The molecular mechanism of vernalization in Arabidopsis and Cereals: Role of Flowering Locus C and its homologs

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Winter varieties of plants can flower only after exposure to prolonged cold. This phenomenon is known as vernalization and has been widely studied in the model plant *Arabidopsis thaliana* as well as in monocots. Through the repression of floral activator genes, vernalization prevents flowering in winter. In *Arabidopsis*, *FLOWERING LOCUS C* or *FLC* is the key repressor during vernalization, while in monocots vernalization is regulated through *VRN1*, *VRN2* and *VRN3* (or *FLOWERING LOCUS T*). Interestingly, *VRN* genes are not homologous to *FLC* but *FLC* homologs are found to have a significant role in vernalization response in cereals. The presence of *FLC* homologs in monocots opens new dimensions to understand, compare and retrace the evolution of vernalization pathways between monocots and dicots. In this review, we discuss the molecular mechanism of vernalization-induced flowering along with epigenetic regulations in *Arabidopsis* and temperate cereals. A better understanding of cold-induced flowering will be helpful in crop breeding strategies to modify the vernalization requirement of economically-important temperate cereals.

Introduction

All living organisms evolve different mechanisms to ensure their survival in local climatic conditions. Being sessile, plants also have evolved different survival mechanisms in response to environmental signals, which allow them to sustain growth in various seasonal changes. Among major environmental

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signals, temperature and photoperiod are the most crucial in determining flowering time. The majority of the plants growing in temperate climates start flowering only after exposure to prolonged cold, a process known as vernalization (Sheldon et al. 2000, Amasino 2004, Trevaskis et al. 2007, Kim et al. 2009) (Fig. 1). For instance, winter cereals, like wheat and barley, are planted in fall and only flower in spring when winter is passed and photoperiod is inductive.

The process of vernalization was originally described in economically-important temperate cereals (Chouard 1960), as it may have a beneficial effect on crop productivity. Winter varieties of temperate cereals remain in a vegetative state without vernalization and their transition to flowering state only occurs when the vernalization requirement is saturated. Most vernalization-dependent plants are long day (LD) plants and they flower when the day length is inductive (Kim et al. 2009, Wang et al. 2014). A combination of the vernalization requirement and inductive photoperiod prevents the plant from flowering precociously when day length is still short and ensures flowering in favorable conditions during spring when days are long and the temperature is rising.

The vernalization requirement may differ among various plant species and also in different ecotypes within a species. The optimal temperature for vernalization and the acquired length of vernalization vary among different species and accessions of the same species. Generally, the optimal temperature ranges for vernalization response are above or around the freezing point (Wollenberg and Amasino 2012, Duncan et al. 2015, Costa and Dean 2019). This difference in the vernalization requirement helps plants to adapt to the local environmental changes as temperature likely represents a crucial factor (Kim et al. 2009). Varying degrees of the vernalization requirement for different cereal accessions provide a perspective on how plants adapt to different temperatures.

This review focuses on molecular aspects of vernalization-mediated flowering in *Arabidopsis thaliana* (Arabidopsis) and cereals. Additionally, we discussed the role of *FLC* in regulating the vernalization response in Arabidopsis and its homologs in temperate cereals. Understanding vernalization in temperate cereals is gaining importance as it could be beneficial in refining breeding efforts of winter varieties of temperate cereals.

Vernalization in *Arabidopsis*

Vernalization is well studied in the model plant Arabidopsis. Summer-annual Arabidopsis ecotypes do not need vernalization to flower, while winter-annuals only flower after fulfilling the vernalization requirement. In Arabidopsis, two key genes conferred the vernalization requirement: *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) (Lee et al. 1993, Clarke and Dean 1994, Koornneef al. 1994, Whittaker and Dean 2017). *FRI* encodes a plant-specific scaffold protein and is required for active transcription of *FLC* (Michaels and Amasino 2001, Jiang et al. 2009). *FRI* regulates the transcription of *FLC* through a large protein complex consisting of SUPPRESSOR OF FRI4 (SUF4), FRI-LIKE1 (FRL1) and FRE ESSENTIAL 1 (FES1) (Choi et al. 2011). This protein complex further recruits the transcription activators and chromatin modifying proteins for *FLC* upregulation (Choi et al. 2011). *FLC* encodes a MADS-box transcription factor that acts as a repressor of flowering (Michales and Amasino 1999, Sheldon et al. 1999). High levels of *FLC* represses the expression of floral integrators genes such as *FLOWERING LOCUS T (FT*), *FD* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) that results in delayed flowering (Michales et al. 2005, Helliwell et al. 2006, Searle et al. 2006, Deng et al. 2011). *FLC* binds to the first intron of *FT* and the promoter of *FD* and *SOC1* (Helliwell et al. 2006, Searle et al. 2006) to perform the repressor function. Expression of *FT* is induced by the circadian clock coordinated gene *CONSTANS* (*CO*) in leaves upon exposure to inductive photoperiods. *FT* in meristem interacts with *FD* and promotes *SOC1* expression and other floral meristem identity genes like *SEPALATA* (*SEP*), *FRUITFUL* (*FUL*) and *APETALA1* (*AP1*) to start the transition to flowering state (Turck et al. 2008, Kim et al. 2009). *SOC1* is responsible for the activation of another floral meristem identity gene: *LEAFY* (*LFY*; Lee et al. 2008; Fig. 2).

In Arabidopsis, repression of *FLC* is mitotically stable and many genes are involved in stable repression and histone modifications of *FLC* such as *AtVRN1*, *AtVRN2*, *VERNALIZATION INSENSITIVE3* (*VIN3)* and *VERNALIZATION5* (*VRN5*; Levy et al. 2002, Gendall et al. 2001, Wood et al. 2006, Bond et al. 2009, Searle et al. 2006, Greb et al. 2007). *AtVRN1* encodes a protein with DNA binding domain and PEST region that are involved in the stable repression of *FLC* (Levy et al. 2002). *AtVRN2* encodes a zinc finger nuclear protein similar to SU(Z)12 in Drosophila and FIS1 and EMF2 in plants (Gendall et al. 2001). *AtVRN2* is part of POLYCOMB GROUP REPRESSIVE COMPLEX 2 (PRC2) and plays an important role in stable maintenance of *FLC* repression after the return to warm conditions (Gendall et al. 2001). *VIN3* encodes a chromatin remodeler Plant Homeodomain finger (PHD) protein and is activated by vernalization (Sung and Amasino 2004). Activation of *VIN3* results in the repression of *FLC* by recruiting the PRC2 complex that catalyzed the H3K27me3 modifications of the *FLC* chromatin (Wood et al. 2006, Bond et al. 2009). VIN3 forms a heterodimer with VRN5 during vernalization and maintains the histone modifications necessary for a stable silencing of *FLC* (Greb et al. 2007, Costa and Dean 2019; Fig. 2). *VIN3* acts as a thermosensor component for the epigenetic silencing of *FLC* and has a key and complex role in influencing the vernalization response in warmer and more variable temperatures (Hepworth et al. 2018). Recent findings in oilseed rape suggested that *FLC* expression declines predominantly in autumn (specifically around October) rather than winter (O'Neill et al. 2019). Also, *FLC* levels in autumn can define the vernalization requirements in different climates (Hepworth et al. 2020), suggesting that vernalization is also influenced by autumn and variable temperatures.

Epigenetic regulation of *FLC* in Arabidopsis

In Arabidopsis, vernalization mediated repression of *FLC* is epigenetically regulated through the PRC2 complex (Wood et al. 2006, De Lucia et al. 2008, Angel et al. 2011). The PRC2 complex alters histones at the *FLC* locus by mediating H3K27me3 and H9K27me3 (trimethylation of histone 3 and 9 on lysine 27 respectively), well established chromatin marks typically associated with a stable epigenetic silencing (Bastow et al. 2004, Sung and Amasino, 2004, Angel et al. 2011). The epigenetic silencing of *FLC* is quantitatively increased with the duration of cold treatment (Angel et al. 2011, Song et al. 2012, Costa and Dean 2019). This epigenetic state is mitotically stable and resets in every next generation to ensure that the vernalization requirement exists for every progeny or next generation (Sheldon et al. 2008, Choi et al. 2009). In Arabidopsis, there are some mutants in which *FLC* is downregulated during vernalization but the downregulation is not stably maintained after return to warm conditions. Interestingly, chromatin-modifying proteins were affected in these mutants (Levy et al. 2002, Mylne et al. 2006, Sung et al. 2006), suggesting that stability in the vernalization state is associated with the chromatin modification of target genes.

The setting of the vernalization response via stable *FLC* repression happens in three phases, i.e. *FLC* level before cold, cold-mediated repression of *FLC* and stable silencing after return to warm temperatures. High *FLC* expression is set during embryogenesis to ensure the vernalization requirement for new progeny (Sheldon et al. 2008, Choi et al. 2009). Before cold, the high *FLC* expression levels in pre-vernalized conditions are associated with transcription-activated histone marks. These include di- or trimethylation on Histone 3 at Lysine 4 (H3K4me2 or H3K4me3) and trimethylation on Histone 3 at Lysine 36 (H3K36me3) together with the substitution of Histone 2A (H2A) with H2AZ Histone variant. The initial upregulation of *FLC* is ensured by RNA polymerase associated factor 1 complex (Paf1 C) and

FRI (Kim et al. 2009, Song et al. 2012). The Paf1C is an elongation factor which associates with the RNA polymerase II and is required for active elongation (Zhang et al. 2009), while *FRI* forms a transcription activation complex that ensures high levels of *FLC* (Choi et al. 2011). Active *FRI* results in ATWDR5a enrichment at *FLC* locus and enhances H3K4me3 and H3K36me3 histone marks (Jiang et al. 2009, Pien et al. 2008, Song et al. 2012). Additionally, PRC2 associates with *FLC* independently of temperatures (De Lucia et al. 2008). In Arabidopsis, the PRC2 complex is constituted of VRN2, SU(Z)12 homolog, SWINGER (SWN), E(z) histone methyltransferase homolog and MSI1 (De Lucia et al. 2008, Song et al. 2012).

Cold-dependent *FLC* repression resulted in the association of the PRC2 complex and PHD (Plant Homeodomain) to form the PHD-PRC2 complex at the transcription start site (Song et al. 2012, Xiao et al. 2017, Zhou et al. 2018, Costa and Dean 2019). The PHD protein complex consists of three known proteins VIN3, VRN5 and VIN3-LIKE2 (VIL2) (Sung and Amasino, 2004, Wood et al. 2006, De Lucia et al. 2008, Zhu et al. 2015). The activity of the PHD-PRC2 complex results in adding H3K27me3 (transcription repressive histone modification) and removing H3K4me3 (activating histone modification) associated with cold-induced *FLC* silencing (Fig. 3) (De Lucia et al. 2008, Angel et al. 2011, Song et al. 2012, Whittaker and Dean 2017, Costa and Dean 2019). Moreover, at the beginning of the cold, the expression of the noncoding antisense transcript *COOLAIR* increases in association with the *FLC* downregulation (Swiezewski et al. 2009, Song et al. 2012, Costa and Dean 2019, Wu et al. 2020). On the other hand, the sense long noncoding transcript COLDAIR (COLD ASSISTED INTRONIC NONCODING RNA) interacts with PRC2 to mediate *FLC* silencing (Heo and Sung, 2011, Song et al. 2012) (Fig. 3). It has been shown that the association of VAL1 (Arabidopsis transcriptional repressor) within the nucleation region during vernalization serves as a trigger for PHD-PRC2 silencing at the *FLC* locus but does not drive the epigenetic silencing (Questa et al. 2016).

When the plants are transferred back to warm conditions after a saturating prolonged cold treatment, the PHD-PRC2 complex spread at the complete *FLC* locus. This leads to an increase in H3K27me3 histone modifications, which further induces the silencing of the *FLC* locus (Angel et al. 2011, Song et al. 2012, Questa et al. 2020). The epigenetic silencing of *FLC* is associated with LIKE HETEROCHROMATIN PROTEIN1 (LHP1) and VRN1 (Mylne et al. 2006). LHP1 likely binds to PRC2 and modifies histones at the *FLC* locus to maintain stable silencing (Mylne et al. 2006, Kim et al. 2009). Stable silencing of *FLC* even after vernalization allows plants to flower in spring. However, *FLC* is reactivated after return to warm conditions in perennial *Brassicaceae* and epigenetic silencing is not stable due to the influence of non-coding SNPs in the nucleation region (Questa et al. 2020).

Vernalization in cereals

The winter varieties of temperate cereals flower only after saturating the vernalization requirement, while spring varieties flower without vernalization. In winter varieties, vernalization is regulated by two main genes, namely *VERNALIZATION1* (*VRN1*) and *VRN2*, that further regulate the floral integrator gene *VRN3* (Dennis and Peacock, 2009, Greenup et al. 2009, Andres et al. 2012). *VRN1* and *VRN2* do not resemble Arabidopsis *VRN* genes suggesting that vernalization pathways may have evolved independently in temperate cereals. *VRN1* functions as a floral promoter (Yan et al. 2003, Trevaskis et al. 2003, Shitsukawa et al. 2007, Dixon et al. 2019) and is central to regulating the vernalization response. It encodes a MADS-box transcription factor and is related to the Arabidopsis floral meristem identity genes *AP1* (*APETALA1*) and *FUL* (Danyluk et al. 2003, Yan et al. 2003, Trevaskis et al. 2003, Preston and Kellogg 2006). In winter varieties of wheat and barley, *VRN1* expression is induced by cold exposure (Yan et al. 2003, Trevaskis et al. 2003). However, in spring varieties, *VRN1* is still expressed even in absence of cold exposure, which reduces or even abolishes their need for vernalization (Trevaskis et al. 2003, Yan et al. 2003). Similarly to Arabidopsis in which *FLC* downregulation is epigenetically-regulated, *VRN1* upregulation is also epigenetically-regulated. *VRN1* upregulation is associated with the inhibition of H3K27me3 (repressive chromatin marks) and the induction of the H3K4me3 level (active chromatin marks; Oliver et al. 2009). *VRN1* promoter region and first intron play a crucial role in maintaining the vernalization response (Yan et al. 2004, Fu et al. 2005). Deletions in the promoter region and first intron of *VRN1* are correlated in spring wheat varieties (Fu et al. 2005, Muterko et al. 2016, Xu et al. 2018). The consecutive upregulation of *VRN1* leads to the low expression of the floral repressor *VRN2* after prolonged cold (Distelfeld et al. 2009, Hemming et al. 2008, Trevaskis et al. 2006, Jarillo and Pineiro. 2011). However, it has been also reported that *VRN1* expression is not mandatory for flowering, or the downregulation of *VRN2* during cold, indicating the possibility that additional genes can be involved in *VRN2* downregulation upon vernalization (Chen and Dubcovsky 2012). *VRN2* encodes a zinc-finger CCT domain protein and there is no homolog found in Arabidopsis (Yan et al. 2004). CCT domain is important for regulating the vernalization requirement and mutations in the CCT domain result in the elimination of vernalization requirement (Distelfeld et al. 2009, Dubcovsky et al. 2005, Li et al. 2017). In wheat and barley, long days in autumn induced *VRN2* that further represses *VRN3* to inhibit flowering (Distelfeld and Dubcovsky 2010, Chen and Dubcovsky 2012). The expression of *VRN1* is regulated by vernalization and independent from photoperiod, contrary to *VRN2* expression that is regulated by photoperiod (Trevaskis et al. 2006, Dubcovsky et al. 2006, Woods et al. 2016, Monteagudo et al. 2019) suggesting that photoperiod also has a significant role in vernalization-induced flowering in cereals. In wheat and barley, the regulation of *VRN1* at higher ambient temperatures controls the spikelet number, floret number and flowering time (Ejaz and Von Korff. 2017, Dixon et al. 2019). Finally, the downregulation of *VRN2* releases *VRN3. VRN3* is a homolog of *FT* (Yan et al. 2004), which induces flowering after vernalization and in response to optimal photoperiod (Distelfeld et al. 2009, Trevaskis et al. 2007, Turner et al. 2005, Yan et al. 2006) (Fig. 4). The floral promoter activity of *VRN3* is activated by long days, similar to *FT* in Arabidopsis (Yan et al. 2006). *VRN3* stimulates the expression of *VRN1* in leaves by a positive feedback loop. *VRN3* increases *VRN1* expression through communicating with another transcription factor (*FDL2*) that binds to the *VRN1* promoter (Li et al. 2008).

Brachypodium distachyon, a temperate grass model system, also exhibit similar vernalization mechanism like wheat and barley (Higgins et al. 2010, Ream et al. 2012). *VRN1* expression is increased upon cold exposure and remains high even after vernalization (Ream et al. 2014, Woods et al. 2016). The high expression level of *VRN1* and *VRN3* after cold induces the transition from vegetative to reproductive state. Recently, it has been shown that inhibition of *VRN1* expression before cold is mediated through *REPRESSOR OF VERNALIZATION1* (*RVR1*), a gene upstream to *VRN1* that has a role in establishing vernalization requirement (Woods et al. 2017b). Interestingly, *VRN2* functions as a floral repressor in *Brachypodium* but, differently from wheat and barley, *VRN2* is not downregulated by cold through *VRN1* (Ream et al. 2014, Woods et al. 2016, Xu et al. 2018). This suggests that the functions of VRN1 and VRN2 as activator and repressor of flowering, respectively, remain conserved even after the diversification of temperate grasses but *VRN1* and *VRN2* regulatory loop do not seem to be conserved across Pooideae (Woods et al. 2016, Woods et al., 2017a). In short day photoperiod, vernalization response is regulated by *FT/VRN3* paralog *FT-like9* (*FTL9*) to confer competency for floral transition (Woods et al., 2019).

Epigenetic regulation of *VRN1* in temperate cereals

Similarly to Arabidopsis, the regulation of vernalization response is epigenetically-regulated in temperate grasses. In wheat and barley, vernalization induced epigenetic modifications of the floral promoter *VRN1*. The expression of *VRN1* is induced by low temperatures and remains high even after return to warm conditions. Epigenetic modifications in *VRN1* promoter and the first intron are responsible for winter memory in wheat and barley (Fu et al. 2005, Oliver et al. 2009, Diallo et al. 2012). Before cold, H3K27me3 repressive histone marks accumulate at the *VRN1* locus and result in the low expression level of *VRN1* (Oliver et al. 2009). During cold, H3K27me3 levels decrease while the active histone marks H3K4me3 and H3K36me3 increased at the *VRN1* chromatin (Fu et al. 2005, Xiao et al. 2014, Oliver et al. 2009, Diallo et al. 2012, Woods et al., 2017b). These histone marks are maintained even after return to warm conditions. H3K27me3 and H3K4me3 histone marks are maintained by the Polycomb and trithorax group complexes (Chittock et al. 2017). In *Brachypodium*, the CURLY LEAF orthologue ENHANCER OF ZESTE like-1 (EZL1) is required for the deposition of H3K27me3 at *VRN1* before cold (Lomax et al. 2018). This reduction in H3K27me3 and enrichment of H3K4me3 at *VRN1* is associated with the stable expression of *VRN1* during vernalization (Distelfeld et al. 2010, Woods et al. 2014, Woods et al. 2017b). In *Brachypodium*, *VRN3 is* also epigenetically-regulated during vernalization and exhibits an alteration in H3K27me3 and H3K4me3 histone marks similar to *VRN1* (Huan et al. 2018). Hence, the winter memory of temperate cereals is maintained through the stable epigenetic regulation of *VRN1* but detailed mechanism on how plants sense cold still need to be explored.

FLC homologs in cereals

In monocots such as wheat and barley, *VERNALIZATION* genes (*VRN1, VRN2* and *VRN3*) are important regulators of the vernalization-mediated flowering. In Arabidopsis, *FRI*-*FLC* are key genes in maintaining the vernalization response. As discussed above, the mechanisms of vernalization and epigenetic regulation of *FLC* and *VRN1* are also different between monocots and eudicots. However, the identification of *FLC* orthologs in monocots, through a combination of phylogenetic reconstructions and genomic synteny, provided a new dimension to understand the vernalization pathway (Ruelens et al. 2013). In temperate cereals, there are three *FLC* homologous genes: *ODDSOC2*, *ODDSOC1* and *MADS37*. MADS-box genes, *ODDSOC1* and *ODDSOC2* belong to *OsMADS51*-like genes, from which several members had already been shown to be vernalization responsive, including the two paralogs

HvODDSOC1 and *HvODDSOC2* from barley (*Hordeum vulgare*) (Greenup et al. 2010, Monteagudo et al. 2019), while *MADS37* belongs to *OsMADS37*-like genes (Ruelens et al. 2013).

In *Brachypodium*, *ODDSOC2* and *MADS37* are responsive to cold while the expression of *ODDSOC1* remains unchanged in response to vernalization in Bd-21, a spring *Brachypodium* accession (Ruelens et al. 2013). Similar to *FLC* in Arabidopsis, the expression of *ODDSOC2* is high before vernalization and low during and after vernalization in *Brachypodium* accessions (Ruelens et al. 2013, Sharma et al. 2017). In barley, the expression of an *FLC* orthologue, *ODDSOC2*, is also downregulated during vernalization and reported to be functional in vernalization response (Greenup et al. 2010). *ODDSOC2* acts as a floral repressor during vernalization (Greenup et al. 2008, Hemming et al. 2012). *ODDSCO2*-overexpressing plants flower late, together with a reduced spike growth, stem and leaf lengths (Greenup et al. 2010). In barley, *VRN1* downregulated the expression of *ODDSOC2* by binding to its promoter and inhibit the expression of *FPF1*-like genes to prevent flowering (Deng et al. 2015, Greenup et al. 2010) (Fig. 4). Recently, it has been shown that *ODDSOC2* (*HvOS2)* appears to have a potential role in regulating vernalization-mediated flowering in barley (Montengudo et al. 2019).

In wheat, four genes were identified as *FLC* homologs: *TaAGL41*, *TaAGL33*, *TaAGL22* and *TaMADS2* (Zhao et al. 2006, Ruelens et al. 2013, Sharma et al. 2017). *FLC* homologs, *TaAGL33* and *TaAGL22* are MADS-box transcripts that exhibit the most similar expression than *FLC* during vernalization and are stably downregulated by vernalization in a diverse set of wheat varieties (Sharma et al. 2017). Similar to *FLC* in Arabidopsis, winter wheat varieties have high a expression level of *TaAGL33* and *TaAGL22*, while their expression levels are low in spring wheat varieties before cold exposure (Sharma et al. 2017, Winfeild et al. 2009; Table 1). The expression of *ODDOSC2* in short days is regulated by temperature and is independent from photoperiod, while *VRN2* expression is dependent on photoperiod suggesting that the repressor activity during vernalization is combinedly maintained by *ODDOC2* and *VRN2* (Hemming et al. 2012, Dixon et al. 2019). These results from *Brachypodium*, barley and wheat indicate that the evolution of vernalization pathways seems to be related to some extent between monocots and eudicots.

FLC homologs can be a good predictor of vernalization

Winter varieties of different plant species have a wide variation in vernalization requirements that helps plants to survive to local climatic conditions and to flower at the optimal time (Kim et al. 2009, Ream et al. 2013, Hepworth et al. 2015, Bloomer and Dean 2017, Bouche et al. 2017). In Arabidopsis, allelic differences at the *FLC* and *FRIGIDA* (*FRI*) locus define the natural variation in vernalization (Clarke and Dean 1994, Shindo et al. 2005, Bloomer et al. 2017, Whittaker & Dean 2017). The early flowering phenotype of spring Arabidopsis ecotypes is found to be associated with the loss of function mutations at the *FLC* locus. In temperate grasses like *Brachypodium*, *ODDSOC2* expression before cold can predict the vernalization requirement (Sharma et al. 2017), suggesting that *ODDSOC2* could function as a potential indicator to define the natural variations of vernalization response. Winter barley and *Brachypodium* accessions have high expression levels of *ODDSOC2*, while expression is low in spring accessions before vernalization (Greenup et al. 2010, Sharma et al. 2017). In winter wheat varieties, allelic variations at *VRN1* are associated with the important determinant for the vernalization requirement (Golovina et al. 2010, Konopatskaia et al. 2016). However, high *ODDSOC2* levels in winter wheat varieties and low *ODDSOC2* levels in spring wheat varieties prior to cold exposure suggested that *ODDSOC2* may also have a potential role in defining the vernalization response in wheat varieties (Sharma et al. 2017).

Conclusion and future perspective

Arabidopsis and temperate cereals are two main systems in which the molecular mechanisms of vernalization have been studied. In both systems, vernalization results in the induction of regulatory genes. In Arabidopsis, cold represses *FLC* that serves as the central regulator while in temperate cereals cold induces *VRN1* expression and initiates the vernalization mechanism. The vernalization pathways in monocots and eudicots are regulated through a completely different set of genes and they are thought to have evolved independently. However, the presence of *FLC* homologs and the functional conservation of *ODDSOC2* in temperate cereals indicate that the evolution of the vernalization pathways is not fully independent.

The fundamental question of the cold sensing mechanism of vernalization still needs to be explored. Vernalization-induced epigenetic modifications at *VRN1* and *ODDSOC2* could provide the key inputs for exploring cold-sensing mechanism in temperate cereals. The current state of research in this domain is generating new questions and promising future findings could be beneficial in breeding efforts to refine vernalization-induced flowering in cereals and to adapt winter crops to a possible changing environment.

Author contributions

The manuscript is written by N.S. All authors edited and finalized the manuscript.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study

References

Amasino R (2004) Vernalization, competence, and the epigenetic memory of winter. Plant Cell 16: 2553-2559

Andres F, Coupland G (2012).The genetic basis of flowering responses to seasonal cues. Nat Rev Genet 13: 627–639

Angel A, Song J, Dean C, Howard M (2011) A Polycomb-based switch underlying quantitative epigenetic memory. Nature 476: 105-108

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C (2004) Vernalization requires epigenetic silencing of FLC by histone methylation. Nature 427: 164-167

Bloomer RH, Dean C (2017) Fine-tuning timing: natural variation informs the mechanistic basis of the switch to flowering in *Arabidopsis thaliana*. Journal of Experimental Botany 68: 5439–5452

Bond DM, Dennis ES, Finnegan EJ (2009) Hypoxia: a novel function for VIN3. Plant Signal Behav 4: 773–776

Bouché F, Woods DP, Amasino RM (2017) Winter memory throughout the plant kingdom: different paths to flowering. Plant Physiology 173: 27-35

Chen A, Dubcovsky J (2012) Wheat TILLING mutants show that the vernalization gene VRN1 down-regulates the flowering repressor VRN2 in leaves but is not essential for flowering. PLoS Genet 8: e1003134

Chittock EC, Latwiel S, Miller T, Mueller CW (2017) Molecular architecture of polycomb repressive complexes. Biochem. Soc. T 45: 193–205

Choi J, Hyun Y, Kang MJ, In Yun H, Yun JY, Lister C, Dean C, Amasino RM, Noh B, Noh YS, Choi Y (2009) Resetting and regulation of Flowering Locus C expression during Arabidopsis reproductive development. Plant J 57: 918-931

Choi K, Kim J, Hwang HJ, Kim S, Park C, Kim SY, Lee I (2011) The FRIGIDA complex activates transcription of *FLC*, a strong flowering repressor in Arabidopsis, by recruiting chromatin modification factors. The Plant Cell 23: 289–303

Chouard P (1960) Vernalization and Its Relations to Dormancy. Annual Review of Plant Physiology and Plant Molecular Biology 11: 191-238

Clarke JH, Dean C (1994) Mapping *FRI*, a locus controlling flowering time and vernalization response in *Arabidopsis thaliana*. Molecular and General Genetics 242: 81–89

Costa S, Dean C (2019) Storing memories: the distinct phases of Polycomb-mediated silencing of Arabidopsis FLC. Biochemical Society Transactions 47: 1187–1196

Danyluk J, Kane NA, Breton G, Limin AE, Fowler DB, Sarhan F (2003) TaVRT-1, a putative transcription factor associated with vegetative to reproductive transition in cereals. Plant Physiol. 132: 1849–1860

De Lucia F, Crevillen P, Jones AM, Greb T, Dean C (2008) A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of FLC during vernalization. Proc Natl Acad Sci USA 105: 16831-16836

Deng W, Casao MC, Wang P , Sato K, Hayes PM, Finnegan E J, Trevaskis B (2015) Direct links between the vernalization response and other key traits of cereal crops. Nat Commun 6: 5882

Deng W, Ying H, Helliwell CA, Taylor JM, Peacock WJ, Dennis ES (2011) FLOWERING LOCUS C (FLC) regulates development pathways throughout the life cycle of *Arabidopsis*. Proc. Natl Acad. Sci. USA 108: 6680–6685

Dennis ES, Peacock WJ (2009) Vernalization in cereals. J Biol 8: 57

Diallo AO, Ali-Benali MA, Badawi M, Houde M, Sarhan F (2012) Expression of vernalization responsive genes in wheat is associated with histone H3 trimethylation. Mol. Genet. Genomics 287: 575–590

Distelfeld A, Dubcovsky J (2010) Characterization of the maintained vegetative phase deletions from diploid wheat and their effect on VRN2 and FT transcript levels. Mol. Genet. Genomics 283: 223–232

Distelfeld A, Li C, Dubcovsky J (2009) Regulation of flowering in temperate cereals. Curr Opin Plant Biol 12: 178-184

Dixon LE, Karsai I, Kiss T, Adamski NM, Liu Z, Ding Y, et al. (2019) VERNALIZATION1 controls developmental responses of winter wheat under high ambient temperatures. Development 146: dev172684

Dubcovsky J (2005) Regulation of flowering time in wheat and barley. Comp Biochem Phys 141: 263–264

Dubcovsky J, Loukoianov A, Fu D, Valarik M, Sanchez A, Yan L (2006) Effect of photoperiod on the regulation of wheat vernalization genes VRN1 and VRN2. Plant Mol. Biol. 60: 469–480

Duncan S, Holm S, Questa J, Irwin J, Grant A, Dean C (2015) Seasonal shift in timing of vernalization as an adaptation to extreme winter. Elife 4: e06620

Ejaz M, VonKorff M (2017) The genetic control of reproductive development under high ambient temperature. Plant Physiol 173: 294–306

Fu DL, Szucs P, Yan L, Helguera M, Skinner JS, von Zitzewitz J, Hayes PM, Dubcovsky J (2005) Large deletions within the first intron in VRN-1 are associated with spring growth habit in barley and wheat. Mol. Genet Genomics 273: 54–65

Gazzani S, Gendall AR, Lister C, Dean C (2003) Analysis of the molecular basis of flowering time variation in Arabidopsis accessions. Plant Physiol 132: 1107-1114

Gendall AR, Levy YY, Wilson A, Dean C (2001) The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. Cell 107: 525–535

Greb, T, Mylne JS, Crevillen P, Geraldo N, An H, Gendall AR, Dean C (2007) The PHD finger protein VRN5 functions in the epigenetic silencing of Arabidopsis FLC. Curr Biol 17: 73–78

Greenup A, Peacock WJ, Dennis ES, Trevaskis B (2009) The molecular biology of seasonal flowering-responses in Arabidopsis and the cereals. Ann Bot 103: 1165-1172

Greenup AG, Sasani S, Oliver SN, Talbot MJ, Dennis ES, Hemming MN, Trevaskis B (2010) ODDSOC2 is a MADS box floral repressor that is down-regulated by vernalization in temperate cereals. Plant Physiol 153: 1062-1073

Helliwell CA, Wood CC, Roberston M, Peacock JW, Dennis ES (2006) The Arabidopsis FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. Plant J 46: 183-192

Hemming MN, Peacock WJ, Dennis ES, Trevaskis B (2008) Low-temperature and daylength cues are integrated to regulate FLOWERING LOCUS T in barley. Plant Physiol 147: 355-366

Hemming MN, Walford SA, Fieg S, Dennis ES, Trevaskis B (2012) Identification of High-Temperature-Responsive Genes in Cereals. Plant Physiology 158: 1439-1450

Heo JB, Sung S (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science 331: 76–79

Hepworth J, Antoniou-Kourounioti RL, Berggren K, Selga C, Tudor E, Yates B, Cox D, Harris BRC, Irwin J, Howard M, Säll T, Holm S, Dean C (2020) Natural variation in autumn FLC levels, rather than epigenetic 2 silencing, aligns vernalization to different climates. BioRxiv doi: doi.org/10.1101/2020.04.19.049148

Hepworth J, Antoniou-Kourounioti RL, Bloomer RH, Selga C, Berggren K, Cox D, Harris BRC, Irwin JA, Holm S, Sall T, Howard M, Dean C (2018) Absence of warmth permits epigenetic memory of winter in Arabidopsis. Nat. Commun. 9: 639

Hepworth J, Dean C (2015) Flowering Locus C's lessons: conserved chromatin switches underpinning developmental timing and adaptation. Plant Physiol 168: 1237–1245

Higgins JA, Bailey PC, Laurie DA (2010) Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate grasses. PLoS ONE 5: e10065

Huan Q, Mao Z, Chong K, Zhang J (2018) Global analysis of H3K4me3/ H3K27me3 in Brachypodium distachyon reveals VRN3 as critical epigenetic regulation point in vernalization and provides insights into epigenetic memory. New Phytol. 219: 1373–1387

Jarrillo JA, Pineiro M (2011) Timing is everything in plant development. The central role of floral repressors. Plant Sci 181: 364-378

Jiang, D, Gu X, He Y (2009) Establishment of the winter-annual growth habit via FRIGIDA- mediated histone methylation at FLOWERING LOCUS C in Arabidopsis. Plant Cell, 21: 1733-1746

Jianng D, Gu X, He Y (2009) Establishment of the winter-annual growth habit via FRIGIDA-mediated histone methylation at FLOWERING LOCUS C in Arabidopsis. Plant Cell 21: 1733-1746

Kim DH, Doyle MR, Sing S, Amasino RM (2009) Vernalization: winter and the timing of flowering in plants. Annu Rev Cell Dev Biol 25: 277-299

Koornneef M, Vries BH, Hanhart C, Soppe W, Peeters T (1994) The phenotype of some late-flowering mutants is enhanced by a locus on chromosome 5 that is not effective in the Landsberg *erecta* wildtype. The Plant Journal 6: 911–919

Lee I, Bleecker A, Amasino R (1993) Analysis of naturally occurring late flowering in *Arabidopsis thaliana*. Molecular and General Genetics 237: 171–176

Lee J, Oh M, Park H, Lee I (2008) SOC1 translocated to the nucleus by interaction with AGL24 directly regulates leafy. Plant J 55: 832–843

Levy YY, Mesnage S, Mylne JS, Gendall AR, Dean C (2002) Multiple roles of Arabidopsis VRN1 in vernalization and flowering time control. Science 297: 243-246

Li C, Dubcovsky J (2008) Wheat FT protein regulates *VRN1* transcription through interactions with FDL2. Plant J. 55: 543–554

Li Y, Xu M (2017) CCT family genes in cereal crops: A current overview. Crop J 5: 449–458

Lomax A, Woods DP, Dong Y, Bouche F, Rong Y, Mayer KS, Zhong X, Amasino RM (2018) An ortholog of CURLY LEAF/ENHANCER OF ZESTE like-1 is required for proper flowering in Brachypodium distachyon. The Plant Journal : For Cell and Molecular Biology 93: 871–882

Michaels SD, Amasino RM (1999) FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11: 949–956

Michaels SD, Amasino RM (2001) Loss of FLOWERING LOCUS C activity eliminates the late-flowering phenotype of FRIGIDA and autonomous pathway mutations but not responsiveness to vernalization. Plant Cell 13: 935–941

Michaels SD, He Y, Scortecci KC, Amasino RM (2003) Attenuation of FLOWERING LOCUS C activity as a mechanism for the evolution of summer-annual flowering behavior in Arabidopsis. Proc Natl Acad Sci U S A 100: 10102-10107

Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM (2005) Integration of flowering signals in winter-annual Arabidopsis. Plant Physiol 137: 149-156

Monteagudo A, Igartua E, Contreras-Moreira B, Gracia MP, Ramos J, Karsai I, Casas AM (2019) Fine-tuning of the flowering time control in winter barley: the importance of HvOS2 and HvVRN2 in non-inductive conditions. BMC Plant Biol 19: 113

Muterko A, Kalendar R, Salina E (2016) Novel alleles of the VERNALIZATION1 genes in wheat are associated with modulation of DNA curvature and flexibility in the promoter region. BMC Plant Biol 16: 9

Mylne JS, Barrett L, Tessadori F, Mesnage S, Johnson L, Bernatavichute YV, Jacobsen SE, Fransz P, Dean C (2006) LHP1, the Arabidopsis homologue of HETEROCHROMATIN PROTEIN1, is required for epigenetic silencing of FLC. Proc Natl Acad Sci U S A 103: 5012-5017

O'Neill CM, Lu X, Calderwood A, Tudor EH, Robinson P, Wells R, Morris R, Penfield S (2019) Vernalization and floral transition in autumn drive winter annual life history in oilseed rape. Curr. Biol. 29: 4300–4306

Oliver SN, Finnegan EJ, Dennis ES, Peacock WJ, Trevaskis B (2009) Vernalization-induced flowering in cereals is associated with changes in histone methylation at the VERNALIZATION1 gene. Proc Natl Acad Sci U S A, 106: 8386-8391

Pien S, Fleury D, Mylne JS, Crevillen P, Inzé D, Avramova Z, Dean C, Grossniklaus U (2008) ARABIDOPSIS TRITHORAX1 dynamically regulates FLOWERING LOCUS C activation via histone 3 lysine 4 trimethylation. Plant Cell 20: 580–588

Preston JC, Kellogg EA (2006) Reconstructing the evolutionary history of paralogous APETALA1/FRUITFULL-like genes in grasses (*Poaceae*). Genetics 174: 421–437

Questa J, Song J, Geraldo N, An H, Dean C (2016) Arabidopsis transcriptional repressor VAL1 triggers Polycomb silencing at FLC during vernalization. Science 353: 485-488

Qüesta JI, Antoniou-Kourounioti RL, Rosa S, Li P, Duncan S, Whittaker C, Howard M, Dean C (2020) Noncoding SNPs influence a distinct phase of Polycomb silencing to destabilize long-term epigenetic memory at Arabidopsis FLC. Genes Dev 34: 446–461

Ream TS, Woods DP, Schwartz CJ, Sanabria CP, Mahoy JA, Walters EM, Kaeppler HF, Amasino RM (2014) Interaction of photoperiod and vernalization determines flowering time of *Brachypodium distachyon*. Plant Physiol 164: 694–709

Ream TS, Woods DP, Amasino RM (2012) The molecular basis of vernalization in different plant groups. Cold Spring Harb. Symp. Quant Biol 77: 105-115

Ruelens P, De Maagd RA, Proost S, Theissen G, Geuten K, Kaufmann K (2013) FLOWERING LOCUS C in monocots and the tandem origin of angiosperm-specific MADS-box genes. Nat Commun 4: 2280

Searle I, He Y, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G (2006) The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. Genes Dev 20: 898-912

Sharma N, Ruelens P, D'Hauw M, Maggen T, Dochy N, Torfs S, Kaufmann K, Geuten K (2017) A Flowering Locus C homolog is a vernalization-regulated repressor in Brachypodium and is cold regulated in wheat. Plant Physiology 173: 1301-1315

Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, et al. (1999) The FLF MADS box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. Plant Cell 11: 445–458

Sheldon CC, Hills MJ, Lister C, Dean C, Dennis ES, Peacock WJ (2008) Resetting of FLOWERING LOCUS C expression after epigenetic repression by vernalization. Proc Natl Acad Sci U S A 105: 2214-2219

Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES (2000) The molecular basis of vernalization: the central role of *FLOWERING LOCUS C* (*FLC*). PNAS 97: 3753–3758

Shindo C, Aranzana MJ, Lister C, Baxter C, Nicholls C, Nordborg M, Dean C (2005) Role of FRIGIDA and FLOWERING LOCUS C in Determining Variation in Flowering Time of Arabidopsis. Plant Physiol 138: 1163-1173

Shitsukawa N, Ikari C, Shimada S, Kitagawa S, Sakamoto K, et al. (2007). The einkorn wheat (*Triticum monococcum*) mutant, maintained vegetative phase, is caused by a deletion in the VRN1 gene. Genes Genet Syst 82: 167–170

Song J, Angel A, Howard M, Dean C (2012) Vernalization - a cold-induced epigenetic switch. J Cell Sci 125: 3723-3731

Sung S, Amasino RM (2004) Vernalization and epigenetics: how plants remember winter. Curr Opin Plant Biol 7: 4-10

Sung S, He Y, Eshoo TW, Tamada Y, Johnson L, Nakahigashi K, Goto K, Jacobsen SE, Amasino RM (2006) Epigenetic maintenance of the vernalized state in Arabidopsis thaliana requires LIKE HETEROCHROMATIN PROTEIN 1. Nature Genetics 38: 706-710

Swiezewski S, Liu F, Magusin A, Dean C (2009) Cold-induced silencing by long antisense transcripts of an Arabidopsis Polycomb target. Nature 462: 799-802

Trevaskis B, Bagnall DJ, Ellis MH, Peacock WJ, Dennis E (2003) MADS box genes control vernalization-induced flowering in cereals. Proc Natl Acad Sci U S A 100: 13099-13104

Trevaskis B, Hemming MN, Dennis ES, Peacock WJ (2007) The molecular basis of vernalization-induced flowering in cereals. Trends Plant Sci 12: 352-357

Trevaskis B, Hemming MN, Peacock WJ, Dennis ES (2006) HvVRN2 responds to daylength, whereas HvVRN1 is regulated by vernalization and developmental status. Plant Physiol 140: 1397-1405

Turck F, Fornara F, Coupland G (2008) Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annu Rev Plant Biol 59: 573-594

Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. Science 310: 1031-1034

Wang Y, Gu X, Yuan W, Schmitz RJ, He Y (2014) Photoperiodic control of the floral transition through a distinct polycomb repressive complex. Dev Cell 28: 727–736

Whittaker C, Dean C (2017) The FLC locus: a platform for discoveries in epigenetics and adaptation. Annual Review of Cell and Developmental Biology 33: 555–575

Winfield MO, Lu C, Wilson ID, Coghill JA, Edwards KJ (2009) Cold and light-induced changes in the transcriptome of wheat leading tophase transition from vegetative to reproductive growth. BMC Plant Biol 9: 55

Wollenberg AC, Amasino RM (2012) Natural variation in the temperature range permissive for vernalization in accessions of *Arabidopsis thaliana*. Plant, Cell and Environment 35: 2181–2191

Wood CC, Robertson M, Tanner G, Peacock WJ, Dennis ES, Helliwell CA (2006) The Arabidopsis thaliana vernalization response requires a polycomb-like protein complex that also includes VERNALIZATION INSENSITIVE 3. Proc Natl Acad Sci U S A 103: 14631-14636

Woods D, Dong Y, Bouche F, Bednarek R, Rowe M, Ream T, Amasino R (2019) A florigen paralog is required for short-day vernalization in a pooid grass. eLife 8: e42153

Woods DP, Bednarek R, Bouche´ F, Gordon SP, Vogel JP, Garvin DF, Amasino RM (2017a) Genetic architecture of flowering-time variation in Brachypodium distachyon. Plant Physiol 173: 269–279

Woods DP, McKeown MA, Dong Y, Preston JC, Amasino RM (2016) Evolution of VRN2/Ghd7-like genes in vernalization mediated repression of grass flowering. Plant Physiol 170: 2124–2135

Woods DP, Ream TS, Bouche F, Lee J, Thrower N, Wilkerson C, Amasino RM. (2017b) Establishment of a vernalization requirement in Brachypodium distachyon requires repressor of vernalization1. PNAS 114: 6623–6628

Wu Z, Fang X, Zhu D, Dean C (2020) Autonomous Pathway: FLOWERING LOCUS C Repression through an Antisense-Mediated Chromatin-Silencing Mechanism. Plant Physiol. 182: 27–37

Xiao J, Xiao J, Jin R, Yu X, Shen M, Wagner JD, et al (2017) Cis and trans determinants of epigenetic silencing by Polycomb repressive complex 2 in Arabidopsis. Nat Genet 49: 1546–1552

Xiao J, Xu S, Li C, Xu Y, Xing L, Niu Y, Huan Q, Tang Y, Zhao C, Wagner D, Gao C, Chong K (2014) O-GlcNAc-mediated interaction between VER2 and TaGRP2 elicits TaVRN1 mRNA accumulation during vernalization in winter wheat. Nat. Commun. 5: 4572

Xu S, Chong K. (2018). Remembering winter through vernalisation. Nat. Plants 4: 997–1009

Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, Sanchez A, Valarik M, Yasuda S, Dubcovsky J (2006) The wheat and barley vernalization gene VRN3 is an orthologue of FT. Proc Natl Acad Sci U S A 103: 19581-19586

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, et al. (2004). The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science 303: 1640–44

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene VRN1. Proceedings of the National Academy of Sciences of the U S A 100: 6263-6268

Zhang Y, Sikes ML, Beyer AL, Schnneider DA (2009) The Paf1 complex is required for efficient transcription elongation by RNA polymerase I. Proc Natl Acad Sci U S A 106: 2153-2158

Zhao T, Ni Z, Dai Y, Yao Y, Nie X, Sun Q (2006) Characterization and expression of 42 MADS-box genes in wheat (Triticum aestivum L.). Mol Genet Genomics 276: 334–350

Zhou Y, Wang Y, Krause K, Yang T, Dongus JA, Zhang Y, Turck F (2018) Telobox motifs recruit CLF/SWN-PRC2 for H3K27me3 deposition via TRB factors in Arabidopsis. Nat Genet 50: 638–644

Zhu D, Rosa S, Dean C (2015) Nuclear organization changes and the epigenetic silencing of *FLC* during vernalization. J Mol Biol 427: 659–669

Figure legends:

Fig. 1. Molecular mechanisms of vernalization-mediated flowering: In Arabidopsis, *FLC* expression? is high before cold (autumn). During winter, prolonged cold exposure results in low *FLC* level and represses *FT*. Later in spring, long days induce *FT* expression and accelerate flowering. In temperate cereals, *VRN2* represses *FT* before cold. In winter, cold-exposure induced the expression of *VRN1* and represses *VRN2*. *VRN1* remains high in spring and allows the induction of *VRN3* to promote flowering.

Fig. 2. Vernalization-induced flowering mechanisms in Arabidopsis: *Flowering Locus C* (*FLC*) is the key repressor of flowering. *FLC* is repressed by prolonged cold exposure results in the downregulation of floral integrators genes such as *FT*, *FD* and *SOC1* that promotes flowering. *FT* is induced by *CO* and photoperiod pathway. *FT* together with *FD* is meristem activates the expression of *SOC1* and floral

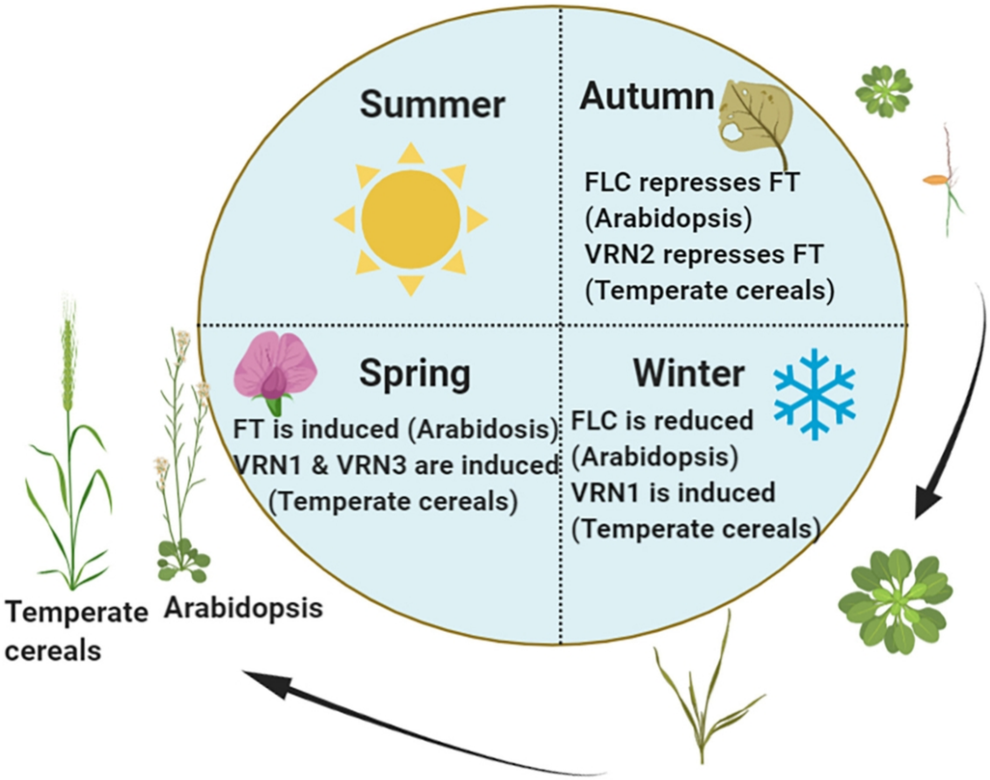
meristem identity genes like *FUL* and *AP1* and accelerate flowering. Gibberellin promotes flowering by inducing *SOC1* and *LFY*.

Fig. 3. Epigenetic modifications in *FLC* chromatin: the expression of *FLC* transcripts is high before cold. Prior to winter (in autumn), high *FLC* levels are maintained through a set of regulators such as *FRI* (upregulates *FLC* expression), ATWDR5a and Paf1C (RNA polymerase associated factor 1 complex), which results in Histone 3 methylation at lysine 4 and 36 (H3K4me and H3K36me). During winter, the silencing of *FLC* is a gradual process regulated by the PHD-PRC2 complex, LHP1 and VAL1 recruitment at FLC, resulting in an increase of H3K27me repressive histone marks in FLC chromatin. Prolonged cold exposure also induced the expression of long noncoding RNA COOLAIR and COLDAIR. COLDAIR helps in PRC2 recruitment by interacting with CURLY LEAF (CLF). After return to warm temperatures, the mitotically-stable silencing of *FLC* is maintained by spreading H3K27me histone marks across the *FLC* chromatin to promote flowering in spring.

Fig. 4. Vernalization-induced flowering in temperate cereals (monocots) and Arabidopsis (eudicots). In temperate cereals (top panel), such as wheat and barley, prolonged cold induces *VRN1* expression through epigenetic modifications in the *VRN1* chromatin. *VRN1* represses the expression of *VRN2* that inhibit *VRN3* transcript. In spring, the long day photoperiod induces *VRN3* expression and *VRN1* expression remains high and accelerates flowering. *VRN1* can also induce *FPF1*-like genes by inhibiting the expression of *ODDSOC2* and therefore promoting flowering. In Arabidopsis (bottom panel), vernalization induced epigenetic silencing of *FLC* mediated through PRC2 and long non-coding RNAs COLDAIR and COOLAIR. *FLC* inhibits the expression of the floral integrator genes such as *FD*, *FT* and *SOC1*. Activation of these floral integrator genes promotes flowering in spring.

Table 1: Different *FLC* homologs in monocots.

S. No.	*FLC* homologs	Description and function	Reference
1.	*BdODDSOC2* (Brachypodium)	Downregulated during winter and a repressor of flowering. Potential predictor of vernalization requirement.	Ruelens et al., 2013 Sharma et al., 2017
2.	*BdODDSOC1* (Brachypodium)	Expression remains unchanged during vernalization.	Ruelens et al., 2013
3.	*BdMADS37* (Brachypodium)	Downregulated during vernalization.	Ruelens et al., 2013
4.	*TaAGL33* (Wheat)	Expression decreases upon prolonged cold exposure. Expression levels before cold can predict the vernalization requirement.	Winfeild et al, 2009 Sharma et al., 2017
5.	*TaAGL22* (Wheat)	Downregulated during vernalization.	Sharma et al., 2017
6.	*HvODDSOC2* (Barley)	Downregulated during vernalization and a repressor of flowering.	Greenup et al., 2010 Ruelens et al., 2013 Monteagudo et al., 2019



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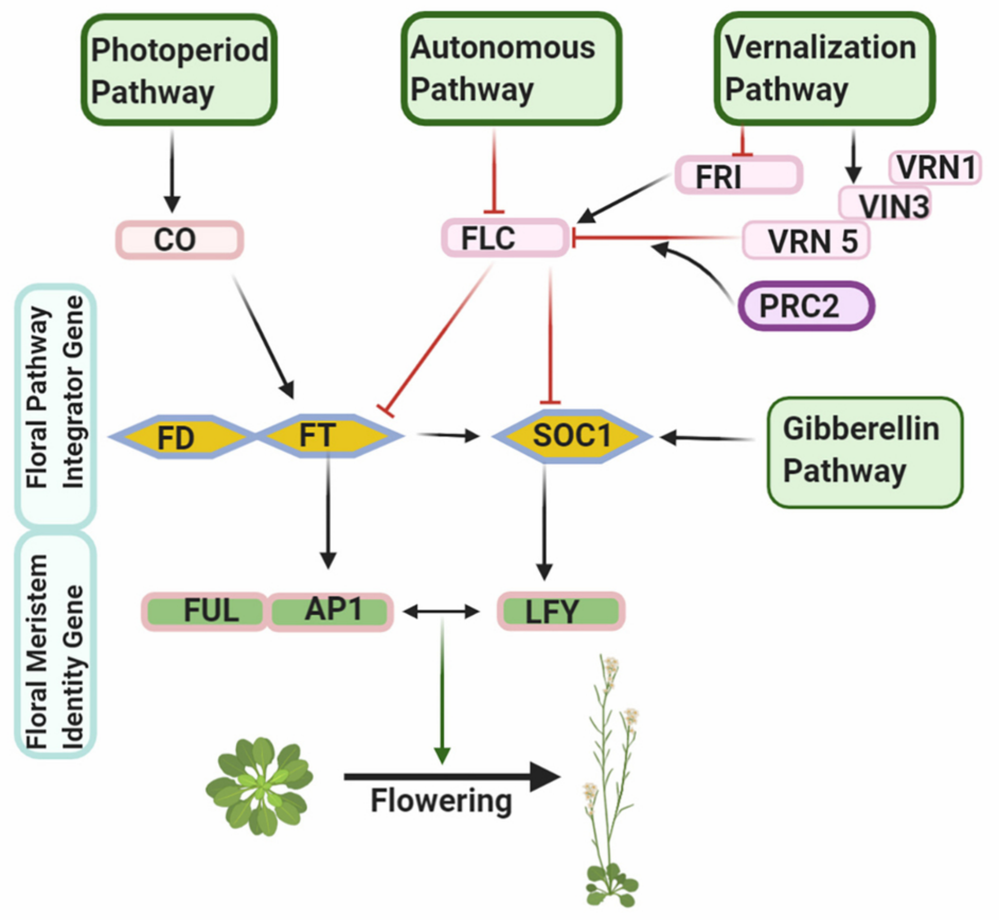

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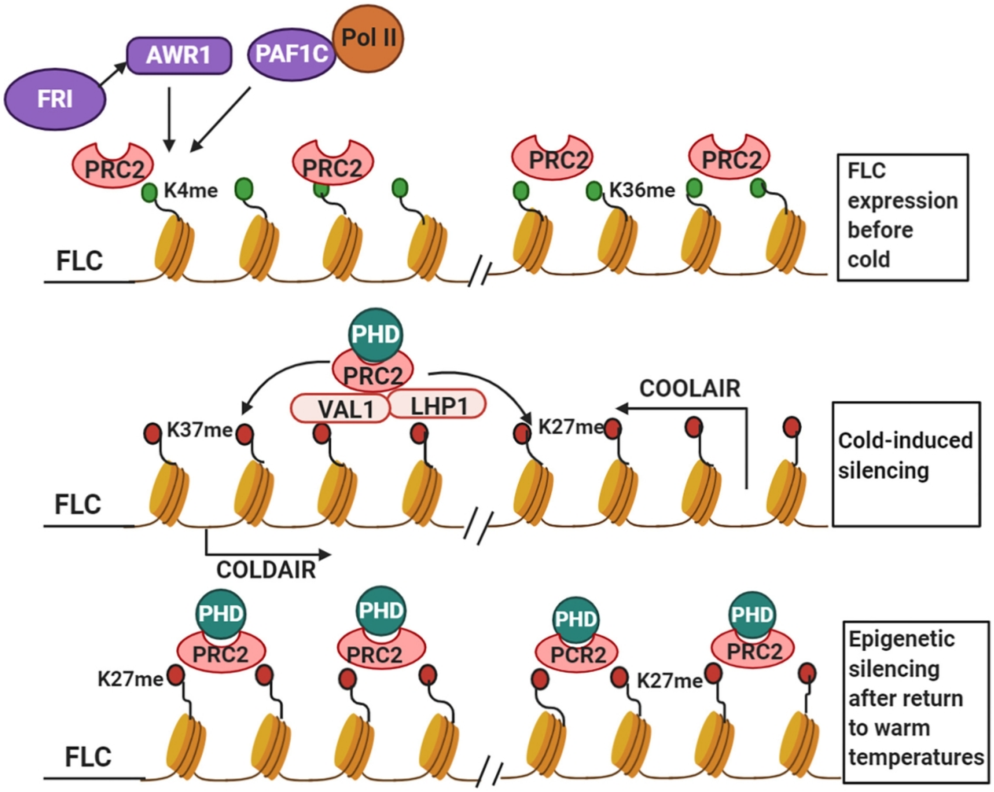

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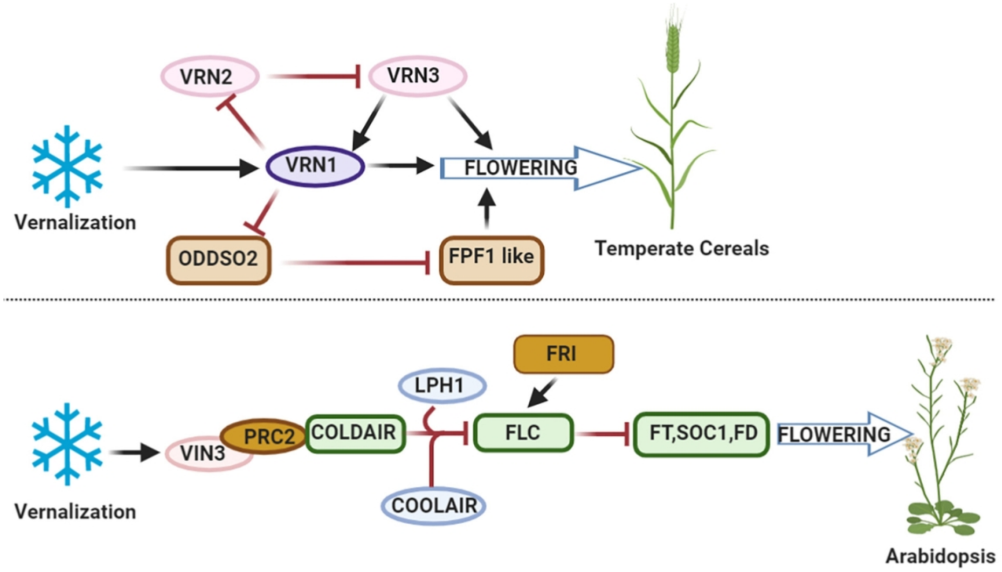

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