 142 radish genes orthologous to flowering-related genes in *A. thaliana*, suggesting that flowering regulation in radish operates through conserved pathways similar to those in *A. thaliana* and other Brassicaceae species (Nie et al., 2016; Wang et al., 2017). *FLC* paralogs mediate a

central, conserved regulatory pathway. Genome sequencing and transcriptome analyses have identified three paralogous *FLC* genes in radish: *RsFLC1*, *RsFLC2*, and *RsFLC3* (Kitashiba et al., 2014; Yi et al., 2014; Jeong et al., 2016). Overexpression of each *RsFLC* gene in *A. thaliana* transgenic plants delayed flowering, demonstrating that all three *RsFLC* genes function as floral repressors (Yi et al., 2014).

4.3.2 Allelic variation and QTL mapping of bolting traits

There is significant variation in vernalization sensitivity among radish cultivars, often driven by structural variations in the paralogous *FLC* genes. A 1,627 bp insertion in the 5' region of the first intron of *RsFLC2* was identified via QTL analysis in an extremely late-bolting radish cultivar requiring over 60 days of cold exposure; this insertion attenuated the repression of *RsFLC2* during vernalization (Wang et al., 2018). Additionally, insertions of 1,910 bp and 1,627 bp in the first introns of *RsFLC1* and *RsFLC2*, respectively, were shown to impair repression of these genes following prolonged cold treatment, resulting in delayed flowering (Mitsui et al., 2023). The combination of the two *RsFLC* alleles carrying these insertions modulates flowering time, with the insertion in *RsFLC2* exerting a particularly strong effect on delayed flowering (Mitsui et al., 2023). While QTLs on chromosomes R02 (*qBT2*) and R07 (*qBT7.2*) implicate *RsFLC2* and *RsFLC1* as primary candidates (Jin et al., 2024), other studies have identified flowering QTLs on chromosome R06 that do not encompass any *FLC* paralogs, suggesting the existence of *FLC*-independent regulatory nodes (Ma et al., 2021).

4.3.3 Local adaptation in wild accessions

Variation in vernalization requirement has also been observed among wild radish accessions collected across Japan, ranging from Hokkaido in the north to Okinawa in the south. While northern regions experience colder climates, daily minimum temperatures in Okinawa (Naha) from October to February range from 14.6 °C to 23.1 °C, which may be above the optimal range for vernalization. Northern accessions failed to flower without vernalization, even under long-day conditions, whereas southern accessions flowered without vernalization, although flowering time varied among accessions. Promotion of flowering by vernalization was also observed in summer accessions. Interestingly, similar levels of *RsFLC* expression were observed in both northern and southern accessions, and *RsFLC* was suppressed by vernalization in both. However, expression levels of *CONSTANS-like 5* (*RsCOL5*), the orthologue of *AtCOL5*—which promotes flowering by increasing *AtFT* expression in short-day-grown *A. thaliana* (Hassidim et al., 2009)—was strongly induced after vernalization, particularly in summer accessions, suggesting that the floral-promoting function of *RsCOL5* may outweigh the floral-repressive function of *RsFLC*. While some suggest a preference for the photoperiod pathway over the vernalization pathway in these cases (Han et al., 2021), whether *RsFLC* functions similarly in summer and winter accessions requires further examination.

4.3.4 Transcriptomic responses to vernalization

Transcriptome analyses conducted under three distinct conditions—non-vernalized, after 40 days of cold treatment, and following a 10-day warm recovery period—revealed differential expression in thousands of genes. Among these, 22 of 78 known flowering-related genes in radish exhibited significant expression changes. Notably, three *RsFLC* paralogs were downregulated following prolonged cold exposure. The expression patterns of *MADS AFFECTING FLOWERING* (*MAF*) genes varied: *RsMAF3* was suppressed only after the transition to warm temperatures following cold treatment, whereas *RsMAF4* expression remained stable across all conditions. Interestingly, *RsVIN3a* and *RsVIN3b* were induced by cold and further upregulated upon transfer to warm conditions (Lee et al., 2024). This contrasts with *AtVIN3* in *A. thaliana*, which typically decreases during warm recovery. While some studies report a drop in *RsVIN3* levels after cold treatment (Mitsui et al., 2023), complementation experiments in *atvin3* mutants confirmed that both *RsVIN3a* and *RsVIN3b* are functionally equivalent to *AtVIN3* (Lee et al., 2024). Further research is required to determine if these divergent patterns are species-wide or line-specific.

On a separate note, GA also influences flowering after the vernalization process. Although GA treatment alone does not induce flowering in radish, it acts synergistically with vernalization to accelerate the process, reducing the required duration of cold exposure. RNA-seq analysis of two lines differing in their cold requirements revealed that GA treatment following 10 days of cold exposure did not produce substantial transcriptional differences between the lines. Nevertheless, both lines showed increased expression of the floral activators *RsFT* and *RsSOC1-1*, suggesting that GA-mediated acceleration of flowering is primarily driven by the induction of these specific genes (Jung et al., 2020).

From a crop quality perspective, vernalization also influences the glucosinolate levels in radish, which are related to the plant's natural defense mechanisms, flavor, and nutrition. Integrated transcriptomic and metabolomic analyses indicate that total glucosinolate (GSL) levels decrease following prolonged cold exposure. The expression of *MYB* transcription factors governing aliphatic GSL biosynthesis—including *RsMYB28a*, *RsMYB28c*, *RsMYB29a*, and *RsMYB29b*—was reduced when plants returned to normal temperatures. In contrast, *MYB* genes involved in indolic GSL biosynthesis showed inconsistent expression patterns; while *MYB34c* was downregulated, *MYB51a* and *MYB51b* were upregulated. This discordance between transcriptomic and metabolomic data suggests a complex or uncoupled regulation of indolic GSL biosynthesis (Nugroho et al., 2021). Therefore, changes in metabolic compounds are additional factors to consider when breeding for desired flowering phenotypes.

4.3.5 Epigenetic regulation of *RsFLC* and chromatin dynamics

The epigenetic silencing of *RsFLC* during vernalization depends critically on RY motifs within its regulatory regions. Specifically, in *RsFLC1*, these motifs are essential for transcriptional repression;

their disruption impairs the accumulation of the repressive histone mark H3K27me3 and delays floral transition. This highlights the critical role of RY motifs in mediating the vernalization response (Lee et al., 2024).

Further epigenetic insights have emerged from comparative analyses between the normal-flowering inbred line ‘Jinjudaepyung’ (‘JD’) and the late-flowering line ‘Simu’ (‘SM’). These studies identified *RsFLC1* and *RsFLC2* as key differentially expressed genes (DEGs). In the normal-flowering line, all three *RsFLC* paralogs were suppressed by cold and remained repressed during the warm recovery phase. Conversely, in the late-flowering ‘SM’ line, *RsFLC2* was not suppressed despite the successful repression of *RsFLC1* and *RsFLC3*. This lack of suppression in the late-flowering line is attributed to two major DNA insertions in *RsFLC2*: a 1,037 bp insertion in the proximal promoter and a 1,627 bp insertion in the first intron. Transformation of this genomic fragment into *A. thaliana* delayed flowering and abolished cold-induced *RsFLC2* suppression, successfully recapitulating the radish phenotype (Lee et al., 2025). Furthermore, chromosome conformation capture (3C) analysis revealed that these insertions disrupt proper intragenic loop formation and impede PRC2-mediated H3K27me3 deposition, thereby neutralizing the vernalization response (Lee et al., 2025).

4.4 The genomic translation from the model annual *A. thaliana* to *Brassica* vegetable crops

The molecular framework underlying *FLC* regulation provides a direct and actionable bridge between mechanistic insights and practical breeding of *Brassica* vegetable crops. From a breeding standpoint, two issues are particularly critical.

First, most cultivars of *Brassica* vegetable crops are grown as F₁ hybrids (Fujimoto et al., 2018), making the synchronization of flowering time between parental lines a key determinant of efficient hybrid seed production. Second, in leafy and root vegetables, premature flowering during cultivation results in severe yield and quality deterioration; therefore, enhancing late-bolting traits is a central breeding objective (Kitamoto et al., 2017). In this context, two molecular parameters emerge as especially informative: the basal expression level of *FLC* prior to vernalization and the efficiency with which *FLC* repression is established and stably maintained during prolonged cold exposure. Elevated pre-vernalization *FLC* expression is strongly associated with delayed bolting in leafy and root vegetables (Takada et al., 2019), whereas incomplete or unstable epigenetic silencing during cold exposure underlies extreme late-flowering phenotypes (Kitamoto et al., 2014; Moon et al., 2026).

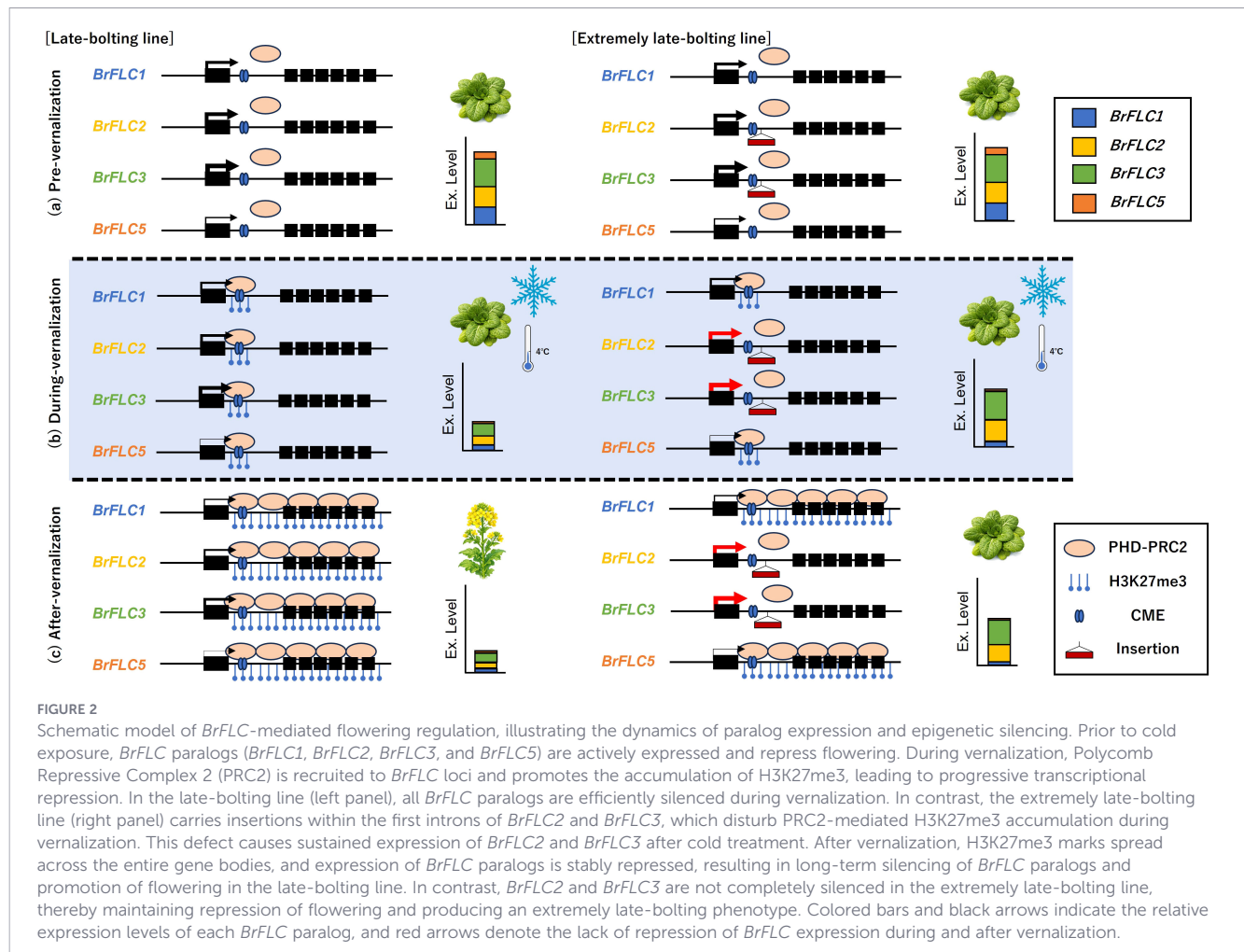
In *Brassica* vegetable crops, flowering time is not governed by a single dominant locus but rather by the combined and context-dependent contributions of multiple *FLC* paralogs (Figure 2). This paralog diversity provides breeders with a tunable genetic system in which flowering behavior can be adjusted incrementally, rather than through binary low- or high-vernalization requirement outcomes. Because the expression levels of individual *FLC* paralogs prior to cold exposure are likely determined primarily by *cis*-regulatory variation in their promoters, one practical strategy may be to select and

pyramid high-expression *FLC* alleles in pre-vernalized states. Alternatively, if *trans*-acting transcription factors that coordinately regulate multiple *FLC* paralogs can be identified, it may be possible to elevate overall *FLC* expression by manipulating a single upstream regulator. Progress in elucidating these regulatory layers will be essential for refining flowering-time control in breeding of *Brassica* vegetable crops. In addition, genome-editing approaches targeting *cis*-regulatory landscapes of promoters (“*cis*-nome editing”) may provide further opportunities to fine-tune *FLC* expression (Saeed et al., 2022). Ultimately, these findings suggest that targeted manipulation of specific *FLC* paralogs—either through marker-assisted selection or genome editing—can fine-tune vernalization requirement without compromising other developmental traits.

A second major breeding target is reduced sensitivity to vernalization. In this regard, the first intron of *FLC* plays a pivotal role and is closely linked to epigenetic regulation. In several *Brassica* vegetable crops, insertions within the first intron prevent *FLC* downregulation even after prolonged cold exposure, resulting in a strong vernalization requirement (Figure 2) (Kitamoto et al., 2014; Li et al., 2022; Mitsui et al., 2023; Lee et al., 2025). This phenotype is most likely caused by impaired recruitment of the PRC2, with the CME representing a key target. The most straightforward strategy to modulate this response is to introduce mutations within the CME, either by identifying naturally occurring variants or through genome editing. Because these are *cis*-regulatory changes, their incorporation into breeding programs via DNA marker-assisted selection is expected to be relatively straightforward. In fact, marker-assisted selection has already been performed using DNA markers targeting insertions in the first intron of *FLC* (Kitamoto et al., 2017). Another emerging possibility is epigenome editing, which has recently been reported to manipulate chromatin states without altering the underlying DNA sequence (Tonosaki et al., 2022; He et al., 2025; Hirata et al., 2025; Nishimura et al., 2025). While this approach holds conceptual promise, precisely regulating multiple paralogs of the target gene may be technically challenging. In addition, substantial regulatory hurdles remain before it can be realistically implemented in practical breeding programs. Importantly, these molecular parameters can be quantified using targeted molecular assays and directly linked to agronomically relevant traits, enabling breeders to move beyond purely phenotypic selection toward genotype-informed prediction and optimization of flowering behavior.

5 Vernalization research beyond the growth chamber

Despite vernalization being an essential and prominent developmental process critical in nature for wild species and the yield and quality of numerous horticultural and staple crops, the overwhelming majority of mechanistic studies rely on highly controlled laboratory assays. This reliance has created a significant knowledge gap, as the complex and variable environmental conditions found in natural habitats and agricultural fields often alter the plant’s response to seasonal cues. To exemplify some notable



outcomes of recent studies that do not rely exclusively on laboratory assays, we categorized such current research into three groups, based on the scope of environmental integration.

The first category comprises validation studies, which aim to verify and contextualize core mechanisms established in the laboratory by comparing molecular and phenotypic outcomes observed under controlled laboratory conditions with those observed under specific, local field environments. Such studies often serve to highlight the limitations of predictive models based solely on chamber experiments. For example, field studies tracking *B. napus* (winter oilseed rape) have revealed that the *FLC* repression occurs predominantly in early autumn, rather than continuously throughout winter—contradicting assumptions based on constant-temperature typically used in chamber experiments to mimic seasonal progression (O'Neill et al., 2019). Further challenging the assumptions of laboratory constancy, work on *A. thaliana* by Burghardt et al. (2016) demonstrated that when plants are subjected to realistic, fluctuating, and warmer cold regimes, the repressive effect of *FLC* on flowering time is significantly weakened. Collectively, these findings highlight that the key molecular event—the stability and effectiveness of *FLC* silencing—is fundamentally shaped by the temporal patterns and variability of cold exposure found outside the laboratory.

The second category comprises mechanistic discovery studies. These studies aim to identify novel molecular or regulatory mechanisms that are triggered or activated only under the complex dynamics of natural field conditions. These studies often employ a hybrid approach, using field data (e.g., temperature fluctuations) to design more realistic, fluctuating cold treatments in the laboratory, leading to the discovery of novel temperature-sensing components or regulatory nodes within the vernalization pathway. Hepworth et al. (2018) investigated how multiple aspects of the temperature profile in diverse European field sites with different winters allow flowering to align with spring conditions. In laboratory vernalization assays, *VIN3*-independent silencing of *FLC* and *VIN3* induction occur as superimposed events during the cold treatment. However, under field conditions, *FLC* silencing can be temporally separated into two distinct phases, each with different kinetics and different *VIN3* regulatory phases. Another study analyzed the induction of *COOLAIR* under field conditions across different *A. thaliana* accessions. These experiments revealed accession-specific temperature sensitivity and identified a critical factor for *COOLAIR* induction, namely the first frost, a characteristic that could be replicated in the laboratory. These findings led the authors to propose that *COOLAIR* could be a key indicator for the arrival of winter (Zhao et al., 2021).

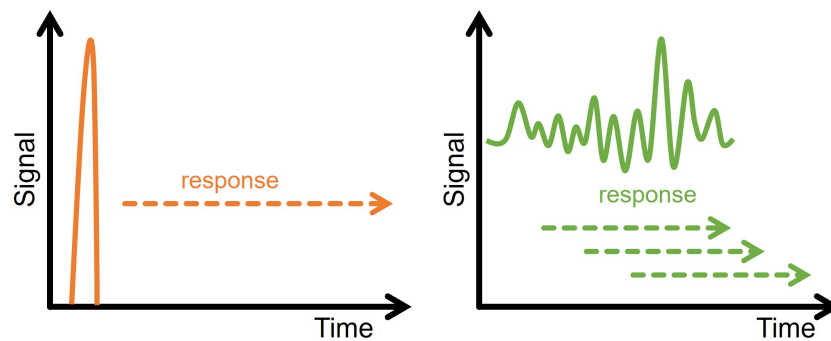


FIGURE 3

Models of information storage in *FLC* regulation. Schematic representation of distinct memory types used to track environmental signals. (Left panel) A traditional switch-like memory where a transient signal triggers a state that persists after the stimulus has ceased. (Right panel) An integrative memory in which a gene tracks fluctuating thermal signals by integrating them over a past temporal window. In this model, gene expression variance is explained by temperatures averaged over time, allowing the plant to capture signal duration as a quantifiable parameter.

Early studies demonstrated that the range of *FLC* expression in a natural population of *Arabidopsis halleri* subsp. *gemmaifera* (*A. halleri*) was substantially broader under natural environmental conditions than in vernalization assays in the laboratory (Aikawa et al., 2010). This finding was recently confirmed by a comparative study between the two perennial life histories of *A. halleri* and *Eutrema japonicum* (*E. japonicum*) (Endo et al., 2025). In both species, the difference in *FLC* expression levels reached as much as 1,000-fold. Furthermore, the duration of *FLC* maintenance—both in its high-expression state (“memory of embryonic state”) and low-expression state (“memory of winter”)—is significantly prolonged in natural settings compared to laboratory conditions. Paradoxically, these results suggest that fluctuating temperatures induce longer-term stability in gene expression than constant cold regimes.

Finally, the last category comprises ecological studies that aim to elucidate how plants—constantly exposed to multiple, often unpredictable factors in natural environments—can generate robust developmental responses. An entire area of research was pioneered through ecological studies of the perennial relative, *A. halleri*, specifically by tracking gene expression and phenotypic changes within wild populations over time (Aikawa et al., 2010; Kudoh, 2016; Nishio et al., 2020).

The traditional concept of “winter memory,” developed primarily from controlled laboratory vernalization assays, depicts *FLC* as a canonical Polycomb/Trithorax target that “remembers” a cellular state across cell divisions. In this context, memory specifically refers to the persistence of *FLC* silencing (maintaining low expression) after the cold stimulus has ceased (after winter ends). However, ecological studies in *A. halleri* revealed a distinct second type of *FLC* memory (Figure 3): one defined by the plant’s integration of a fluctuating thermal signal over a past temporal window (Aikawa et al., 2010; Endo et al., 2025). The evergreen perennial life history of *A. halleri* uniquely permits the continuous monitoring of *AhGFLC* levels across the full annual cycle in mitotically active leaf cells. The foundational study by Aikawa et al. (2010) revealed a cyclic expression pattern in which *AhGFLC* maintenance and de-silencing states are reiterated over the four seasons in this cellular lineage. Notably, despite the influence of

multiple environmental cues on perennial flowering (Madrid et al., 2021), temperature alone accounted for approximately 80% of *AhGFLC* expression variance across the annual cycle when averaged over a six-week window, thereby revealing that *FLC* integrates thermal inputs over time. The underlying molecular mechanism—a *cis*-acting chromatin switch elaborated in annual life histories (Angel et al., 2011; Berry and Dean, 2015)—also operates in the perennial life history (Nishio et al., 2020; Buzas et al., 2021). Importantly, in addition to evidencing this unique memory type, these studies simultaneously developed empirical methods to capture the duration of this memory as a quantifiable parameter. This was achieved by statistically regressing gene expression against past temperature windows and incorporating bootstrap analyses to robustly define best-fit intervals (Aikawa et al., 2010; Nishio et al., 2020). Recent evidence indicates that this type of integrative memory is conserved in divergent perennials such as *E. japonicum* (Endo et al., 2025).

Overall, while translational validation studies have confirmed the relevance of laboratory findings and revealed some differences, and mechanistic discovery studies have identified new regulatory nuances under fluctuating conditions, molecular ecology studies have begun to transform the study of vernalization. This emerging approach advances the field from a system-specific description into a comprehensive framework capable of bridging molecular and ecological scales to understand plant seasonal responses under real-world climate variability.

6 Perspective: from translation to reciprocity

While *A. thaliana* provided the foundational blueprint for elucidating the vernalization pathway, the “multidimensional landscape” described here suggests that the research relationship between the model and the *Brassica* vegetable crops has developed beyond a one-way translation. The vast natural variation inherent in *Brassica* morphotypes offers an exhaustive “treasure trove” of regulatory diversity—ranging from tandem gene duplications to

cell-type-specific responses—that often holds more immediate agricultural applicability than the study of *A. thaliana* natural accessions. This shift has been accelerated by the increasing accessibility of sophisticated molecular technologies beyond traditional model systems. With the advent of pangenomics and CRISPR-Cas9 technologies, *Brassica* species are transitioning from mere “recipients of knowledge” to primary models for investigating complex genome-environment interactions. This transition will enable the direct engineering of phenological traits, ultimately positioning *Brassica* vegetable crops as the frontier for understanding the plasticity of flowering in a changing global climate.

Author contributions

SY: Writing – original draft. AC: Writing – original draft. AA: Writing – original draft. RF: Writing – original draft, Supervision, Writing – review & editing. DB: Writing – original draft, Conceptualization, Writing – review & editing. KO: Writing – review & editing, Writing – original draft.

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References

- Abuyusuf, M., Nath, U. K., Kim, H. T., Islam, M. R., Park, J. I., and Nou, I. S. (2019). Molecular markers based on sequence variation in *BoFLC1.C9* for characterizing early- and late-flowering cabbage genotypes. *BMC Genet.* 20, 42. doi: 10.1186/s12863-019-0740-1
- Ahn, J. Y., Subburaj, S., Yan, F., Yao, J., Chandrasekaran, A., Ahn, K. G., et al. (2024). Molecular evaluation of the effects of *FLC* homologs and coordinating regulators on the flowering responses to vernalization in cabbage (*Brassica oleracea* var. *capitata*) genotypes. *Genes* 15, 154. doi: 10.3390/genes15020154
- Aikawa, S., Kobayashi, M. J., Satake, A., Shimizu, K. K., and Kudoh, H. (2010). Robust control of the seasonal expression of the *Arabidopsis FLC* gene in a fluctuating environment. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11632–11637. doi: 10.1073/pnas.0914293107
- Akter, A., Itabashi, E., Kakizaki, T., Okazaki, K., Dennis, E. S., and Fujimoto, R. (2021). Genome triplication leads to transcriptional divergence of *FLOWERING LOCUS C* genes during vernalization in the genus *Brassica*. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.619417
- Akter, A., Kakizaki, T., Itabashi, E., Kunita, K., Shimizu, M., Akter, M. A., et al. (2023). Characterization of *FLOWERING LOCUS C 5* in *Brassica rapa* L. *Mol. Breed.* 43, 58. doi: 10.1007/s11032-023-01405-0
- Akter, A., Miyazaki, J., Shea, D. J., Nishida, N., Takada, S., Miyaji, N., et al. (2020). Gene expression analysis in response to vernalization in Chinese cabbage (*Brassica rapa* L.). *Hort. J.* 89, 268–277. doi: 10.2503/hortj.UTD-150
- Akter, A., Takahashi, S., Deng, W., Shea, D. J., Itabashi, E., Shimizu, M., et al. (2019). The histone modification H3 lysine 27 tri-methylation has conserved gene regulatory roles in the triplicated genome of *Brassica rapa* L. *DNA Res.* 26, 433–443. doi: 10.1093/dnares/dsz021
- Angel, A., Song, J., Dean, C., and Howard, M. (2011). A Polycomb-based switch underlying quantitative epigenetic memory. *Nature* 476, 105–108. doi: 10.1038/nature10241
- Aranda, S., Mas, G., and Di Croce, L. (2015). Regulation of gene transcription by Polycomb proteins. *Sci. Adv.* 1, e1500737. doi: 10.1126/sciadv.1500737
- Berry, S., and Dean, C. (2015). Environmental perception and epigenetic memory: mechanistic insight through *FLC*. *Plant J.* 83, 133–148. doi: 10.1111/tpj.12869
- Binenbaum, J., Adamkova, V., Fryer, H., Xu, L., Gorringer, N., Wlodzimierz, P., et al. (2025). CRISPR targeting of H3K4me3 activates gene expression and unlocks centromere-proximal crossover recombination in *Arabidopsis*. *Nat. Commun.* 16, 9587. doi: 10.1038/s41467-025-65167-3

- Burghardt, L. T., Runcie, D. E., Wilczek, A. M., Cooper, M. D., Roe, J. L., Welch, S. M., et al. (2016). Fluctuating, warm temperatures decrease the effect of a key floral repressor on flowering time in *Arabidopsis thaliana*. *New Phytol.* 210, 564–576. doi: 10.1111/nph.13799
- Buzas, D. M. (2017). Capturing environmental plant memories in DNA, with a little help from chromatin. *Plant Cell Physiol.* 58, 1302–1312. doi: 10.1093/pcp/pcx092
- Buzas, D. M., Nishio, H., and Kudoh, H. (2021). The flowering season-meter at *FLOWERING LOCUS C* across life histories in crucifers. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.640442
- Buzas, D. M., Robertson, M., Finnegan, E. J., and Helliwell, C. A. (2011). Transcription-dependence of histone H3 lysine 27 trimethylation at the *Arabidopsis* polycomb target gene *FLC*. *Plant J.* 65, 872–881. doi: 10.1111/j.1365-3113X.2010.04471.x
- Buzas, D. M., Tamada, Y., and Kurata, T. (2012). *FLC*: a hidden polycomb response element shows up in silence. *Plant Cell Physiol.* 53, 785–793. doi: 10.1093/pcp/pcr163
- Calderwood, A., Lloyd, A., Hepworth, J., Tudor, E. H., Jones, D. M., Woodhouse, S., et al. (2021). Total *FLC* transcript dynamics from divergent paralogue expression explains flowering diversity in *Brassica napus*. *New Phytol.* 229, 3534–3548. doi: 10.1111/nph.17131
- Camargo, L. E. A., and Osborn, T. C. (1996). Mapping loci controlling flowering time in *Brassica oleracea*. *Theor. Appl. Genet.* 92, 610–616. doi: 10.1007/BF00224565
- Chalhoub, B., Denoeud, F., Liu, S., Parkin, I. A., Tang, H., Wang, X., et al. (2014). Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. *Science* 345, 950–953. doi: 10.1126/science.1253435
- Cheng, F., Mandáková, T., Wu, J., Xie, Q., Lysak, M. A., and Wang, X. (2013). Deciphering the diploid ancestral genome of the Mesohexaploid *Brassica rapa*. *Plant Cell* 25, 1541–1554. doi: 10.1105/tpc.113.110486
- Cheng, F., Wu, J., and Wang, X. (2014). Genome triplication drove the diversification of *Brassica* plants. *Hortic. Res.* 1, 14024. doi: 10.1038/hortres.2014.24
- Dai, Y., Gao, X., Zhang, S., Li, F., Zhang, H., Li, G., et al. (2024). Exploring the regulatory dynamics of *BrFLC*-associated lncRNA in modulating the flowering response of Chinese cabbage. *Int. J. Mol. Sci.* 25, 1924. doi: 10.3390/ijms25031924
- Del Olmo, I., Poza-Viejo, L., Piñeiro, M., Jarillo, J. A., and Crevillén, P. (2019). High ambient temperature leads to reduced *FT* expression and delayed flowering in *Brassica rapa* via a mechanism associated with H2A.Z dynamics. *Plant J.* 100, 343–356. doi: 10.1111/tpl.14446
- Deng, W., Buzas, D. M., Ying, H., Robertson, M., Taylor, J., Peacock, W. J., et al. (2013). *Arabidopsis* Polycomb Repressive Complex 2 binding sites contain putative GAGA factor binding motifs within coding regions of genes. *BMC Genomics* 14, 593. doi: 10.1186/1471-2164-14-593
- Ding, Y., Ndamukong, I., Xu, Z., Lapko, H., Fromm, M., and Avramova, Z. (2012). *ATX1*-generated H3K4me3 is required for efficient elongation of transcription, not initiation, at *ATX1*-regulated genes. *PLoS Genet.* 8, e1003111. doi: 10.1371/journal.pgen.1003111
- Endo, Y., Nishio, H., Oguchi, T., Yamane, K., Eesele, V. F., Frederica, C. F., et al. (2025). Conserved gene- and network-level thermal memory intervals in two divergent perennial crucifers in nature. *bioRxiv*. doi: 10.1101/2025.10.31.685746
- Fang, H., Shao, Y., and Wu, G. (2021). Reprogramming of histone H3 lysine methylation during plant sexual reproduction. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.782450
- Fu, W., Huang, S., Gao, Y., Zhang, M., Qu, G., Wang, N., et al. (2020). Role of *BrSDG8* on bolting in Chinese cabbage (*Brassica rapa*). *Theor. Appl. Genet.* 133, 2937–2948. doi: 10.1007/s00122-020-03647-4
- Fujimoto, R., Uezono, K., Ishikura, S., Osabe, K., Peacock, W. J., and Dennis, E. S. (2018). Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems. *Breed. Sci.* 68, 145–158. doi: 10.1270/jsbbs.17155
- Godwin, J., and Farrona, S. (2022). The importance of networking: plant Polycomb Repressive Complex 2 and its interactors. *Epigenomes* 6, 8. doi: 10.3390/epigenomes6010008
- Guo, D. P., Ali Shah, G., Zeng, G. W., and Zheng, S. J. (2004). The interaction of plant growth regulators and vernalization on the growth and flowering of cauliflower (*Brassica oleracea* var. *botrytis*). *Plant Growth Regul.* 43, 163–171. doi: 10.1023/B:GROW.0000040114.84122.11
- Guo, N., Wang, S., Wang, T., Duan, M., Zong, M., Miao, L., et al. (2024). A graph-based pan-genome of *Brassica oleracea* provides new insights into its domestication and morphotype diversification. *Plant Commun.* 5, 100791. doi: 10.1016/j.xplc.2023.100791
- Han, Q., Sakaguchi, S., Wakabayashi, T., and Setoguchi, H. (2021). Association between *RsFT*, *RsFLC* and *RsCOL5* (A&B) expression and flowering regulation in Japanese wild radish. *Aob Plants* 13, plab039. doi: 10.1093/aobpla/plab039
- Hassidim, M., Harir, Y., Yakir, E., Kron, I., and Green, R. M. (2009). Over-expression of *CONSTANS-LIKE 5* can induce flowering in short-day grown *Arabidopsis*. *Planta* 230, 481–491. doi: 10.1007/s00425-009-0958-7
- He, L., Yao, Y., You, Y., Wei, X., Ma, Y., Yuan, W., et al. (2025). Versatile molecular tools enabling customizable DNA methylation editing in *Arabidopsis*. *Nat. Commun.* 17, 251. doi: 10.1038/s41467-025-66933-z
- Hepworth, J., Antoniou-Kourouni, R. L., Bloomer, R. H., Selga, C., Berggren, K., Cox, D., et al. (2018). Absence of warmth permits epigenetic memory of winter in *Arabidopsis*. *Nat. Commun.* 9, 639. doi: 10.1038/s41467-018-03065-7
- Hirata, S., Ozono, T., Kawai, K., Machida, C., Kobayashi, K., Ikeda, Y., et al. (2025). Development of a simple and locus-restricted DNA methylation editing system using direct fusion of a nickase-type SpCas9 and DNA methylation-related enzymes in *Arabidopsis thaliana*. *Plant Cell Physiol.* pcaf162. doi: 10.1093/pcp/pcf162
- Hou, J., Cai, X., Xu, Y., Kang, J., Wang, X., and Wu, J. (2025). Identification of *BoFLC2* as a major gene controlling flowering time in cabbage (*Brassica oleracea* var. *capitata*). *Sci. Hortic.* 351, 114407. doi: 10.1016/j.scienta.2025.114407
- Huang, S., Hou, L., Fu, W., Liu, Z., Li, C., Li, X., et al. (2020). An insertion mutation in *Bra032169* encoding a histone methyltransferase is responsible for early bolting in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.00547
- Irwin, J. A., Lister, C., Soumpourou, E., Zhang, Y., Howell, E. C., Teakle, G., et al. (2012). Functional alleles of the flowering time regulator *FRIGIDA* in the *Brassica oleracea* genome. *BMC Plant Biol.* 12, 21. doi: 10.1186/1471-2229-12-21
- Irwin, J. A., Soumpourou, E., Lister, C., Lighthart, J. D., Kennedy, S., and Dean, C. (2016). Nucleotide polymorphism affecting *FLC* expression underpins heading date variation in horticultural brassicas. *Plant J.* 87, 597–605. doi: 10.1111/tpl.13221
- Itabashi, E., Shea, D. J., Fukino, N., Fujimoto, R., Okazaki, K., Kakizaki, T., et al. (2019). Comparison of cold responses for orthologs of cabbage vernalization-related genes. *Hort. J.* 88, 462–470. doi: 10.2503/hortj.UTD-059
- Jeong, Y. M., Kim, N., Ahn, B. O., Oh, M., Chung, W. H., Chung, H., et al. (2016). Elucidating the triplicated ancestral genome structure of radish based on chromosome-level comparison with the *Brassica* genomes. *Theor. Appl. Genet.* 129, 1357–1372. doi: 10.1007/s00122-016-2708-0
- Jin, Y., Luo, X., Li, Y., Peng, X., Wu, L., Yang, G., et al. (2024). Fine mapping and analysis of candidate genes for *qBT2* and *qBT7.2* locus controlling bolting time in radish (*Raphanus sativus* L.). *Theor. Appl. Genet.* 137, 4. doi: 10.1007/s00122-023-04503-x
- Jung, H., Jo, S. H., Jung, W. Y., Park, H. J., Lee, A., Moon, J. S., et al. (2020). Gibberellin promotes bolting and flowering via the floral integrators *RsFT* and *RsSOC1-1* under marginal vernalization in radish. *Plants (Basel)* 9, 594. doi: 10.3390/plants9050594
- Jung, H., Lee, A., Jo, S. H., Park, H. J., Jung, W. Y., Kim, H. S., et al. (2021). Nitrogen signaling genes and *SOC1* determine the flowering time in a reciprocal negative feedback loop in Chinese cabbage (*Brassica rapa* L.) based on CRISPR/Cas9-mediated mutagenesis of multiple *BrSOC1* homologs. *Int. J. Mol. Sci.* 22, 4631. doi: 10.3390/ijms22094631
- Jung, H., Park, H. J., Jo, S. H., Lee, A., Jung, M., Kim, Y. S., et al. (2025). Introducing gene-edited copies of *BrSOC1* genes into late-bolting inbred Chinese cabbage lines effectively delays bolting. *Planta* 262, 104. doi: 10.1007/s00425-025-04822-x
- Kakizaki, T., Kato, T., Fukino, N., Ishida, M., Hatakeyama, K., and Matsumoto, S. (2011). Identification of quantitative trait loci controlling late bolting in Chinese cabbage (*Brassica rapa* L.) parental line Nou 6 gou. *Breed. Sci.* 61, 151–159. doi: 10.1270/jsbbs.61.151
- Kawanabe, T., Osabe, K., Itabashi, E., Okazaki, K., Dennis, E. S., and Fujimoto, R. (2016). Development of primer sets that can verify the enrichment of histone modifications, and their application to examining vernalization mediated chromatin changes in *Brassica rapa* L. *Genes Genet. Syst.* 91, 1–10. doi: 10.1266/ggs.15-00058
- Kim, D. H. (2025). Current understanding of the vernalization-mediated floral transition process in *Arabidopsis* and several *Brassica* crop plants. *Hort. J.* doi: 10.2503/hortj.SZD-R010
- Kim, S., Kim, J. A., Kang, H., and Kim, D. H. (2022). A premature stop codon in *BrFLC2* transcript results in early flowering in oilseed-type *Brassica rapa* plants. *Plant Mol. Biol.* 108, 241–255. doi: 10.1007/s11103-021-01231-y
- Kinoshita, Y., Motoki, K., and 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. doi: 10.1007/s00122-023-04311-3
- Kitamoto, N., Nishikawa, K., Tanimura, Y., Urushibara, S., Matsuura, T., Yokoi, S., et al. (2017). Development of late-bolting F1 hybrids of Chinese cabbage (*Brassica rapa* L.) allowing early spring cultivation without heating. *Euphytica* 213, 292. doi: 10.1007/s10681-017-2079-x
- Kitamoto, N., Yui, S., Nishikawa, K., Takahata, Y., and Yokoi, S. (2014). A naturally occurring long insertion in the first intron in the *Brassica rapa FLC2* gene causes delayed bolting. *Euphytica* 196, 213–223. doi: 10.1007/s10681-013-1025-9
- Kitashiba, H., Li, F., Hirakawa, H., Kawanabe, T., Zou, Z., Hasegawa, Y., et al. (2014). Draft sequences of the radish (*Raphanus sativus* L.) genome. *DNA Res.* 21, 481–490. doi: 10.1093/dnares/dsu014
- Kudoh, H. (2016). Molecular phenology in plants: In natura systems biology for the comprehensive understanding of seasonal responses under natural environments. *New Phytol.* 210, 399–412. doi: 10.1111/nph.13733
- Lee, A., Jung, H., Park, H. J., Jo, S. H., Jung, M., Kim, Y. S., et al. (2023). Their C-termini divide *Brassica rapa* FT-like proteins into FD-interacting and FD-independent proteins that have different effects on the floral transition. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.1091563

- Lee, S. W., Kim, H., and Kim, D. H. (2025). Insertional mutations in *RsFLC2* disrupt vernalization-induced intragenic looping and H3K27me3 enrichment in radish. *Physiol. Plant* 177, e70314. doi: 10.1111/pp.70314
- Lee, S. W., Nugroho, A. B. D., Park, M., Moon, H., Kim, J., and Kim, D. H. (2024). Identification of vernalization-related genes and cold memory element (CME) required for vernalization response in radish (*Raphanus sativus* L.). *Plant Mol. Biol.* 114, 5. doi: 10.1007/s11103-023-01412-x
- Li, P., Filiault, D., Box, M. S., Kerdaffrec, E., van Oosterhout, C., Wilczek, A. M., et al. (2014). Multiple *FLC* haplotypes defined by independent *cis*-regulatory variation underpin life history diversity in *Arabidopsis thaliana*. *Genes Dev.* 28, 1635–1640. doi: 10.1101/gad.245993.114
- Li, Q., Peng, A., Yang, J., Zheng, S., Li, Z., Mu, Y., et al. (2022). A 215-bp indel at intron I of *BoFLC2* affects flowering time in *Brassica oleracea* var. *capitata* during vernalization. *Theor. Appl. Genet.* 135, 2785–2797. doi: 10.1007/s00122-022-04149-1
- Li, X., Zhang, S., Bai, J., and He, Y. (2016). Tuning growth cycles of *Brassica* crops via natural antisense transcripts of *BrFLC*. *Plant Biotechnol. J.* 14, 905–914. doi: 10.1111/pbi.12443
- Li, X., Zheng, Y., Luo, L., Chen, Q., Yang, T., Yang, Y., et al. (2024). The evolution and functional divergence of *FT*-related genes in controlling flowering time in *Brassica rapa* ssp. *rapa*. *Plant Cell Rep.* 43, 86. doi: 10.1007/s00299-024-03166-2
- Lin, S. I., Wang, J. G., Poon, S. Y., Su, C. L., Wang, S. S., and Chiou, T. J. (2005). Differential regulation of *FLOWERING LOCUS C* expression by vernalization in cabbage and *Arabidopsis*. *Plant Physiol.* 137, 1037–1048. doi: 10.1104/pp.104.058974
- Lu, F., Cui, X., Zhang, S., Liu, C., and Cao, X. (2010). JM14 is an H3K4 demethylase regulating flowering time in *Arabidopsis*. *Cell Res.* 20, 387–390. doi: 10.1038/cr.2010.27
- Ma, Y., Chhakekar, S. S., Rameneni, J. J., Kim, S., Gan, T. H., Choi, S. R., et al. (2021). Identification of QTLs and candidate genes related to flower traits and bolting time in radish (*Raphanus sativus* L.). *Agronomy* 11, 1623. doi: 10.3390/agronomy11081623
- Mabry, M. E., Turner-Hissong, S. D., Gallagher, E. Y., McAlvay, A. C., An, H., Edger, P. P., et al. (2021). The evolutionary history of wild, domesticated, and feral *Brassica oleracea* (Brassicaceae). *Mol. Biol. Evol.* 38, 4419–4434. doi: 10.1093/molbev/msab183
- Madrid, E., Chandler, J. W., and Coupland, G. (2021). Gene regulatory networks controlled by *FLOWERING LOCUS C* that confer variation in seasonal flowering and life history. *J. Exp. Bot.* 72, 4–14. doi: 10.1093/jxb/eraa216
- Maple, R., Zhu, P., Hepworth, J., Wang, J. W., and Dean, C. (2024). Flowering time: From physiology, through genetics to mechanism. *Plant Physiol.* 195, 190–212. doi: 10.1093/plphys/kiad109
- Marquardt, S., Raitskin, O., Wu, Z., Liu, F., Sun, Q., and Dean, C. (2014). Functional consequences of splicing of the antisense transcript *COOLAIR* on *FLC* transcription. *Mol. Cell* 54, 156–165. doi: 10.1016/j.molcel.2014.03.026
- McAlvay, A. C., Ragsdale, A. P., Mabry, M. E., Qi, X. S., Bird, K. A., Velasco, P., et al. (2021). *Brassica rapa* domestication: untangling wild and feral forms and convergence of crop morphotypes. *Mol. Biol. Evol.* 38, 3358–3372. doi: 10.1093/molbev/msab108
- Mehraj, H., Takahashi, S., Miyaji, N., Akter, A., Suzuki, Y., Seki, M., et al. (2021). Characterization of histone H3 lysine 4 and 36 tri-methylation in *Brassica rapa* L. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.659634
- Mitsui, Y., Shimomura, M., Komatsu, K., Namiki, N., Shibata-Hatta, M., Imai, M., et al. (2015). The radish genome and comprehensive gene expression profile of tuberous root formation and development. *Sci. Rep.* 5, 10835. doi: 10.1038/srep10835
- Mitsui, Y., Yokoyama, H., Nakaegawa, W., Tanaka, K., Komatsu, K., Koizuka, N., et al. (2023). Epistatic interactions among multiple copies of *FLC* genes with naturally occurring insertions correlate with flowering time variation in radish. *Arab. Plants* 15, plac066. doi: 10.1093/aobpla/plac066
- Moon, H., Kim, H., and Kim, D. H. (2025). A histone methyltransferase, *EARLY FLOWERING IN SHORT DAYS* (EFS) is required for reproductive organs development via histone H3K36 methylation in the oil-seed type *Brassica rapa* (*B. rapa* var. *trilobularis*) plant. *Hortic. Environ. Biotechnol.* 66, 701–714. doi: 10.1007/s13580-025-00681-3
- Moon, H., Park, M., and Kim, D. H. (2026). Epigenetic suppression of *Brassica rapa* *FLC* via a COLD MEMORY ELEMENT-like motif during vernalization. *Mol. Breed.* 46, 1. doi: 10.1007/s11032-025-01615-8
- Nie, S., Li, C., Xu, L., Wang, Y., Huang, D., Muleke, E. M., et al. (2016). *De novo* transcriptome analysis in radish (*Raphanus sativus* L.) and identification of critical genes involved in bolting and flowering. *BMC Genomics* 17, 389. doi: 10.1186/s12864-016-2633-2
- Nishikawa, M., Tamiru-Oli, M., Hara, M., Segawa, T., Saiga, S., Makita, N., et al. (2023). Non-vernalization requirement for flowering in *Brassica rapa* conferred by a dominant allele of *FLOWERING LOCUS T*. *Theor. Appl. Genet.* 136, 132. doi: 10.1007/s00122-023-04378-y
- Nishimura, K., Miyazaki, J., Takada, Y., Akter, M. A., Sarker, K. K., Chowdhury, A. P., et al. (2025). Epigenetic regulation in horticultural crops. *Hort. J.* doi: 10.2503/hortj.SZD-R005
- Nishio, H., Buzas, D. M., Nagano, A. J., Iwayama, K., Ushio, M., and Kudoh, H. (2020). Repressive chromatin modification underpins the long-term expression trend of a perennial flowering gene in nature. *Nat. Commun.* 11, 2065. doi: 10.1038/s41467-020-15896-4
- Noh, B., Lee, S. H., Kim, H. J., Yi, G., Shin, E. A., Lee, M., et al. (2004). Divergent roles of a pair of homologous Jumonji/Zinc-finger-class transcription factor proteins in the regulation of *Arabidopsis* flowering time. *Plant Cell* 16, 2601. doi: 10.1105/tpc.104.025353
- Nugroho, A. B. D., Kim, S., Lee, S. W., and Kim, D. H. (2023). Transcriptomic and epigenomic analyses revealed that polycomb repressive complex 2 regulates not only developmental but also stress responsive metabolism in *Brassica rapa*. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1079218
- Nugroho, A. B. D., Lee, S. W., Pervitasari, A. N., Moon, H., Choi, D., Kim, J., et al. (2021). Transcriptomic and metabolic analyses revealed the modulatory effect of vernalization on glucosinolate metabolism in radish (*Raphanus sativus* L.). *Sci. Rep.* 11, 24023. doi: 10.1038/s41598-021-03557-5
- O'Neill, C. M., Lu, X., Calderwood, A., Tudor, E. H., Robinson, P., Wells, R., et al. (2019). Vernalization and floral transition in autumn drive winter annual life history in oilseed rape. *Curr. Biol.* 29, 4300–4306.e2. doi: 10.1016/j.cub.2019.10.051
- Okazaki, K., Sakamoto, K., Kikuchi, R., Saito, A., Togashi, E., Kuginuki, Y., et al. (2007). Mapping and characterization of *FLC* homologs and QTL analysis of flowering time in *Brassica oleracea*. *Theor. Appl. Genet.* 114, 595–608. doi: 10.1007/s00122-006-0460-6
- Payá-Milans, M., Poza-Viejo, L., Martín-Uriz, P. S., Lara-Astiaso, D., Wilkinson, M. D., and Crevillén, P. (2019). Genome-wide analysis of the H3K27me3 epigenome and transcriptome in *Brassica rapa*. *Gigascience* 8, giz147. doi: 10.1093/gigascience/giz147
- Poza-Viejo, L., Payá-Milans, M., San Martín-Uriz, P., Castro-Labrador, L., Lara-Astiaso, D., Wilkinson, M. D., et al. (2022). Conserved and distinct roles of H3K27me3 demethylases regulating flowering time in *Brassica rapa*. *Plant Cell Environ.* 45, 1428–1441. doi: 10.1111/pce.14258
- Poza-Viejo, L., Payá-Milans, M., Wilkinson, M. D., Piñeiro, M., Jarillo, J. A., and Crevillén, P. (2024). *Brassica rapa* CURLY LEAF is a major H3K27 methyltransferase regulating flowering time. *Planta* 260, 27. doi: 10.1007/s00425-024-04454-7
- Qiu, Q., Mei, H., Deng, X., He, K., Wu, B., Yao, Q., et al. (2019). DNA methylation repels targeting of *Arabidopsis* REF6. *Nat. Commun.* 10, 2063. doi: 10.1038/s41467-019-10026-1
- Ridge, S., Brown, P. H., Hecht, V., Driessen, R. G., and Weller, J. L. (2015). The role of *BoFLC2* in cauliflower (*Brassica oleracea* var. *botrytis* L.) reproductive development. *J. Exp. Bot.* 66, 125–135. doi: 10.1093/jxb/eru408
- Roudier, F., Ahmed, I., Berard, C., Sarazin, A., Mary-Huard, T., Cortijo, S., et al. (2011). Integrative epigenomic mapping defines four main chromatin states in *Arabidopsis*. *EMBO J.* 30, 1928–1938. doi: 10.1038/emboj.2011.103
- Saban, J. M., Romero, A. J., Ezard, T. H. G., and Chapman, M. A. (2023). Extensive crop-wild hybridization during *Brassica* evolution and selection during the domestication and diversification of *Brassica* crops. *Genetics* 223, iyad027. doi: 10.1093/genetics/iyad027
- Saeed, S., Usman, B., Shim, S. H., Khan, S. U., Nizamuddin, S., Saeed, S., et al. (2022). CRISPR/Cas-mediated editing of *cis*-regulatory elements for crop improvement. *Plant Sci.* 324, 111435. doi: 10.1016/j.plantsci.2022.111435
- Searle, I. R., Pontes, O., Melnyk, C. W., Smith, L. M., and Baulcombe, D. C. (2010). JM14, a JmjC domain protein, is required for RNA silencing and cell-to-cell movement of an RNA silencing signal in *Arabidopsis*. *Genes Dev.* 24, 986–991. doi: 10.1101/gad.579910
- Sequeira-Mendes, J., Araguez, I., Peiro, R., Mendez-Giraldez, R., Zhang, X., Jacobsen, S. E., et al. (2014). The functional topography of the *Arabidopsis* genome is organized in a reduced number of linear motifs of chromatin states. *Plant Cell* 26, 2351–2366. doi: 10.1105/tpc.114.124578
- Shea, D. J., Itabashi, E., Takada, S., Fukai, E., Kakizaki, T., Fujimoto, R., et al. (2017). The role of *FLOWERING LOCUS C* in vernalization of *Brassica*: the importance of vernalization research in the face of climate change. *Crop Pasture Sci.* 69, 30–39. doi: 10.1071/CP16468
- Shea, D. J., Nishida, N., Takada, S., Itabashi, E., Takahashi, S., Akter, A., et al. (2019). Long noncoding RNAs in *Brassica rapa* L. following vernalization. *Sci. Rep.* 9, 9302. doi: 10.1038/s41598-019-45650-w
- Shivaraj, S. M., Dhakate, P., Mayee, P., Negi, M. S., and Singh, A. (2014). Natural genetic variation in *MIR172* isolated from *Brassica* species. *Biol. Plant* 58, 627–640. doi: 10.1007/s10535-014-0441-6
- Shu, J., Liu, Y., Zhang, L., Li, Z., Fang, Z., Yang, L., et al. (2018). QTL-seq for rapid identification of candidate genes for flowering time in broccoli × cabbage. *Theor. Appl. Genet.* 131, 917–928. doi: 10.1007/s00122-017-3047-5
- Song, X., Wei, Y., Xiao, D., Gong, K., Sun, P., Ren, Y., et al. (2021). *Brassica carinata* genome characterization clarifies U's triangle model of evolution and polyploidy in *Brassica*. *Plant Physiol.* 186, 388–406. doi: 10.1093/plphys/kiab048
- Sri, T., Gupta, B., Tyagi, S., and Singh, A. (2020). Homeologs of *Brassica* *SOC1*, a central regulator of flowering time, are differentially regulated due to partitioning of evolutionarily conserved transcription factor binding sites in promoters. *Mol. Phylogenet. Evol.* 147, 106777. doi: 10.1016/j.ympev.2020.106777

- Su, T., Wang, W., Li, P., Zhang, B., Li, P., Xin, X., et al. (2018). A genomic variation map provides insights into the genetic basis of spring Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) selection. *Mol. Plant* 11, 1360–1376. doi: 10.1016/j.molp.2018.08.006
- Sung, S., and Amasino, R. M. (2004). Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature* 427, 159–164. doi: 10.1038/nature02195
- Takada, S., Akter, A., Itabashi, E., Nishida, N., Shea, D. J., Miyaji, N., et al. (2019). The role of *FRIGIDA* and *FLOWERING LOCUS C* genes in flowering time of *Brassica rapa* leafy vegetables. *Sci. Rep.* 9, 13843. doi: 10.1038/s41598-019-50122-2
- Tan, C., Ren, J., Wang, L., Ye, X., Fu, W., Zhang, J., et al. (2021). A single amino acid residue substitution in *BraA04g017190.3C*, a histone methyltransferase, results in premature bolting in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). *BMC Plant Biol.* 21, 373. doi: 10.1186/s12870-021-03153-9
- Tao, Z., Shen, L., Gu, X., Wang, Y., Yu, H., and He, Y. (2017). Embryonic epigenetic reprogramming by a pioneer transcription factor in plants. *Nature* 551, 124–128. doi: 10.1038/nature24300
- Tonosaki, K., Fujimoto, R., Dennis, E. S., Raboy, V., and Osabe, K. (2022). Will epigenetics be a key player in crop breeding? *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.958350
- U, N. (1935). Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Jpn. J. Bot.* 7, 389–452.
- Uptmoor, R., Li, J., Schrag, T., and Stützel, H. (2012). Prediction of flowering time in *Brassica oleracea* using a quantitative trait loci-based phenology model. *Plant Biol.* 14, 179–189. doi: 10.1111/j.1438-8677.2011.00478.x
- Wang, J., Qiu, Y., Cheng, F., Chen, X., Zhang, X., Wang, H., et al. (2017). Genome-wide identification, characterization, and evolutionary analysis of flowering genes in radish (*Raphanus sativus* L.). *BMC Genomics* 18, 981. doi: 10.1186/s12864-017-4377-z
- Wang, J., Zhang, B., Guo, H., Chen, L., Han, F., Yan, C., et al. (2023). Transcriptome analysis reveals key genes and pathways associated with the regulation of flowering time in cabbage (*Brassica oleracea* L. var. *capitata*). *Plants* 12, 3413. doi: 10.3390/plants12193413
- Wang, Q., Zhang, Y., and Zhang, L. (2018). A naturally occurring insertion in the *RsFLC2* gene associated with late-bolting trait in radish (*Raphanus sativus* L.). *Mol. Breed.* 38, 137. doi: 10.1007/s11032-018-0897-8
- Wei, Q., Hu, T., Xu, X., Tian, Z., Bao, C., Wang, J., et al. (2022). The new variation in the promoter region of *FLOWERING LOCUS T* is involved in flowering in *Brassica rapa*. *Genes (Basel)* 13, 1162. doi: 10.3390/genes13071162
- Woodhouse, S., He, Z., Woolfenden, H., Steuernagel, B., Haerty, W., Bancroft, I., et al. (2021). Validation of a novel associative transcriptomics pipeline in *Brassica oleracea*: identifying candidates for vernalisation response. *BMC Genomics* 22, 539. doi: 10.1186/s12864-021-07805-w
- Xi, X., Wei, K., Gao, B., Liu, J., Liang, J., Cheng, F., et al. (2018). BrFLC5: a weak regulator of flowering time in *Brassica rapa*. *Theor. Appl. Genet.* 131, 2107–2116. doi: 10.1007/s00122-018-3139-x
- Xin, X., Li, P., Zhao, X., Yu, Y., Wang, W., Jin, G., et al. (2024). Temperature-dependent jumonji demethylase modulates flowering time by targeting H3K36me2/3 in *Brassica rapa*. *Nat. Commun.* 15, 5470. doi: 10.1038/s41467-024-49721-z
- Yang, H., Hu, J., Chen, M., Lan, M., and He, J. (2025). Transcriptome and metabolome analyses revealed mechanisms underlying vernalization-induced bolting in two contrasting genotypes of cabbage (*Brassica oleracea* var. *capitata*). *J. Plant Biochem. Biotechnol.* 34, 969–983. doi: 10.1007/s13562-025-01000-8
- Yang, J., Liu, D., Wang, X., Ji, C., Cheng, F., Liu, B., et al. (2016). The genome sequence of allopolyploid *Brassica juncea* and analysis of differential homoeolog gene expression influencing selection. *Nat. Genet.* 48, 1225–1232. doi: 10.1038/ng.3657
- Yaschenko, A. E., Alonso, J. M., and Stepanova, A. N. (2025). *Arabidopsis* as a model for translational research. *Plant Cell* 37, koae065. doi: 10.1093/plcell/koae065
- Yi, G., Park, H., Kim, J. S., Chae, W. B., Park, S., and Huh, J. H. (2014). Identification of three *FLOWERING LOCUS C* genes responsible for vernalization response in radish (*Raphanus sativus* L.). *Hortic. Environ. Biotechnol.* 55, 548–556. doi: 10.1007/s13580-014-1151-x
- Yuan, W., Luo, X., Li, Z., Yang, W., Wang, Y., Liu, R., et al. (2016). A *cis* cold memory element and a trans epigenome reader mediate Polycomb silencing of *FLC* by vernalization in *Arabidopsis*. *Nat. Genet.* 48, 1527–1534. doi: 10.1038/ng.3712
- Zhang, X., Jia, Q., Jia, X., Li, J., Sun, X., Min, L., et al. (2025a). *Brassica* vegetables—an undervalued nutritional goldmine. *Hortic. Res.* 12, uhac302. doi: 10.1093/hr/uhac302
- Zhang, X., Menard, R., Li, Y., Coruzzi, G. M., Heitz, T., Shen, W. H., et al. (2020). *Arabidopsis* SDG8 potentiates the sustainable transcriptional induction of the *Pathogenesis-related* genes *PR1* and *PR2* during plant defense response. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.00277
- Zhang, Z., Cai, X., Liang, J., Liu, J., Guo, J., Yang, W., et al. (2025b). Time-resolved single-cell atlas identifies the spatiotemporal transcription dynamics in vernalization response in *Brassica rapa*. *Cell Rep.* 44, 115725. doi: 10.1016/j.celrep.2025.115725
- Zhao, Z., Tan, C., Zhang, J., Zhang, L., Hou, Q., Tang, T., et al. (2025). *BrSWN* mutation reduces the H3K27me3 level at the *BrFLC2* and *BrFLC3* loci and confers a late-bolting phenotype in Chinese cabbage. *Plant J.* 122, e70151. doi: 10.1111/tpj.70151
- Zhao, Y., Zhu, P., Hepworth, J., Bloomer, R., Antoniou-Kourounioti, R. L., Doughty, J., et al. (2021). Natural temperature fluctuations promote *COOLAIR* regulation of *FLC*. *Genes Dev.* 35, 888–898. doi: 10.1101/gad.348362.121
- Zheng, Y., Gao, Z., Luo, L., Wang, Y., Chen, Q., Yang, Y., et al. (2021). Divergence of the genetic contribution of *FRIGIDA* homologues in regulating the flowering time in *Brassica rapa* ssp. *rapa*. *Gene* 796–797, 145790. doi: 10.1016/j.gene.2021.145790
- Zhou, Y., Wang, Y., Krause, K., Yang, T., Dongus, J. A., Zhang, Y., et al. (2018). Telobox motifs recruit CLF/SWN-PRC2 for H3K27me3 deposition via TRB factors in *Arabidopsis*. *Nat. Genet.* 50, 638–644. doi: 10.1038/s41588-018-0109-9