Exogenous Gibberellins Induce Wheat Spike Development under Short Days Only in the Presence of *VERNALIZATION1*[1][C][W][OPEN]

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The activation of the meristem identity gene *VERNALIZATION1* (*VRN1*) is a critical regulatory point in wheat (*Triticum* spp.) flowering. In photoperiod-sensitive wheat varieties, *VRN1* is expressed only under long days (LDs), but mutants carrying deletions in a regulatory element in its promoter show *VRN1* transcription and early spike development under short days (SDs). However, complete spike development is delayed until plants are transferred to LDs, indicating the existence of an additional regulatory mechanism dependent on LDs. We show here that exogenous gibberellin (GA) application accelerates spike development under SDs, but only in wheat lines expressing *VRN1*. The simultaneous presence of GA and *VRN1* results in the up-regulation of the floral meristem identity genes *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1-1* and *LEAFY*, whereas inhibition of GA biosynthesis with paclobutrazol precludes the LD induction of these two genes. The inductive role of GA on wheat flowering is further supported by the up-regulation of GA biosynthetic genes in the apices of plants transferred from SDs to LDs and in photoperiod-insensitive and transgenic wheat plants with increased *FLOWERING LOCUS T* transcription under SDs. The up-regulation of GA biosynthetic genes was not observed in the leaves of the same genetic stocks. Based on these observations, we propose a model in which FLOWERING LOCUS T is up-regulated in the leaves under LDs and is then transported to the shoot apical meristem, where it simultaneously induces the expression of *VRN1* and GA biosynthetic genes, which are both required for the up-regulation of the early floral meristem genes *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1-1* and *LEAFY* and the timely development of the wheat spike.

During plant development, the timing of the transition from vegetative to reproductive growth is critical for reproductive success and, in cultivated cereals, to maximize seed production. Upon undergoing this transition, annual plants are committed to flower and therefore have evolved a series of mechanisms by which flowering time can be precisely coordinated with environmental cues. Temperate grasses, which include barley (*Hordeum.* spp.) and wheat (*Triticum* spp.), evolved in regions with marked seasonal changes and developed mechanisms to initiate and undergo the reproductive phase under favorable conditions. The ancestral forms in this group of species have a winter growth habit (accelerated flowering after vernalization) and are photoperiod sensitive (accelerated flowering by long days [LDs]).

Vernalization is the requirement for an extended period of low temperature to accelerate flowering. This helps to ensure that varieties with a winter growth habit will not flower until after winter, protecting the delicate floral meristems from frost damage. This is in contrast to spring-sown varieties, which carry mutations in key vernalization genes that abolish this requirement. In temperate cereals, such as wheat and barley, the main genes regulating the vernalization response are *VERNALIZATION1* (*VRN1*; Danyluk et al., 2003; Trevaskis et al., 2003; Yan et al., 2003), *VRN2* (Yan et al., 2004; Distelfeld et al., 2009b), and *VRN3* (Yan et al., 2006; Nitcher et al., 2013). These genes are interconnected by complex epistatic interactions whereby an allele for spring growth habit in any one of these genes is epistatic to the alleles for winter growth habit in the other two genes (Takahashi and Yasuda, 1971; Trevaskis et al., 2007; Distelfeld et al., 2009a). The MADS-box gene *VRN1*, a homolog of the Arabidopsis (*Arabidopsis thaliana*) meristem identity gene *APETALA1* (*AP1*), is central to the vernalization response (Danyluk et al., 2003; Trevaskis

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et al., 2003; Yan et al., 2003). The induction of *VRN1* transcription by vernalization coincides with cold-induced changes in histone methylation and acetylation patterns in its regulatory regions (Oliver et al., 2009). The up-regulation of *VRN1* in the leaves is important to maintain low transcript levels of the flowering repressor *VRN2* in the spring (Loukoianov et al., 2005; Chen and Dubcovsky, 2012), which facilitates the up-regulation of *VRN3* and the initiation of the transition to the reproductive phase (Distelfeld et al., 2009a). The *VRN3* gene in wheat and barley and the *Heading date 3a* (*Hd3a*) gene in rice (*Oryza sativa*) have been shown to encode proteins structurally and functionally similar to Arabidopsis FLOWERING LOCUS T (Yan et al., 2006; Tamaki et al., 2007) and therefore will hereafter be referred to as FT.

The second major environmental signal to modulate flowering time is the variation in daylength during the growing season (photoperiod). It has been demonstrated in Arabidopsis and rice that exposure to an inductive photoperiod results in the rapid up-regulation of *FT* in the leaves (Kobayashi et al., 1999; Kojima et al., 2002). This gene encodes a small mobile protein that is part of the florigen signal that travels via the phloem to the shoot apical meristem (Corbesier et al., 2007; Tamaki et al., 2007). In Arabidopsis, upon arriving at the shoot apical meristem, FT interacts with the bZIP transcription factor FLOWERING LOCUS D (FD) and binds the promoter of *AP1*, activating its expression and inducing the transition from the vegetative to reproductive growth phase (Abe et al., 2005; Wigge et al., 2005). A recent study in rice demonstrated that the interaction between FT-FD and the *OsMADS15* promoter, a homolog of *AP1*, requires the formation of a floral activation complex comprising FT, FD, and 14-3-3 linker proteins (Taoka et al., 2011). The wheat *FT* gene is induced under LDs (Yan et al., 2006) and encodes a protein that interacts with FD-LIKE2 (FDL2), which has the ability to interact with the wheat *VRN1* promoter (Li and Dubcovsky, 2008). These results suggest that these components of the photoperiod pathway are largely conserved between monocots and dicots.

In the temperate cereals, the *PHOTOPERIOD1* (*PPD1*) gene plays a major role in the activation of *FT* under LDs, ensuring that it is induced only under inductive photoperiods (Turner et al., 2005). However, deletions in the *PPD1* promoter or differences in *PPD1* copy number result in photoperiod-insensitive plants, which exhibit *FT* expression and flowering in both short days (SDs) and LDs (Beales et al., 2007; Wilhelm et al., 2009; Díaz et al., 2012). In photoperiod-sensitive wheat varieties, *FT* transcription in the leaves is prevented by VRN2 in the fall but not in the spring, when *VRN2* transcripts are maintained at low levels by the up-regulation of *VRN1* during the winter (Chen and Dubcovsky, 2012).

An interesting *VRN1* allele was identified in *Triticum monococcum*, a photoperiod-sensitive diploid wheat with a genome closely related to the A-genome of tetraploid and hexaploid wheat. This allele carries a natural deletion in a MADS-box binding site (CArG box) within the *VRN1* promoter (henceforth, *Vrn1g* allele, GenBank DQ146422) and is associated with the up-regulation of *VRN1* transcription and early spike development under SDs (Dubcovsky et al., 2006). By contrast, other photoperiod-sensitive wheats do not show *VRN1* expression in the leaves or apices under SDs, regardless of their growth habit (Dubcovsky et al., 2006). The spikes in plants carrying the *Vrn1g* allele develop beyond the double ridge stage and form glume primordia, but complete spike development and subsequent flowering is restricted while plants are maintained under SD conditions. However, when these plants are transferred to LDs, rapid and full spike development is triggered (Dubcovsky et al., 2006). This observation demonstrates that the induction of *VRN1* alone is not sufficient for normal spike development and that exposure to an inductive LD photoperiod is required to induce additional genes involved in this developmental process.

Because of their role in flowering induction in other species, we hypothesized that GAs may be involved in this additional LD-dependent regulatory process during wheat spike development. GAs are a family of tetracyclic diterpenoid carboxylic acids, which include several bioactive forms that regulate multiple aspects of a plant's development, including the initiation and promotion of flowering (Mutasa-Göttgens and Hedden, 2009). It has long been known that GA is able to induce bolting and flowering (Lang, 1957), a function that appears to be conserved in the grasses because exogenous GA application is sufficient to accelerate flowering development in vernalized *Lolium perenne* plants (Macmillan et al., 2005). In wheat, GA has previously been shown to accelerate flowering in spring varieties grown under LDs (Evans et al., 1995) and in winter varieties that have undergone vernalization (Razumov et al., 1960).

The rate of GA biosynthesis is modulated by the activity of a series of enzymes, which result in the conversion between different forms of GA, of which only a subset exhibit bioactive function (e.g. GA_1, GA_3, and GA_4; Yamaguchi, 2008). Three families of 2-oxoglutarate-dependent dioxygenases (2-ODD genes) catalyze the latter stages of the GA biosynthetic pathway and are critical in regulating the overall rate of GA biosynthesis. The GA 20-oxidase (GA20ox) family consists of at least four members in wheat and catalyzes three successive steps late in the GA biosynthetic pathway (Fig. 1). GA 3-oxidases (GA3ox genes) exist as a smaller family, members of which catalyze the conversion of several precursors synthesized by GA20ox to bioactive forms of GA. A third 2-ODD gene family, the GA 2-oxidases (GA2ox genes), catalyze the conversion of both bioactive and precursor GAs to inactive forms and thus act to reduce bioactive GA levels (Fig. 1). The rate of GA biosynthesis is controlled by feedback regulatory mechanisms among the genes encoding these biosynthetic enzymes. There is some evidence suggesting that plants respond to environmental cues by alterations in the expression of GA biosynthetic genes, particularly the *GA20ox* genes, which are thought to catalyze the rate-limiting steps in this pathway (Webb et al., 1998). In both *L. perenne* and *Lolium temulentum*, exposure to an

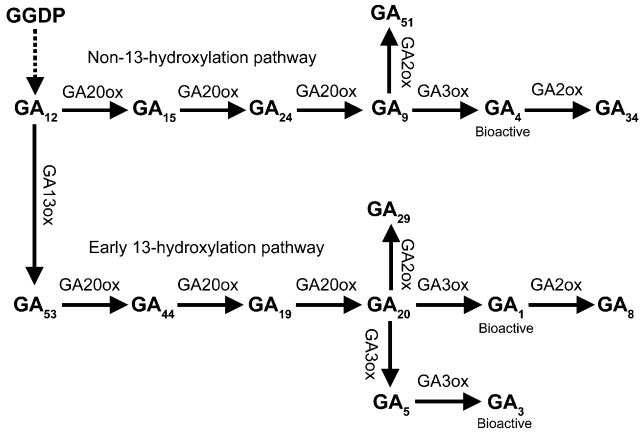

Figure 1. Simplified GA biosynthetic pathway including the final stages of the 13-hydroxy and non-13-hydroxy pathways. Adapted from Yamaguchi (2008).

inductive LD photoperiod results in the up-regulation of *GA20ox1* and an increase in bioactive GA concentrations in the leaves (Macmillan et al., 2005; King et al., 2006).

In Arabidopsis, GA influences the transition from vegetative to reproductive growth phases by regulating the expression of two transcription factors, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) and *LEAFY* (*LFY*). GA induces the expression of *SOC1*, a MADS-box gene that integrates signals from the GA (Moon et al., 2003), photoperiod (Hepworth et al., 2002), and vernalization pathways (Searle et al., 2006). The homologous gene in maize (*Zea mays*), *ZmMADS1*, exhibits a similar expression profile, suggesting that this function has been retained in the monocots (Heuer et al., 2001). In wheat, there are three genes in the *SOC1*-like clade (*SOC1-1*, *SOC1-2*, and *SOC1-3*), although their specific developmental roles have yet to be determined (Zhao et al., 2006; Paolacci et al., 2007). In Arabidopsis, SOC1 activates *LFY* expression through interactions with AGAMOUS-LIKE24 (AGL24; Lee et al., 2008), but GA can also induce *LFY* expression independently of *SOC1* (Achard et al., 2004). *LFY* is a floral meristem identity gene widely conserved among both monocots and dicots (Bomblies et al., 2003) whose transgenic overexpression results in the conversion of shoot apical meristems to flower meristems and accelerated flowering in Arabidopsis (Weigel and Nilsson, 1995). A single wheat homolog of *LFY* has been identified and shown to be expressed in the developing flowers during the early stages of spike development (Shitsukawa et al., 2006).

The roles and interactions between *SOC1*, *LFY*, and the GA pathway in the temperate grasses are poorly understood. Studies in wheat are further complicated by the presence of different levels of polyploidy, which include diploid einkorn wheat (*T. monococcum*, $2n = 14$, genomes A^mA^m), allotetraploid pasta wheat (*Triticum turgidum*, $2n = 28$, genomes AABB), and allohexaploid common wheat (*Triticum aestivum*, $2n = 42$, genomes AABBDD), which will be referred to generically as wheat (for review, see Dubcovsky and Dvorak, 2007). Furthermore, previous attempts to dissect the GA and photoperiod pathways in the temperate cereals have been complicated by the simultaneous induction of GA biosynthesis and *FT* expression by LDs. In this study, we take advantage of a set of *VRN1* promoter mutants in diploid wheat that express *VRN1* under SDs, photoperiod-sensitive and -insensitive sister lines in tetraploid wheat, and transgenic hexaploid wheat lines overexpressing *FT* to decouple the effects of LDs, FT, and exogenous GA applications. Using these genetic tools, we show that both *VRN1* and GA are required for the up-regulation of *LFY* and *SOC1-1* and the acceleration of spike development. We also show that the induction of *FT* both under LDs and SDs is associated with the up-regulation of GA biosynthetic genes in the apices, but not in the leaves. Based on these results, we present a model that integrates the role of GA into the vernalization and photoperiod pathways of wheat flowering regulation.

RESULTS

Exogenous GA Application Accelerates Flowering Development in Wheat Only in the Presence of *VRN1*

Plants from three *T. monococcum* accessions carrying the wild-type *vrn1* allele (not expressed under SD, winter growth habit) remained in the vegetative stage when grown under SDs (Fig. 2A). In agreement with previous results (Dubcovsky et al., 2006), the apices of the other three *T. monococcum* accessions carrying the *Vrn1g* allele (spring growth habit) progressed to the double ridge stage, but their spike development was restricted while maintained under SDs (Fig. 2B).

We applied 7×10^{-5} mol GA_3 in 95% (v/v) ethanol to the base of the most recently expanded leaf of 12 plants in each genotype, while treating 12 control plants with 10 μL 95% (v/v) ethanol. GA treatment had a limited effect on apical development in the three *T. monococcum* accessions carrying the wild-type *vrn1* allele. After eight applications of GA over 2 weeks (Fig. 2A), the apices of these plants were at the same developmental stage as the control lines treated with 95% (v/v) ethanol. By contrast, the apices of plants carrying the *Vrn1g* allele showed a clear response to the exogenous GA and progressed to the terminal spikelet stage and the formation of glume and lemma primordia (Fig. 2B). Apical development in tillers that did not receive the exogenous GA was unaffected by the addition of GA to a neighboring tiller in the same plant.

To confirm that the differences in the responses to the GA application were linked to the different *VRN1* alleles, we selected 16 plants homozygous for the *vrn1* allele and 14 homozygous for the *Vrn1g* allele from the *T. monococcum* F_2 population described in "Materials and Methods" and grew them under the same SD conditions. Application of exogenous GA to one-half

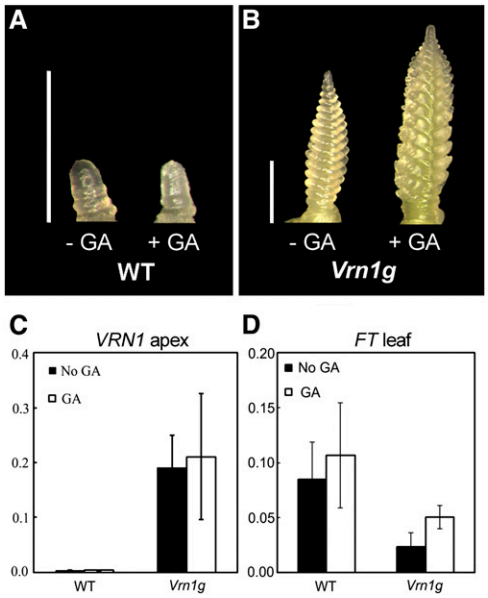

Figure 2. Effect of exogenous GA application in the presence and absence of *VRN1*. *T. monococcum* lines carrying the wild type (WT) *vrn1* allele for winter growth habit (DV92, PI 306540, PI 428175; A) and the *Vrn1g* allele for spring growth habit (PI 349049, PI 326317, PI 418582; B) grown under SDs for 8 weeks. In GA-treated plants (+GA), exogenous GA_3 was applied to the base of the most recently fully expanded leaf on the main stem, while control plants (–GA) were treated with 10 μL of 95% (v/v) ethanol. Twelve plants of each genotype, four from each accession, were treated with GA, and 12 were used as controls. Bar = 1 mm. C, Transcript levels of *VRN1* in the apices. D, Transcript levels of *FT* in the leaves. The *y* axis scale is expression in fold-*ACTIN* levels (number of molecules of target gene/number of molecules of *ACTIN*). Error bars indicate SE of the means of three different accessions. [See online article for color version of this figure.]

of the plants from each genotype confirmed the results from the initial six accessions. All plants with the *vrn1* allele remained in the vegetative stage irrespective of GA application, whereas all plants carrying the *Vrn1g* allele advanced to the double ridge stage in the absence of exogenous GA and to a more advanced spike developmental stage with the addition of exogenous GA (Supplemental Fig. S1). This result confirmed that the differences in apical development in response to GA are genetically linked to the *VRN1* gene, as demonstrated before for the response to SDs (Dubcovsky et al., 2006).

The requirement of both GA and *VRN1* in spike development was further validated using the *maintained vegetative phase* (*mvp*) mutants, *T. monococcum* plants that carry a deletion of the entire *VRN1* gene (Shitsukawa et al., 2007). Consistent with previous results, *mvp* mutants did not respond to GA application and their apices remained in the vegetative stage even after 2 weeks of exogenous GA application (Supplemental Fig. S2). Taken together, these results demonstrate that the acceleration of apical development in response to GA application is both genetically linked to the *Vrn1g* allele and that *VRN1* expression is a prerequisite for this response.

We then tested the effects of GA application on the expression levels of *VRN1* and *FT* in the apices of plants carrying a wild-type or *Vrn1g* allele. This analysis confirmed that, under SDs, *VRN1* expression in the apex is significantly higher in genotypes with a *Vrn1g* allele but also revealed that GA application had no significant effect on the transcript levels of this gene (Fig. 2C). *FT* transcripts were undetectable in the apices of either genotype and, in the leaves, were present at very low levels that are insufficient to induce flowering (<0.1-fold *ACTIN*; Fig. 2D). This is an expected result in photoperiod-sensitive plants grown under SD conditions, because *FT* induction is known to require exposure to LDs, under which conditions *FT* can reach greater than 10-fold *ACTIN* levels (Chen and Dubcovsky, 2012). Similarly, GA treatment had no significant effect on *FT* transcript levels in either genotype. These results suggest that GA acts to accelerate apical development through pathways that are downstream of these flowering-promoting genes.

Despite the initial acceleration in spike development to the terminal spikelet stage and the initiation of stem elongation, the continual application of GA to SD-grown plants did not result in normal flower development. The resulting spikes had an unusual morphology and a reduced number of spikelets that had elongated glumes compared with those from normal spikes (Supplemental Fig. S3).

Transcript Level Changes of GA Biosynthetic Genes in Wheat Apices upon Exposure to LDs

To test the role of endogenous GA biosynthesis in spike development, we compared the apical expression levels of four members of the *GA20ox* biosynthetic gene family as well as the GA biosynthesis gene *GA3ox2* and the GA catabolic gene *GA2ox1* (Fig. 1) in *T. monococcum* *Vrn1g* plants maintained under SDs with those transferred to LDs. Plants were grown under SDs for 6 weeks and then one-half of the plants were transferred to LDs for 2 weeks and one-half were maintained under SDs. Plants moved to LDs exhibited significantly higher *FT* expression than those maintained under SDs ($P < 0.05$; Fig. 3A). Transcript levels of *GA20ox1*, *GA20ox2*, and *GA20ox4* were all significantly up-regulated ($P < 0.05$) in the apices of plants transferred to LDs compared with those kept under SDs (Fig. 3B). No significant difference in expression was found for *GA20ox3* (Fig. 3B) or for *GA3ox2* (Fig. 3C). In addition, a significant decrease ($P < 0.05$) in *GA2ox1* transcript levels was observed in plants transferred to LDs (Fig. 3D).

Overexpression of *FT* under SDs Is Associated with the Up-Regulation of GA Biosynthetic Genes in Wheat Apices

To test if the observed changes in GA biosynthetic gene expression profiles in the apex were dependent on the LD up-regulation of *FT*, we compared two sets of isogenic pairs of lines that differ in *FT* expression under SDs. The first pair is the hexaploid *T. aestivum*

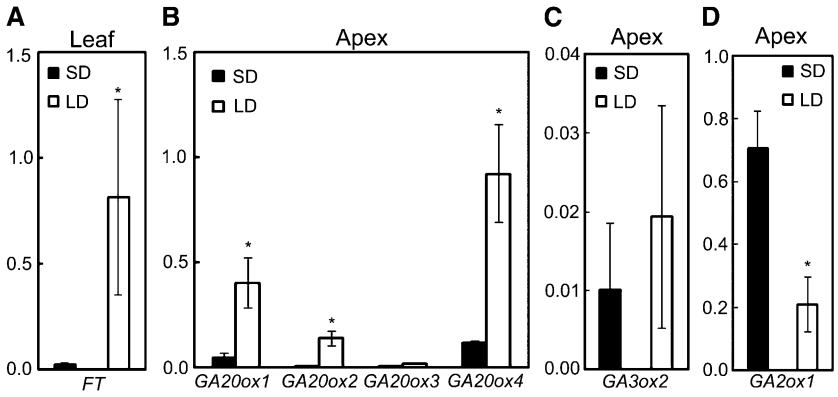

Figure 3. Effect of photoperiod on the transcript levels of *FT* and GA biosynthetic genes in *T. monococcum* accessions carrying the *Vrn1g* allele (PI 349049, PI 326317, PI 418582). A, Transcript levels of *FT* in the leaves. Transcript levels of *GA20ox1*, *GA20ox2*, *GA20ox3*, and *GA20ox4* (B), *GA3ox2* (C), and *GA2ox1* (D) in the shoot apical meristem. SD indicates 8 weeks under SDs. LD indicates 6 weeks under SDs followed by two weeks under LDs. The *y* axis scale is expression in fold-*ACTIN* levels (number of molecules of target gene/number of molecules of *ACTIN*). Error bars indicate SE of the means based on three different accessions. $*P < 0.05$ (differences between SDs and LDs).

variety Jagger (winter growth habit, photoperiod sensitive) and the corresponding transgenic *FT* overexpressing line, hereafter referred to as 'Jagger' *FT-OE* (Yan et al., 2006). These lines were developed through transformation with a highly expressed *FT* allele (see "Materials and Methods"). The second pair is the tetraploid *T. turgidum* variety Kronos (spring growth habit, *Ppd-A1a* photoperiod-insensitive allele) and its backcross sister line carrying the corresponding wild-type photoperiod-sensitive allele (Wilhelm et al., 2009).

'Jagger' FT Overexpressing Transgenic Lines

We previously showed that the 'Jagger' *FT-OE* transgenic lines exhibit greatly accelerated flowering under LDs (Yan et al., 2006). We show in this study that even under SDs, these plants exhibit a significant up-regulation of *FT* in the leaves ($P < 0.05$; Fig. 4A) and flowered earlier than the nontransgenic control plants. On average, in 'Jagger' *FT-OE* plants, spikes emerged in 83.4 ± 1.5 d and set seeds under SDs, whereas the nontransgenic control plants remained in the vegetative stage. Eight-week-old 'Jagger' *FT-OE* plants already showed advanced apical development compared with wild-type 'Jagger' plants (Supplemental Fig. S4A). Expression analysis by quantitative reverse transcription (qRT)-PCR showed that the 'Jagger' *FT-OE* shoot apical meristems exhibited significantly higher transcript levels of *VRN1* ($P < 0.01$; Fig. 4B) and of the GA biosynthetic genes *GA20ox2* and *GA20ox4* ($P < 0.05$) than the wild-type 'Jagger' plants (Fig. 4C). *GA20ox1* and *GA20ox3* showed the same trend but the differences were not significant (Fig. 4C). Expression of the GA catabolic gene *GA2ox1* was significantly lower in *FT-OE* plants ($P < 0.05$; Fig. 4E), while *GA3ox2* expression was unchanged (Fig. 4D).

Photoperiod-Insensitive Tetraploid Wheat Lines

Using RNA extracted from leaves harvested every 4 h over a 24-h SD period, we found that in the photoperiod-insensitive 'Kronos,' *FT* transcript levels are highly up-regulated in the leaves during daylight hours (8 AM to 4 PM), peaking at the middle of the day (12 PM, 6-fold *ACTIN*) before falling to lower levels later in the day and in the dark (Fig. 4F). In the SD-grown photoperiod-sensitive sister lines, *FT* transcript levels were undetectable at all stages of the day (Fig. 4F). After 8 weeks growth under SDs, apices of the photoperiod-insensitive plants had already begun to develop lemma and glume primordia, whereas photoperiod-sensitive plants remained in the vegetative stage (Supplemental Fig. S4B). The apical transcript levels of *VRN1* ($P < 0.05$; Fig. 4G) and the GA biosynthetic genes *GA20ox1* and *GA20ox4* ($P < 0.05$; Fig. 4H) were significantly higher in the photoperiod-insensitive than in the photoperiod-sensitive plants, correlating again with advanced apical development. *GA20ox2* and *GA20ox3* showed the same trend, but the differences were not significant (Fig. 4H). Consistent with results comparing 'Jagger' and *FT-OE* lines, *GA2ox1* expression was significantly lower in photoperiod-insensitive plants ($P < 0.05$; Fig. 4J), while *GA3ox2* expression was not significantly different (Fig. 4I).

Taken together, these results indicate that the up-regulation of *FT* expression under SDs, either through transgenic overexpression or photoperiod insensitivity, is sufficient for the up-regulation of the biosynthetic *GA20ox* genes and the down-regulation of the catabolic *GA2ox1* gene in the apex and, in the presence of *VRN1*, for normal spike development and seed production.

Wheat *LFY* and *SOC1-1* Are Induced in Developing Apices by LDs, Exogenous GA Application, and *FT* Overexpression

We then compared apical transcript levels of the wheat homologs of *SOC1* and *LFY*, genes which, in Arabidopsis and other grass species, play important roles in the promotion of flowering by integrating the

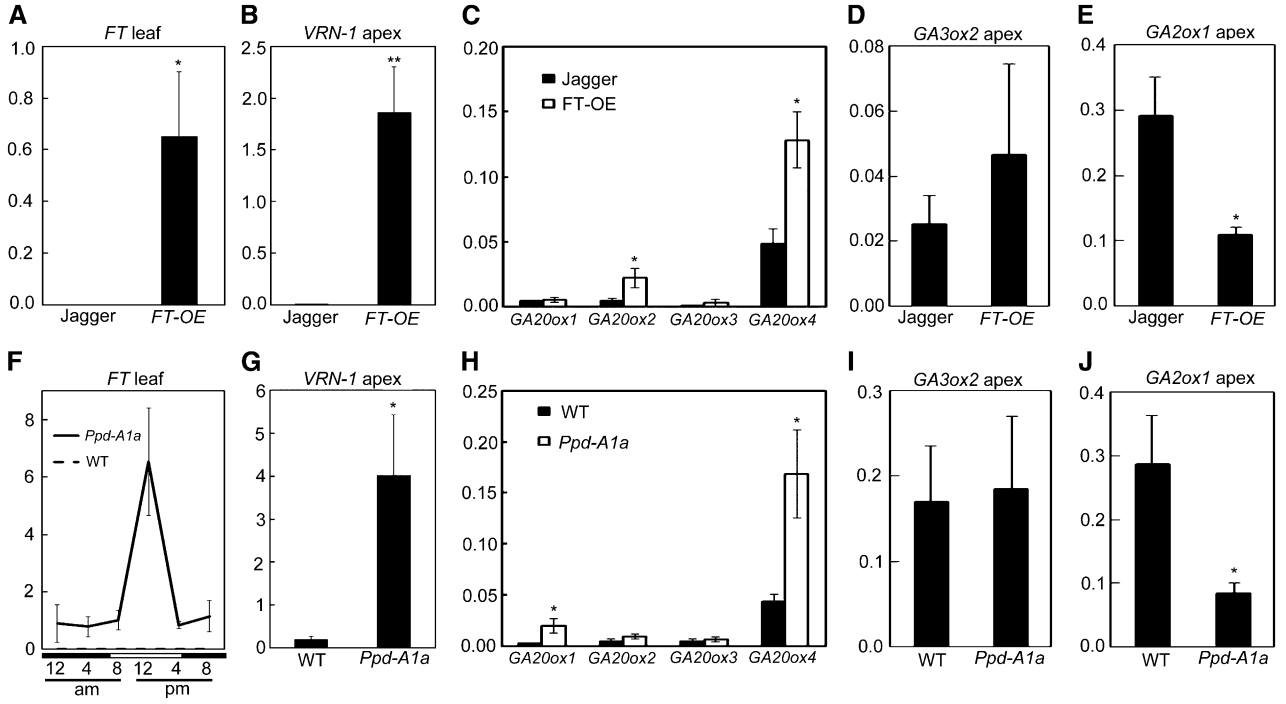

Figure 4. Effect of *FT* overexpression and misexpression under SDs on GA biosynthetic gene transcript levels. Relative expression levels of *FT* in the leaves (A and F) and *VRN1* (B and G), *GA20ox1-4* (C and H), *GA3ox2* (D and I), and *GA2ox1* (E and J) in the apices of 8-week-old 'Jagger' wild-type and transgenic 'Jagger' *FT-OE* plants (A–E) and in photoperiod-sensitive (WT) and -insensitive (*Ppd-A1a*) plants (F–J). Both sets of plants were grown under SDs. The *y* axis scale is expression in fold-*ACTIN* levels. Error bars indicate SE of the means based on six (A and F), five (B–E), and four (G–J) biological replicates respectively. The bar below F indicates the duration of the dark (black) and light (white) periods. $*P < 0.05$, $**P < 0.01$.

GA and photoperiod pathways. The expression levels of these genes were first compared in the apices of SD- and LD-grown wild-type and *Vrn1g* plants. One-half of the SD plants were treated with GA_3 for 2 weeks, while one-half of the LD plants were treated with the GA biosynthesis inhibitor paclobutrazol (PAC) before exposure to LDs. The four resulting treatments are designated SD, SD + GA, LD, and LD + PAC. We found that the expression of *SOC1-1* ($P < 0.05$), *SOC1-2* ($P < 0.05$), and *LFY* ($P < 0.01$) were significantly higher in *Vrn1g* plants than in wild-type plants under SD conditions without GA treatment (Fig. 5, A, B, and D). This result indicates that *VRN1* has an effect, either direct or indirect, on the expression of these genes. *SOC1-3* transcript levels showed a similar pattern but the differences were not significant ($P = 0.10$; Fig. 5C).

Within the *Vrn1g* plants, both *SOC1-1* and *LFY* were significantly up-regulated in the apices ($P < 0.05$) in response to both LD and GA treatment (Fig. 5, A and D). For the same treatments, we detected no significant differences in the expression of *SOC1-2* or *SOC1-3* (Fig. 5, B and C). This result suggests that following *VRN1* expression, the presence of GA is sufficient to induce the expression of *SOC1-1* and *LFY*.

The importance of GA in the regulation of *SOC1-1* and *LFY* was also observed in the reciprocal experiment, in which GA biosynthesis was blocked by the addition of PAC before the plants were transferred from SDs to LDs. The inhibition of the GA biosynthetic pathway abolished the observed increase in *SOC1-1* and *LFY* transcript levels, providing further evidence of the importance of GA in the up-regulation of these genes.

As in previous experiments, where the presence of GA alone was not sufficient to induce spike development in the absence of *VRN1*, neither *SOC1-1* nor *LFY* were significantly up-regulated in the wild-type plants carrying the *vrn1* allele (not expressed under SDs; Fig. 5, A and D). These results suggest that, in wheat, both *VRN1* and GA are required for the induction of *SOC1-1* and *LFY*.

We found that both *SOC1-1* and *LFY* were also significantly up-regulated ($P < 0.05$) in the apices of SD-grown hexaploid 'Jagger' *FT-OE* (Fig. 5E) and photoperiod-insensitive tetraploid 'Kronos' (Fig. 5F) plants compared with their respective wild-type controls, which showed no expression of *FT*. Also consistent with the previous analysis, we found no changes in the expression levels of *SOC1-3* in these plants, although *SOC1-2* showed the opposite profile, with a significant decrease ($P < 0.05$) in expression in 'Jagger' *FT-OE* plants when compared with wild-type 'Jagger' (Fig. 5E). The up-regulation of *SOC1-1* and *LFY* in the apices in response to the misexpression or overexpression of *FT*

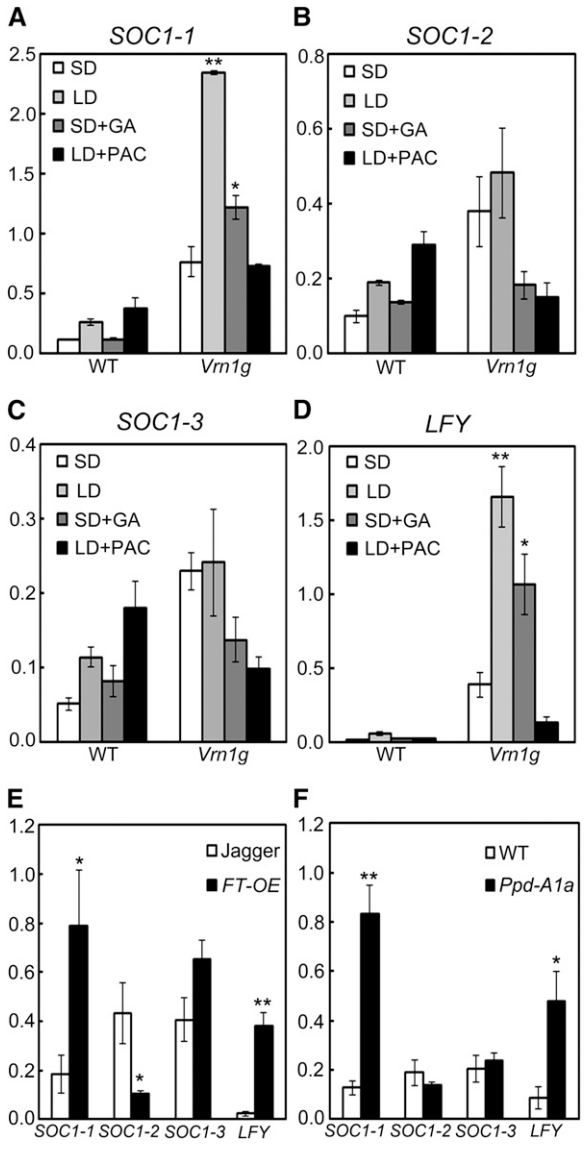

Figure 5. Effect of photoperiod, exogenous GA application, and *FT* overexpression and misexpression under SDs on *LFY* and *SOC1* transcript levels in wheat shoot apical meristems. Transcript levels of *SOC1-1* (A), *SOC1-2* (B), *SOC1-3* (C), and *LFY* (D) in apices of wild-type (WT) and *Vrn1g* plants grown for 8 weeks under SDs, grown for 6 weeks under SD with 2 weeks of exogenous GA_3 application (SD+GA), and grown for 6 weeks under SDs followed by 2 weeks under LDs both with (LD+PAC) and without (LD) PAC treatment prior to transfer. E and F, Effect of the overexpression of *FT* under SDs on the transcript levels of *LFY* and the three *SOC1* paralogs. E, 'Jagger' compared with transgenic 'Jagger' overexpressing *FT* under SDs (*FT-OE*). F, Photoperiod-sensitive (WT, no *FT* expression) compared with photoperiod-insensitive (*Ppd-A1a*, *FT* expression) plants. The *y* axis scale is expression in fold-*ACTIN* levels (number of molecules of target gene/number of molecules of *ACTIN*). Error bars indicate SE of the means based on three (A–D), five (E), and four (F) biological replicates, respectively. $*P < 0.05$, $**P < 0.01$.

in the leaves under SDs suggests that *FT* is sufficient to either induce the presence of GA in the apices or to bypass the GA requirement.

Accelerated Apical Development Is Not Associated with a Significant Increase in *GA20ox* Transcript Levels in the Leaves

The experiments described above show that the induction of *FT* in the leaves is associated with an up-regulation of *VRN1* and GA biosynthetic genes in the apex. We used qRT-PCR to determine if the up-regulation of *FT* is also associated with an up-regulation of GA biosynthetic genes in the leaves. Using leaf tissues harvested every 4 h from 8-week-old tetraploid photoperiod-sensitive and -insensitive sister lines grown under SDs, we found that all four *GA20ox* genes showed variation in transcript levels throughout the day. However, at no point was the expression of any of the *GA20ox* genes significantly different between the photoperiod-sensitive and -insensitive sister lines (Fig. 6, A–D), despite the clear acceleration in apical development observed in the photoperiod-insensitive lines (Supplemental Fig. S4B).

We then compared expression levels of these genes in diploid *T. monococcum* plants with the *Vrn1g* allele grown under SDs for 6 weeks and then transferred to LDs. Samples were taken 1, 2, 4, 7, and 14 d after transferring the plants to LDs. The complete time course for the four *GA20ox* genes is presented in Supplemental Figure S5, and the final time point (14 d after transfer to LDs) is presented in Figure 6E. In each case, leaf tissue was harvested at 8 AM. No significant differences in the expression of any *GA20ox* gene in the leaves was detected between the plants transferred to LDs and those that remained under SDs as a control (Fig. 6E; Supplemental Fig. S5).

To further validate this result, we also compared the expression of the four *GA20ox* genes in the leaves of 8-week-old hexaploid 'Jagger' and transgenic 'Jagger' *FT-OE* hexaploid wheat plants grown under SDs. As in the SD-LD experiment, no significant differences in the expression of any *GA20ox* gene in the leaves was observed between the transgenic and the control lines (Fig. 6F), despite the significant differences in expression of these genes detected in the apices (Fig. 4C).

In summary, within the resolution of our experiments, we found no significant evidence for an up-regulation of GA biosynthetic gene expression in the leaves of diploid, tetraploid, and hexaploid wheat in response to LDs or misexpression or overexpression of *FT*, despite the increase in *GA20ox*, *LFY*, and *SOC1-1* transcript levels in the apices of these plants and the acceleration of spike development.

DISCUSSION

Normal Spike Development in Wheat Requires the Simultaneous Presence of GA and *VRN1*

To ensure a timely transition between vegetative and reproductive growth stages, plants integrate signals from

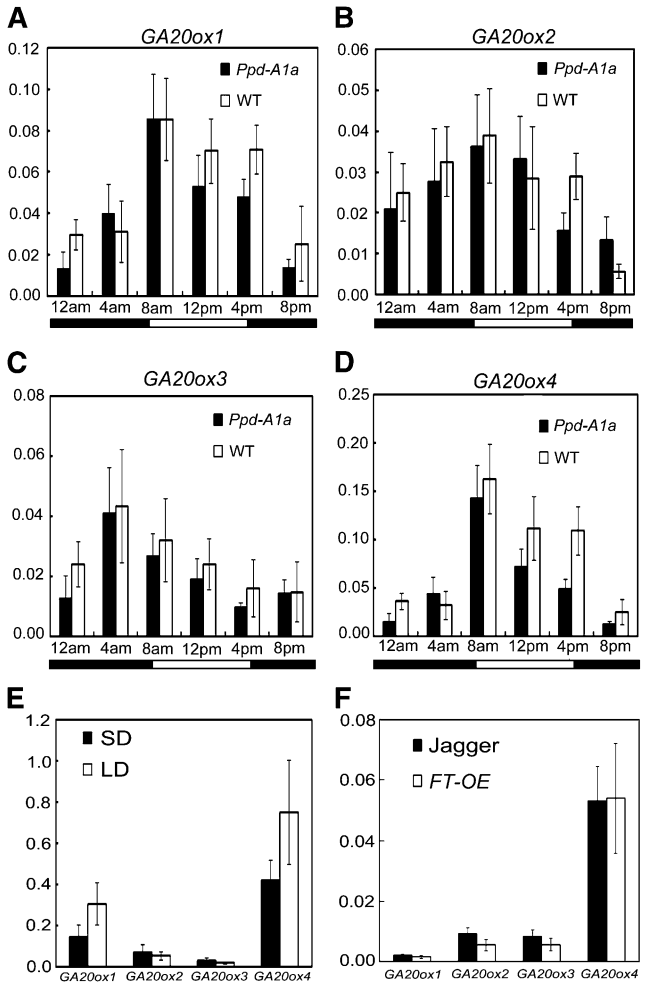

Figure 6. GA biosynthetic gene expression in the leaves. Transcript levels of *GA20ox1* (A), *GA20ox2* (B), *GA20ox3* (C), and *GA20ox4* (D) in the leaves of photoperiod-sensitive (WT) and -insensitive (*Ppd-A1a*) plants grown under SDs for 8 weeks. RNA was extracted from leaf tissues harvested every 4 h. Lights in growth chambers were on between 8 AM and 4 PM (white line below graph). *GA20ox* transcript levels in *Vrn1g* plants grown under SDs for 8 weeks compared with plants grown for 6 weeks under SDs followed by 2 weeks under LDs (E) and 8-week-old SD-grown 'Jagger' and transgenic 'Jagger' plants overexpressing *FT* under SDs (*FT-OE*; F). The *y* axis scale is expression in fold-*ACTIN* levels (number of molecules of target gene/number of molecules of *ACTIN*). Error bars indicate SE of the means based on six (A–D), three (E), and five (F) biological replicates, respectively.

multiple regulatory pathways by which they monitor and respond to different environmental cues. In wheat, the photoperiod and vernalization pathways monitor the main seasonal signals, and their interactions have been well characterized (Trevaskis et al., 2007; Distelfeld et al., 2009a). However, little is known regarding the role of GA in these processes.

In this study, we show that the application of bioactive GA can accelerate spike development in photoperiod-sensitive diploid wheat plants grown under SDs, but only in those genotypes that express the meristem identity gene *VRN1* under SDs (*Vrn1g* allele; Fig. 2B). By contrast, inhibition of the GA biosynthetic pathway by the addition of PAC delayed spike development in *T. monococcum* plants transferred from SDs to LDs.

These observations are consistent with previous experiments in *T. aestivum*, *L. perenne*, and *Phleum pratense*. In *T. aestivum*, the exogenous application of GA was shown to induce flowering in winter varieties, but only after they have been vernalized (Razumov et al., 1960), and in spring varieties, in which the up-regulation of *VRN1* does not require vernalization (Evans et al., 1995). Similarly, in *L. perenne*, exogenous applications of GA accelerate flowering development, but only after the induction of *VRN1* by vernalization (Macmillan et al., 2005). In the perennial grass *P. pratense*, while primary and regrowth stems all undergo elongation, only those exposed to vernalization, which induces expression of a *VRN1* homolog, go on to develop inflorescences (Seppänen et al., 2010). The uncoupling of the stem elongation and floral development in this perennial grass can be tentatively explained by the results from this study. Although the presence of GA would be sufficient to induce stem elongation in primary and regrowth stems, flower development would only occur in the vernalized tillers where GA and *VRN1* are present together. These results from *L. perenne* and *P. pratense* suggest that the requirement for the simultaneous presence of *VRN1* and GA at the apex for normal spike development might be common to other temperate grass species.

In photoperiod-sensitive wheat varieties, a LD photoperiod is required to induce the transcription of *FT* and its direct target *VRN1* to the levels required for the initiation of the reproductive stage. The induction of *VRN1* in the absence of *FT* is sufficient to initiate the reproductive transition of the vegetative apices to the double ridge stage, as shown in *T. monococcum Vrn1g* plants grown under SD (Fig. 2B; Dubcovsky et al., 2006). However, when plants are maintained under SDs, spike development is restricted. Addition of exogenous GA or transfer of the plants from SDs to LDs is sufficient to overcome this block in development, suggesting that this LD-regulated process is mediated by GA.

Although the continuous addition of GA to SD-grown *Vrn1g* plants accelerated the initial spike development, it was not sufficient for the development of fertile spikes (Supplemental Fig. S3). This incomplete flower development could be the result of an inappropriate dose of GA_3, the incorrect localization of the exogenous GA signal, or the existence of GA-independent floral regulatory mechanisms regulated by FT or LDs. In support of this final point, in Arabidopsis, a subset of floral homoeotic genes was shown to be regulated independently of GA (Yu et al., 2004).

The Simultaneous Presence of *VRN1* and GA Is Required for the Up-Regulation of *SOC1-1* and *LFY*

In Arabidopsis, GA has different roles under SDs and LDs (Galvão et al., 2012; Porri et al., 2012). Under

SDs, GA promotes the expression of *SOC1* and *LFY* at the shoot apical meristem by independent DELLA-mediated pathways, which results in the induction of flowering (Mutasa-Göttgens and Hedden, 2009). Interestingly, young Arabidopsis seedlings grown under SDs are less competent than older plants to respond to the same level of GA_4 (Eriksson et al., 2006). This suggests that, as in wheat, there are additional genes in Arabidopsis that are required for the plant to respond fully to exogenous GA application. Under LDs, GA is not required to activate *SOC1* but it still plays an important role in the activation of downstream flowering promoters in the apical meristem and in the transcriptional activation of *FT* in the phloem (Galvão et al., 2012; Porri et al., 2012).

In photoperiod-sensitive wheat plants grown under SDs, the addition of exogenous GA is not sufficient to induce *FT* expression in the leaves (Fig. 2D), a result consistent with observations in *L. temulentum* (King et al., 2006). However, *T. monococcum* plants carrying the *Vrn1g* allele and grown under SDs did show a significant increase in *SOC1-1* and *LFY* transcript levels in the shoot apical meristem in response to GA treatment or transfer to LDs (Fig. 5, A and D). Taken together, these results indicate that GA can induce *SOC1-1* and *LFY* expression in the apices independently of FT. The central role of GA on the transcriptional regulation of *SOC1-1* and *LFY* in the wheat apical meristem was further demonstrated by the inhibitory effect of PAC in the induction of these two genes when diploid wheat plants were transferred from SDs to LDs (Fig. 5, A and D).

In wheat, there are three closely related *SOC1* genes, and while they are all up-regulated by *VRN1*, only *SOC1-1* was induced by the addition of GA or by the transfer of plant from SDs to LDs (Fig. 5, A–C). Consistent results were observed in the 'Jagger' *FT-OE* transgenic lines and 'Kronos' photoperiod-insensitive lines grown under SDs, which showed a significant up-regulation of *SOC1-1* but not of the other two *SOC1* paralogs. Taken together, these results indicate that *SOC1-1* is the critical *SOC1* paralog linking the GA signaling pathway with the induction of *LFY* and downstream flowering genes.

In Arabidopsis, SOC1 proteins form heterodimers with AGL24 that are translocated into the nucleus, where they activate *LFY* expression, through direct interactions with its promoter (Lee et al., 2008). If wheat SOC1-1 has a similar mechanism, it may explain the good correlation observed between the transcription profiles of *SOC1-1* and *LFY*. Transcript levels of these two genes show simultaneous increases in response to exogenous GA applications, transfer from SDs to LDs, or presence of photoperiod-insensitive or *FT*-overexpressing alleles in plants grown under SDs (Fig. 5, A and D–F).

In Arabidopsis, LFY and AP1 act in tandem at two distinct stages of development. First, interactions between these two genes are critical for the transition of the vegetative to the reproductive shoot apical meristem (Liljegren et al., 1999). Then, later in development, these two proteins bind to the promoters and activate the expression of homeotic genes that specify the outer whorl during floral organ specification (Parcy et al., 1998; Busch et al., 1999). This outer whorl in the Arabidopsis flower (sepals) corresponds to the lemma and palea in the grass spikelet (Ambrose et al., 2000). In situ hybridization experiments have shown that the homologs of *AP1* and *LFY* in wheat and *L. temulentum* are expressed in these organs during spikelet development (Gocal et al., 2001; Shitsukawa et al., 2006; Kinjo et al., 2012). These results suggest that the role these genes have in determining the outer floral organs may be conserved between Arabidopsis and the grass species.

However, the early role of *LFY* and *AP1* in the coordination of the initial transition from the vegetative to the reproductive apical meristem in Arabidopsis (Wagner et al., 1999) does not seem to exist in the grasses. In *L. temulentum*, the expression of *LtLFY* in spikelet meristems is induced much later than the initial induction of *LtMADS1* and *LtMADS2* (homologs of *AP1*; Gocal et al., 2001). This is consistent with our finding that in wheat, the induction of *LFY* by GA application or exposure to LDs occurs only after the expression of *VRN1* (Fig. 5D). Therefore, in both wheat and *L. temulentum*, the activation of *LFY* occurs later in reproductive development than in Arabidopsis.

GA Biosynthesis in the Wheat Apex Is Correlated with the Induction of *FT* in the Leaves

Experiments in this study describing the effects of exogenous GA application or blocking GA biosynthesis with PAC demonstrate that GA plays a critical role in normal wheat spike development. Therefore, a mechanism should exist by which bioactive GA is transported to or synthesized in the apices during the early stages of wheat spike development. In *Lolium* spp., both *GA20ox* expression and bioactive GA levels increase in the leaves in response to an inductive photoperiod. Based on these results, and on experiments tracking radioactively labeled GA, it was suggested that bioactive GA is produced in the leaf and is subsequently transported to the apex where it induces floral development (King et al., 2001, 2006; Eriksson et al., 2006).

These studies were published before the demonstration that *FT* is the central component of the florigen, a graft-transmissible signal produced in the leaves under inductive photoperiods that is then transported to the shoot apical meristem where it induces flowering (Zeevaart, 2006). Since then, the mobility of the FT protein through the phloem has been demonstrated in a number of different species including rice (Tamaki et al., 2007). Upon arriving at the developing shoot apical meristem, FT is incorporated into a floral activation complex that also includes FD and 14-3-3 linker proteins, which binds to target gene promoters, activating their expression (Taoka et al., 2011).

Our results show that, in wheat, the misexpression or overexpression of *FT* under SDs is sufficient for the transcriptional activation of *GA20ox* biosynthetic genes and the down-regulation of *GA2ox1*, a gene involved

in the turnover of bioactive GA into inactive forms. Although we found no significant changes in expression of *GA3ox2*, which catalyzes the conversion to bioactive GA, the changes in *GA20ox* transcript levels should be sufficient to alter the levels of GA because this enzyme is thought to catalyze the rate-limiting steps of the GA biosynthetic pathway (Fig. 1). By contrast, we found no evidence for the up-regulation of *GA20ox* expression in the leaves of diploid, tetraploid, or hexaploid wheat. Photoperiod-sensitive diploid wheat lines transferred from SDs to LDs for 1 to 14 d showed no significant induction of any *GA20ox* gene in the leaves after exposure to LDs (Supplemental Fig. S5). There was also no significant increase in *GA20ox* transcript levels in the leaves in photoperiod-insensitive tetraploid lines misexpressing *FT* or in transgenic hexaploid lines overexpressing *FT* under SDs relative to their respective controls (Fig. 6). Instead, under the same conditions, we observed a significant up-regulation of *GA* biosynthetic genes (*GA20ox*) and down-regulation of genes involved in GA inactivation (*GA2ox1*) in the apices, consistent with a sustained increase in the rate of GA biosynthesis in this tissue. Based on these results, we favor the hypothesis that, in wheat, localized endogenous GA biosynthesis in the apices plays a more critical role than the transport of bioactive GA from the leaves.

Our data does not eliminate the possibility that some of the GA present in the wheat apices is the result of transport from the leaves. In fact, the acceleration of developing spikes under SDs in *Vrn1g* diploid wheat plants by exogenous applications of GA_3 to the base of the leaves suggests that GAs can, at least over short distances, be transported from the leaves to the apices in wheat. It is also possible that the relative importance of GA biosynthesis in the apices and GA transport from the leaves differs among species, with transport playing a larger role in *L. temulentum* (King et al., 2001) and local biosynthesis a larger role in wheat.

CONCLUSION

In this study, we demonstrate that both VRN1 and GA are required in the wheat shoot apical meristem for the up-regulation of *SOC1-1* and *LFY* and for the acceleration of spike development under SDs. We also show that *FT* acts upstream of the GA signal, by activating several different *GA20ox* genes and repressing *GA2ox1* expression, changes in transcription likely associated with increased levels of bioactive GA in the developing apex. Based on these results, we propose a model in which the up-regulation of *FT* in the leaves and its transport to the apex is sufficient for the induction of both *VRN1* and the genes involved in the synthesis of bioactive GA, which is required for normal spike development (Fig. 7).

The direct measurement of bioactive GA forms in the wheat apices is complicated by their low abundance and their potential heterogeneous concentrations in different regions of the shoot apical meristem. In maize, for example, a *GA2ox1* gradient has been detected in the shoot apical meristem, which presumably prevents GA from being synthesized in the meristematic zone but allows GA to reach the basal, expanding area of the stem, to favor cell expansion and differentiation (Bolduc and Hake, 2009). The methods to determine bioactive GA concentration we had access to (Chiwocha et al., 2003, 2005) require the pooling of hundreds of apices to generate enough material and therefore are not appropriate to detect localized differences within the apices.

Even though we did not provide a direct measurement of bioactive GA forms in the apices, we confirmed that the addition of bioactive GA was sufficient to induce the spike development under SDs (in the presence of *VRN1*) and that the repression of GA biosynthesis was sufficient to block spike development under LDs. The requirement of GA for normal spike development and the regulation of the GA biosynthesis by FT ensures that, even in wheat lines expressing *VRN1* under SDs (e.g. *Vrn1g T. monococcum* lines), floral meristems do not fully develop until the longer days of spring, thus protecting them from potential frost damage.

The critical roles played by GA biosynthetic genes *SOC1-1* and *LFY* during the early stages of spike development make them interesting targets to engineer developmental changes in the wheat spike to improve wheat yield potential.

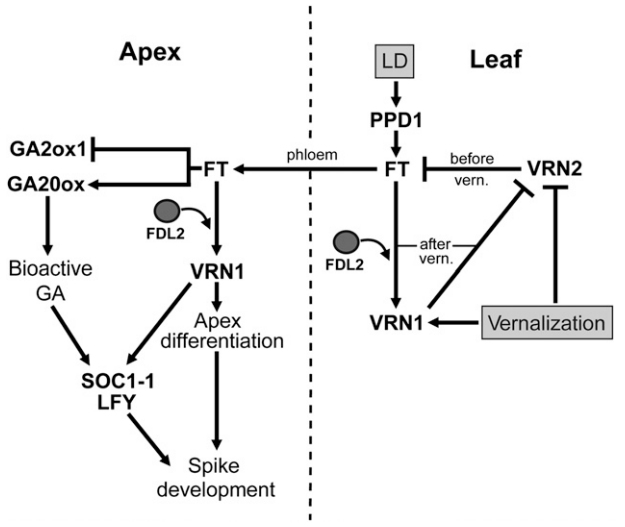

Figure 7. Regulation of flowering time in temperate cereals. A regulatory positive feedback loop including *VRN1*, *VRN2*, and *FT* (*VRN3*) operates in the leaves to modulate the levels of FT. The FT protein travels through the phloem to the shoot apical meristem where it interacts with FDL2 to induce *VRN1* transcription. We propose that once in the apices, FT also up-regulates GA biosynthetic *GA20ox* genes and down-regulates the catabolic *GA2ox* gene (directly or indirectly). The resulting increases in bioactive GA, in the presence of *VRN1*, result in the up-regulation of *SOC1-1* and *LFY*, which are required for normal spike development.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The following plant materials were used in our experiments:

T. monococcum Lines

Plants carrying the "wild-type" *vrn1* allele (DV92, PI 306540, PI 428175) and the *Vrn1g* allele (PI 349049, PI 326317, PI 418582) were described previously (Dubcovsky et al., 2006). Both genotypes carry the homozygous recessive *vrn2* allele, which confers a spring growth habit. The *Vrn1g* allele has a 34-bp deletion encompassing a putative CArG box in the *VRN1* promoter resulting in its expression under SDs.

F_2 Population of Vrn1g Mutants

To validate the linkage between the differential response to exogenous addition of GA to the *VRN1* locus, we made a cross between spring *Triticum monococcum* accessions DV92 (*vrn1 vrn2a*) with PI 326317 (*Vrn1g vrn2b*). The resulting F_1 plants were self-pollinated to generate a segregating F_2 population. Sixteen and 14 homozygous F2 lines for the *vrn1* and *Vrn1g* allele, respectively, were selected for the SD-GA experiment using the marker for the 34-bp deletion encompassing the CArG box in the *VRN1* promoter described in Dubcovsky et al. (2006).

Photoperiod-Sensitive and -Insensitive Sister Lines

We derived BC_3F_2 sister lines from two *Triticum turgidum* spp. *durum* (AABB) varieties, 'Kronos' (photoperiod insensitive) and 'Durelle' (photoperiod sensitive). 'Kronos' was used as the recurrent parent, and the photoperiod-insensitive *Ppd-A1a* allele was selected in each generation using markers described previously (Wilhelm et al., 2009).

'Jagger' FT-OE

Photoperiod-sensitive winter 'Jagger' (*Triticum aestivum*) plants were transformed using the dominant *FT* allele from the variety Hope, which contains a retroelement insertion in the promoter, resulting in the overexpression of *FT* (Yan et al., 2006). These were compared with wild-type 'Jagger' lines as a control.

Growth Conditions

All plants were grown under controlled conditions in a PGR15 growth chamber (Conviron) with combined fluorescent and halogen incandescent lamps providing 400 to 500 μM m^{-2} s^{-1}. Lights were on for 8 h per day under SDs and for 16 h per day under LDs with temperatures ranging from 18°C to 23°C.

GA Treatments

Bioactive GA_3 (Gold Biotechnology) was prepared as a 7.2 mM stock solution in 95% (v/v) ethanol. For each treatment, 7×10^{-5} mol of GA_3 was applied to the base of the most recently expanded leaf on the main tiller, which was tagged. Control plants were treated with 10 μL 95% (v/v) ethanol. Plants were treated every two days at 10 AM for 2 weeks.

PAC Treatment

PAC (1.7×10^{-5} mol; Sigma-Aldrich) was applied in a 10-mL volume to the roots of each plant every 48 h, beginning 4 d before transfer to LD conditions and maintained for the remainder of the experiment.

qRT-PCR Experiments

For analysis of apical expression, approximately 20 to 25 apices were harvested per biological replicate. For expression studies in the leaf, the basal 4 cm was used. Immediately after harvest, tissues were frozen in liquid nitrogen and ground into a fine powder. RNA was extracted using the Spectrum Plant Total RNA Kit (Sigma-Aldrich). The quality and concentration of each RNA sample was checked using a Nanodrop spectrophotometer. First-strand complementary DNA (cDNA) was synthesized from 1 μg of total RNA with the QuantiTect Reverse Transcription Kit (Qiagen) and diluted to a concentration of 10 ng μL^{-1}. qRT-PCR reactions were performed using an ABI 7500 Fast Real-Time PCR System (Applied BioSystems) with Fast SYBR Green Master Mix. Each 20-μL reaction was comprised of 10 μL 2x Fast SYBR Green Master Mix, 0.5 μL of forward and reverse primers (10 μM), 8 μL distilled, deionized H_2O, and 1 μL diluted cDNA (10 ng). All expression values are presented as fold-*ACTIN* (number of target molecules/number of *ACTIN* molecules), calculated using the ΔCt (Cycle threshold) method as described in Chen and Dubcovsky (2012). Primers for *VRN1* (Yan et al., 2003), *FT* (Yan et al., 2006), and *ACTIN* (Distelfeld and Dubcovsky, 2010), which was used as an endogenous control, have all been described previously. All other qRT-PCR primers listed in Supplemental Table S1 were designed to amplify all homeologs for each target gene. Primer efficiencies were calculated from the slope of Ct values generated from five 4-fold cDNA dilutions (1, 1:4, 1:16, 1:64, and 1:256), each carried out in duplicate. Efficiencies for all primer pairs in this study were above 90% (Supplemental Table S1). Specificity was tested by analyzing dissociation curves of all amplified products, in addition to monitoring for amplification in a negative control with no cDNA template. All selected primers showed amplification of a single product in dissociation curves and no amplification in the negative controls.

GA20ox Gene Sequences

qRT-PCR primers were designed using publicly available sequences of *SOC1-1* (AM502861), *SOC1-2* (AM502883), *SOC1-3* (AM502887), and *LFY* (AM502887). Sequences of *TaGA20ox2*, *TaGA20ox3*, and *TaGA20ox4* were isolated by searching available wheat (*Triticum* spp.) EST and sequence databases using the rice (*Oryza sativa*) homologs of these genes (*OsGA20ox2* = Os01g0883800, *OsGA20ox3* = Os07g0169700, *OsGA20ox4* = Os05g0421900) as queries. The resulting homologous sequences were used to develop qRT-PCR primers for expression analysis. qRT-PCR primers for *GA20ox1* and *GA3ox2* were described previously (Pearce et al., 2011), and those for *GA2ox1* were provided by Andy Phillips and Yidan Li (Rothamsted Research).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Effect of exogenous GA_3 on apical development in the presence and absence of VRN1.

Supplemental Figure S2. Effect of exogenous GA_3 on apical development in *T. monococcum mvp* mutants.

Supplemental Figure S3. Spike and spikelet morphology of *Vrn1g* plants exposed to LD and treated with GA_3 for 18 weeks.

Supplemental Figure S4. Apical morphology of SD-grown 'Jagger' and 'Jagger' *FT-OE* plants, and 'Kronos' photoperiod-sensitive and -insensitive plants.

Supplemental Figure S5. Timecourse of *GA20ox* expression in the leaves of *T. monococcum Vrn1g* plants exposed to LD.

Supplemental Table S1. qRT-PCR primers and their efficiencies.

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