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RESEARCH PAPER

Identification of genomic regions determining the phenological development leading to floral transition in wheat (*Triticum aestivum* L.)

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Abstract

Autumn-seeded winter cereals acquire tolerance to freezing temperatures and become vernalized by exposure to low temperature (LT). The level of accumulated LT tolerance depends on the cold acclimation rate and factors controlling timing of floral transition at the shoot apical meristem. In this study, genomic loci controlling the floral transition time were mapped in a winter wheat (*T. aestivum* L.) doubled haploid (DH) mapping population segregating for LT tolerance and rate of phenological development. The final leaf number (FLN), days to FLN, and days to anthesis were determined for 142 DH lines grown with and without vernalization in controlled environments. Analysis of trait data by composite interval mapping (CIM) identified 11 genomic regions that carried quantitative trait loci (QTLs) for the developmental traits studied. CIM analysis showed that the time for floral transition in both vernalized and non-vernalized plants was controlled by common QTL regions on chromosomes 1B, 2A, 2B, 6A and 7A. A QTL identified on chromosome 4A influenced floral transition time only in vernalized plants. Alleles of the LT-tolerant parent, Norstar, delayed floral transition at all QTLs except at the 2A locus. Some of the QTL alleles delaying floral transition also increased the length of vegetative growth and delayed flowering time. The genes underlying the QTLs identified in this study encode factors involved in regional adaptation of cold hardy winter wheat.

Key words: Cold acclimation, final leaf number, floral transition, low temperature tolerance, QTL, *T. aestivum*, vernalization.

Introduction

The timing of flowering is a key factor in plant adaptation to seasonal changes. This important developmental step in a plant life cycle has evolved in wild species to ensure that seed production and dispersal occur under the most favourable conditions. Winter cereals cultivated in Canada are seeded in the autumn, overwinter as seedlings, and produce seed the following summer. The development towards the reproductive phase in winter cereals can be divided into two stages, where the first step occurs in the autumn when low but non-freezing temperatures (LTs) induce vernalization and cold acclimation processes in the plants. Vernalization causes a developmental switch at the shoot apical meristem, which after weeks of LT exposure ceases to produce leaf primordia and starts to initiate floral primordia. Phytohormones and their interaction with various transcription factors play important roles in the initiation of leaf primordia and subsequent lateral organs at the flanks of the shoot apical meristem (see review by Shani *et al.*, 2006). The timing of floral transition determines the total number of leaf primordia induced, which subsequently affects the total number of leaves developed on the main stem before flowering (Kirby, 1990). During cold acclimation, the plants' ability to withstand freezing temperatures is dramatically increased, and maximum LT tolerance is generally

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Abbreviations: AFLP, amplified fragment length polymorphism; CIM, composite interval mapping; cM, centimorgan; DArT, Diversity Array Technology®; DH, doubled haploid; *eps*, earliness *per se*; FLN, final leaf number; *FT*, *FLOWERING LOCUS T*; LD, long-day; LOD, log-likelihood; LT, low temperature; MIM, multiple interval mapping; QTL, quantitative trait locus; SSR, simple sequence repeat.

obtained at the vernalization saturation point (Fowler *et al.*, 1996*a*). A faster rate and/or a longer time to floral transition increases the total LT tolerance accumulated in crown tissues, and thus enhances the plants' ability to survive winter (Fowler *et al.*, 1996*b*; Mahfoozi *et al.*, 2001). The second stage towards flowering occurs in the spring when warm temperatures and long day (LD) conditions induce development of floral organs and florets.

To improve winter survival in cereals, the timing of floral transition and developmental rate during the vegetative phase of meristem development needs to be optimized. Both internal and environmental cues regulate the switch from the vegetative to reproductive phase in plants. The mechanisms involved in the process have been best understood in the LD dicot *Arabidopsis*. Four pathways, each involving the autonomous flowering, photoperiod response, vernalization requirement, and gibberellic acid metabolism/response genes, are known to regulate flowering time in *Arabidopsis* (see reviews by Bernier and Périlleux, 2005; Bäurle and Dean, 2006; Kobayashi and Weigel, 2007). The signals mediated by these pathways converge at different floral integrators that induce meristem identity genes to promote flowering. Cereals carry genes homologous to those of flowering pathways in *Arabidopsis* (see recent reviews by Cockram *et al.*, 2007; Trevaskis *et al.*, 2007*a*), but limited knowledge exists about their individual role, allelic variation, and interactions.

The vernalization requirement for flowering in winter wheat involves three major factors encoded by *VRN1* (e.g. *Vrn-A1*, *Vrn-B1* and *Vrn-D1*), *VRN2* (e.g. *Vrn-A2*), and *VRN3* (formerly *Vrn-B4* or *Vrn5*) loci as determined by genetic mapping in hexaploid and diploid wheat (reviewed by Laurie *et al.*, 2004; Trevaskis *et al.*, 2007*a*). The three *VRN1* loci on the group 5 chromosomes encode proteins related to the meristem identity APETALA1-like (AP1) MADS-box factors in *Arabidopsis* (Danyluk *et al.*, 2003; Murai *et al.*, 2003; Trevaskis *et al.*, 2003; Yan *et al.*, 2003). Winter wheat lines carry recessive *vrn-A1*, *vrn-B1*, and *vrn-D1* loci, which are induced upon vernalization, whereas spring wheat lines carry at least one dominant *VRN1* allele promoting flowering with little (*Vrn-B1* and *Vrn-D1*) or no (*Vrn-A1*) vernalization. *VRN1* induces flowering competency during the vernalization process and is also implicated in inflorescence meristem development during the second stage towards flowering (Preston and Kellogg, 2008). The vernalization factor VRN2, encoded by a gene located at the end of chromosome arm 5AL in diploid and hexaploid wheat, acts as a floral repressor in non-vernalized plants grown under LD conditions (Yan *et al.*, 2004). VRN3 is encoded from the 7B chromosome in wheat and is homologous to the flowering integrator *FLOWERING LOCUS T* (*FT*) gene in *Arabidopsis* (Yan *et al.* 2006). *FT* in *Arabidopsis* is induced in leaves and encodes a signal that is transported via phloem to the shoot apex where it promotes floral development (Corbesier *et al.*, 2007). The wheat *VRN3* gene, *TaFT*, is induced in leaves under LD conditions and promotes early flowering when overexpressed in winter wheat (Yan *et al.*, 2006). Differences in flowering time are associated with allelic differences for the three homeologous *FT* loci on the group 7 chromosomes (Yan *et al.*, 2006; Bonnin *et al.*, 2008).

The photoperiod insensitivity to LD conditions in wheat is controlled by three dominant loci: *Ppd-A1*, *Ppd-B1*, and *Ppd-D1*, all located on the short arms of group 2 chromosomes (see review by Snape *et al.*, 2001). Most winter wheat cultivars grown in temperate climates carry one or several recessive *ppd* loci to provide enough photosensitivity to delay flower development until the risk of freezing temperatures is overcome in the spring.

The earliness *per se* (*eps*) genes in wheat are presumed to modify flowering time independently of photoperiod and vernalization, and are mainly responsible for the fine tuning of flowering time (Hoogendoorn, 1985). Genes affecting the number and/or the rate of leaf primordia produced on the shoot apex (plastochron) or genes affecting the rate of leaf emergence on the stem (phyllochron) are expected to underlie some of the e*ps* loci in wheat and barley. A number of mutants showing altered plastochron and/or altered phyllotaxy have been identified in plants, and the phenotype for these mutants is often complex where both the vegetative and the reproductive phases are affected. Genes associated with leaf initiation and vegetative phase change in *Arabidopsis* and rice include, for example, a microRNA (miRNA) and its targets, the *SQUAMOSA PROMOTER BINDING PROTEIN*-like (*SPL*) transcripts (Wu and Poethig, 2006; Xie *et al.*, 2006). In addition, RNA-binding proteins such as the TERMINAL EAR 1 in maize (Veit *et al.*, 1998) are involved in leaf initiation, as well as members of a cytochrome P450 family, CYP78A11 in rice (Miyoshi *et al.*, 2004) and CYP78A5/CYP78A7 in *Arabidopsis* (Wang *et al.*, 2008). Although many *eps* loci have been reported for wheat (Snape *et al.*, 2001) and barley (Laurie *et al.*, 1995; Ivandic *et al.*, 2002), no association with phyllochron genes in rice, maize, or *Arabidopsis* has been demonstrated.

In this study traits associated with floral transition, length of vegetative growth, and flowering time have been assessed in a winter wheat doubled haploid (DH) population derived from a Norstar×winter Manitou cross (Båga *et al.*, 2007). The population segregates for LT tolerance and was found to segregate for final leaf number (FLN), days to FLN (dFLN), and days to anthesis (dANT) when grown in both vernalized and non-vernalized environments. Genomic regions affecting the developmental traits were mapped to the Norstar genome.

Materials and methods

Plant material

A DH population of 142 lines derived from a Norstar×winter Manitou cross (Båga *et al.*, 2007) was used in the study. Norstar is an LT-tolerant (LT_{50}=–20.7 °C) winter wheat that produces a relatively high number of leaves before fully vernalized plants will flower (FLN=13)

(Fowler and Limin, 2004). Fully vernalized winter Manitou plants produce ~10 leaves before flowering (FLN=10) and are damaged or killed at warmer temperatures (LT_{50}=–14.3 °C). Winter Manitou (*vrn-A1*, *vrn-B1*, and *vrn-D1*) carries the genetic background of a spring wheat cultivar, Manitou (*Vrn-A1*, *vrn-B1*, and *vrn-D1*), but has the winter growth habit *vrn-A1* allele introgressed from Norstar (Limin and Fowler, 2002). Differences in spring/winter growth habit of Manitou and Norstar are determined by alleles at the *Vrn-A1* locus (Brule-Babel and Fowler, 1988).

Determination of vegetative to flowering transition in controlled environments

Seeds were imbibed in water and held in the dark for 4 d at 4 °C, 1 d at 20 °C, and then planted into 6-inch pots containing Redi-earth (W.R. Grace and Co. of Canada, Ltd, Ajax, ON, Canada). Plants for non-vernalized measurements were grown under a 20 h day and 4 h night at 20 °C. Plants for vernalization were placed in a 20 h day and 4 h night photoperiod at 4 °C for 49 d and then transferred to 20 °C. Plants were uniformly fertilized with 'Osmocote' (Chisso-Asahi Fertilizer Co., Tokyo, Japan) and a nutrient-complete solution ('Tune-up'™, Plant Products Ltd, Brampton, ON, Canada) as required. Leaves were numbered and the plants were grown until the FLN on the main shoot could be determined and until date of anthesis could be recorded. These experiments were repeated three times in time and space, and average values obtained from the three trials were used for quantitative trait locus (QTL) mapping.

QTL analysis

A composite wheat map derived from mapping data of Norstar×winter Manitou and Norstar×Cappelle-Desprez DH populations has been previously reported (Båga *et al.*, 2007). In this study an updated version of the Norstar map was used where 488 Diversity Array Technology® (DArT) markers were added. The DArT genotype data were obtained from 90 Norstar×Winter Manitou and 90 Norstar×Cappelle-Desprez DH lines genotyped by Triticarte Pty Ltd, Australia. The genotype data for the Norstar× winter Manitou population were extended to 142 lines that were fully genotyped using a total of 316 simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers as previously described (Båga *et al.*, 2007). The updated map contained 29 linkage groups, and 977 markers derived from the Norstar×winter Manitou population, and covered 3111 cM. The marker order and distance for SSR and AFLP markers were essentially the same as the published map with a few minor shifts. The chromosome maps for the Norstar×winter Manitou population were used for QTL analysis, and chromosomes related to traits analysed in this study are presented in Fig. 2.

The QGENE software (Nelson, 1997) was used to determine the Pearson's correlation coefficients between trait data. Composite interval mapping (CIM; Zeng, 1994) was performed using the Windows QTL Cartographer 2.5 software (Wang *et al.*, 2007). The CIM standard model with forward stepwise regression and backward elimination, 0.05 probabilities into and out of the model, and a walk speed of 1 cM was used to search for the main QTLs. Threshold log-likelihood (LOD) scores for significant QTLs were determined by tests of 1000 permutations. Multiple interval mapping (MIM) was initiated with identified main QTLs and searching for additional QTLs and epistatic interactions. Since there is no established method to calculate the threshold LOD for MIM mapping, the threshold LOD score (P <0.05) determined by CIM was used as the *ad hoc* critical value for MIM as suggested (Kao *et al.*, 1990).

Results

Trait analysis

A total of 142 DH lines of the Norstar×winter Manitou population were grown under controlled conditions and analysed for FLN, dFLN, and dANT. The traits were assessed on (i) non-vernalized plants grown at 20 °C; and (ii) plants that had undergone full vernalization at 4 °C followed by growth at 20 °C. The mapping population was specially designed to neutralize the effects of the vernalization genes positioned on the group 5 chromosomes (e.g. *vrn-A1*, *vrn-B1*, and *vrn-D1*) to allow detection of loci with minor effects on floral transition time. In addition, the study was conducted under uniform LD conditions to neutralize any differences between parental alleles for the major photoperiod-determining genes.

Analysis of trait data revealed significant ($P \leqslant 0.001$) correlations between time for floral transition (FLN), length of vegetative growth (dFLN), and flowering time (dANT) values in both non-vernalized and vernalized plants (Table 1). However, timing of floral transition was a better predictor of flowering time in non-vernalized plants (r=0.76) as compared with vernalized plants (r=0.36). A comparison of previously determined LT tolerance values determined for the mapping population (Båga *et al.*, 2007) with the trait values in this study revealed a low but significant ($P \leqslant 0.001$) correlation between LT tolerance and FLN in both non-vernalized (r=0.30) and vernalized plants (r=0.32; Table 1). These observations agree with previous cold acclimation studies showing that the timing of floral transition is an important factor that affects LT tolerance levels in winter wheat (Limin and Fowler, 2002).

Assessment of vegetative growth in vernalized and non-vernalized lines

Non-vernalized plants of the cold-hardy parent Norstar reached the flag leaf stage after an average of 133 d, whereas the less cold-hardy winter Manitou plants produced a flag leaf after 90 d. To reach anthesis, Norstar needed an additional 42 days compared with winter Manitou (147 d versus 105 d). Norstar also produced more leaves than winter Manitou before flowering (22 versus 16 leaves). After considering that three leaves on the stem are

derived from the embryo (Kirby and Appleyard, 1987), the average number of new leaves emerging per day was similar for Norstar (0.14) and winter Manitou (0.15), suggesting no major difference in phyllochron when plants were grown without vernalization.

The non-vernalized DH lines of the Norstar×winter Manitou population showed FLN values ranging from 16 to 24 leaves, dFLN varied from 90 d to 147 d, and dANT values were from 103 d to 151 d (Fig. 1). The trait values for the DH lines followed a normal distribution, with slight transgressive segregation towards the Norstar values.

As expected, vernalization of parent and DH lines greatly reduced the length of the vegetative growth phase (Fig. 1). The time to FLN in vernalized Norstar was 29 d whereas only 21 d were required for vernalized winter Manitou to reach the flag leaf stage. On average, 13 leaves emerged in Norstar and 10 leaves in winter Manitou before flowering. In vernalized winter Manitou, anthesis occurred after 33 d, which was 9 d earlier than for vernalized Norstar (42 d). As for the non-vernalized parental lines, a large part of the difference in time to anthesis was due to the difference in the length of the vegetative growth phase. The trait data for the vernalized DH lines showed FLN values that ranged from 10 to 13 leaves, dFLN varied from 21 d to 35 d, and dANT ranged from 31 d to 44 d. Distribution of dFLN values for vernalized plants tended to be skewed toward the Norstar parent.

Genetic mapping of trait data

The data were analysed by CIM to identify chromosomal regions associated with each of the six traits considered in this study. The threshold LOD score for CIM analysis was determined by tests of 1000 permutations and was found to be relatively consistent among the different traits (LOD=3.2–3.3; Table 2). The significant (P <0.05) QTLs identified by CIM are listed in Table 2 and their location on the Norstar consensus map is shown in Fig. 2. The QTLs detected for each trait were tested by MIM, which fitted most QTLs detected by CIM into a model using the CIM

Table 1. Correlation coefficients between trait values determined for the Norstar×winter Manitou DH population

Trait	Vernalized	Abbreviation	FLNnv	dFLNnv	dANTnv	FLNv	dFLNv	dANTv	LTtol
Final leaf number	No	FLNnv		0.83[a]	0.76[a]	0.62[a]	0.40[a]	0.29[a]	0.30[a]
Days to final leaf number	No	dFLNnv			0.89[a]	0.47[a]	0.45[a]	0.34[a]	0.27[b]
Days to anthesis	No	dANTnv				0.54[a]	0.49[a]	0.40[a]	0.28[a]
Final leaf number	Yes	FLNv					0.51[a]	0.36[a]	0.32[a]
Days to final leaf number	Yes	dFLNv						0.89[a]	0.16
Days to anthesis	Yes	dANTv							0.10
Low temperature tolerance (LT_{50} value)[c]	Yes	LTtol							

[a] Significance of Pearson correlation coefficient $P \leqslant 0.001$.
[b] Significance of Pearson correlation coefficient $P \leqslant 0.01$.
[c] From Båga *et al.* (2007).

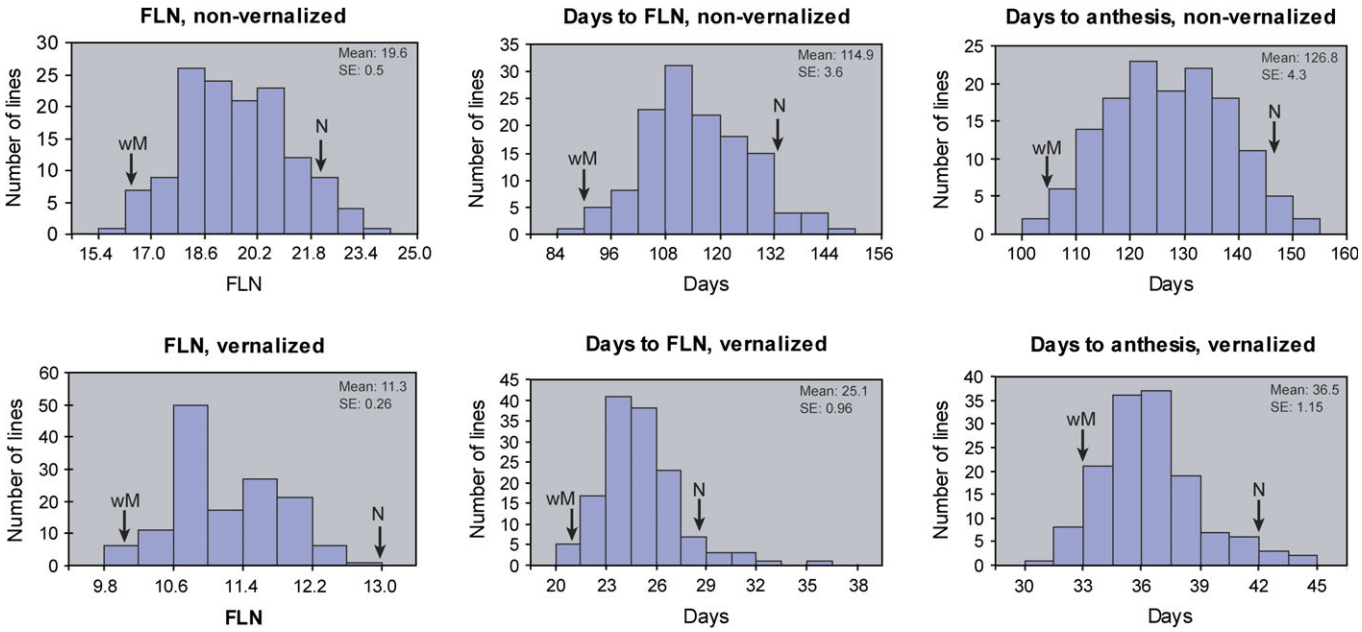

Fig. 1. Frequency distribution of average trait values in the Norstar×winter Manitou DH population. Trait values for parents, Norstar (N), and winter Manitou (wM) are indicated.

Table 2. Estimated genetic effects of QTLs and epistatic interactions for nine traits analysed for the Norstar×winter Manitou population

	CIM mapping					QTL model—MIM mapping[a]					
QTL	**Chromosome region**	**Interval (cM)**	**Peak (cM)**	**LOD**	**Allele**	**Chromosome region**	**Peak (cM)**	**Nearest marker**	**LOD**	**Additive effect[b]**	**Expl.[c] (%)**
*QFLNnv_*	1B.1	62–87	74	8.8	N	1B.1	74	E37M59_134	11.5	0.66	15.8
LOD_{thr}=3.3	2A	84–100	88	4.6	wM	2A	88	wmc206	6.7	–0.48	8.0
	2B.2	84–98	95	5.5	N	2B.2	95	E37M60_145	4.9	0.41	10.9
						5A	88	barc197	3.5	0.34	3.5
	6A	105–135	115	10.1	N	6A	106	barc353	10.9	0.63	17.0
	7A	53–66	58	3.9	N	7A	58	wmc247	5.0	0.41	6.2
						5A×7A			3.8	–0.35	5.9
											Total 67.3
*QdFLNnv_*	1B.1	79–81	79	3.4	N	1B.1	79	wmc419	4.0	3.05	7.5
LOD_{thr}=3.2						1B.2	173	E32M48_118	3.7	–3.15	7.1
	2B.1	57–58	58	3.3	wM	2B.1	58	gwm374	4.7	–3.67	3.0
	2B.2	84–96	95	4.6	N	2B.2	95	wPt-1068	5.3	3.98	10.6
						4A.2	83	gwm494	3.2	3.13	7.9
	6A	106–135	135	4.8	N	6A	134	gwm427	3.3	3.08	9.8
						6D	92	cfd45	3.5	–2.90	6.7
*QdANTnv_*	2B.2	84–99	95	5.5	N						Total 52.6
LOD_{thr}=3.3	4A.1	0–12	4	4.8	N	4A.1	4	wmc173	4.6	4.00	14.0
	6A	105–135	127	5.6	N	6A	127	gwm617	5.6	4.22	15.8
											Total 29.8
*QFLNv_*	1B.1	69–84	74	4.4	N	1B.1	73	E37M59_134	6.6	0.21	11.0
LOD_{thr}=3.2	2A	91–92	91	3.2	wM						
	2B.2	75–100	93	5.3	N	2B.2	85	E37M49_400	4.5	0.17	11.0
	4A.1	3–16	12	3.7	N	4A.1	12	cfa2121	4.5	0.17	8.8
						5A	94	CBF	3.2	0.14	4.9
	6A	105–135	127	5.6	N	6A	127	gwm617	5.9	0.19	12.2
	7A	57–74	63	4.6	N	7A	60	wmc247	4.7	0.17	9.7
											Total 57.6
*QdFLNv_*						1B.1	62	wPt-0260	3.5	0.59	6.6
LOD_{thr}=3.2	2B.2	78–101	94	5.4	N	2B.2	100	wPt-2415	5.3	0.75	14.0
	4A.1	0–13	5	4.4	N						
	4A.2	81–90	86	3.7	N	4A.2	83	gwm494	3.5	0.61	8.4
	6B	68–85	76	5.1	N	6B	78	wmc265	4.9	0.70	8.4
	6D	82–89	88	3.5	wM	6D	85	cfd5	4.4	–0.73	10.7
											Total 56.2
*QdANTv_*	2B.2	89–100	100	6.6	N	2B.2	91	wPt-5680	6.2	0.79	12.9
LOD_{thr}=3.2	3B	21–29	23	3.4	N	3B	29	wPt-5064	3.9	0.63	8.7
						4A.1	2	wmc173	3.4	0.57	6.4
	4A.2	76–100	88	10.4	N	4A.2	88	wmc283	9.2	0.95	18.9
	6B	71–80	76	4.2	N	6B	76	E36M47_81	7.0	0.84	11.2
						7D	88	cfd30	4.1	0.67	5.4
											Total 63.5

[a] The treshold LOD score determined by CIM is used as the cut-off value for assignment of QTLs and interactions in the MIM model
[b] Positive value indicates Norstar allele increased trait value, whereas negative value indicates winter Manitou allele increased trait value.
[c] Percentage trait value explained by QTL or QTL interaction.

threshold LOD as an *ad hoc* cut-off value. Since the threshold LOD for MIM cannot be precisely determined (Kao *et al.*, 1990), the suggested additional QTLs and epistatic interaction detected (Table 2) should be regarded as tentative QTLs until validated by further studies.

Identification of QTLs for FLN

QTLs for FLN in non-vernalized DH lines of the Norstar×winter Manitou population were mapped to five chromosome regions, 1B.1, 2A, 2B.2, 6A, and 7A, by CIM (Fig. 2, Table 2). The increased FLN in the DH population was associated with Norstar alleles at all regions, except for the chromosome 2A locus, *QFLNnv-2A*, where winter Manitou alleles increased FLN. The largest additive effects on FLN were provided by *QFLNnv-1B.1* positioned near the centromere on chromosome 1B and *QFLNnv-6A* located towards the end of chromosome arm 6AL (Fig. 2). All five *QFLNnv* loci identified by CIM could be incorporated into a MIM

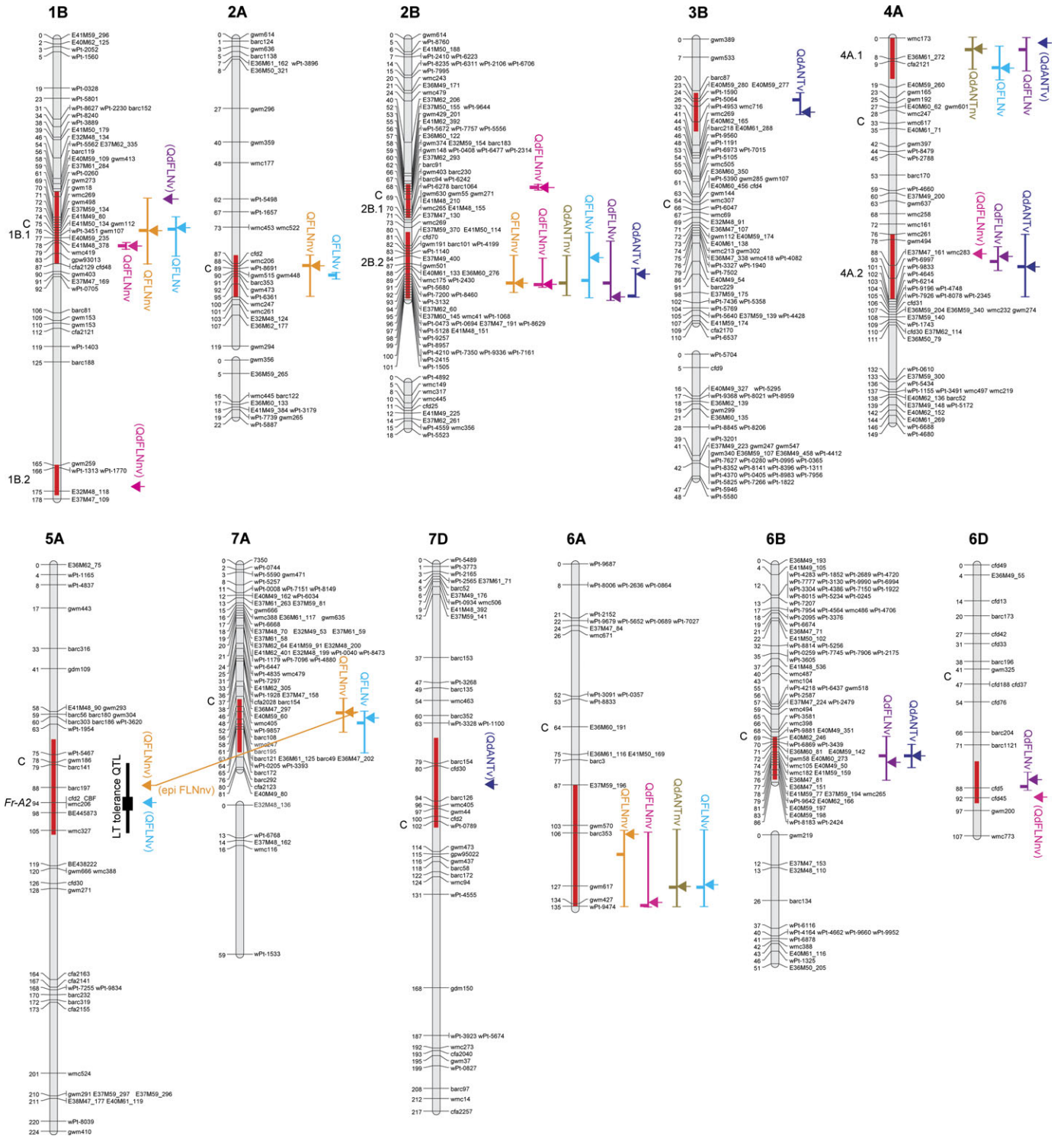

Fig. 2. Genetic map of chromosomes associated with floral transition, length of vegetative growth, and flowering time. Genetic markers scored for the Norstar×winter Manitou population are indicated on the Norstar chromosome maps. The approximate position of the centromere region (C) is shown, and regions carrying QTLs (Table 2) are indicated by a red segment within the chromosome bar. Significant ($P \leqslant 0.05$) QTLs identified by CIM are indicated by a vertical trait bar on the right-hand side of the chromosome. The position of the peak LOD score determined by CIM is indicated by a box on the left side of the trait bar, whereas the position of the QTL peak proposed by MIM is indicated by a horizontal arrow (Table 2). QTLs only suggested by MIM are shown within brackets, and these loci should be regarded as tentative QTLs.

model, which also suggested an additional locus, *QFLNnv-5A*. The *QFLNnv-5A* locus was mapped to the *Fr-A2* locus associated with a main QTL for LT tolerance in winter wheat (Båga *et al.*, 2007). A weak interaction between the *QFLNnv-5A* and 7A locus, *QFLNnv-7A*, was predicted by the MIM model (Table 2; Fig. 2). When this interaction involved a winter Manitou allele, the FLN was reduced by 0.35 leaves and floral transition occurred earlier. The

Norstar alleles at *QFLNnv-5A* or the *QFLNnv-7A* loci individually increased the FLN by 0.34 and 0.41 leaves, respectively, consistent with a delay in floral transition. The QTL model predicted for FLN in non-vernalized plants explained 67.3% of the total variation for the trait.

CIM mapping of FLN in vernalized DH lines identified six significant QTLs on chromosome regions 1B.1, 2A, 2B.2, 4A.1, 6A, and 7A (Table 2; Fig. 2). With the exception of the *QFLNv-4A.1* locus, all QTLs for FLN in vernalized plants coincided reasonably well to QTLs predicted for FLN in non-vernalized plants. The MIM model for FLN in vernalized plants included five of the six significant QTLs and added the chromosome 5A locus, *QFLNv-5A* (Table 2). In contrast to non-vernalized plants, no interaction between *QFLNv-5A* and *QFLNv-7A* was modelled for vernalized plants. All QTLs in the FLNv model were contributed by Norstar alleles, explaining 57.6% of the total variation for FLN.

Identification of QTLs associated with time for vegetative growth

Genomic regions that significantly affected the length of the vegetative phase in non-vernalized plants (dFLNnv) were identified on chromosomes 1B, 2B (two loci), and 6A by CIM mapping (Table 2). The significant QTLs could be incorporated into a MIM QTL model that also added loci on chromosomes 1B (*QdFLNnv_1B.2*), 4A (*QFLNnv_4A.2*), and 6D (*QFLNnv_6D*). Norstar alleles for QTLs on chromosome regions 1B.1, 2B.2, 4A.2, and 6A contributed to a longer vegetative phase, whereas winter Manitou alleles at the 1B.2, 2B.1, and 6D regions reduced the time to FLN. The MIM model for FLNnv explained 52.6% of the phenotypic variation in the population.

Significant QTLs for length of the vegetative phase in vernalized plants (dFLNv) were identified by CIM on chromosome regions 2B.2, 4A.1, 4A.2, 6B, and 6D (Table 2; Fig. 2), but only the 2B.2 region was coincident with QTLs for dFLN in non-vernalized plants. MIM analysis of dFLNv included all QTLs identified by CIM except the *QdFLNv_4A.1* locus, and added the *QdFLNv_1B.1* locus to the model. Norstar alleles at all loci except at the *QdFLNv_6D* locus lengthened the vegetative phase, and the model explained 56.2% of the total variance for dFLNv.

QTLs associated with flowering time

Significant QTLs for flowering time measured by time to anthesis in non-vernalized (dANTnv) DH lines were located to chromosome regions 2B.2, 4A.1, and 6A, where Norstar alleles delayed flowering (Table 2; Fig. 2). Only *QdANT_4A.1* and *QdANT_6A* loci were included into the MIM model, which explained 29.8% of trait values. For vernalized plants, Norstar alleles at chromosome regions 2B.2, 3B, 4A.2, and 6B were found to be significantly correlated with delayed flowering (Table 2). MIM analysis incorporated four significant QTLs and added two suggestive QTLs positioned on chromosome regions 4A.1 and 7D into the model. The MIM model for dANTv explained 63.5% of trait values.

Discussion

Vernalization accelerates flowering in winter wheat and is most effective in young plants, but the treatment can be substituted by plant age (Wang *et al.*, 1995). As demonstrated in this study, winter wheat lines grown without exposure to LT show an extended vegetative growth as compared with vernalized plants and produce a high FLN (Fig. 1). The timing of floral transition is one of the determining factors for LT tolerance in winter wheat (Fowler and Limin, 2004), and genes increasing FLN have potential use for development of lines with enhanced winter hardiness.

The timing of floral transition in non-vernalized and vernalized plants is mainly controlled by common factors

The QTL analysis by CIM presented in this study showed that five genomic regions (1B.1, 2A, 2B.2, 6A, and 7A) significantly affected the number of leaves produced on the main stem of the Norstar×winter Manitou population when grown without vernalization (Fig. 3). The same five regions and a region on chromosome 4A (4A.1) were significantly involved in determining FLN in vernalized plants. The map position of the FLN QTLs suggested that the same genes in vernalized and non-vernalized plants underlie each of the FLN 1B, 2A, 2B.2, 5A, 6A, and 7A.2 QTLs (Fig. 2). Thus, many of the genes controlling floral transition at the shoot apical meristem under normal growth temperature (20 °C) appeared to be involved in regulating floral transition in plants grown at LT (4 °C). As the 4A.1 region was only implicated in FLN for vernalized plants (Table 2; Fig. 3), this suggested that the underlying gene is under strict temperature control.

The difference in FLN observed between lines when grown with and without vernalization reflects the difference in developmental rate affecting the number of leaf primordia produced at the shoot apical meristem before floral transition (Hay and Ellis, 1998). At each growth temperature, the FLN is influenced by the rate of two different processes occurring simultaneously at the shoot apical meristem; the initiation of leaf meristems and the acquisition of flowering competence. Although both processes drive development towards floral transition, they are differentially regulated by temperature. Thus, the variation in FLN seen between genotypes may to a large extent be caused by differences in temperature sensing and/or signalling affecting plastochron and/or vernalization rates. The parent lines of the mapping population used in this study differ in their induction temperature of cold acclimation (Fowler, 2008). Like other LT-tolerant wheat, rye, and barley lines, Norstar starts to cold-acclimate at higher temperatures (~14 °C) as compared with less hardy lines such as winter Manitou (~9 °C). Thus, it is tempting to speculate that pathways involved in induction of cold-regulated genes also play a role in controlling floral transition at the meristem. Components of this

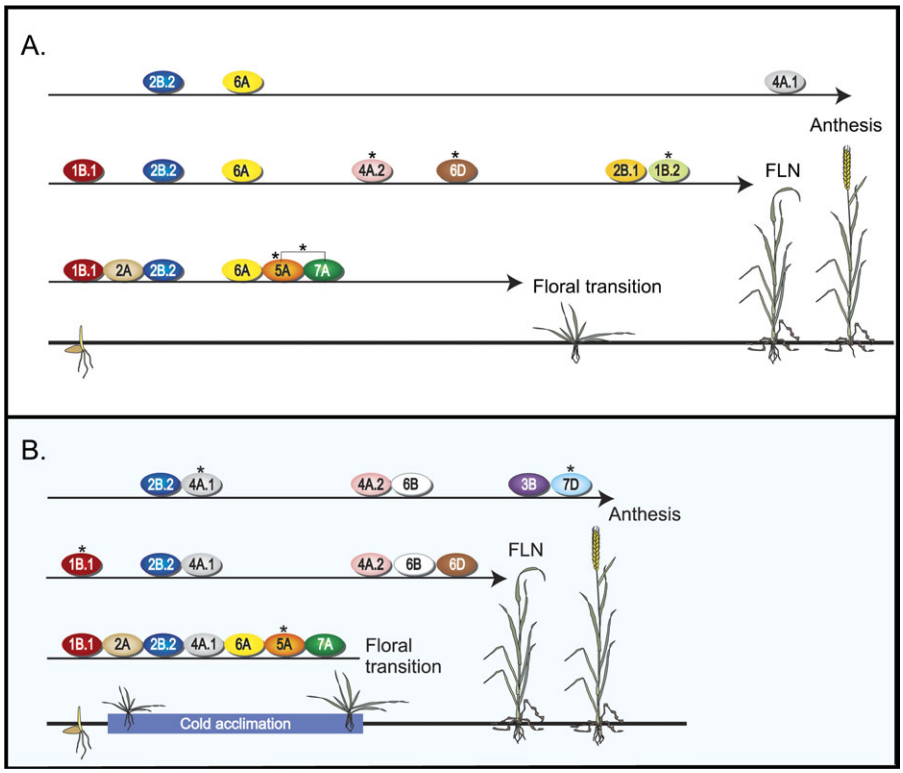

Fig. 3. Schematic illustration of chromosomal regions associated with floral transition, length of vegetative growth, and flowering time. Chromosomal regions (Fig. 2) influencing time to floral transition at the shoot apical meristem (*QFLN*), length of vegetative growth (*QdFLN*), and time to anthesis (*QdANT*) in the Norstar×winter Manitou DH lines grown under non-vernalized (A) and vernalized (B) controlled conditions. The illustration shows all significant QTLs identified by CIM, and possible QTLs only identified by MIM are indicated by an asterisk.

temperature-regulated pathway are encoded from the *Fr-A2* region on chromosome 5A (Fig. 2), which carries the major QTL for LT tolerance explaining >50% of LT tolerance in the Norstar×winter Manitou population (Båga *et al.*, 2007). Involvement of the *Fr-A2* region in floral transition was suggested by the MIM model for FLN, which predicted a role for the *QFLN_5A* locus in both non-vernalized and vernalized plants (Table 2).

In rice, three *PLASTOCHRON* genes that appear to act redundantly in apical meristem maintenance have been identified. The *PLA1* locus encodes a cytochrome P450 protein, CYP78A11, associated with both leaf and internode growth and is suggested to regulate plant hormone levels (Miyoshi *et al.*, 2004). The *PLA2* gene encodes an RNA-binding protein produced in the leaf primordia where it slows down leaf maturation (Kawakatsu *et al.*, 2006), and the *PLA3* locus codes for a glutamate decarboxylase II with similarity to a human *N*-acetyl α-linked acidic dipeptidase (Kawakatsu *et al.*, 2009). It remains to be determined if any of the QTLs identified in the present study correspond to plastochron loci identified in rice.

FLN loci affecting the time for vegetative growth and flowering time

Many QTL alleles increasing FLN could be associated with increased length of vegetative growth and delayed flowering time (Fig. 3). The most consistent association between the three traits was found for the 2B.2 region positioned below the centromere on chromosome 2B (Fig. 2). The position of this locus suggests that it corresponds to *eps* loci mapped to the 2BL chromosome arm in different wheat (Worland, 1996; Hanocq *et al.*, 2004) and barley (Laurie *et al.*, 1995; Boyd *et al.*, 2003) populations.

Another locus that controlled FLN, dFLN, and dANT was positioned within the 6A QTL region, where Norstar alleles increased the values of all three traits in non-vernalized plants, but only increased FLN in vernalized plants (Table 2; Fig. 3). In vernalized plants, it was noted that Norstar alleles at the *QdANTv_6B* locus mapped close to the chromosome 6B centromere (Fig. 2) delayed flowering. The map positions of *QdANTnv_6A* and *QdANTv_6B* loci controlling flowering time in non-vernalized and vernalized plants, respectively, suggest that they correspond to flowering loci *eps6L.1* and *eps6L.2* identified in barley (Laurie *et al.*, 1995). The presence of ear emergence loci on group 6 chromosomes in wheat is known from studies of Chinese Spring diteleosomic lines (Islam-Faridi *et al.*, 1996), in which increased dosages of group 6 flowering alleles delay flowering. Thus, it has been speculated that the group 6 chromosomes encode flowering inhibitor(s) that are inactivated by vernalization (Islam-Faridi *et al.*, 1996). Whether any of the group 6 QTLs identified in this study are regulated by vernalization remains to be determined,

but their different roles in non-vernalized and vernalized plants suggest this may be the case.

A flowering time locus in vernalized plants was suggested by MIM modelling to be positioned close to the centromere on chromosome 7D (*QdANTv_7D*; Table 2; Fig. 2). This locus maps close to flowering time QTLs mapped in wheat by Huang *et al.* (2004) and Cuthbert *et al.* (2008). A recent study has shown a correlation between differences in flowering time and polymorphism for *FT* loci encoded from the group 7 chromosomes (Bonnin *et al.*, 2008). The *TaFTD* allele is positioned ~10 cM distal to LOD peaks for QTLs associated with flowering time in the present study (Fig. 2), and thus could underlie *QdANTv_7D*. A gene encoding an activator of *FT* in barley, *HvCO1*, has also been mapped close to the chromosome 7H centromere (Griffiths *et al.*, 2003). Another candidate gene for the 7D flowering time locus is the floral repressor gene, *TaVRT-2* (Kane *et al.*, 2005), which has been mapped ~10 cM above the 7H centromere in barley (Szücs *et al.* 2006). The barley gene, *HvVRT-2*, inhibits floral meristem identity and is expected to have a role in repressing floral development during the winter (Trevaskis *et al.*, 2007*b*). Additional studies are needed to determine if any of candidate genes for *QdANTv_7D*, *TaFTD*, *CONSTANS*, and/or *TaVRT-2* are involved in controlling flowering time in the Norstar× winter Manitou population.

Putative phyllochron loci in wheat

The 4A.2, 6B, and 6D regions in vernalized plants showed a large effect on the time for vegetative growth, but did not have a significant influence on floral transition time (Fig. 3). Thus, these regions appear to carry genes that affect only the phyllochron, which is influenced by photoperiod and light quality (far/red ratio) in addition to ambient growth temperature. Differences in light perception and/or signalling between parent lines may underlie some of the putative phyllochron loci in the present study.

Several recent studies have implicated RNA splicing factors and various small mobile RNAs including miRNA in the regulation of floral transition, vegetative growth, and flowering time in plants, primarily from studies in *Arabidopsis* (see recent reviews by Bäurle and Dean, 2006; Terzi and Simpson, 2008), maize (Wu and Poethig, 2006), and rice (Xie *et al.*, 2006). Very little is known about small RNA action in wheat, although miRNAs known to target transcripts encoding floral pathway integrators, AP2-like floral repressors, and flowering-inducing SQUAMOSA PROMOTER BINDING PROTEIN-like factors are known to be produced (Yao *et al.*, 2007). Thus, small RNAs and splicing activities are likely to control traits analysed in this study and will be the focus of further studies aimed at identifying genes causing variation for developmental traits in the Norstar×winter Manitou population.

Practical implications of the study

Neutralization of both the vernalization and the photoperiod requirements in the experimental design of this study allowed for detection of loci with minor effects on the timing of floral transition in winter wheat. Norstar alleles at the five genomic regions 1B.1, 2B.2, 4A.1, 6A, and 7A and winter Manitou alleles at the chromosome 2A region were all associated with delay of floral transition in vernalized plants (Figs 2, 3). The timing of floral transition has a direct influence on the accumulation and maintenance of LT tolerance and the length of vegetative growth and the timing of flowering. Identification of genes underlying these QTLs will reveal important factors involved in regional adaptation of winter wheat.

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References

Båga M, Chodaparambil SV, Limin AE, Pecar M, Fowler DB, Chibbar RN. 2007. Identification of quantitative trait loci and associated candidate genes for low-temperature tolerance in cold-hardy winter wheat. *Functional and Integrative Genomics* **7,** 53–68.

Bäurle I, Dean C. 2006. The timing of developmental transitions in plants. *Cell* **125,** 655–664.

Bernier G, Périlleux C. 2005. A physiological overview of the genetics of flowering time control. *Plant Biotechnology Journal* **3,** 3–16.

Bonnin I, Rousset M, Madur D, Sourdille P, Dupuits C, Brunel D, Goldringer I. 2008. FT genome A and D polymorphisms are associated with the variation of earliness components in hexaploid wheat. *Theoretical and Applied Genetics* **116,** 383–394.

Boyd WJR, Li CD, Grime CR, *et al*. 2003. Conventional and molecular genetic analysis of factors contributing to variation in the timing of heading among spring barley (*Hordeum vulgare* L.) genotypes grown over a mild winter growing season. *Australian Journal of Agricultural Research* **54,** 1277–1301.

Brule-Babel AL, Fowler DB. 1988. Genetic control of cold hardiness and vernalization requirement in winter wheat. *Crop Science* **28,** 879–884.

Cockram J, Jones H, Leigh FJ, O'Sullivan D, Powell W, Laurie DA, Greenland AJ. 2007. Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. *Journal of Experimental Botany* **58,** 1231–1244.

Corbesier L, Vincent C, Jang S, *et al*. 2007. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science* **316,** 1030–1033.

Cuthbert JL, Somers DJ, Brûlé-Babel AL, Brown PD, Crow GH. 2008. Molecular mapping of quantitative trait loci for yield and yield components in spring wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* **117,** 595–608.

Danyluk J, Kane NA, Breton G, Limin AE, Fowler DB, Sarhan F. 2003. *TaVRT-1*, a putative transcription factor associated with vegetative to reproductive transition in cereals. *Plant Physiology* **13,** 1849–1860.

Fowler DB. 2008. Cold acclimation threshold induction temperatures in cereals. *Crop Science* **48,** 1147–1154.

Fowler DB, Chauvin LP, Limin AE, Sarhan F. 1996*b*. The regulatory role of vernalization in the expression of low-temperature-induced genes in wheat and rye. *Theoretical and Applied Genetics* **93,** 554–559.

Fowler DB, Limin AE. 2004. Interactions among factors regulating phenological development and acclimation rate determine low-temperature tolerance in wheat. *Annals of Botany* **94,** 717–724.

Fowler DB, Limin AE, Wang S-Y, Ward RW. 1996*a*. Relationship between low-temperature tolerance and vernalization response in wheat and rye. *Canadian Journal of Plant Science* **76,** 37–42.

Griffiths S, Dunford RP, Coupland G, Laurie DA. 2003. The evolution of *CONSTANS*-like gene families in barley, rice, and Arabidopsis. *Plant Physiology* **131,** 1855–1867.

Hanocq E, Niarquin M, Heumez E, Rousset M, Le Gouis J. 2004. Detection and mapping of QTL for earliness components in a bread wheat recombinant inbred lines population. *Theoretical and Applied Genetics* **110,** 106–115.

Hoogendoorn J. 1985. A reciprocal F_1 monosomic analysis of the genetic control of time of ear emergence, number of leaves and number of spikelets in wheat (*Triticum aestivum* L.). *Euphytica* **34,** 545–558.

Huang XQ, Kempf H, Ganal MW, Röder MS. 2004. Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* **109,** 933–943.

Islam-Faridi MN, Worland AJ, Law CN. 1996. Inhibition of ear-emergence time and sensitivity to day-length determined by the group 6 chromosomes of wheat. *Heredity* **77,** 572–580.

Ivandic V, Hackett CA, Nevo E, Keith R, Thomas WTB, Forster BP. 2002. Analysis of simple sequence repeats (SSRs) in wild barley from the Fertile Crescent: associations with ecology, geography and flowering time. *Plant Molecular Biology* **48,** 511–527.

Kane NA, Danyluk J, Tardif G, Quellet F, Laliberté J-F, Limin AE, Fowler DB, Sarhan F. 2005. *TaVRT-2*, a member of the *St*MADS-11 clade of flowering repressors, is regulated by vernalization and photoperiod in wheat. *Plant Physiology* **138,** 2354–2363.

Kao C-H, Zeng Z-B, Teasdale RD. 1990. Multiple interval mapping for quantitative trait loci. *Genetics* **152,** 1203–1216.

Kawakatsu T, Itoh J-I, Miyoshi K, Kurata N, Alvarez N, Veit B, Nagato Y. 2006. *PLASTOCHRON2* regulates leaf initiation and maturation in rice. *The Plant Cell* **18,** 612–625.

Kawakatsu T, Taramino G, Itoh J-I, *et al*. 2009. *PLASTOCHRON3/GOLIATH* encodes a glutamate carboxypeptidase required for proper development in rice. *The Plant Journal* Epub ahead of print. PMID 19228340.

Kirby EJM. 1990. 1990 Co-ordination of leaf emergence and leaf and spikelet primordium initiation in wheat. *Field Crops Research* **25,** 253–264.

Kirby EJM, Appleyard M. 1987. *Cereal development guide*, 2nd edn. Stoneleigh, Kenilworth, UK: Arable Unit, National Agricultural Centre.

Kobayashi Y, Weigel D. 2007. Move on up, it's time for change—mobile signals controlling photoperiod-dependent flowering. *Genes and Development* **21,** 2371–2384.

Laurie DA, Griffiths S, Dunford RP, Christodoulou V, Taylor SA, Cockram J, Beales J, Turner A. 2004. Comparative genetic approaches to the identification of flowering time genes in temperate cereals. *Field Crops Research* **90,** 87–99.

Laurie DA, Pratchett N, Bezant JH, Snape JW. 1995. RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter×spring barley (*Hordeum vulgare* L.) cross. *Genome* **38,** 575–585.

Limin AE, Fowler DB. 2002. Developmental traits affecting low-temperature tolerance response in near-isogenic lines for the vernalization locus *Vrn-A1* in wheat (*Triticum aestivum* L. em Thell). *Annals of Botany* **89,** 579–585.

Mahfoozi S, Limin AE, Fowler DB. 2001. Influence of vernalization and photoperiod responses on cold hardiness in winter cereals. *Crop Science* **41,** 1006–1011.

Miyoshi K, Ahn B-O, Kawakatsu T, Ito Y, Itoh J-I, Nagato Y. 2004. *PLASTOCHRON1*, a timekeeper of leaf initiation in rice, encodes cytochrome P450. *Proceedings of the National Academy of Sciences, USA* **101,** 875–880.

Murai K, Miyamae M, Kato H, Takumi S, Ogihara Y. 2003. *WAP1*, a wheat *APETALA1* homolog, plays a central role in the phase transition from vegetative to reproductive growth. *Plant and Cell Physiology* **44,** 1255–1265.

Nelson JC. 1997. QGENE: software for marker-based genomic analysis and breeding. *Molecular Breeding* **3,** 239–245.

Preston JC, Kellogg EA. 2008. Discrete developmental roles for temperate cereal grass *VERNALIZATION1/FRUITFULL*-like genes in flowering competency and the transition to flowering. *Plant Physiology* **146,** 265–276.

Shani E, Yanai O, Ori N. 2006. The role of hormones in shoot apical meristem function. *Current Opinion in Plant Biology* **9,** 484–489.

Snape JW, Butterworth K, Whitechurch E, Worland AJ. 2001. Waiting for fine times: genetics of flowering time in wheat. *Euphytica* **119,** 185–190.

Szücs P, Karsai I, von Zitzewitz J, Mészáros K, Cooper LLD, Gu YQ, Chen THH, Hayes PM, Skinner JS. 2006. Positional relationships between photoperiod response QTL and photoreceptor and vernalization genes in barley. *Theoretical and Applied Genetics* **112,** 1277–1285.

Terzi LC, Simpson GG. 2008. Regulation of flowering time by RNA processing. *Current Topics in Microbiology and Immunology* **326,** 201–218.

Trevaskis B, Bagnall DJ, Ellis MH, Peacock WJ, Dennis ES. 2003. MADS box genes control vernalization-induced flowering in cereals. *Proceedings of the National Academy of Sciences, USA* **100,** 13099–13104.

Trevaskis B, Hemming MN, Dennis ES, Peacock WJ. 2007*a*. The molecular basis of vernalization-induced flowering in cereals. *Trends in Plant Science* **12,** 352–357.

Trevaskis B, Tadege M, Hemming MN, Peacock WJ, Dennis ES, Sheldon C. 2007*b*. *Short vegetative phase*-like MADS-box genes inhibit floral meristem identity in barley. *Plant Physiology* **143,** 225–235.

Veit B, Briggs SP, Schmidt RJ, Yanofsky MF, Hake S. 1998. Regulation of leaf initiation by the *terminal ear 1* gene of maize. *Nature* **393,** 166–168.

Wang J-W, Schwab R, Czech B, Mica E, Weigel D. 2008. Dual effects of miR156-targeted *SPL* genes and *CYP78A5/KLUH* on plastochron length and organ size in *Arabidopsis thaliana*. *The Plant Cell* **20,** 1231–1243.

Wang S, Basten CJ, Zeng Z-B. 2007. *Windows QTL Cartographer 2.5*. Raleigh, NC: Department of Statistics, North Carolina State University (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Wang S-Y, Ward RW, Ritchie JT, Fischer RA, Schulthess U. 1995. Vernalization in wheat. I. A model based on the interchangeability of plant age and vernalization duration. *Field Crops Research* **41,** 91–100.

Worland AJ. 1996. The influence of flowering time genes on environmental adaptability in European wheats. *Euphytica* **89,** 49–57.

Wu G, Poethig RS. 2006. Temporal regulation of shoot development in *Arabidopsis thaliana* by *miR156* and its target *SPL3*. *Development* **133,** 3539–3547.

Xie K, Wi C, Xiong L. 2006. Genomic organization, differential expression, and interaction of SQUAMOSA promoter-binding-like transcription factors and microRNA156 in rice. *Plant Physiology* **142,** 280–293.

Yao Y, Guo G, Ni Z, Sunkar R, Du J, Zhu J-K, Sun Q. 2007. Cloning and characterizaion of microRNAs from wheat (*Triticum aestivum* L.). *Genome Biology* **8,** R96.

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J. 2003. Positional cloning of the wheat vernalization gene *VRN1*. *Proceedings of the National Academy of Sciences, USA* **100,** 6263–6268.

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J. 2004. The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science* **303,** 1640–1644.

Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, Sanchez A, Valarik M, Yasuda S, Dubcovsky J. 2006. The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. *Proceedings of the National Academy of Sciences, USA* **103,** 19581–19586.

Zeng Z-B. 1994. Precision mapping of quantitative trait loci. *Genetics* **136,** 1457–1468.