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ORIGINAL PAPER

Genetic loci in the photoperiod pathway interactively modulate reproductive development of winter wheat

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Abstract Responses to photoperiod and low temperature are the two primary adaptive mechanisms which enable wheat plants to synchronize developmental processes with changes in seasonal climate. In this study, the developmental process was characterized at two stages: stem length during the onset of stem elongation and heading date. These two developmental events were monitored and mapped in recombinant inbred lines (RILs) of a population generated from a cross between two complementary and locally adapted hard winter wheat cultivars. 'Intrada' undergoes stem elongation earlier but reaches heading later, whereas 'Cimarron' undergoes stem elongation later but reaches heading earlier. Variation in the developmental process in this population was associated with three major QTLs centered on *Xbarc200* on chromosome 2B, *PPD-D1* on chromosome 2D, and *Xcfd14* on chromosome 7D. The Intrada *Xbarc200* and *Xcfd14* alleles and the Cimarron *PPD-D1* allele accelerated both stem elongation and heading stages, or the Cimarron *Xbarc200* and *Xcfd14* alleles and the Intrada *PPD-D1* allele delayed both stem elongation and heading stages. Integrative effects of the three QTLs accounted for 43% (initial stem length) and 68% (heading date) of the overall phenotypic variation in this population. *PPD-D1* is a reasonable candidate gene for the QTL on chromosome 2D, *PPD-B1* could be associated with the QTL on chromosome 2B, but *VRN-D3* (=*FT-D1*) was not linked with the QTL on chromosome 7D, suggesting that this is a novel locus involved in winter wheat development. Because the *PPD-D1* QTL was observed to interact with other two QTLs, all of these QTLs could play a role in the same pathway as involved in photoperiod response of winter wheat.

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Introduction

Responses to temperature and photoperiod are two the key factors which enable synchrony of wheat development with changes in seasonal climate (Angus et al. 1981; Baker et al. 1986; Fowler et al. 2001; Kirby et al. 1999; Laurie 1997; Laurie et al. 1995; Snape et al. 2001a, b). For a winter wheat cultivar that requires a period of low temperature to accelerate the ability to flower—a process known as vernalization (Amasino 2005)—temperature plays a critical role in the rate of reproductive development relative to a spring wheat cultivar that has no vernalization requirement (Flood and Halloran 1986; Griffiths et al. 2003; Hemming et al. 2008; Kane et al. 2005; Pugsley 1972). When the vernalization requirement in any winter wheat cultivar has been satisfied, its reproductive developmental rate will be mainly affected by genes in the photoperiod pathway, as it occurs in spring wheat (Beales et al. 2007; Snape et al. 2001a). To attain a vernalization saturation point, nearly all contemporary winter wheat cultivars in the US southern Great Plains are a winter wheat type that may require exposure for 2–6 weeks to minimum air temperatures of 2–8°C; except for some years in extreme southern areas such as south Texas, this range of vernalization requirements is met under natural field conditions (Berry et al. 1980; Wang et al. 1995a, b). Even with the fulfilled vernalization requirement, wide variation in the timing of stem elongation and heading is often observed among winter wheat cultivars across years (Edwards et al. 2007), implying that photoperiod genes

may play a major role in regulating the developmental process in winter wheat cultivars.

Stem elongation, prior to jointing, precisely represents the timing of transition from vegetative to reproductive development as it occurs immediately following the transition but prior to heading, a trait that is usually used to describe the effect of vernalization or photoperiod on the developmental transition (Chen et al. 2009). An earlier stem elongation stage is desirable for lengthening the floret developmental phase to increase the number of fertile florets (Goncharov 2003), whereas a later stem elongation stage is needed to avoid late-winter freeze and early spring frost injuries, produce more forage resource in the dual-purpose system (Redmon et al. 1996). Initiation of both stem elongation and heading represents critical agronomic traits for evaluating adaptation to dual-purpose or grain-only wheat production systems in the southern Great Plains (Carver et al. 2001; Manupeerapan et al. 1992). Depending on the investigator and/or application, stem elongation may be defined as when the first internode on the main stem reaches 1.5 cm in length, also called first-hollow-stem stage (Redmon et al. 1996), or as the appearance of the first node one inch (2.54 cm) above the soil surface, also called jointing stage (McMaster 2009). Heading date is defined when the plant spike emerges above the collar of the flag leaf and first becomes visible above the canopy (McMaster 2009). Non-precocious initiation of stem elongation with minimal delay of heading is a desirable phenotype in locales where sowing date is often adjusted to accommodate the possibility of grazing and grain production on the same crop.

An extended vegetative period, i.e., delayed initiation of stem elongation, provides an obvious benefit to a dual-purpose management system via longer grazing duration (Khalil et al. 2002). Later onset of stem elongation, however, could result in a later heading date that may expose the wheat grain-filling stage to greater risk of drought and heat-stressed conditions and hence loss of grain yields (Edwards et al. 2007). Precise control of this developmental progression by means of specific combinations of genes and their alleles controlling respective developmental events is necessary to optimize the timing of stem elongation and heading for maximum grain yield (Carver et al. 2001; Holliday 1956; Krenzer 2000; MacKown and Carver 2005).

Genetic factors in the vernalization and photoperiod pathways have been extensively studied in wheat (Hay and Ellis 1998; Laurie et al. 1995; Law and Worland 1997; Snape et al. 2001b). Three vernalization genes, *VRN1* (Yan et al. 2003), *VRN2* (Yan et al. 2004b), and *VRN3* (Yan et al. 2006), have been cloned from diploid wheat and barley using a positional cloning strategy. Winter growth habit in hexaploid wheat is mainly attributed to a recessive allele *vrn-1* at all of three orthologous loci, for which expression is induced by vernalization to accelerate flowering (Danyluk et al. 2003; Fu et al. 2005; Loukoianov et al. 2005; Murai et al. 2003; Trevaskis et al. 2003; Yan et al. 2004a). *VRN1* was isolated based on variation in vernalization requirement between spring and winter wheat, but it was recently found linked to a major locus *QSte.osu-5A* that was mapped associated with variation in initial stem length in a population of recombinant inbred lines (RILs) generated from two winter wheat cultivars (Chen et al. 2009). Using the marker for *QSte.osu-5A*, however, no allelic variation was found between two locally adapted winter wheat cultivars, 'Intrada' and 'Cimarron', even though their reproductive development patterns have been known to differ. Intrada undergoes stem elongation earlier but reaches heading later, whereas Cimarron undergoes stem elongation later but reaches heading earlier. The expected segregation for these two traits in their progeny population has provided an excellent opportunity to map new genetic loci (excluding *QSte.osu-5A*) involved in reproductive development in winter wheat cultivars. In the present study, we report that variation in stem elongation initiation and heading date between Cimarron and Intrada is controlled by three major QTLs, one associated directly with *PPD-D1* and another two interacting with the *PPD-D1* QTL.

Materials and methods

Plant materials

A population of 115 F_7-derived RILs was developed from the cross Intrada/Cimarron by the single-seed descent method. Intrada and Cimarron are both winter wheat cultivars released by Oklahoma State University, USA in 1990 and 2000, respectively. Cimarron was bred in a typical grain-only breeding program, and its pedigree is 'Payne'*2/CO725052. Intrada was selected for better adaptation to a dual-purpose management system, and its pedigree is 'Rio Blanco'/'TAM 200' (Carver et al. 2003).

Phenotypic data collection

Field experiments were carried out at the Oklahoma State University Agronomy Research Station, Stillwater, OK, for 3 years from 2005 to 2007. All 115 RILs and both parents were planted in a replicates-in-sets experimental design, with a plot size of 1.3 × 3.0 m, in late September each year. Plots were managed according to local recommendations for dual-purpose wheat production (Royer and Krenzer 2000). The seeds were planted within 1 week around 20 September of each year. The initial internode length was measured during 1–10 March, depending on when all of the RILs had produced 1.5 cm hollow stem. Heading dates were recorded during the middle of April. The day length at

stem elongation and heading time in the experimental sites was about 11.5 and 13 h, respectively. As the experiments were performed with local cultivars and in the field, the requirement of the plants for vernalization and photoperiod could have been satisfied.

The initial internode length was measured using the methods previously described by Redmon et al. (1996), Krenzer (2000), and Chen et al. (2009). Approximately, 20 plants of each line from the field plot were sampled and separated. Ten largest main stem or tillers were measured for the length between the crown and the end of the hollow stem. Longer hollow stem corresponded to earlier onset of stem elongation. Heading date of each line was scored when approximately 50% of the heads had emerged completely from the boot.

A subset of 94 RILs and the two parental lines were first used for mapping to match the number of samples limited to a plate with 96 wells. Once a QTL was located on specific chromosomes, more SSR markers were added to increase marker density by comparison with consensus maps in GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml). Finally, out of 935 SSR markers, 241 polymorphic markers were mapped in the population.

PCR reactions for SSR markers were performed in a touchdown PCR program (Xu et al. 2005) with these modifications: 94°C for 5 min, 5 cycles of 94°C for 45 s, 68°C decreasing to 60°C for 5 min, and 72°C for 1 min, 5 cycles of 94°C for 45 s, 58°C decreasing to 50°C for 5 min, and 72°C for 1 min, 30 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 30 s, followed by 10 min of 72°C final extension. PCR products were separated on 6.5% acrylamide/bisacrylamide (19:1) gel at 350 V for 3–4 h. Gels were stained with ethidium bromide, and polymorphic bands were scored according to allelic patterns of the two parental lines.

A single nucleotide polymorphism (SNP) in EST clone BF201235 was mapped to increase marker density on chromosome 2B where a QTL was found. BF201235 is in bin 2BS3-0.84–1.00, based on the Wheat SNP Database (http://wheat.pw.usda.gov/SNP/new/index.shtml). Forward primer BF201235-F3 5′-GGAGTTTGAGAACGCCAGAG-3′ and reverse primer BF201235-R3 5′-GGGTGGTCGTAGTCTGATGA-3′ were used to amplify genomic DNA fragments from Intrada and Cimarron. The PCR amplification consisted of 94°C for 5 min, 40 cycles of 94°C for 45 s, 55°C for 1 min, and 72°C for 45 s, and 72°C 10 min for final extension. The PCR products were purified and directly sequenced.

The sequences of three photoperiod *PPD1* genes in wheat were determined based on the orthologous *PPD-H1* gene in barley (Beales et al. 2007). A forward primer PPD-D1_F and the mixture of two reverse primers PPD-D1_R1 and PPD-D1_R2 were used to amplify genomic fragments of *PPD-D1* from Intrada and Cimarron. Six pairs of primers specific to *PPD-B1* were designed to cover the complete *PPD-B1* gene, but no polymorphism was found between the two parental lines (data not shown). No attempt was made to isolate *PPD-A1*, since no QTL was mapped on chromosome 2A in the population.

A pair of primers, forward primer VRN-D3-F6 5′-CTTCTATTCACATGTTTCGTTCATG-3′ and reverse primer VRN-D3-R8 5′-ACGAGCACGAAGCGATGGATCGC-3′, was used to specifically amply *VRN-D3* (Chen and Yan, unpublished data). The PCR products were directly digested with restriction enzyme *Nco*I, and the Intrada *VRN-D3* allele showed 375 and 27-bp bands, whereas the Cimarron *VRN-D3* allele showed a 401-bp band. The marker for *VRN-D3* was used to map in 96 RILs of the Intrada × Cimarron population.

Linkage map construction and QTL discovery

In order to simplify the process to find possible genetic linkage groups, polymorphic SSR markers were tentatively categorized by their chromosomal locations published on GrainGenes. Markers were grouped and ordered via MapMaker/EXP 3.0b. Mapped markers were then employed as "anchor" markers to search for all markers using the "assign" command. Assigned markers were grouped and ordered again until all possible markers in a linkage group had been precisely positioned. Map distance was based on the Kosambi function.

For the QTL analysis, the association between a given trait and the presence/absence of polymorphic SSR markers was first analyzed by PROC CORR using the 'WITH' statement of SAS (SAS Institute, Raleigh, NC), rather than by a one-way analysis of variance; no linkage information was required and all computations could be realized in a single procedure. Intrada- and Cimarron-alleles were assigned values of '1' and '2', respectively, for the correlation analysis. Markers with a large coefficient of correlation were given higher priority in mapping and QTL analysis. Subsequently, interval mapping (IM) was conducted by Windows QTL Cartographer version 2.5 (North Carolina State University, Raleigh, NC, USA). Intervals of 1–2 cM were chosen to walk across a certain chromosome. A QTL was claimed if its LOD value exceeded 2.5, a default threshold that is used for QTL analysis.

All major QTLs declared for a trait by IM were set in the initial model in multiple interval mapping (MIM) to assess proportions of the total phenotypic variation explained by these QTLs for each year. Interaction effects were searched and further tested for significance using the default BIC criterion with $c(n)=\log(n)$. Non-significant terms were removed from the final model. Combined effects of all QTLs were represented by multiple R^2 values.

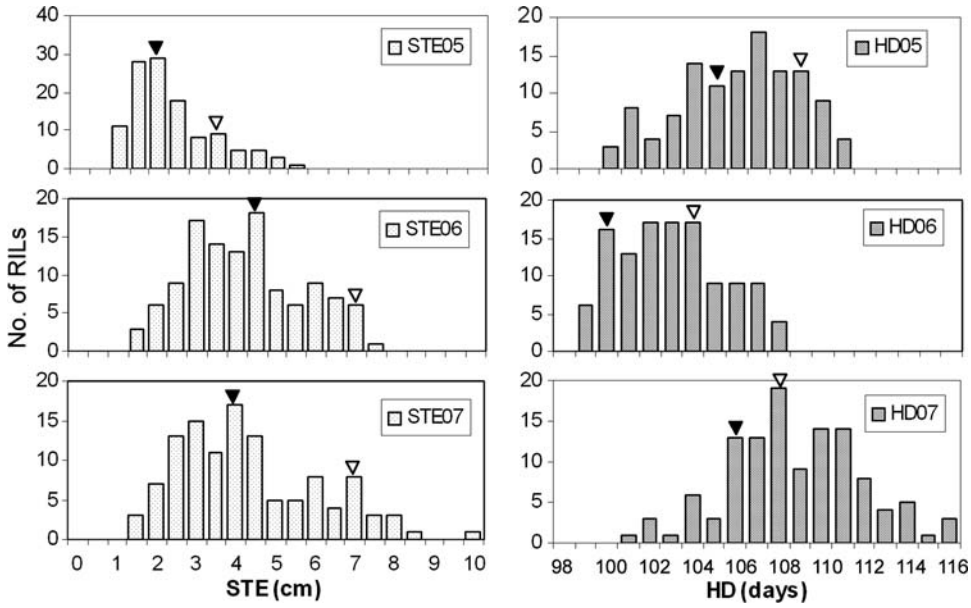

Fig. 1 Frequency distributions for initial stem elongation (STE) and heading date (HD) in the RIL population of Intrada/Cimarron evaluated in 3-year trials. *Open triangle* indicates the parent Intrada; *bold triangle* indicates Cimarron

The difference between two classes of homozygous alleles was calculated to indicate the genetic effect of each QTL, which is twice as much as the additive effect in the case of RILs. Statistical test was conducted using PROC TTEST in SAS. Two- or three-way QTL interactions averaged over 3 years were assessed with PROC GLM in SAS. RILs were further classified into marker genotypes by the closest marker flanking each QTL. Mean was compared using PROC GLM in SAS to assure inferences were made between marker genotypes with significant differences.

Results

Segregation of initial stem length and heading date

The parental line Cimarron initiated stem elongation later but reached heading earlier than the parental line Intrada across 3 years (Fig. 1). Some RILs in the Intrada × Cimarron population showed either earlier stem elongation or later heading date than both parental lines, demonstrating transgressive segregation in the population. The difference in initial stem length between the two parental lines was 1.5, 2.2, and 3.1 cm in year 2005, 2006, and 2007, respectively; the range for the population of RILs was 5.5, 6.3, and 10.1 cm in the corresponding year. Similarly, the respective RIL range in heading date in year 2005, 2006, and 2007 was 13.0, 9.3, and 16.0 days, much larger than the corresponding parental difference of 3.0, 3.5, and 2.5 days.

Phenotypic distributions for initial stem length and heading date among 115 RILs were monomodal and continuous but not consistent in pattern across years (Fig. 1). Segregation for these two traits was likely controlled by multiple genes with additive effects and influenced by changes in environmental factors across years.

Although the two parental lines showed a negative association between initial stem length and heading date (i.e., Cimarron had later stem elongation stage but earlier heading date), a positive correlation ($P < 0.01$) was observed between initial stem length and heading date at the population level across 3 years (Table 1). Krenzer (2000) reported that initial stem length did not necessarily relate to heading date phenologically among a limited set of cultivars. However, Edwards et al. (2007) later reported from a broader genetic sample of cultivars that delayed stem elongation was associated with later heading in thermal-time units, though the cultivar range for initiation of stem elongation in actual calendar days was much larger than the range in heading date. Statistical analysis at the segregating population level could provide valuable information on the genetic basis of this phenological relationship, given that random inbred lines from the population represent random assortment of genes controlling one or both traits. Such analysis cannot be extracted from genetic samples derived from fixed gene combinations selected in cultivars.

Construction of a linkage map

A total of 935 SSR markers were used for screen polymorphisms between two parental lines, and 241 polymorphic markers (25.8%) were used for mapping in the population. The low rate of polymorphism that occurred at the regions of interest inhibited construction of a genetic map based merely on SSR markers. Twenty SNP markers (data not shown) recently developed in wheat (http://wheat.pw.usda.gov/SNP/new/index.shtml) were also tested to develop

Table 1 Phenotypic correlation between initial stem elongation (STE) and/or heading date (HD) in years 2005, 2006, and 2007

	STE06	STE07	HD05	HD06	HD07
STE05	0.74	0.76	−0.64	−0.70	−0.50
STE06		0.58	−0.56	−0.68	−0.40
STE07			−0.61	−0.63	−0.45
HD05				0.90	0.66
HD06					0.66

All correlation coefficients were significant with p values less than 0.0001. Greater stem elongation signified earlier initiation of reproductive development, whereas lower values for heading date indicated earlier maturity. Hence a negative correlation coefficient for these traits signified a positive association

markers, but only two showed polymorphisms between Cimarron and Intrada. One SNP marker (BF201235) was mapped associated with a QTL found in this study.

In addition, severe distortion in segregation ratios was detected in this population. For example, *Xcfa2174*, *Xwmc702*, *Xcfd14*, and *Xgwm437* formed a group spanning 20.7 cM on chromosome 7D, but only about 25% of the RILs was of the Intrada type. A χ^2 showed that their segregation deviated from the expected 1:1 ratio ($P < 0.0001$). Another linkage group of 21.4 cM consisting of *Xgdm130*, *Xcfd31*, *Xwmc438*, and *Xbarc352* fit the expected 1:1 segregation ratio ($P > 0.05$). All eight markers have been reported to be located on the short arm of chromosome 7D (http://wheat.pw.usda.gov), but the two linkage groups failed to be assembled into the same linkage group in the Intrada × Cimarron population.

The existence of the segregation distortions and lack of polymorphic DNA markers in this population prompted an alternative strategy to find QTLs for target traits. Effects of individual markers were first analyzed when 140 SSR markers were mapped using a larger coefficient of correlation and a significance level of 0.001 as thresholds. When any marker was found to have a significant effect, it was assumed that the initial marker was associated with a putative QTL. Based on the consensus map in wheat, additional reported SSR markers surrounding the initial marker were screened and polymorphic markers were mapped to saturate the putative QTL region. Correlation analysis was carried out repeatedly to examine association of new markers with the traits. Eventually, three QTLs were found associated with segregation of initial stem length and heading date.

QTLs for initial stem length and heading date

The first QTL was positioned on the short arm of chromosome 2B by a linkage group consisting of nine SSR markers (Fig. 2a). This QTL was centered on SSR marker *Xbarc200* and spanned approximately 25 cM between two SSR markers, *Xwmc770* and *Xwmc25*. A pair of primers specific to EST BF201235 in bin 2BS3-0.84-1.00 was used to amplify genomic DNA fragments from Intrada to Cimarron. Sequencing results indicated that PCR products were from a single-gene copy. After digestion with restriction enzyme *Rsa* I, polymorphic bands were observed between Intrada (269 + 215 bp) and Cimarron (269 + 119 + 96 bp). This SNP marker was located 2 cM from SSR marker *Xwmc25*, validating the physical location of this group of SSR markers on chromosome 2B. Gene *PPD-B1* was reported to reside on the short arm of chromosome 2B, but no allelic variation was found between Intrada and Cimarron by sequencing six PCR fragments of this gene (data not shown). This QTL on chromosome 2B explained 8–25% of the phenotypic variation in initial stem length with significant LOD values of 2.75 (2005), 4.93 (2006), and 1.36 (2007). This QTL also explained 8–26% of the phenotypic variation in heading date, with significant LOD values of 2.33 (2007) to 5.22 (2006) (Table 2).

A second QTL was located on the short arm of chromosome 2D, based on an assembled group of ten SSR markers and one gene marker positioned on chromosome 2D (Fig. 2b). This QTL was initially mapped in association with SSR locus *Xcfa2201*. Given its similar chromosomal location, the PCR marker for *PPD-D1* previously developed by Beales et al. (2007) was tested and showed the same polymorphic bands between Intrada (414-bp) and Cimarron (288-bp) as reported (data not shown). This result indicated that Intrada carried an allele conferring sensitivity to long days, whereas Cimarron carried an allele conferring insensitivity to long days. This gene marker for *PPD-D1* was mapped to the center of the QTL peak (Fig. 2b); hence *PPD-D1* is a reasonable candidate for this QTL. Approximately, 15% of the phenotypic variation in initial stem length in year 2007 could be explained by this QTL, but its effect on this trait was not significant in year 2005 or 2006. Apparently, the contribution of this QTL to stem elongation was masked by changes in environmental conditions across years. In contrast, this QTL accounted for 24–38% of the phenotypic variation in heading date, constituting the largest effect on this trait among all of three QTLs (Table 2).

Table 2 Putative QTLs for initial stem elongation (STE) and heading date (HD), their LOD scores, the phenotypic variation explained, and means for each allele class

Marker	Trait	Year	LOD	R^2	Means by allele		
					Intrada	Cimarron	Difference
Xbarc200	STE	05	2.75	0.16	2.72	1.99	0.73**
		06	4.93	0.25	4.83	3.47	1.36**
		07	1.36	0.12	4.72	3.82	0.90*
	HD	05	3.41	0.18	105.84	108.08	−2.24**
		06	5.22	0.26	101.84	104.13	−2.29**
		07	2.33	0.08	107.52	109.69	−2.07**
PPD-D1	STE	05	0.26	0.00	2.21	2.53	−0.32ns
		06	0.03	0.00	4.06	4.21	−0.15ns
		07	2.32	0.15	3.80	5.02	−1.22**
	HD	05	9.56	0.38	108.23	104.81	3.43**
		06	5.71	0.27	103.90	101.48	2.42**
		07	4.32	0.24	109.55	107.03	2.52**
Xcfd14	STE	05	6.10	0.26	3.11	2.02	1.09**
		06	4.86	0.22	5.01	3.73	1.37**
		07	2.15	0.12	5.18	3.85	1.33**
	HD	05	4.36	0.18	105.10	107.81	−2.71**
		06	5.81	0.25	101.24	103.78	−2.54**
		07	1.88	0.10	107.19	109.27	−2.08**

Significance level for comparing mean difference, * < 0.05; ** < 0.01

A third QTL was located on the short arm of chromosome 7D (Fig. 2c) in a 14-cM interval centered on *Xcfd14* and flanked by SSR markers *Xwmc702* and *Xgwm437*. The vernalization gene *VRN-B3* (=*FT-B*) on the short arm of chromosome 7B has been cloned and identified as an orthologue of the *FT* gene in *Arabidopsis* and gene *Hd3a* in rice (Yan et al. 2006). To determine if the homoeologous *VRN-D3* (=*FT-D1*) on the short arm of chromosome 7D was associated with this QTL, we mapped the *VRN-D3* gene. However, it was located 42 cM apart away from *Xcfd14*, excluding the possibility that *VRN-D3* is a candidate gene for the QTL on chromosome 7D. This QTL had the largest effect on initial stem length among all the three QTLs, explaining 12–26% of the phenotypic variation. It also accounted for 10–25% of the phenotypic variation in heading date.

The combined effects of the three single QTLs, including the significant interactions, were estimated using MIM for two traits in 3 years, respectively. For initial stem elongation, they could account for 40, 51, and 38% of the total variation in 2005, 2006, and 2007, respectively. As for heading date, they accounted for 69, 66, and 41% of the total variation in 2005, 2006, and 2007, respectively. Significant environmental effects on initial stem length and heading date were observed among the years as expected.

Interactive effects of the three QTLs

A significant interaction effect on initial stem elongation was found between *Xbarc200* and *PPD-D1* QTLs ($P < 0.0001$), as well as between *Xcfd14* and *PPD-D1* QTLs ($P < 0.0001$) No significant interaction effect on initial stem length was detected between *Xbarc200* and *Xcfd14* QTLs. When RILs did not possess both the Intrada *Xbarc200* allele (I2B) and the Cimarron *PPD-D1* allele (C2D), they had the most delayed stem elongation. The other three genotypes showed little difference (Fig. 3a). This suggested that I2B and C2D alleles had the same function to promote stem elongation. Provided that the Cimarron *PPD-D1* allele (C2D) was present, RILs having the Intrada *Xcfd14* allele (I7D) showed the earliest stem elongation compared to the other three genotypes (Fig. 3b). On the contrary, without the presence of C2D, RILs with either I7D or C7D alleles did not differ significantly. This implied that C2D allele was required for the expression of I7D allele. Although all three QTLs showed a significant effect on heading date, interactive effects between any pair of QTLs detected at the stem elongation stage dissipated at heading ($P > 0.05$).

Intrada carried an *Xbarc200* allele on chromosome 2B that conferred earlier stem elongation, as well as an earlier heading date with an average effect of −2.2 days (Table 2). Likewise, Intrada carried the *Xcfd14* allele on chromosome 7D for earlier stem elongation and with an average effect for accelerating heading date by −2.4 days in the population. Conversely, Cimarron carried a *PPD-D1* allele that also accelerated heading by −2.4 to −3.4 days in the population, and Cimarron *Xbarc200* and *Xcfd14* alleles delayed the developmental process characterized at these two stages.

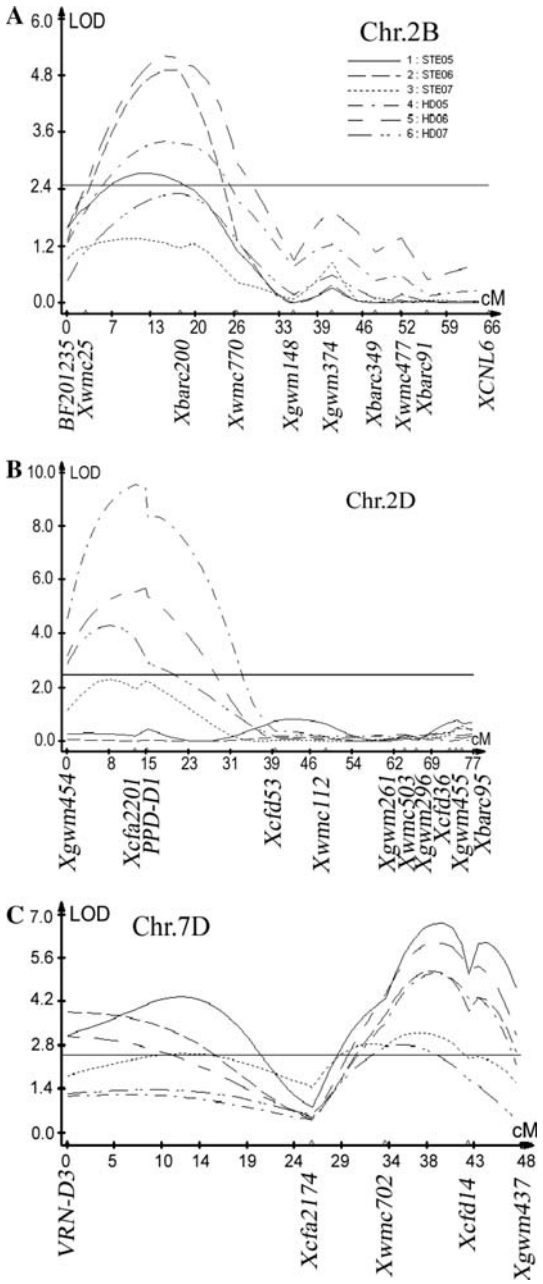

Fig. 2 Location of three QTLs for initial stem elongation (STE) and heading date (HD) suggested by interval mapping of WinQTLCart 2.5. **a** QTL on chromosome 2B, **b** QTL on chromosome on 2D, and **c** QTL on chromosome 7D

Various combinations of different alleles with different effects, as well as gene interactions, determined various stem elongation and heading date phenotypes in the Intrada × Cimarron population. Net effects of the Intrada allele and Cimarron allele at each QTL can be described in the order of BARC200_PPD-D1_CFD14 for eight genotypes from the population of 94 RILs (Fig. 4). Two genotypes (C2B_I2D_I7D and C2B_I2D_C7D) produced the latest stage for both stem elongation and heading date. The extreme genotype C2B_C2D_I7D showed the earliest stem

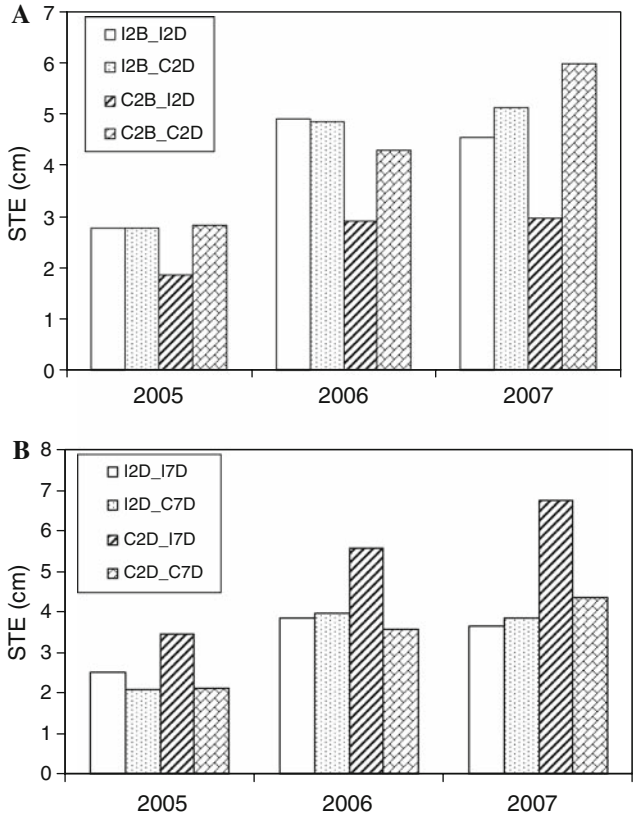

Fig. 3 Mean initial stem elongation of marker genotypes to show two-QTL interactions. **a** Interactions between *Xbarc200* and *PPD-D1* QTLs, **b** Interactions between *PPD-D1* and *Xcfd14* QTLs

elongation, whereas the genotype I2B_C2D_I7D showed the earliest heading date. These profiles are consistent with the genetic basis of the two parental phenotypes: the I2B_I2D_I7D genotype for Intrada had an earlier stem elongation stage with a later heading date, whereas the C2B_C2D_C7D genotype for Cimarron had a later stem elongation stage but an earlier heading date.

Discussion

Construction of a complete and high-density linkage map along all chromosomes is necessary to find all QTLs responsible for target traits (Benfey and Mitchell-Olds 2008). Common wheat, however, has three homologous genomes, and many SSR markers have multiple copies, complicating specific location of each SSR marker (Somers et al. 2004). On the other hand, the two parents i.e., Intrada and Cimarron are local cultivars that are likely to have some common ancestries. The polymorphisms were quite uneven across genome. In this study, we used single-marker correlation analysis and interval mapping alternatively with the increase of markers. Once a single marker

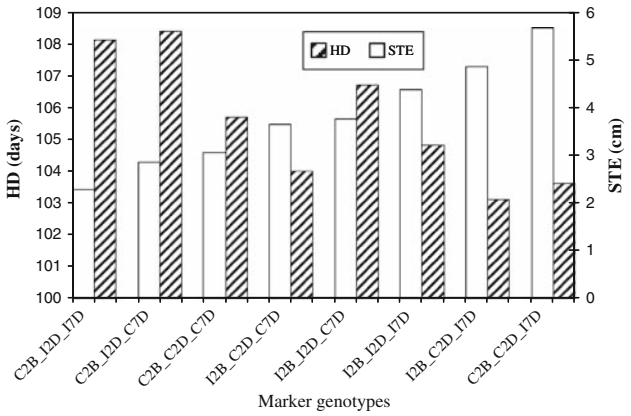

Fig. 4 Mean initial stem elongation (STE) and heading date (HD) of eight genotypes classified by the closest marker for each QTL. In the symbol of genotypes, the allele is denoted by the *first letter* of parent plus chromosome name. For instance, I2B and C2B represent the Chromosome 2B QTL alleles from Intrada and Cimarron, respectively

was found to have a significant effect on the target trait, all potential markers close to the initial marker were explored to saturate the region of interest. In spite of low genome-wide coverage from 241 SSR markers, we were able to find three major QTLs explaining a substantial portion of the phenotypic variance in the population. Of particular importance, is that, we found one QTL associated with *PPD-D1* and another two QTLs interacting with *PPD-D1*.

Vernalization and photoperiod are principal mechanisms that regulate flowering time in temperate cereals (Dubcovsky et al. 2006; Hemming et al. 2008; Kane et al. 2005; Laurie et al. 1995; Snape et al. 2001b). Vernalization usually has the largest effect on flowering time among genetic factors or among non-genetic factors such as temperature, light, nutrient supply, or plant age. Variation in initial stem length and heading date in the Intrada × Cimarron population was not found to be associated with a major QTL *QSte.osu-5A* for stem elongation and winter dormancy release, which is located on chromosome 5A in a region encompassing *VRN-A1* in a different HRW population, Jagger × 2174 (Chen et al. 2009). No polymorphism was found at *VRN-A1* between Intrada and Cimarron, and no QTL was found associated with SSR markers on chromosome 5A, including *Xcfa2163*, a SSR marker that was mapped 15.6 cM to *VRN-A1* (Xue et al. 2008). Hence in this population, the major genetic locus *VRN-A1* in the vernalization pathway was likely fixed for the same allele from Intrada to Cimarron. Therefore, variation in initial stem length and heading date in the Intrada × Cimarron population was found to be controlled by other genetic loci. Because of the tight association of *PPD-D1* with the QTL on chromosome 2D, *PPD-D1* naturally was an excellent candidate gene for this QTL. Furthermore, it suggests that the *PPD-D1* and genes in the interacting QTLs play a key role in differentiating among winter wheat genetic backgrounds with varying ontogeny.

Wheat is generally a long-day (LD, usually 16 h light) plant, because LD accelerates flowering in spring wheat and vernalized winter wheat (Levy and Peterson 1972; Pinthus and Nerson 1984). However, it has been found that when unvernalized plants of some photoperiod-sensitive cultivars of winter wheat are treated with several weeks of SD and then transferred to LD, they will flower earlier than plants treated with continuous LD (Evans 1987; Krekule 1964; McKenney and Sando 1935). This observation led to the hypothesis that wheat was initially a SD/LD plant (Aamlid et al. 2000; Dubcovsky et al. 2006; Heide 1994; Humphreys et al. 2006). Wheat cultivars can be divided into sensitive and insensitive types based on their response to photoperiod. For spring wheat or winter wheat that has been fully vernalized and/or treated with SD, LD treatment accelerates flowering. This LD-sensitivity is a wild-type response that mutated into insensitivity, enabling earlier flowering without LD treatment (Laurie et al. 1995; Law and Worland 1997; Snape et al. 2001a). The *PPD-D1a* allele that confers insensitivity to photoperiod and early flowering in SD or LD plants contains a 2-kb deletion upstream from the coding region of the wheat *PRR* gene (pseudo-response regulator) on chromosome 2D (Beales et al. 2007). Our study indicated that Cimarron has a photoperiod-insensitive allele and thus reached heading earlier than Intrada, which has a photoperiod-sensitive allele. The mutation at *PPD-D1* was found in 11 out of 19 cultivars adapted to the US Central Plains (Chen and Yan, unpublished data).

Photoperiod insensitivity caused by *PPD-B1* on chromosome 2B is due to a mutation outside the sequenced region or to a closely linked gene (Beales et al. 2007). All attempts failed to map *PPD-B1* in the Intrada × Cimarron population due to lack of polymorphism in the reported sequenced region 600 bp upstream from the translation start codon and 800 bp downstream from the stop codon. Based on comparative maps, however, *PPD-B1* may be associated with the QTL, we found in the short arm of chromosome 2B that was validated by the SNP marker for BF201235 in bin 2BS3-0.84-1.00. A QTL for flowering time was linked with SSR makers *Xgwm148* and X*wmc770* in the Renan × Recital winter wheat population (Gervais et al. 2003). This QTL was believed to be linked with *PPD-B1* (Gervais et al. 2003; Hanocq et al. 2007; Hanocq et al. 2004). *Xgwm148* was also very close to the QTL on chromosome 2B mapped in our population. The phenotypic data showed that the potential *PPD-B1* QTL and *PPD-D1* QTL have opposite effects in Intrada and Cimarron, suggesting that it is likely that there is a photoperiod insensitive allele on 2B in Intrada and on 2D in Cimarron.

In this study, *VRN-D3* (=*FT-D*) was mapped on the short arm of chromosome 7D. Our attempt to find SSR

markers closely flanking *VRN-D3* was unsuccessful. A significant genetic effect of a single *VRN-D3* locus was detected on initial stem length ($P < 0.0001$) and heading date ($P < 0.0001$). *VRN-H3* was cloned in barley based on variation in flowering time in spring × winter populations (BG213 × *H. spontaneum* and BG213 × 'Igri'), which have a recessive *vrn-H1* (winter) genetic background (Yan et al. 2006). The orthologous *VRN-B3* was observed to be responsible for single-gene segregation of heading date in a wheat CS × CS (Hope7B) population (Yan et al. 2006), which had a dominant spring allele *Vrn-D1* in its background (Pugsley 1972). The significant effect of *VRN-3* promoting flowering was detected in the previous spring wheat population and the vernalized winter wheat population used in this study, indicating an important role of *VRN-3* in regulation of developmental process in wheat. However, no significant effect of *VRN-3* was detected in the unvernalized winter wheat population (unpublished data), which differed from the effect of *VRN-H3* detected in the winter barley population. Further work will be needed to understand how *VRN-3* plays a different role in overcoming vernalization requirement between winter wheat and barley.

The QTL on the short arm of chromosome 7D was not associated with *VRN-D3* (=*FT-D1*) but with *Xcfd14* in the same genomic region, suggesting this is a new locus involved in regulating reproductive development in winter wheat. Interaction of this QTL with *PPD-D1* QTL suggested that they might play a regulatory role in the same pathway. The short arms of the group 7 chromosomes have been reported to carry genes for heading date or flowering time using substitution lines or populations segregating for heading date or flowering or maturity time QTLs, for example, 7A (Halloran 1967; Hanocq et al. 2007; Hyne et al. 1994; Kuchel et al. 2006; Law and Worland 1997; Quarrie et al. 1994), 7B (Flood and Halloran 1983; Halloran 1967; Hanocq et al. 2007; Hoogendoorn 1985; Kuchel et al. 2006; Law and Wolfe 1966; Quarrie et al. 1994; Sourdille et al. 2003), and 7D (Borner et al. 2002; Gervais et al. 2003; Hyne et al. 1994). The expression of these genes or QTLs may be affected by vernalization or photoperiod treatment, or both of them, or neither of them. The positional cloning of *VRN-3* in two temperate species (Yan et al. 2006) greatly benefits from a complete sequence of the rice collinear region, including the heading date gene *Hd3a*, an *FT* orthologue in rice (Kojima et al. 2002). This gene-cloning strategy based on comparative maps should facilitate fine mapping and cloning of the gene responsible for the QTL on chromosome 7D found in this study.

This study provided genetic evidence that the three QTLs interactively modulate the developmental transition time, resulting in various stem lengths on a fixed sampling date. It is the various allelic combinations of multiple loci and the duration of their effect, from stem elongation to heading, which establishes variation in reproductive development in winter wheat. The genetic effects of all three QTLs on the developmental process were extended to initiation time of stem elongation, which contributed to variation in heading date. The two parental cultivars Intrada and Cimarron each contained genes with opposite effects on initiation of stem elongation and heading date, i.e., earlier onset of stem elongation coupled with later heading date, or vice versa. Substitution of the Intrada *Xbarc200* allele on chromosome 2B in the Cimarron background would accelerate heading date by about 2 days at the expense of a slightly earlier stem elongation than Cimarron. Late initiation of stem elongation might be achieved without greatly delaying heading date on the basis of the three QTLs identified so far. The intermediate phenotypes of the two locally adapted cultivars represent the acceptable ranges for these traits for wheat production in the southern Great Plains.

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