The Role of FLOWERING LOCUS C Relatives in Cereals

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FLOWERING LOCUS C (FLC) is one of the best characterized genes in plant research and is integral to vernalization-dependent flowering time regulation. Yet, despite the abundance of information on this gene and its relatives in Arabidopsis thaliana, the role FLC genes play in other species, in particular cereal crops and temperate grasses, remains elusive. This has been due in part to the comparative reduced availability of bioinformatic and mutant resources in cereals but also on the dominant effect in cereals of the VERNALIZATION (VRN) genes on the developmental process most associated with FLC in Arabidopsis. The strong effect of the VRN genes has led researchers to believe that the entire process of vernalization must have evolved separately in Arabidopsis and cereals. Yet, since the confirmation of the existence of FLC-like genes in monocots, new light has been shed on the roles these genes play in both vernalization and other mechanisms to fine tune development in response to specific environmental conditions. Comparisons of FLC gene function and their genetic and epigenetic regulation can now be made between Arabidopsis and cereals and how they overlap and diversify is coming into focus. With the advancement of genome editing techniques, further study on these genes is becoming increasingly easier, enabling us to investigate just how essential FLC-like genes are to modulating flowering time behavior in cereals.

Keywords: flowering time, cereals, FLOWERING LOCUS C, vernalisation, ambient temperature

INTRODUCTION

FLOWERING LOCUS C (FLC) genes are a clade of MADS-box transcription factors in plants and are major regulators in many aspects of plant development. They are mostly associated with vernalization-regulated flowering but also have important roles in seed dormancy (Chen et al., 2014; Chen and Penfield, 2018), ambient temperature regulated development (Balasubramanian et al., 2006; Lee et al., 2013), germination (Chiang et al., 2009), as well as being associated with other processes like bud dormancy, circadian rhythm, water use efficiency, and indirect defense against herbivory (McKay et al., 2003; Edwards et al., 2006; Kumar et al., 2016; Mohammadin et al., 2017). In fact, there are over 500 FLC binding sites in the Arabidopsis thaliana (henceforth Arabidopsis) genome indicating that FLC is involved in much more than vernalization (Deng et al., 2011). In flowering time regulation, FLC acts as a repressor protein and acts mainly by repressing the activation of key floral promoting genes such as FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1; Searle et al., 2006; Deng et al., 2011).
The existence of FLC-like genes in cereals remained elusive for many years while the wealth of information on Arabidopsis FLC continued to accumulate. Many believed that FLC was restricted to eudicots and that monocot plants evolved separate mechanisms to regulate development and flowering time. However, a turning point came when it was concretely established through genome synteny analysis and phylogenetic reconstructions that FLC relatives did indeed exist in cereals (Ruelens et al., 2013). In this pivotal publication, it was shown that a clade of genes within monocots were phylogenetically related to the FLC genes of Arabidopsis. Within this monocot FLC clade, there are two subclades: the OsMADS51 and OsMADS37 subclades, so called after the representation of these rice genes within each clade. The OsMADS51 subclade is subsequently divided into two groups: the ODDSOC1-like and ODDSOC2-like groups. The name “ODDSOC” came from their weak similarity to the flowering time gene SOC1 (Greenup et al., 2010). Members of the ODDSOC2 clade of genes are the most characterized out of all FLC-like genes in monocots thus far. The details of these relationships and their relationship to the Arabidopsis FLC genes can be seen in Figure 1. It must be noted that although the Arabidopsis and monocot FLC clades are related, it is likely that the ancestral gene function was partitioned differently within the groups. Therefore, direct comparisons of individual members across groups are not completely accurate.

Furthermore, FLC-like genes in cereals are Type II MADS-domain proteins despite having previously been annotated as Type I MADS-domain proteins, and have the typical MIKC protein structure (Zhao et al., 2006; Schilling et al., 2020).

Due to the advancement of genome sequencing technology, genetic mapping and genome editing methods, the nature of the function of FLC-like genes in cereals is coming into focus. Furthermore, avenues are now opening to further advance our knowledge on these genes which may reveal diversification of their function from their Arabidopsis homologs. This review aims to highlight the key findings over the last two decades of the role FLC relatives play in cereals and how progress made in biotechnology will further our understanding of the molecular control of plant development, perhaps leading us to utilize these genes as biotechnological tools for crop improvement.

**EVIDENCE FOR CONSERVED FUNCTION: WHAT DO WE ALREADY KNOW?**

FLCs Are Involved in Vernalization in Grasses

In cereals, the main determinants of vernalization-regulated flowering are the VERNALIZATION (VRN) genes VRN1, VRN2, and VRN3 (Yan et al., 2003, 2004, 2006). Generally, vernalization results in the upregulation of the floral promoter VRN1 which downregulates the floral repressor VRN2, alleviating its repressive effect on the flowering promoter VRN3, an orthologue of FT (reviewed in Trevaskis et al., 2007; Distelfeld et al., 2009; Ream et al., 2012). VRN3 then positively regulates VRN1 expression resulting in a positive feedback loop which induces flowering. This feedback loop in general determines the flowering habit of cereals with mutations in any of these proteins leading to altered spring or winter growth habit (Yan et al., 2004, 2006; Fu et al., 2005). Yet, variation in vernalization response can still remain in cultivars which have shared alleles of these genes (Rizza et al., 2016), opening up the potential for other genes to have functional significance in this process. The identification of FLC-like genes in cereal research remained elusive for many years leading to the conclusion that the vernalization pathway of Arabidopsis and cereals evolved completely separately (Yan et al., 2004; Winfield et al., 2009; Greenup et al., 2010). Incorporating the finding that an FLC clade exists in monocots, it appears more likely that the ancestral species of dicots and monocots contained both FLC and AP1/VRN1-like genes, and each group was differentially recruited during the evolution of vernalization responsiveness. As AP1 retains a role in regulating flowering in Arabidopsis, so too do FLC homologs play a role in similar processes in cereals. This section aims to highlight these roles FLC-like genes play in the vernalization process and flowering time regulation in crop species.

FLOWERING LOCUS C homologs were described as being involved in vernalization in cereals almost 20 years ago where Trevaskis et al. (2003) described TaMX23, a MADS-box gene repressed by vernalization in winter wheats. TaMX23 increases in abundance in early vegetative development and the effect of vernalization on TaMX23 expression depended on whether the cultivar was a spring or winter variety (Trevaskis et al., 2003). TaMX23 shares homology with both TaODDSOC2 (TaOS2; also known as TaAGL33) and TaAGL42 (Winfield et al., 2009). Although it is more similar in sequence to TaAGL42, its reported expression pattern reflects that of TaOS2.

TaOS2 is the most described FLC-like gene in wheat so far. Like FLC, all three homologs of TaOS2 are downregulated by vernalization and repression is maintained 2 weeks post- vernalization (Winfield et al., 2009; Sharma et al., 2017; Appels et al., 2018). In winter varieties, TaOS2 expression is initially high in leaf tissue and gradually declines throughout development as temperature decreases, yet its expression is constitutively low in spring lines, indicating that the function is cultivar-dependent and relevant to the flowering habit of these lines (Winfield et al., 2009; Sharma et al., 2017). Creating premature stop codons using CRISPR/Cas9 gene editing in the D-homeolog revealed an effect of this gene on flowering time, as mutants flower 3 days earlier than wild type (Appels et al., 2018). It is encouraging that a knockout of a single homeolog in hexaploid wheat reveals a phenotype. A 3-day alteration in flowering time is no mean feat in wheat breeding and can have great implications on yield in a region-specific manner. Validating this phenotype in the field will be enlightening to discover whether the phenotype is maintained and in which environments are the greatest effects found. It is also possible that functional redundancy is at play and multiple mutations in all homeologs of TaOS2 might reveal more striking phenotypes to uncover the roles of these genes in flowering time regulation in wheat.
A second FLC-like gene, \textit{TaAGL42} (or \textit{TaODDSOC1}), has also been described as being regulated by vernalization in wheat (Winfield et al., 2009; Sharma et al., 2017); however, \textit{TaAGL42} is upregulated in winter cultivars and is downregulated or stably expressed in spring varieties. Additionally, \textit{TaAGL42} expression was shown to increase rapidly in response to a sudden drop in temperature in two winter varieties, suggesting that this gene could be involved in cold acclimation and tolerance in these lines (Winfield et al., 2009). In conclusion, although the gene is cold-regulated in a variety-specific manner, the function of \textit{TaAGL42} remains unclear in wheat.

Relatives of these genes have also been described in barley, where their identification came about as a result of a desire to characterize new genes responsive to vernalization. Through the analysis of homologs of \textit{TaMX23} (Trevaskis et al., 2003), two genes were identified: one sharing homology to \textit{TaMX23}
and another sharing homology with TaOS2 (Treviskis et al., 2003; Winfield et al., 2009; Greenup et al., 2010). The two homologs were named HvODDSOC1 (HvOS1) and HvODDSOC2 (HvOS2), respectively, due to their weak sequence similarity to SOC1 in Arabidopsis. HvOS1 expression increased in response to vernalization, consistent with its homolog in wheat (Winfield et al., 2009). HvOS2 expression was repressed in response to vernalization in both the leaves and apices, and this repression was maintained post-vernalization (Greenup et al., 2010). The expression of HvOS2 was also strongest in winter barley varieties pre-vernalization and was dramatically reduced upon exposure to prolonged cold, while expression remained low and constant in spring varieties. This highlights the importance of choice of cultivar when studying FLC homologs in cereals. Overexpressing HvOS2 in the spring barley resulted in delayed flowering in these lines, strongly suggesting that HvOS2 acts as a repressor of the floral transition. In contrast, no phenotype was observed for HvOS2 knockdown lines created using RNA interference (RNAi); however, this is to be expected in a spring line where HvOS2 is low naturally and vernalization is not required. In a separate study, differences in the rate of reproductive development under insufficient vernalization conditions was also explained by a difference in HvOS2 expression levels between two winter varieties (Monteagudo et al., 2019), further supporting the idea of HvOS2 as a vernalization-dependent regulator of the floral transition.

Aside from the crops themselves, research has been conducted on FLC-like genes in the model temperate grass Brachypodium distachyon (henceforth Brachypodium). In fact, Brachypodium was the organism chosen to first analyze the activity of the FLC-like genes so far from basic research on flowering time regulation and vernalization in cereals. Therefore, many of these findings have been related to or are based on descriptions of the activities of the diploid relative Triticum monococcum, barley, and Brachypodium. In general, evidence exists to suggest that VRN1 is required to repress ODDSOC2 post-vernalization to enable rapid flowering.

FLC and VRN1 Activities Are Entwined in Cereals

Much of what we have learned about FLC-like genes so far comes from basic research on flowering time regulation and vernalization in cereals. Therefore, many of these findings have been related to or are based on descriptions of the activities of the diploid relative Triticum monococcum, barley, and Brachypodium. In general, evidence exists to suggest that VRN1 is required to repress ODDSOC2 post-vernalization to enable rapid flowering.

In T. monococcum, it was observed that TmOS2 levels rose post-vernalization in mutant lines lacking functional TmVRN1 while levels remained low in wild type lines. Analysis of TmOS2 levels pre- and during vernalization showed that there was no difference in expression between wild type and mutant lines. It is only post-vernalization TmOS2 levels that are affected by TmVRN1 loss of function, suggesting that TmVRN1 is required to repress TmOS2 post-vernalization but not to reduce its activity initially (Greenup et al., 2010).

Similar to T. monococcum, HvOS2 expression is lowest in barley lines with dominant, active VRN1 alleles, consistent with the hypothesis that VRN1 represses OS2 in temperate cereals (Greenup et al., 2010). Supporting this hypothesis, it was subsequently reported that HvVRN1 binds to the HvOS2 promoter in the spring variety Golden Promise (Deng et al., 2015). Several HvVRN1 binding sites have also been identified throughout the HvOS2 locus (Monteagudo et al., 2019). Likewise, there is an antagonistic relationship between BdOS2 and BdVRN1 expression patterns in Brachypodium. BdOS2 expression is elevated in BdVRN1 knockout lines (Woods et al., 2016). As well as that, BdOS2 expression is elevated in lines overexpressing BdVRN2, associated with low BdVRN1 expression and delayed flowering (Woods et al., 2016). Interestingly, BdOS2 expression patterns are not significantly influenced by overexpression of BdVRN1 or knockdown of BdVRN2, indicating that the response of BdOS2 to BdVRN1 expression is qualitative and not dosage dependent.

This relationship between VRN1 and ODDSOC2 is conserved in hexaploid wheat, where TaOS2 expression post-vernalization is linked to the nature of VRN1 alleles found in a given
cultivar (Dixon et al., 2019). This relationship has implications for flowering time behavior which will be discussed in more detail in the next section.

**ODDSOC2 and the Balancing Act of Vernalization and Ambient Temperature**

Temperature is a key environmental signal which regulates many facets of plant development. Flowering time in both *Arabidopsis* and cereals is regulated by ambient temperature, with increasing temperatures generally resulting in earlier flowering times (McMaster and Wilhelm, 2003; Balasubramanian et al., 2006; Ejaz and von Korff, 2017; Dixon et al., 2018). Underlying this trait in *Arabidopsis* are the activities of FLC and its relative FLOWERING LOCUS M (FLM). FLM, like FLC, negatively regulates the floral transition, however, it is mostly involved in ambient temperature-dependent flowering (Balasubramanian et al., 2006; Lee et al., 2013; Posé et al., 2013). FLM functions as part of a repressor complex with another MADS-domain transcription factor, SHORT VEGETATIVE PHASE (SVP). This complex represses the activities of flowering promoters under cold temperatures to delay flowering and the stability of the complex and of the proteins themselves are affected by increasing temperature, reducing their repressive effects in warm conditions (Lee et al., 2013; Posé et al., 2013; Capovilla et al., 2017). Temperature-dependent alternative splicing of *FLM* is integral to this response, where the relative abundance of certain transcripts compared to others determines the flowering phenotype in response to temperature (Capovilla et al., 2017; Lutz et al., 2017).

High levels of *FLC* itself also result in thermal unresponsiveness, therefore, suggesting that FLC suppresses thermal induction of flowering (Balasubramanian et al., 2006). These findings could suggest that vernalization is the dominant process that must be realized to allow *Arabidopsis* to be receptive to temperature, likely to prevent precocious flowering in winter.

In cereals, the activities of *ODDSOC2* can also be linked to ambient-temperature regulated flowering. *ODDSOC2* has been shown to be responsive to ambient temperature in both wheat and barley, which both show earlier flowering phenotypes in response to increasing temperature (McMaster and Wilhelm, 2003; Ejaz and von Korff, 2017; Dixon et al., 2018). In wheat, however, it was shown that certain cultivars exhibited delayed flowering in response to increasing ambient temperature (Dixon et al., 2019). It was revealed that this trait arose from the incomplete vernalization of this cultivar, leading to the re-activation of floral repressors including VRN2 and TaOS2. The increase in *TaOS2* expression was linked to the *VRN1* allele found in this specific cultivar, which were unable to maintain repression of *TaOS2* after incomplete vernalization, explaining in part the delayed flowering phenotype (Dixon et al., 2019).

*HvOS2* was also shown to be responsive to ambient temperature in barley. *HvOS2* expression increases under high temperature conditions, particularly under short day photoperiods – conditions which result in the slowest development of the shoot apex (Hemming et al., 2012; Ejaz and von Korff, 2017). Like *TaOS2*

in wheat, the response of *HvOS2* is influenced by the *VRN1* allele present in the variety analyzed, with lines with the winter *Hvvrn1* allele having higher *HvOS2* expression levels in response to high ambient temperature (Ejaz and von Korff, 2017). *Hvvrn1* itself is downregulated under high temperature conditions, highlighting further the negative correlation between *VRN1* and *OS2* expression in cereals. Plants overexpressing *HvOS2* also exhibited delayed reproductive development under both cool and high temperatures while lines with a RNAi-mediated knockdown of *HvOS2* underwent more rapid reproductive growth at higher temperatures compared to wild type plants (Hemming et al., 2012). This is reflective of phenotypes obtained when *FLM* expression is modified in *Arabidopsis* (Posé et al., 2013).

Taken together, this evidence suggests that *ODDSOC2* functions in winter cereal varieties to repress the reproductive transition under warm temperatures until the vernalization requirement is completely saturated. It can be speculated that this is an adaptation to prevent precocious flowering during the winter should a brief period of warmth occur. Interestingly, this ecologically significant process is linked to *FLC* gene activity across both *Arabidopsis* and cereals; however, unlike FLM, the mode of action of *ODDSOC2* remains unknown. There is no evidence so far to suggest that *ODDSOC2* is alternatively spliced to influence this process. It is also unknown whether this increase in *ODDSOC2* expression occurs in varieties which can be induced to flower using warm temperatures and short-day conditions (Evans, 1987). Regardless, parallels can be drawn on the roles these *FLC* genes play in fine-tuning flowering time in a temperature-specific manner in both *Arabidopsis* and cereals.

A summary of the roles *FLC* genes play in vernalization, ambient temperature, and their relationship to *VRN1* are outlined in Figure 2 and Table 1.

**FLC Is Relevant Outside of Temperate Cereals**

Rice and maize diverged from the Pooidae roughly 64 million years ago and their flowering times are regulated in different ways to the temperate cereals (Wang et al., 2015). Reflective of the tropical regions in which they evolved, these species have no vernalization requirement and flowering is promoted by short day photoperiods. Nonetheless, both rice and maize contain *FLC* homologs which have been shown to regulate flowering time. *OsMADS51*, a homolog of *ODDSOC1*, acts as a flowering promoter under short days in rice (subsp. Japonica; Kim et al., 2007). Knockout of this protein correlates with the downregulation of *AP1* and *FT* homologs, which may explain the mode of action of this protein. In maize, *ZmMADS69* acts as a flowering promoter under both long and short days, and is thought to have been a target of selection to expand the cultivation zone of maize (Liang et al., 2019). Therefore, despite paucity of information on *FLC*-like genes in cereals, it is clear that flowering time regulation is a fundamental feature of these genes across the cereals.
Epigenetic Regulation of FLC-Like Genes in Cereals

The epigenetic regulation of FLC in *Arabidopsis* is well described and FLC can be considered a model gene for the study of epigenetics in general (Whittaker and Dean, 2017). FLC activity is regulated through the chromatin environment at the FLC locus and via RNA-mediated silencing mechanisms. Chromatin modification to promote FLC activity is regulated mainly through the actions of the FRIGIDA (FRI) complex (FRI-C). The FRI-C increases levels of active chromatin markers, such as H3K36me3 and H3K4me3, through the recruitment of chromatin modification proteins (Choi et al., 2011; Li et al., 2018). These markers are targeted during vernalization, where they are removed, and replaced with H3K9me3 and H3K27me3, resulting in a silenced chromatin state (Bastow et al., 2004; Finnegan and Dennis, 2007; Angel et al., 2011; Whittaker and Dean, 2017). The accumulation of chromatin silencing markers is mediated by the PHD-PRC2 complex (Plant Homeodomain-Polycomb Repression Complex 2; Wood et al., 2006; De Lucia et al., 2008). Silencing of FLC is additionally associated with the action of long non-coding RNAs (lncRNAs) and components of the autonomous pathway (Sheldon et al., 2000; Ietswaart et al., 2012; Whittaker and Dean, 2017). RNA binding proteins of the autonomous pathway function to process *COOLAIR*, a set of lncRNAs transcribed antisense of FLC (Sewiezewski et al., 2009; Hornyik et al., 2010; Whittaker and Dean, 2017). The significance of these lncRNAs in the regulation of vernalization remains controversial (Helliwell et al., 2011; Luo et al., 2019); however, much data have been gathered to indicate a functional if not essential role. The physical association of *COOLAIR* with FLC chromatin is associated with the reduction of H3K36me3 and H3K4me3, rendering the chromatin inactive (Csorba et al., 2014; Fang et al., 2020). *COOLAIR* is also induced by vernalization to assist with the inactivation of FLC (Sewiezewski et al., 2009; Kim and Sung, 2017). The silencing of FLC is associated with two other lncRNAs, *COLDWRAP* and *COLDAIR*, which recruit the PHD-PRC2 complex to specific chromatin regions (Heo and Sung, 2011; Kim and Sung, 2017).

Due to the relatively recent discovery of FLC-like genes in cereals, no research has been done to test whether FRI or the FRI-C functions in cereal plants. Studies have shown that homologs of the various FRI-C components can be detected in monocots (Choi et al., 2011) and rice FRI-like genes form distinct clades with *Arabidopsis* FRI-like genes (Michaels et al., 2004). According to the plant genome database EnsemblPlants, 25 and 13 proteins have been annotated as FRI-like for *Triticum aestivum* (cv. Chinese Spring) and * Hordeum vulgare* (cv. Morex), respectively. Homologs can also be identified for *Brachypodium distachyon* (9), *Oryza sativa subsp. japonica* (12), *Sorghum bicolor* (10), *Triticum dicoccoides* (16), *Triticum turgidum* (15), and *Zea mays* (13). It is possible that these uncharacterized proteins may act as scaffold proteins similar to FRI but for other pathways and functions.

In *Arabidopsis*, FLC is silenced during vernalization via a series of histone modifications by the PHD-PRC2 complex (De Lucia et al., 2008). The utilization of this complex in plants as a method to regulate vernalization-dependent flowering is conserved across *Arabidopsis* and cereals. The major regulator of vernalization in cereals, VRN1, acts as a promoter of flowering, rather than a repressor like FLC. Before vernalization, H3K27me3 repressive marks are deposited at the VRN1 locus in wheat and barley (Oliver et al., 2009; Diallo et al., 2012). During vernalization, H3K27me3 decreases while the active markers H3K4me3 and H3K36me3 increase (Oliver et al., 2009; Diallo et al., 2012). The same mode of epigenetic regulation of VRN1 is conserved in *Brachypodium*, and is regulated by ENHANCER OF ZESTE-LIKE 1 (EZLI), a homolog of CURLY LEAF (CLF), and a methyltransferase in the PRC2 complex in *Arabidopsis* (Lomax et al., 2018). VRN3/FT is also regulated in the same way in wheat and *Brachypodium* (Oliver et al., 2009; Huan et al., 2018).

Recruitment of the PRC2 to epigenetically regulate the vernalization response evolved in both *Arabidopsis* and cereals. However, the nature of the chromatin modifiers deposited at FLC and VRN1 is different due to their nature as a repressor and promoter, respectively. Therefore, this recruitment likely evolved after the independent evolution of the vernalization response pathway in monocots and dicots. Yet, some evidence exists to suggest that FLC-like genes are also regulated by
the PRC2 in a similar manner to FLC in Arabidopsis. To analyze the effect of vernalization on histone modifications at HvOS2 in barley, H3K27me3 marks were analyzed at the presumed transcriptional start site of HvOS2 in plants with or without 7 weeks of vernalization (Greenup et al., 2010). There was no significant difference in H3K27 trimethylation at this region indicating that perhaps repression of HvOS2 post-vernalization is regulated in a different way to FLC. The region tested by Greenup et al. begins ~100 bp upstream of the transcriptional start site (TSS). Although H3K27me3 deposits increase during vernalization at the TSS of FLC in Arabidopsis, the greatest increase is at the exon 1/intron 1 junction termed the “nucleation region” (Bastow et al., 2004; Finnegan and Dennis, 2007; Yuan et al., 2016). On return to warmth, H3K27me3 spreads from the nucleation region across the FLC locus. Future experiments targeting other regions within the HvOS2 locus could reveal more similarities in the epigenetic regulation of both FLC and HvOS2. Regulation via other markers such as H3K9me3 or H3 acetylation levels may provide insight into the regulation of HvOS2 to vernalization.

In contrast to barley, BdOS2 in Brachypodium showed high levels of H3K27me3 after vernalization in both spring and winter accessions (Sharma et al., 2017). H3K27me3 was enriched at the BdOS2 locus after vernalization for Bd21 and BdTR3C – spring and winter accessions, respectively, and the enrichment was maintained 1-week post-vernalization. For the winter allele of BdOS2 in BdTR3C, H3K27me3 can be found spanning the entire locus post-vernalization. The extensive methylation marks of H3K27 in the locus of BdTR3C compared to Bd21 may explain the mechanism as to how BdOS2 is stably repressed in the winter but not spring accession. It is possible that in strong winter varieties, FLC genes have evolved increasingly stringent or more complex methods of silencing to ensure flowering time is synchronized most optimally with the environment (Shindo et al., 2006; Hepworth et al., 2020). Analysis of winter varieties of FLC homologs in other cereal crops may reveal that the epigenetic regulation of FLC-like genes is more conserved than currently realized.

Brachypodium has also been shown to encode IncRNAs, similar to COOLAIR, which target FLC-like genes for downregulation during vernalization (Jiao et al., 2019). Two high confidence IncRNAs were detected for BdOS2, while one IncRNA could be detected for BdOS1. These IncRNAs were termed BdCOOLAIR1 and BdCOOLAIR2 for BdOS1 and BdOS2, respectively, as although they are not homologous to the AtCOOLAIR sequence, their position relative to their sense counterparts is similar. Expression of these IncRNAs is induced by vernalization, and their induction is significantly higher in a winter accession compared to a facultative accession, while their expression is absent in a spring accession. Knockdown of BdCOOLAIR2 via RNAi also affects the rate of silencing of BdOS2 in BdTR3C, though it is not essential for the complete silencing of BdOS2 (Jiao et al., 2019). This information suggests that IncRNAs complement the mechanisms which silence FLC-like genes in grasses, in a similar fashion to FLC regulation in Arabidopsis, although this mode of regulation is accession dependent. In addition, IncRNAs have been annotated for FLC

| Species                  | Gene          | Observation                                                                 | Citation                                         |
|--------------------------|---------------|-----------------------------------------------------------------------------|-------------------------------------------------|
| *Brachypodium distachyon*| BdOS1         | Upregulated by vernalization                                                | Ruelens et al. (2013)                           |
|                          | BdOS2         | Downregulated by vernalization                                              | Ruelens et al. (2013); Sharma et al. (2017)     |
|                          |               | Negatively regulated by VRN1                                                | Woods et al. (2016)                             |
|                          | BdMADS37      | Downregulated by vernalization                                              | Ruelens et al. (2013)                           |
| *Hordeum vulgare*        | HvOS1         | Induced by ABA and JA                                                       | Kapazoglou et al. (2012)                        |
|                          |               | Role in seed development                                                    |                                                 |
|                          |               | Upregulated by vernalization                                                |                                                 |
|                          |               | Downregulated by high temperature                                           |                                                 |
|                          | HvOS2         | Induced by JA                                                               |                                                 |
|                          |               | Role in seed development                                                    |                                                 |
|                          |               | Downregulated by vernalization                                              |                                                 |
|                          |               | Negatively regulated by VRN1                                                |                                                 |
|                          |               | Regulates cell elongation                                                    |                                                 |
|                          |               | VRN1 binds to its promoter                                                  | Deng et al. (2015)                              |
|                          |               | Possible negative regulator of early reproductive development               | Hemming et al. (2012); Monteagudo et al. (2019) |
| *Oryza sativa*           | OsMADS51      | Short-day flowering promoter                                                |                                                 |
| *Triticum aestivum*      | TaAGL33/TaOS2 | Down-regulated by vernalization                                             |                                                 |
|                          |               | Knockout of D-homeolog causes earlier flowering                            |                                                 |
|                          | TaAGL42/TaMX23/TaOS1 | Upregulated under high ambient temperature                              |                                                 |
|                          |               | Gradual increase in expression throughout development                       |                                                 |
| *Triticum monococcum*    | TmODDSOC2     | Negatively regulated by VRN1                                                | Greenup et al. (2010)                           |
| *Zea mays*               | ZmMADS69      | Flowering promoter                                                          | Liang et al. (2019)                             |
genes in 6 other grass species, including wheat, although these still need to be experimentally verified.

DIVERSIFICATION OF FLC FUNCTION IN CROPS

Although thoroughly studied for its involvement in vernalization-dependent regulation of flowering time, FLC function is implicated in many other aspects of plant growth and development. Analysis of expression levels of FLC-like genes in cereals during development and under various experimental treatments also suggests that homologs of FLC play diverse roles in cereal physiology. For example, in the early gene expression experiments of Zhao et al. (2006), it was shown that at least one of the genes analyzed is expressed at, at least, one of the various life stages and in at least one of the various tissue types throughout wheat development. Expression can be detected from initial embryo imbibition to seed development post-anthesis while other genes are predominantly expressed in roots.

Curiously, in the dataset of both Zhao et al. (2006) and Schilling et al. (2020), TaAGL41 could not be detected at significant levels but could be detected by Sharma et al. (2017) for several cultivars. Further analysis using the Wheat Expression Browser (Ramirez-González et al., 2018) indicates that TaAGL41 is indeed expressed throughout development but the extent of its expression is cultivar-specific. This could suggest a role for this gene in fine tuning development in a cultivar-specific manner. The Wheat Expression Browser highlights that TaAGL41 is downregulated by cold in the spring cultivar Manitou (Li et al., 2015), yet the main stimulus which affected TaAGL41 across the dataset was infection by the wheat yellow rust pathogen Puccinia striiformis f. sp. Tritici (Dobon et al., 2016). Expression of two other high confidence FLC homologs in wheat, TaFLC.4A1 and TaFLC.4A2, could not be detected at significant levels in the developmental time course analyzed by Schilling et al. (2020), and their expression does not change considerably across the different varieties available on the Wheat Expression Browser. Rather, these genes appear to be mainly influenced by drought stress (Liu et al., 2015; Ramirez-González et al., 2018). The relationship between flowering time and stress adaptation is complex and the molecular mechanisms determining this relationship are still not fully understood; however, a link between flowering time regulators and stress is found in plants (reviewed in Kazan and Lyons, 2016). It is possible that FLC-like genes not only play roles regulating development but also that their function has diversified to fine tune other developmental and growth processes. This reflects findings in the Brassicaceae that although the core function of FLC across species is the regulation of flowering time, different members of the FLC clade have been recruited for species-specific roles typically within stress response pathways (Mateos et al., 2017). Further investigation into these expression patterns as well as generation of knockout mutants may reveal a novel role for these genes in stress response pathways in cereals.

Additionally, HvOS2 has been shown to negatively influence cell length and, therefore, leaf, internode, and spike length (Greenup et al., 2010). The data suggest that HvOS2 downregulation by vernalization allows the process of stem elongation and bolting as secondary regulation of the reproductive process.

As well as being expressed during seed development stages in wheat, FLC-like genes have been implicated in seed development in barley. HvOS1 and HvOS2 are differentially expressed in cultivars of different seed size and at different stages of seed development (Kapazoglou et al., 2012). Analysis of their expression patterns revealed that HvOS1 expression is induced more substantially in early seed development in cultivars with large seeds, while HvOS2 levels are significantly higher in later developmental stages in cultivars with small seeds. This pattern could suggest an association between the expression of FLC genes and seed size in barley and that each gene is important for different stages of development – either endosperm cellularization or seed maturation. Additionally, both genes contained the endosperm-specific element GCN4 in their promoters, along with elements for responses to abscisic acid, an important phytohormone for seed maturation as well as abiotic stress (Takaiwa et al., 1996; Finkelstein et al., 2002; Kapazoglou et al., 2012). Taken together, these data implicate a role of FLC homologs in seed development and suggest that perhaps there is an association between them and seed size. An association study including more cultivars with a variety of seed sizes could be undertaken to fully determine whether FLC-like genes regulate this important agronomic trait.

Outside of the temperate cereals, ZmAGL19, an FLC homolog in maize, is targeted by OPAQUE11, a central regulator of endosperm development and nutrient metabolism (Feng et al., 2018). OPAQUE11 is specifically expressed in the endosperm and positively regulates ZmAGL19 expression, suggesting that ZmAGL19 might be part of the seed development regulation process in maize.

FUTURE DIRECTIONS

Much has been learned about the roles FLC genes play in cereals, mostly indirectly through the study of flowering time in these species. The scientific community is now able to study FLC genes further due to the dramatically improved genetic resources available. Reference genome assemblies are now available for several hexaploid wheat cultivars, as well as tetraploid wheat, diploid progenitor species, and 2- and 6-row barley (Ling et al., 2013; Fox et al., 2014; Luo et al., 2017; Mascher et al., 2017; Appels et al., 2018; Maccalferri et al., 2019). Genes are also annotated to include SNP variations which can be easily identified using the online platform EnsemblPlants. Identifying homologs and SNP-variants across cultivars has never been easier for researchers without bioinformatics training. Access to tools such as these will increase the pace at which genes are identified and studied, increasing the potential to finally characterize the once enigmatic FLC gene family.

Additionally, populations of mutant plants have been created for widespread use in both hexaploid and tetraploid wheat and barley (Krasileva et al., 2017; Schreiber et al., 2019). TILLING lines containing homeolog-specific mutations in genes of interest
can be ordered and crossed, creating specific combinations to study gene function and redundancy. In a more targeted approach, protocols for wheat transformation and mutation via virus-induced gene silencing and CRISPR/Cas9 are available (see wheat-training.com for resources). In combination with speed breeding, it is possible to fully characterize the effect of mutations in both model and crop plants in considerably less time (Watson et al., 2018). At this moment in time, comparable resources to the model plant Arabidopsis from which most of our information on FLC genes comes from are available. This review also highlights how relevant Brachypodium is as a model for basic and translational research for temperate cereals and that research using this small grass will continue to be a valuable option to study FLC genes. It is possible within the next few years that we will see a greater increase in FLC-related knowledge outside of Arabidopsis. The availability of these resources provides hope that much more knowledge can be gained on FLC function in cereals in years to come.

**AUTHOR CONTRIBUTIONS**

AK prepared the outline and wrote the manuscript. KG contributed to discussions and critical revision of the manuscript.

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All authors contributed to the article and approved the submitted version.

**FUNDING**

AK and The Geuten Lab are supported by KU Leuven grant C24/17/037 and FWO grant G065713N.

**ACKNOWLEDGMENTS**

We thank Emma Doyle (University College Dublin), Sam Balzarini (KU Leuven), Philip Ruelens (WUR), and Daniel Woods (UC Davis) for their helpful comments, suggestions, and insights. We also thank three reviewers of this article for their valuable comments.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.617340/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.