

Effect of the Hope *FT-B1* Allele on Wheat Heading Time and Yield Components

REBECCA NITCHER, STEPHEN PEARCE, GABRIELA TRANQUILLI, XIAOQIN ZHANG, AND JORGE DUBCOVSKY

From the Department of Plant Sciences, University of California, Davis, One Shields Avenue, Davis, CA 95616 (Nitcher, Pearce, Tranquilli, Zhang, and Dubcovsky); the Instituto de Recursos Biológicos, Instituto Nacional de Tecnología Agropecuaria, Buenos Aires, Argentina (Tranquilli); and the Howard Hughes Medical Institute, Chevy Chase, MD (Dubcovsky).

Address correspondence to Jorge Dubcovsky at the address above, or e-mail: jdubcovsky@ucdavis.edu.

Abstract

Precise regulation of flowering time is critical for plant reproductive success and, in cereals, to maximize grain yields. Seasonal cues including temperature and day length are integrated to regulate the timing of flowering. In temperate cereals, extended periods of cold (vernalization) release the repression of *FLOWERING LOCUS T1* (*FT1*), which is upregulated in the leaves in response to inductive long-day photoperiods. *FT1* is a homolog of rice *HD3a*, which encodes a protein transported from leaves to the shoot apical meristem to induce flowering. A rare *FT-B1* allele from the wheat variety “Hope” has been previously shown to be associated with an early flowering phenotype under long-day photoperiods. Here, we demonstrate that the Hope *FT-B1* allele accelerates flowering even under short days, and that it is epistatic to the *VERNALIZATION 1* (*VRN1*) gene. On average, the introgression of Hope *FT-B1* into 6 genetic backgrounds resulted in 2.6 days acceleration of flowering ($P < 0.0001$) and 4.1% increase in spike weight ($P = 0.0093$), although in one variety, it was associated with a decrease in spike weight. These results suggest that the Hope *FT-B1* allele could be useful in wheat breeding programs to subtly accelerate floral development and increase adaptation to changing environments.

Subject areas: Gene action, regulation, and transmission

Key words: flowering, *FT*, *Triticum*, *VRN3*, wheat

In temperate cereals such as wheat (*Triticum* spp.), the precise control of flowering time is critical to ensure that the plant's reproductive development coincides with optimal environmental conditions, thus maximizing grain yields. Several genes with important roles in regulating wheat flowering development in response to long exposures to cold temperatures (vernalization) or changes in day length (photoperiod) have been cloned and characterized, including *VERNALIZATION 1* (*VRN1*) (Danyluk et al. 2003; Trevaskis et al. 2003; Yan et al. 2003), *VRN2* (Yan et al. 2004b), *VRN3* (Yan et al. 2006), and *PHOTOPERIOD 1* (*PPD1*) (Turner et al. 2005; Beales et al. 2007).

VRN1 is a homologue of the *Arabidopsis* floral meristem identity gene *APETALA1* (*AP1*) and is central to the vernalization response in temperate cereals. In varieties with a winter growth habit, vernalization induces the gradual increase of *VRN1* transcript levels and results in competence to flower (Danyluk et al. 2003; Trevaskis et al. 2003; Yan et al. 2003). Large deletions and insertions in the regulatory regions

of *VRN1* reduce or eliminate vernalization requirement in wheat and barley and result in a spring growth habit (Yan et al. 2004a; Fu et al. 2005; Hemming et al. 2009).

It was recently demonstrated that a critical role of *VRN1* is to repress the flowering repressor *VRN2* in the leaves during the spring (Chen and Dubcovsky 2012). The *VRN2* locus consists of duplicated genes encoding CCT-domain proteins (*ZCCT1* and *ZCCT2*) and simultaneous deletion of, or non-functional mutation in, both copies is sufficient to confer a spring growth habit (Yan et al. 2004b). Under long days (LD), *VRN2* suppresses the induction of *VRN3*, a gene encoding a phosphatidylethanolamine-binding protein (PEBP) structurally and functionally similar to *FLOWERING LOCUS T* (*FT*) in *Arabidopsis* (Yan et al. 2006). This gene, henceforth referred to as *FT1*, is one of at least 6 *FT*-like paralogs which have been identified in cereals (Faure et al. 2007; Lv et al. 2014). Of these, *FT1* shows the most robust induction of flowering when transformed into rice and wheat (Yan et al. 2006; Kikuchi et al. 2009).

Before vernalization, the induction of *FT1* by LD photoperiod is blocked by *VRN2*. However, following the vernalization-induced upregulation of *VRN1* and its subsequent repression of *VRN2*, *FT1* is upregulated inducing flowering in the spring. Under LD, the induction of *FT1* is mediated by the photoperiod gene *PPD1* (Turner et al. 2005; Beales et al. 2007).

The *FT1* ortholog in rice, *HEADING DATE 3a* (*Hd3a*), has been demonstrated to act as a graft-transmissible mobile signal, which is transported via the phloem to the shoot apical meristem (Tamaki et al. 2007). After transport to the apex, *HD3a* is incorporated into a floral activation complex which also comprises *FD* and 14-3-3 linker proteins. This complex binds to the promoters of target genes such as *OsMADS15* (rice ortholog of *Arabidopsis AP1*), thus activating their expression and inducing the irreversible transition from vegetative to reproductive growth of the shoot apical meristem (Taoka et al. 2011). In wheat, the interaction between *FT1* and *FDL-2* and the binding of *FDL-2* to the *VRN1* promoter *in vitro* have also been demonstrated, suggesting that this mechanism is conserved among grass species (Li and Dubcovsky 2008).

A large number of natural *VRN1* alleles have been described, and many have been incorporated into wheat breeding programs to alter both growth habit and flowering time (Yan et al. 2004a; Fu et al. 2005; Chu et al. 2011; Díaz et al. 2012). In contrast, limited natural variation has been described for the wheat *FT1* gene. In barley, several haplotypes have been identified, and a dominant *FT1* allele for early flowering has been shown to be associated with increased copy number (Nitcher et al. 2013). In wheat, sequence variation has been reported for *FT-A1* (a SNP resulting in an amino acid substitution in exon 3) and *FT-D1* (a single base pair insertion-deletion in the third exon), which results in small variations in flowering time for both loci (Bonnin et al. 2008). In *FT-B1*, an allele dominant for spring growth habit has been identified in the *Triticum aestivum* variety “Hope,” derived from a cross between the wild emmer “Yaraslov” (*Triticum dicoccum*) and the Canadian *T. aestivum* variety “Marquis” (Clark et al. 1926; McFadden 1930).

The Hope *FT-B1* allele is defined by the insertion of a 5295 bp retrotransposon element in the promoter region and is associated with early flowering under LD photoperiods (Yan et al. 2006). This association was confirmed by the transformation of the winter hexaploid variety “Jagger” with the Hope *FT-B1* allele, which resulted in greatly increased *FT1* transcript levels and acceleration of flowering even in the absence of vernalization (Yan et al. 2006). The *FT-B1* allele with the repetitive element insertion is not common among modern tetraploid durum and hexaploid bread wheat varieties. The narrow distribution of this allele and the lack of information regarding its effects on heading date and agronomic performance in different genetic backgrounds under field conditions have limited its utilization in wheat breeding programs.

In the current study, we demonstrate that the Hope *FT-B1* allele is capable of accelerating flowering in a photoperiod-sensitive winter wheat variety even under short-day (SD)

photoperiod, and that it is epistatic to *VRN1* under both greenhouse and field environments. Using 6 pairs of near-isogenic sister lines with and without the Hope *FT-B1* allele, we show that this allele significantly reduces days to heading (DTH) in the field in some varieties, and that in general, it has no significant negative effects on yield components, demonstrating its potential utility as a tool to adjust flowering time in wheat breeding programs.

Materials and Methods

Materials and Growing Conditions

Two chromosome substitution lines in the *T. aestivum* “Chinese Spring” (CS) background were used in this study: CS (Hope 7B) (Kuspira and Unrau 1957) and CS (Cheyenne 5D) (Morris et al. 1966). In CS (Hope 7B), CS chromosome 7B, carrying the wild-type *FT-B1* allele, was replaced by chromosome 7B from “Hope,” which carries the highly expressed *FT-B1* allele with a retroelement insertion in the promoter. In CS (Cheyenne 5D), CS chromosome 5D carrying the *Vrn-D1* allele (spring growth habit) was replaced by Cheyenne chromosome 5D, which carries the *vrn-D1* allele (winter growth habit). Since the *Vrn-D1* allele is the only *VRN1* allele for spring growth habit in CS, the CS (Cheyenne 5D) plants have a winter growth habit.

The substitution lines CS (Hope 7B) and CS (Cheyenne 5D) were crossed to produce a population segregating for Hope *FT-B1* and *Vrn-D1*. Except for the segregating chromosomes 7B and 5D, the genetic background of these lines is expected to be identical. F_2 individuals were genotyped for these 2 genes (see below), and those with different combinations of homozygous alleles were selected to produce F_3 families for further evaluations. The first experiment included 29 F_3 families ($N = 109$), representing the 4 genotypic classes expected from the segregation of these 2 genes, and the second experiment included derived F_4 lines ($N = 111$). Plants were grown in a greenhouse under nonvernalizing, LD controlled conditions, and were periodically randomized. DTH was recorded as the number of days from germination to heading.

Transgenic plants obtained from the bombardment of the photoperiod-sensitive winter variety “Jagger” with a genomic copy of the Hope *FT-B1* allele including its natural promoter (henceforth referred to as transgenic *FT1*_{HOPE} lines) were described before (Yan et al. 2006). Transgenic and wild-type Jagger were compared under SD (8h light/16h dark) and LD (16h light/8h dark) photoperiods at 16 °C with 16% humidity in Conviron CMP3244 growth chambers (Conviron, Pembina, ND). Seeds were germinated in Petri dishes, incubated at 4 °C for 24h, and then transferred to 25 °C for 48h before transplanting into 6-inch pots in the growth chambers. These transgenic plants showed segregation for *FT1* expression level, which is likely due to the segregation of multiple functional and nonfunctional transgene insertion events during bombardment. For this experiment, we selected 7 lines grown under LD and 11 lines grown under SD carrying the transgene (confirmed by PCR) and exhibiting *FT1* expression

greater than or equal to 2 times the average expression of the Jagger lines in each respective photoperiod.

To determine the effect of the Hope *FT-B1* allele on heading time and yield components in the field, the CS (Hope 7B) chromosome substitution line was used as a donor to introgress the Hope *FT-B1* allele into 1 tetraploid (UC1113) and 4 hexaploid varieties (UC1041, UC1110, Kern, and Clear White), all with a spring growth habit. The recurrent parents differ in the combinations of *VRN1* alleles responsible for their spring growth habit. The tetraploid breeding line UC1113 (Chicaiza et al. 2006) carries the *Vrn-A1c* allele (first intron deletion), whereas the hexaploid breeding line UC1110 (Lowe et al. 2011) carries the *Vrn-A1a* allele (insertion and duplication in promoter region, Yan et al. 2004a). The bread wheat variety Clear White (PI 635044) carries *Vrn-B1a* and *Vrn-D1a* alleles for spring growth habit (both with first intron deletions, Fu et al. 2005), whereas an isogenic sister line designated as Clear White-*vrn-B1* carries only the *Vrn-D1a* allele. The variety Kern (PI 612142) and the breeding line UC1041 (Uauy et al. 2009) also have *Vrn-D1a* as the only allele for spring growth habit (Fu et al. 2005). The Hope *FT-B1* allele was introgressed into these 6 lines using marker-assisted backcrossing. After 4 generations of backcrossing, near-isogenic sister lines were selected, which are expected to be approximately 97% identical.

The BC₄F₂ lines were grown in the greenhouse and lines homozygous for the different *VRN1* genotypes and for wild-type or Hope *FT-B1* alleles were selected (see *FT-B1* and *VRN1* Genotyping section below). Selected BC₄F₃ (2011–2012 growing season) and BC₄F₄ (2012–2013 growing season) sister lines were grown in single rows at the University of California, Davis, CA (38°32'N, 121°46'W). Both experiments were planted in a Split-Plot Randomized Complete Block Design (10 blocks). These rows were cultivated following typical growing practices for the region as previously described (Brevis and Dubcovsky 2010). DTH was recorded as number of days from sowing to heading. We also recorded yield components including grains per spike (GpS), thousand kernel weight (TKW), spikelets per spike (SpS), and weight of the grain per spike (SW). The number of GpS was determined as the average of 5 spikes harvested randomly per row. SW was estimated from GpS and TKW values. Following the second field season, samples of each line (BC₄F₃ seed) were submitted to the National Small Grains Collection for use as germplasm. The PI numbers are as follows: UC1113 + Hope *FT-B1*, PI 671995; UC1113, PI 638741; UC1041 + Hope *FT-B1*, PI 671996; UC1041, PI 671997; UC1110 + Hope *FT-B1*, PI 671998; UC1110, PI 671999; Kern + Hope *FT-B1*, PI 672000; Kern, PI 672001; Clear White + *vrn-B1* + Hope *FT-B1*, PI 672002; Clear White + *vrn-B1*, PI 672003; Clear White + Hope *FT-B1*, PI 672004; Clear White, PI 672005.

FT-B1 and *VRN1* Genotyping

Wild-type and Hope *FT-B1* alleles were assessed in field lines using the codominant marker described previously (*Typ509I* assay, Yan et al. 2006). Transgenic *FTI*_{HOPE} lines and CS populations were characterized using allele-specific (dominant) *FT-B1* PCR markers (Yan et al. 2006), to detect the

presence and absence of the retrotransposon insertion in the promoter region.

Allelic variants in the promoter of hexaploid *Vrn-A1a* were determined with a codominant marker, using a combination of primers developed in this and previous studies (Yan et al. 2004a) (Table 1). Two complementary sets of allele-specific primers were used to develop a codominant marker for the first intron deletion present in the *Vrn-A1c* allele. Specifically, the absence of the deletion in the wild-type allele was tested with primers described by Fu et al. (2005), while new primers were designed to detect the presence of the intron deletion (Table 1). Similarly, 2 pairs of complementary allele-specific primers were used to characterize the intron 1 deletions present in the *Vrn-B1a* and *Vrn-D1a* alleles (Fu et al. 2005) (Table 1).

Statistical Analysis

Data from the greenhouse experiments were analyzed with a 2-way factorial Anova including *FT1* and *VRN1* alleles, while data from field experiments were analyzed with a 3-way factorial Anova including *FT1* alleles, variety, and year. Type III sum of squares and Least Squares means were used to compensate for the unequal number of plants in the different genotyping classes. Means were compared using Tukey's test. Due to the presence of significant interactions in the field experiments, DTH was also tested separately by variety and yield components were tested separately by year and variety. All statistical analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC).

Quantitative Reverse Transcription-PCR

Leaf tissue from Jagger and transgenic *FTI*_{HOPE} plants was harvested (SD at 5 weeks and 14 weeks and LD at 5 weeks) and immediately frozen in liquid nitrogen before grinding into a fine powder. Tissue was harvested from the youngest, fully deployed leaf or from the flag leaf of flowering plants at 10 a.m. (4h of light). Total RNA was extracted using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich, St Louis, MO) and 1 μg of the RNA equivalent of cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Foster City, CA). Quantitative reverse transcription-PCR (qRT-PCR) reactions were performed in 20 μl final volume including 1× USB® VeriQuest™ SYBR® Green qPCR Master Mix (Affymetrix, Santa Clara, CA) 0.5 μM of F and R primers and 10 ng of cDNA. qRT-PCR was carried out using an Applied Biosystems 7500 Fast Real-Time PCR System machine (Applied Biosystems). *ACTIN* was used as an endogenous control. Primers for *FT1* (Shimada et al. 2009) and *ACTIN* (Fu et al. 2007) have been described previously. Transcript levels for all genes and experiments presented in this study are expressed as linearized fold-*ACTIN* levels calculated by the formula $2^{(ACTIN\ CT - TARGET\ CT)} \pm$ standard error. The resulting number indicates the ratio between the initial number of molecules of the target gene and the number of molecules of *ACTIN* and therefore, the *Y* scales are comparable across genes and experiments.

Table 1 Primers used for genotyping *VRN1* and *FT1* alleles

Locus	Forward primer	Reverse primer	Product size (bp) spring/winter
<i>Vrn-A1a</i> promoter ^a	TGAAAGGAAAATTCGTCTCGT	GCAGGAATCGAAATCGAA ^b	945/713
<i>Vrn-A1c</i> first intron (deletion) ^c	TTTGGTAGATCCGTTTGCC	TGTTGGGAAAATAGAGTGACCC	942/NA
<i>Vrn-A1</i> first intron (WT) ^d	GCACCTCAACCACCTAAC	TCATCCATCATCAAGGCAAA	NA/1068
<i>Vrn-B1a</i> first intron (deletion) ^d	CAAGTGGAAACGGTTAGGACA	CTCATGGCAAAAATTGAAAGATGA	709/NA
<i>Vrn-B1</i> first intron (WT) ^d	CAAGTGGAAACGGTTAGGACA	CAAAATGAAAAGGAATGAGAGCA	NA/1149
<i>Vrn-D1a</i> (deletion) ^d	GTTGTCTGCCITCAICAAATCC	GGTACATGGTGGTCTGTGTC	1671/NA
<i>Vrn-D1</i> (WT) ^d	GTTGTCTGCCITCAICAAATCC	AAATGAAAAGGAACGAGAGCG	NA/997
<i>FT-B1</i> Hope ^e	CATAATGCCAAGCCGGTGAAGTAC	ATGCTGCGCAATAGCTAGC	1200/NA
<i>FT-B1</i> WT ^e	ATGCTTCCGCTTGCCATCC	CTATCCCTACCGCCATTAG	NA/1140
<i>FT-B1</i> ^{ef}	TCCTAGCAAGCACACCGATAA	CGTGCACATCATGATCAAAAG	120/154

NA, no amplification; WT, wild type.

^aAnnealing temperature 60 °C.^bYan et al. (2004a).^cAnnealing temperature 56 °C.^dFu et al. (2005).^eYan et al. (2006).^fDigest by *Tsp509I*.

Results

Characterization of the Hope *FT-B1* Allele Under Different Photoperiods

To analyze the effect of the Hope *FT-B1* allele on heading time under different photoperiods, we compared wild-type Jagger with transgenic *FT1*_{HOPE} lines in controlled growth chamber experiments under SD and LD photoperiods. Under LD conditions, *FT1* transcript levels were low or undetectable in 5-week-old Jagger plants (average fold-*ACTIN* = 0.016 ± 0.004, Figure 1A). This is an expected result for unvernallized plants from a winter variety and is consistent with previous observations (Yan et al. 2006). By contrast, *FT1* transcript levels were almost 5-fold higher than *ACTIN* (4.92 ± 2.14, Figure 1A) in the 7 transgenic plants selected for the presence of the active *FT1*_{HOPE} transgene and grown under LD photoperiod. These plants headed significantly earlier (62.7 ± 3.2 days) than the wild-type Jagger plants (155 ± 1.5 days, *P* < 0.0001, Figure 1B). This result confirms the association between increased *FT1* expression and the acceleration in flowering time under LD.

Jagger plants flowered significantly later under SD (171 days) than they did under LD (155 days, *P* < 0.0001, Figure 1B). As expected for a photoperiod-sensitive line grown under SD, *FT1* transcript levels of 5-week-old Jagger plants were almost undetectable. In transgenic Hope *FT-B1* plants of the same age, we also found very low levels of *FT1* expression, so we repeated the expression analysis in 14-week-old plants to match the developmental stage of the LD samples (initiation of heading). The 11 transgenic plants selected for the presence of the active *FT1*_{HOPE} allele grown in a SD photoperiod showed *FT1* transcript levels 38-fold higher (0.269 ± 0.129-fold-*ACTIN*) than those observed in the nontransgenic Jagger plants (0.007 ± 0.003-fold-*ACTIN*). The increased *FT1* transcript levels in the transgenic plants was associated with a significantly earlier (*P* = 0.0003) flowering time (131.6 ± 7.5 days, Figure 1B) than that observed for the Jagger wild-type plants (171.75 ± 2.6 days). This result demonstrates that the Hope *FT-B1* allele is able to accelerate flowering in a photoperiod-sensitive genotype under noninductive SD photoperiod. However, *FT1* transcript levels detected in the transgenic plants grown under SD were lower (*P* = 0.073) than those observed in the transgenic plants grown under LD, which was also reflected in later flowering under SD than under LD (*P* < 0.0001, Figure 1B).

Epistatic Interactions Between *VRN-D1* and *FT-B1*

To better understand the effect of the Hope *FT-B1* allele in spring and winter backgrounds, we analyzed the epistatic interactions between *FT-B1* and *VRN-D1*. In F₃ families derived from the cross between chromosome substitution lines CS (Hope 7B) and CS (Cheyenne 5D), which were homozygous for different *VRN-D1/FT-B1* allelic combinations, we observed a significant interaction between these 2 loci (*P* = 0.028, Figure 2A). This interaction, validated in an independent experiment performed

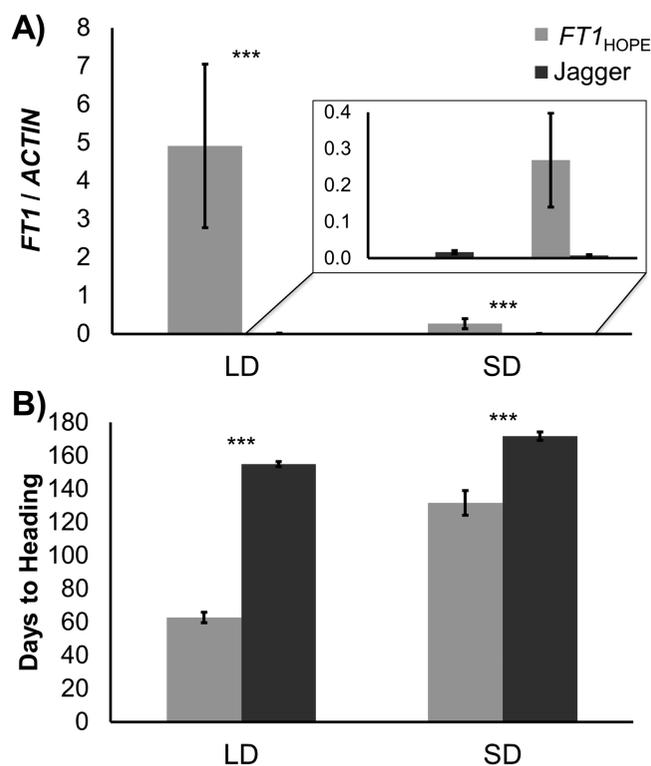


Figure 1. (A) *FT1* transcript levels (fold-*ACTIN* \pm standard error) of 7–10 biological replicates at flowering (5 weeks LD, 14 weeks SD) of transgenic *FT1*_{HOPE} and wild-type Jagger lines. (B) DTH in *FT1*_{HOPE} transgenic plants and wild-type Jagger under LD and SD photoperiods. *** $P \leq 0.0001$, comparison of *FT1*_{HOPE} phenotype to wild-type Jagger phenotype in designated photoperiod.

with derived F_4 lines ($P = 0.0003$, Figure 2B), indicates that the effect of each gene is affected by the allele present in the other gene. Therefore, we describe the simple effects of each gene within each of the classes of the other gene. The Hope *FT-B1* allele significantly accelerated flowering in the winter *vrn-D1* background, both in the F_3 (Hope *FT-B1* 44 days earlier, $P < 0.0001$) and F_4 populations (Hope *FT-B1* 38.2 days earlier, $P = 0.003$). However, the effect was not significant within the plants carrying the *Vrn-D1* allele (spring growth habit) either in the F_3 (Hope *FT-B1* 16.9 days earlier, $P = 0.104$) or F_4 populations (Hope *FT-B1* 9.3 days later, $P = 0.508$). The reciprocal analysis of *VRN1* effects showed that the difference in heading time between the plants carrying the *Vrn-D1* and *vrn-D1* alleles was significant within the wild-type *FT-B1* class (52 days F_3 , 56 days F_4 , $P < 0.0001$) but not within the Hope *FT-B1* class (25 days F_3 , 9 days F_4 , $P > 0.05$, Figure 2). Taken together, these results indicate that the Hope *FT-B1* and *Vrn-D1* alleles for early flowering are epistatic to the alleles for late flowering in the other gene.

Effect of the Hope *FT-B1* Allele on Heading Time and Yield Components Under Field Conditions

Using 6 pairs of near-isogenic sister lines for the wild-type and Hope *FT-B1* alleles, we detected significant differences in heading time that were consistent across years (Table 2)

and small differences in yield components that were significant only for one of the 2 years (Table 3). Plants carrying the Hope *FT-B1* allele flowered significantly earlier than the sister lines carrying the wild-type *FT-B1* ($P < 0.01$) in both years. No significant interactions were detected between year and the other factors in the statistical model, so the results from both years were combined (Table 2). On average, plants carrying the Hope *FT-B1* allele flowered 2.6 days earlier than those carrying the wild-type allele ($P < 0.0001$, Table 2). A significant interaction was detected between genotype and *FT-B1* ($P = 0.0003$), so we also tested the effect of this locus on heading date separately for each genotype. Plants carrying the Hope *FT-B1* allele flowered 4–5 days earlier than those carrying the wild-type allele and these differences were highly significant in UC1113, Clear White-*vrn-B1*, and Kern, and marginally nonsignificant in UC1110 ($P = 0.057$). The last 2 varieties, UC1041 and Clear White, showed less than 1 day difference in heading time between sib-lines with and without the Hope *FT-B1* allele, and the differences were not significant ($P > 0.56$, Table 2).

To assess the impact of *FT-B1* alleles on yield components, we recorded GpS, TKW, and SpS, and we calculated the SW (Table 3). Overall, we observed a significant increase in GpS ($P = 0.0036$) and SW ($P = 0.0093$) and a marginally nonsignificant increase in SpS ($P = 0.0733$). Differences in TKW were not significant. Interestingly, the interaction

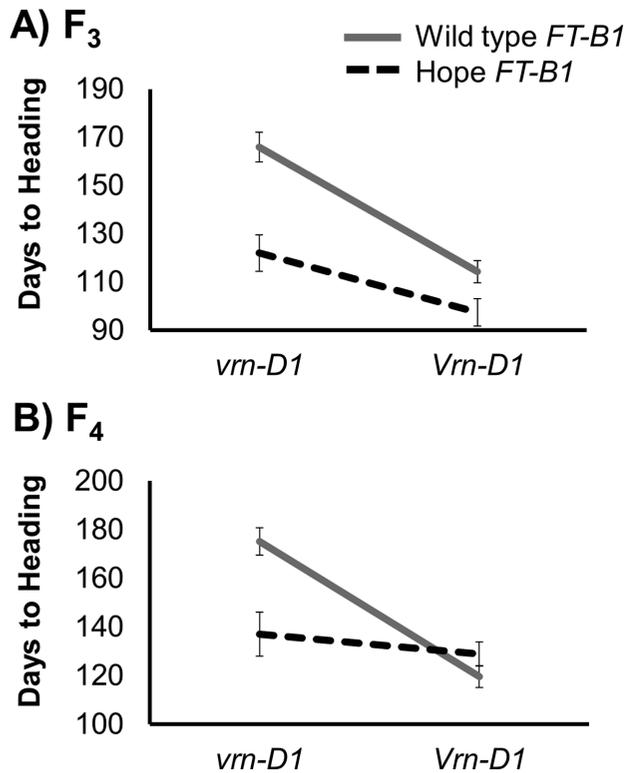


Figure 2. Interaction graphs for heading time in the CS (Cheyenne 5D) × CS (Hope 7B) (A) F₃ and (B) F₄ populations segregating for both *FT-B1* and *VRN-D1*. Nonparallel lines indicate a strong interaction (nonadditive effects).

between *FT-B1* alleles and year was not significant for any of the traits ($P > 0.55$), indicating that the responses were consistent across both years.

For all 4 traits, we found significant interactions between lines and year (GpS: $P = 0.0158$; TKW: $P = 0.0001$; SpS: $P < 0.0001$; SW: $P = 0.0033$), as well as between line and *FT-B1* (2013—GpS: $P = 0.0088$; TKW: $P = 0.0424$; SpS: $P < 0.0001$; SW: $P = 0.0256$). Therefore, comparisons between the wild-type and Hope *FT-B1* alleles are presented separately by year and line (Table 3). An average increase of 4–5% in grain number per spike and of 3–5% in spike weight was observed across years, but when considered separately, the differences were significant only for the second year (2013 GpS: $P = 0.0017$; 2013 SW: $P = 0.0021$). Interestingly, this increase in grain number was not associated with a significant decrease in TKW (2012–2013, $P = 0.7611$; Table 3). The analysis by individual lines showed a heterogeneous response for all 4 traits (significant interactions line × *FT-B1* allele; Table 3). Except in the variety Kern, the presence of the *FT1* Hope allele was associated with slight increases in GpS, SpS, or SW, which were significant only in 6 of the 30 comparisons ($P < 0.05$, Table 3). By contrast, Kern showed slightly lower GpS, SpS, and SW values across both years in the sister lines carrying the *FT1* Hope allele, a decrease that was significant only in 2 of the 6 comparisons ($P < 0.05$, Table 3). When the 2 years were combined, Kern showed a significant decrease in SpS ($P = 0.0027$), whereas UC1113 and UC1041 showed significant increases in this parameter ($P = 0.0232$ and $P = 0.0015$ respectively; Table 3). A similar trend was observed for GpS, which is correlated with SpS (Table 3). It is interesting to point out that in Clear White-*vrn-B1*, the presence of the Hope *FT-B1* allele was

Table 2 Effect of *FT-B1* alleles on average heading time in 6 pairs of near-isogenic sister lines

		<i>FT-B1</i>	DTH	<i>P</i> value
<i>FT-B1</i> Effect across line		WT	139.72	<0.0001
		Hope	137.13	
Line by <i>FT-B1</i> interaction				0.0003
Line	<i>VRN1</i>	<i>FT-B1</i>	DTH	<i>P</i> value
UC1113 (tetraploid)	<i>Vrn-A1c</i>	WT	149.80	0.0005
		Hope	145.80	
UC1041 (hexaploid)	<i>Vrn-D1a</i>	WT	137.60	0.6970
		Hope	138.05	
UC1110 (hexaploid)	<i>Vrn-A1c</i> (promoter)	WT	137.15	0.0571
		Hope	133.58	
Kern (hexaploid)	<i>Vrn-D1a</i>	WT	138.10	<0.0001
		Hope	134.40	
Clear White- <i>vrn-B1</i> (hexaploid)	<i>Vrn-D1a</i>	WT	139.25	<0.0001
		Hope	134.65	
Clear White (hexaploid)	<i>Vrn-B1a/D1a</i>	WT	135.56	0.5687
		Hope	135.94	

P values indicate the significance of the effects over 2 years of field experiments using a 3-way factorial Anova. Values are also presented separately by line due to the presence of a significant interaction between the *FT-B1* locus and the line. WT, wild type.

Table 3 Effect of *FT-B1* alleles on yield components in 6 pairs of near-isogenic sister lines in 2 field experiments

	<i>FT-B1</i>	GpS			TKW			SpS			SW		
		2012	2013	2012–2013	2012	2013	2012–2013	2012	2013	2012–2013	2012	2013	2012–2013
Across lines													
<i>FT-B1</i> effect across lines	WT	61.71	63.92		41.89	40.56		21.01	20.92		2.58	2.60	
	Hope	64.41	66.92		41.75	40.80		21.32	21.24		2.66	2.73	
	<i>P</i> value	0.1220	0.0017	0.0036	0.7945	0.6867	0.7611	0.2844	0.0865	0.0733	0.2002	0.0021	0.0093
Line by <i>FT-B1</i> interaction	<i>P</i> value	0.4069	0.0088	0.0236	0.2291	0.0424	0.0323	0.2973	<0.0001	<0.0001	0.3642	0.0256	0.0263
Year by line interaction	<i>P</i> value			0.0158			0.0001						0.0033
Year by <i>FT-B1</i> interaction	<i>P</i> value			0.8178			0.8355						0.5529
By line													
UC1113 (tetraploid)	WT	65.56	70.00		47.11	49.77		20.26	20.58		3.48	3.09	
	Hope	68.48	78.22		47.03	48.17		21.22	21.96		3.77	3.21	
	<i>P</i> value	0.5690	0.0084	0.0584	0.9580	0.0428	0.3237	0.2100	0.0528	0.0232	0.6314	0.0409	0.1454
UC1041 (hexaploid)	WT	58.16	57.14		35.15	35.45		21.98	19.52		2.02	2.05	
	Hope	65.50	61.74		33.21	34.16		22.72	21.22		2.10	2.16	
	<i>P</i> value	0.0394	0.0485	0.0043	0.1904	0.3917	0.1206	0.2167	0.0007	0.0015	0.2672	0.4370	0.1729
UC1110 (hexaploid)	WT	52.91	55.18		46.39	43.14		20.29	20.14		2.40	2.47	
	Hope	54.18	57.68		47.47	43.51		20.91	20.48		2.51	2.55	
	<i>P</i> value	0.5358	0.2675	0.8267	0.6349	0.9757	0.6371	0.6537	0.3053	0.3218	0.6739	0.3672	0.7627
Kern (hexaploid)	WT	56.04	63.22		45.05	41.25		20.44	21.04		2.61	2.52	
	Hope	53.12	58.96		43.55	41.99		19.66	19.46		2.47	2.32	
	<i>P</i> value	0.4130	0.0402	0.0778	0.0817	0.2254	0.4375	0.1923	0.0031	0.0027	0.2523	0.0934	0.0794
Clear White- <i>mtt-B1</i> (hexaploid)	WT	66.58	66.64		38.54	36.55		21.44	21.84		2.44	2.57	
	Hope	70.52	71.10		39.88	39.37		21.48	22.28		2.80	2.81	
	<i>P</i> value	0.3111	0.0784	0.0667	0.0606	0.0298	0.0040	0.9342	0.3730	0.4797	0.1293	0.0054	0.0029
Clear White (hexaploid)	WT	75.77	71.34		38.03	36.89		21.93	22.4		2.63	2.90	
	Hope	79.80	73.82		38.70	37.26		22.30	22.04		2.74	3.10	
	<i>P</i> value	0.4668	0.3682	0.1997	0.2621	0.7116	0.1623	0.4702	0.4802	0.9600	0.4045	0.3145	0.1577

Averages and *P* values are presented for the effects over 2 years of field experiments as determined by 3-way factorial Anova, as well as separately by year and line due to the presence of significant interactions. WT, wild type.

associated with a highly significant increase in SW, as a result of simultaneous increases in GpS ($P = 0.0667$) and TKW ($P = 0.0040$).

Discussion

Hope *FT-B1* and Photoperiod

The rare *FT-B1* allele identified in the variety Hope offers the opportunity to expand the genetic tools available to breeders to modulate wheat flowering time. This allele was previously shown to be expressed at higher levels than the wild-type allele under LD conditions, resulting in an early flowering phenotype (Yan et al. 2006). In the current study, we demonstrate that the Hope *FT-B1* allele is also expressed at higher levels than the wild-type allele under SD and that this is sufficient for the acceleration of flowering under SD. However, *FT1* expression was detected at a much later stage of the plant's development and to a lower level under SD (Figure 1A), and this was correlated with later flowering under SD than under LD ($P < 0.0001$, Figure 1B). This observation suggests that while the increased expression of the transgenic Hope *FT-B1* allele is sufficient to accelerate flowering in wheat, the mechanisms which result in low *FT1* transcription during SD are not fully eliminated by the insertion of the retroelement in the promoter region of the Hope *FT-B1* allele.

Interaction Between *FT-B1* and Other Flowering Loci

It was previously demonstrated in barley that increased *FT1* copy number, and the associated earlier expression of *FT1*, is sufficient to induce flowering of winter barley varieties in the absence of vernalization (Nitcher et al. 2013). Likewise, previous studies have shown that the transgenic Hope *FT-B1* allele is able to overcome the repressed state of the *VRN1* locus in the winter wheat variety Jagger in the absence of vernalization. The natural Hope *FT-B1* allele was also shown to increase *FT1* transcript levels in spring lines (Yan et al. 2006) but has not been tested before in winter wheat varieties.

In this study, we found that the natural Hope *FT-B1* allele significantly accelerates flowering of plants carrying the *vrn-D1* allele for winter growth habit but had no significant effect in plants carrying the dominant *Vrn-D1a* allele for spring growth habit in the CS background (Figure 2). Similar epistatic interactions have also been reported recently between Hope *FT-B1* and the *VERNALIZATION 4* (*VRN4*) gene, where the dominant *Vrn-D4* and Hope *FT-B1* alleles for early flowering were epistatic to the wild-type alleles for late flowering (Kippes et al. 2014). A possible explanation for these epistatic interactions could be the existence of an upper threshold of *FT1* or *VRN1* expression beyond which further increases in expression do not result in additional acceleration of flowering.

Taken together, these results suggest that the increased expression of the natural Hope *FT-B1* allele is sufficient to overcome the need for vernalization to induce the expression of *vrn1* alleles, and that its effects are smaller in plants that have other dominant alleles for spring growth habit. The

accelerated reproductive development associated with the presence of the Hope *FT-B1* allele in winter varieties under LD and in photoperiod-sensitive varieties under SD may limit the utilization of this allele in winter wheat programs or in those that use photoperiod-sensitive varieties, particularly in regions with long and severe winters.

Effect of the Hope *FT-B1* Allele Under Field Conditions

To determine the potential utility of the Hope *FT-B1* allele to slightly accelerate flowering in spring wheat breeding programs, we evaluated its effect on heading time and yield components in near-isogenic sister lines in 5 spring hexaploid backgrounds and 1 spring tetraploid background under field conditions. Although the overall average effect of the Hope *FT-B1* allele was to accelerate flowering by 2.6 days, the responses were heterogeneous among varieties (Table 2).

It is interesting to point out that the Hope *FT-B1* allele accelerated flowering relative to the wild type by 4.6 days in the Clear White-*vrn-B1* line ($P < 0.0001$) carrying only *Vrn-D1a* but had no effect in the Clear White variety carrying both *Vrn-B1a* and *Vrn-D1a*. Since these 2 lines are isogenic, the differential effect of the Hope *FT-B1* allele on flowering time can be attributed to the additional *Vrn-B1a* allele. The presence of the additional *Vrn-B1a* allele in Clear White accelerates heading time by 3.7 days relative to Clear White-*vrn-B1* (in *FT-B1* wild-type allele background) and may have eliminated any residual vernalization requirement. Following the hypothesis presented above, the resulting *VRN1* transcript levels may have exceeded the threshold beyond which further increases have no additional effect on heading time. As in our epistatic experiment, the Hope *FT-B1* allele was associated with accelerated heading time only within the late heading class (in this case, Clear White-*vrn-B1*). In addition to Clear White-*vrn-B1*, Kern and UC1041 also carry *Vrn-D1a* as the only *Vrn1* allele for spring growth habit. However, whereas Kern shows highly significant differences in heading time between *FT-B1* alleles (3.6 days, $P < 0.0001$), UC1041 showed no significant differences (0.45 days, $P = 0.6970$). This result suggests that additional genetic factors other than *VRN1* can modulate the effects of the *FT-B1* alleles. As an example of the complexity of the regulation of this critical flowering gene, posttranscriptional regulation of *FT1* by microRNAs has been recently reported in *Brachypodium distachyon* (Wu et al. 2013).

Taken together, our results indicate that the differences in heading time associated with the Hope *FT-B1* allele are highly dependent on the genetic background. For example, the introgression of the Hope *FT-B1* allele in winter wheat varieties can result in a spring growth habit and consequently, very large differences in heading time (in nonvernalized plants). Within the spring varieties, the differences in heading time between the *FT-B1* alleles are expected to be smaller than those in the winter NILs. Even within the spring varieties, the Hope *FT-B1* allele is expected to have a larger effect on heading time in those varieties that still have a residual vernalization requirement (e.g., those carrying only the *Vrn-B1a* or the *Vrn-D1a* alleles for early flowering). However, in

lines with identical *Vrn1* alleles, additional unknown genes can modulate the effect of the *FT-B1* alleles, as shown in the *Vrn-D1a* varieties Kern, Clear White-*vrn-B1*, and UC1041 (Table 2). These results indicate that breeders interested in using the Hope *FT-B1* allele to accelerate flowering would need to validate experimentally the effect of this allele in their selected genetic background and environment.

To provide additional information to wheat breeders interested in using the Hope *FT-B1* allele to engineer earlier-flowering varieties, we evaluated the effect of this allele on GpS, TKW, SpS, and spike weight. We selected these traits based on their high heritability (Kisana et al. 1982; Ali et al. 2008; Al-Tabbal and Al-Fraihat 2012) and the possibility to evaluate them with limited amounts of seed. Although these traits are correlated with yield in common wheat and are useful predictors of total grain yield (Sokoto et al. 2012), yield trials with replicated small plots at multiple locations will be required to confirm the positive trends on yield components detected in some of the lines.

Previous studies have shown that *FT1* transcript levels affect wheat spike development by upregulating gibberellin biosynthetic genes in the apical region (Pearce et al. 2013). It would be interesting to investigate if these changes in gibberellin biosynthetic genes are related to the increase in the number of SpS and GpS observed in many of the Hope *FT-B1* lines. However, this general trend should be considered with caution since the yield component traits showed a variable response across lines. Although in most lines we observed a trend toward an increase in SpS, GpS, and SW, the variety Kern showed the opposite trend (Table 3).

The effects on TKW were more variable but interestingly were not always negatively correlated with the differences in grain number. For example, Clear White-*vrn-B1* showed simultaneous increases in GpS and TKW, that when combined in the calculation of SW, resulted in significant increases in the combined Anova across years ($P = 0.0029$, Table 3). In summary, the absence of a consistent negative effect on yield components associated with the Hope *FT-B1* allele is encouraging for the utilization of this allele to subtly accelerate flowering of commercial spring wheat varieties.

To help breeders test the effects of the Hope *FT-B1* allele in their particular environments, the backcross lines developed in this study have been released as publicly available germplasm to the National Small Grains Collections (see Material and Methods for PI numbers). This might be particularly interesting for spring durum wheat breeding program, where the Hope *FT-B1* allele has not been available before.

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