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Genetic regulation of developmental phases in winter wheat

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Abstract The orderly development of winter wheat through its life cycle can be marked at three stages: stem elongation, heading date, and physiological maturity. The duration of a developmental phase between two stages is important in yield component generation. In this study the three developmental stages were characterized and 350 markers were mapped in a population of recombinant inbred lines (RILs) generated from a cross between two winter wheat cultivars ('Jagger' and '2174'). Three major QTLs were found to control variation in developmental process, and each of them was tightly associated with a known flowering gene, *VRN-A1* on chromosome 5A, *PPD-D1* on chromosome 2D, and *VRN-D3* on chromosome 7D. The average contribution of the gene marker for each QTL to the total phenotypic variation (R^2) was evaluated over 3 years. The effect of *VRN-A1* ranged from 21.5% at stem elongation to 17.4% at physiological maturity. The effect of *PPD-D1* was minor (6.7%) at stem elongation but increased to 29.7% at heading and 20.1% at physiological maturity. The effect of *VRN-D3* was not detected at stem elongation but increased to 14.6% at heading and to 20.5% at physiological maturity. Hence, the *VRN-A1* locus, the *PPD-D1* locus, and the *VRN-D3* locus had greatest impact on development at stem elongation, heading date, and physiological maturity, respectively. Whereas the Jagger *VRN-A1* and *VRN-D3* alleles accelerated development, the Jagger *PPD-D1* allele delayed the developmental process due to its sensitivity to photoperiod. Our findings suggest that through the appropriate combination of alleles at these three loci one would be able to regulate the various developmental phases to accommodate different agricultural needs.

Keywords Stem elongation · Flowering time · Physiological maturity, vernalization, photoperiod

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Introduction

The orderly development and growth of wheat from germination to maturity can be defined through several developmental events that can be visibly characterized based on morphological changes, such as emergence, stem elongation prior to jointing, booting, heading, flowering, grain filling, and maturity (Haun 1973; Zadoks et al. 1974; Hay and Kirby 1991; McMaster 2009). The development and growth of the wheat plant can be divided into three phases: germination to stem elongation (GE-STE), stem elongation to heading time (STE-HD), and heading time to physiological maturity (HD-PM) (Hay and

Kirby 1991; Snape et al. 2001; Gonzalez et al. 2002). A strong genetic component determines the timing and duration of these phases (McMaster 2009). Variation in duration of a developmental phase, however, is often observed among wheat cultivars that serve different purposes in agricultural production. For instance, a longer GE-STE phase is selected to generate more biomass for cattle grazing in dual-purpose production systems (Redmon et al. 1996). An extended STE-HD phase is required to increase the number of fertile florets (Gonzalez et al. 2003), whereas a longer HD-PM phase will benefit grain filling to increase grain weight (Whitechurch and Slafer 2001). The timing of a developmental stage is also important in wheat production. A relatively later stem elongation time is desired to avoid frost damage frequently occurring during early spring (Fowler et al. 2001), whereas a relatively early maturity time is desired to avoid the hot and dry summer season, or as global climate shifts toward warmer temperatures.

In the model plant Arabidopsis, the growth and development of the plant may be divided into the vegetative phase (V), the first-inflorescence phase (I1), and the second-inflorescence phase (I2) (Ratcliffe et al. 1998). These developmental phases are believed to be controlled by a group of flowering time genes in the vernalization, photoperiod, and autonomous pathways (Ratcliffe et al. 1998; Amasino 2004). Approximately 80 genes have been reported to affect flowering time in diploid Arabidopsis (Levy et al. 2002; Tasma and Shoemaker 2003). Due to the presence of three homoeologous genomes in hexaploid wheat (*T. aestivum*, $2n$=6x=42, AABBDD), many more genes are expected to affect flowering time in this species compared with Arabidopsis (Laurie et al. 1995; Law and Worland 1997; Hay and Ellis 1998; Snape et al. 2001; Yan 2009).

So far, only a few genes regulating wheat flowering time have been utilized to describe reproductive development in wheat. In the wheat vernalization pathway, three major genes have been cloned: *VRN1* (Yan et al. 2003), *VRN2* (Yan et al. 2004), and *VRN3* (Yan et al. 2006). A dominant allele at each of *VRN1* and *VRN3* is responsible for spring growth habit, and a dominant allele at *VRN2* is responsible for winter growth habit; therefore, the only gene recombination of recessive *vrn1*, recessive *vrn3* and dominant *Vrn2* determines the winter growth habit due to interactions among these three genes (Pugsley 1971; Tranquilli and Dubcovsky 2000; von Zitzewitz et al. 2005; Yan et al. 2006; Szűcs et al. 2007). All three genes were cloned in populations generated from crosses between spring wheat and winter wheat. These genetic and molecular mechanisms pertaining to the difference between winter and spring growth habit by *VRN1*, *VRN2* and *VRN3* cannot be used to explain variation in the developmental process among winter wheat genotypes. The underlying genetic mechanisms determining variation in flowering time in winter wheat are not well understood.

Previous studies have indicated that the known vernalization gene loci may have pleiotropic effects (Fowler et al. 2001; Košner and Pánková 1998; Snape et al. 2001; Whitechurch and Slafer 2002). Recently, *VRN-A1* (*VRN1* on genome A of hexaploid wheat) has been shown to affect heading date in a population generated from a cross between two spring wheat cultivars (Kuchel et al. 2006), and it was also shown to have tight association with variation in the initiation of stem elongation, in a population generated from a cross between two winter wheat cultivars Jagger and 2174 (Chen et al. 2009). When *VRN-A1* was fixed for the same allele from the recombinant inbred lines (RILs) of Intrada and Cimarron, both winter wheat cultivars, variation in initial stem length and heading date was found to be controlled by genetic loci in the photoperiod pathway including *PPD-D1* on chromosome 2D (Wang et al. 2009). When vernalization requirement in winter wheat cultivar has been satisfied, its reproductive developmental rate will be mainly affected by *PPD* genes, as occurs in spring wheat (Beales et al. 2007; Snape et al. 2001).

Previous studies, however, were limited in explaining the genetic basis of a development stage, because only a single trait, such as the arrival time of stem elongation, or heading or flowering time, was described in a segregating population. No information is available about how the duration of a developmental phase is genetically regulated in wheat. In a recent study, we reported that the initiating time of stem elongation was controlled by *VRN-A1* and *PPD-D1* loci in the Jagger × 2174 population (Chen et al. 2009), but no analyses on heading date and physiological maturity were conducted, as more environments were needed to validate the genetic model for regulation of those two traits.

In this present study we report that variation in the three developmental stages was predominantly controlled by three major QTLs, and each of them was tightly associated with a known flowering gene, *VRN-A1*, *PPD-D1*, and *VRN-D3*. Pleiotropic effects of these gene loci at different developmental stages characterized in the same population in this study have allowed us to better understand how various developmental phases are predetermined in winter wheat.

Materials and methods

Two winter wheat cultivars, Jagger and 2174, showed a significant difference in the timing of stem elongation (Chen et al. 2009; Edwards et al. 2007) and heading date (Edwards et al. 2007). Ninety-six recombinant inbred lines (RILs) of the Jagger × 2174 population were tested at the Agronomy Research Station in Stillwater, Oklahoma in 2006 and 2008, and at the North Central Agronomy Research Station near Lahoma, OK in 2007. The same set of $F_{6:8}$ RILs was tested in all three experiments. Fertilizer N was applied each year in amounts considered, after adjusting for residual mineral N, adequate for a grain yield of 3,000 kg ha^{-1}.

Initial reproductive development, or the degree of stem elongation (STE), was measured by the length of hollow stem of the main tiller on a given date for each year, as described by Chen et al. (2009). During the following periods, heading date (HD) was monitored daily and visually scored when 50% of the heads in a plot had completely emerged from the boot: 10–21 April 2006, 21–30 April 2007, and 21 April to 5 May 2008. Physiological maturity (PM) was scored in the first year (18 May 2006) using a visual scale of 1 to 5, for which '1' indicated development beyond physiological maturity (very few green heads, yellow peduncles) and '5' indicated completely green canopy. Values between 1 and 5 represented gradual stages of physiological maturity. Based on the visual scale in 2006, major QTLs were found associated with physiological maturity, and thus the trait was more precisely scored in 2007 (from May 27 to June 4) and 2008 (from May 25 to June 4) as the actual date of physiological maturity when the spike peduncle and plant internodes of at least 50% of the plants in a given line lost all green color.

In addition to the previous 246 SSR markers mapped in this population by Chen et al. (2009), 104 new SSR markers were added to fill gaps in the previous linkage groups or to saturate the QTL regions corresponding to heading date and physiological maturity. In addition to gene markers for *VRN-A1* and *PPD-D1* mapped by Chen et al. (2009), two new gene markers, *Xcdo708* and *VRN-D3* were also mapped in this study.

A PCR marker was developed for RFLP marker *Xcdo708* that was mapped 0.7 cM distal to *VRN-A1* on chromosome 5A in *T. monococcum* (Yan et al. 2003). The *Xcdo708* sequence (GenBank accession number AY245605) was used to search for ESTs deposited in GenBank databases, and all of the ESTs were assembled into three groups that could be derived from genomes A, B, and D of hexaploid wheat. A pair of primers (CDO708-F1 5′-AGTGGT CAATTTGTTGGTGTTGCCG-3′ and CDO708-R1 5′-ACTGCGCTCTTCTCCTTCTCATCAA-3′) was used to amplify orthologous fragments from the three genomes in Jagger and 2174. Sequencing plasmid DNAs cloned from these PCR products showed three types of genomic fragments. One genomic fragment showed variation between Jagger and 2174 due to the presence of a 16-bp insertion/deletion (indel). A new pair of primers, CDO708-F5 5′-CAAACAGCCGTG ACACACAAGAG-3′ and CDO708-R5 5′-TAAAGG AACATACAAAGTATTAAC-3′ was used to amplify internal genomic fragments for mapping based on the 16-bp indel polymorphism that was distinguished on a 9% acrylamide/bisacrylamide gel. PCR was performed for 40 cycles (90°C for 30 s, 55°C for 30 s, and 72°C for 1 min per cycle) followed by a 10-min final extension at 72°C.

A PCR marker was developed to detect allelic variation in *VRN-D3* on chromosome 7D between Jagger and 2174. Based on the multiple alignment of *VRN-3* (*FT*) sequences, *VRN-A3* (=*FT-A*) (GenBank accession EF428115), *VRN-B3* (*FT-B*) (GenBank accession EF428112), and *VRN-D3* (=*FT-D*) (GenBank accession EF428114) (Bonnin et al. 2008), a pair of primers VRN-D3-F1 5′-TCAGGGTGAC CTTCGGGAAC-3′ and VRN-D3-R1 5′-TCGCTTC GTGCTCGTGCTCTTCC-3′ was designed to specifically amplify *VRN-D3*. A 1-bp deletion was found in *VRN-D3* in 2174 but not in Jagger. A new pair of primers, forward primer VRN-D3-F6 5′-CTTCTA TTCACATGTTTCGTTCATG-3′ and reverse primer

VRN-D3-R8 5′-ACGAGCACGAAGCGATGGATC GC-3′, was used to specifically amplify *VRN-D3* from the 96 RILs. The PCR products were directly digested with restriction enzyme *Nco*I, and the Jagger *VRN-D3* allele showed 375- and 27-bp bands, whereas the 2174 *VRN-D3* allele showed a 401-bp band.

All markers (354) were assembled into linkage groups using MapMaker 3.0 (Whitehead Institute for Biomedical Research, Cambridge, MA). Functions of interval mapping (IM) and composite interval mapping (CIM) of WinQTLCart 2.5 (North Carolina State University, Raleigh, NC, USA) were then used to search for QTLs by phenotypic data against linkage groups. The LOD value, the contribution of a QTL to the total phenotypic variation (R^2) of a QTL was each calculated using WinQTLCart 2.5. Unlinked markers were also analyzed using the CORR procedure of SAS to determine if they produced significant correlation coefficients at a less stringent significance level ($P < 0.01$). Means were compared using PROC GLM in SAS to assure inferences were made between marker genotypes with significant differences.

Results

Phenotypic distributions in the RIL population

Both Jagger and 2174 have winter growth habit, but their RIL progeny varied widely in arrival time for specific reproductive development stages under field conditions. The three developmental traits exhibited similar phenotypic distributions in the RIL population (Fig. 1). As reported for the stem elongation stage (Chen et al. 2009), heading date and physiological maturity produced continuous distributions, suggesting these three traits were controlled by multiple genes.

Jagger reached each developmental stage earlier than 2174 (Fig. 1). Whereas many lines produced phenotypes similar to the parental lines, a few lines with extreme traits were generated. Considering the restricted phenotypic range for the two parental lines, gene dispersion was apparent in the parents, and hence transgressive genotypes were created due to recombination among multiple loci in the progeny plants. This single segregating population provided an opportunity to identify multiple genes relevant to selection for specific developmental phases.

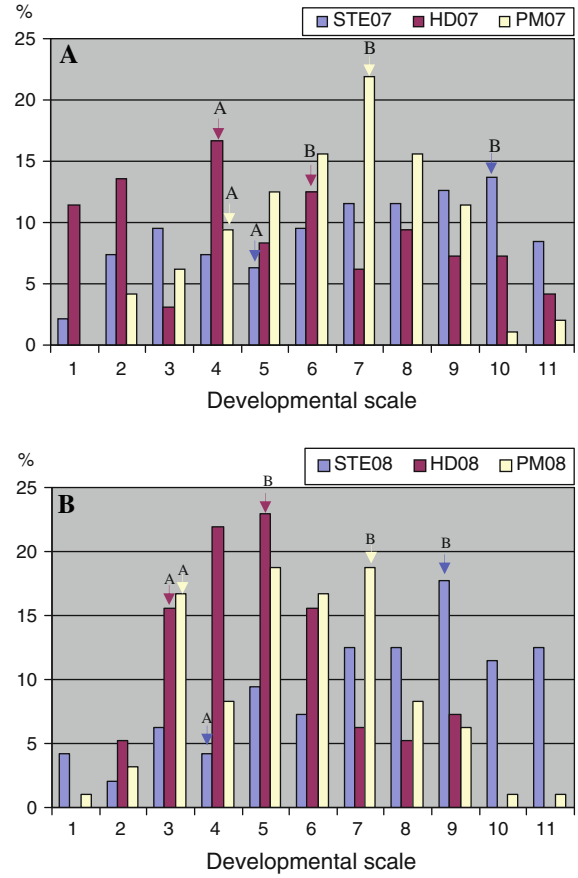

Fig. 1 Frequency distribution for STE, HD, and PM scored in 2007 and 2008. *Arrows* indicate parental lines Jagger (**a**) and 2174 (**b**). The *vertical axis* indicates frequency of RILs (in percentage) tested in the Jagger × 2174 population. The *horizontal axis* indicates the phenotypic scale of 1 to 11. For stem elongation (cm): (*1*) ≥6.5; (*2*) 6–6.5; (*3*) 5.5–6; (*4*) 5–5.5; (*5*) 4.5–5; (*6*) 4–4.5; (*7*) 3.5–4; (*8*) 3–3.5; (*9*) 2.5–3; (*10*) 2–2.5; (*11*) For heading date (days, using 1 April as day 1): (*1*) ≤20; (*2*) 21; (*3*) 22; (*4*) 23; (*5*) 24; (*6*) 25; (*7*) 26; (*8*) 27; (*9*) 28; (*10*) 29; (*11*) ≥30. For physiological maturity (days, using 1 May as day 1): (*1*) ≤26; (*2*) 27; (*3*) 28; (*4*) 29; (*5*). 30; (*6*) 31; (*7*) 32; (*8*) 33; (*9*) 34; (*10*) 35; (*11*) ≥36

Statistical analysis was conducted to detect genotype × environment interactions during years 2007 and 2008 for the phenotypic data, since a different scale was applied for these traits in 2006. Genetic components of variance were estimated to determine broad-sense heritability, as any dominance effect was considered negligible among RILs. Broad-sense heritability for stem elongation, heading date, and physiological maturity was 63.9, 92.1, and 85.7%, respectively.

QTL associations with *VRN-A1*, *PPD-D1*, and *VRN-D3* genes

Using a previous linkage map for stem elongation as a foundation (Chen et al. 2009), a higher density genetic map with 350 SSR markers was established in this study, with an emphasis on saturating genomic regions associated with heading date and physiological maturity. Three major QTLs were associated with variation in the developmental process. No other single marker outside of these three major QTLs was found to produce significant genetic effects across years, even using the relaxed criteria of a larger R^2 and significance level of 0.01. Especially intriguing was the finding that three genes previously reported to be involved in regulation of flowering time in vernalization and photoperiod pathways were each tightly mapped with these QTLs (Fig. 2).

The first QTL co-located with the *VRN-A1* (*AP1-A*) locus on chromosome 5A (Fig. 2a). This locus not only influenced stem elongation as reported in the previous study of this population (Chen et al. 2009) but also strongly affected heading date and physiological maturity. Besides five SSR markers and *VRN-A1* in this group, a new PCR marker for RFLP marker *Xcdo708* was developed and mapped with the QTL on chromosome 5A. *Xcdo708* was reportedly linked with *VRN1* in a high-density genetic map previously used to clone *VRN1* (Yan et al. 2003).

The second QTL co-located with the *PPD-D1* locus on chromosome 2D (Fig. 2b). This locus had a minor effect on stem elongation, but no QTL was previously named by Chen et al. (2009) since its LOD value did not exceed the threshold value. The mapping of *PPD-D1* in the same group as 11 SSR markers on chromosome 2D validated the chromosomal location of this QTL. A genetic distance of 26 cM separated *PPD-D1* and *Xwms261*, which might have resulted in a skewed curve of the QTL peak toward the right side (Fig. 2b). The attempt to fill the large gap between *PPD-D1* and *Xwms261* using SSR markers was not successful, due to lack of polymorphic markers in this region.

The third QTL co-located with the *VRN-D3* (*FT-D*) locus on chromosome 7D (Fig. 2c). Nine SSR markers reported on the wheat consensus map were mapped encompassing *VRN-D3*, validating the chromosomal location of this QTL.

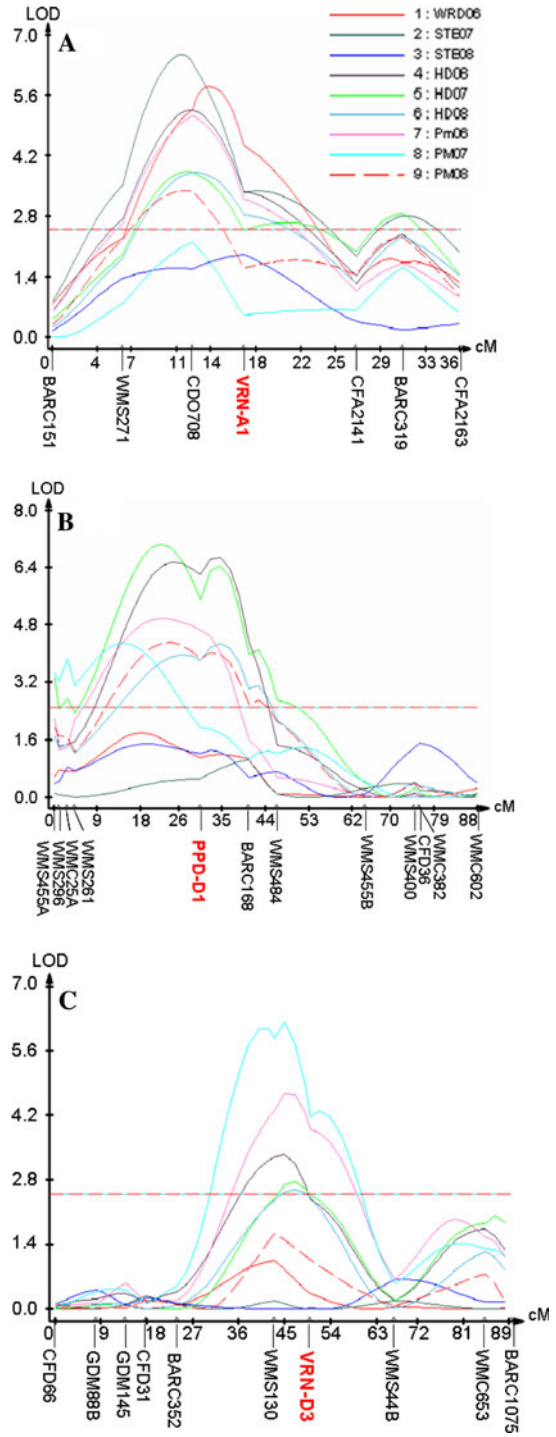

Fig. 2 Chromosome locations of QTLs for STE, HD, and PM in the population of RILs derived from the cross Jagger × 2174. The traits were scored in 2006, 2007, and 2008. The QTLs on 5A, 2D, and 7D peaked at *VRN-A1*, *PPD-D1*, and *VRN-D3*, respectively. *Horizontal dashed line* indicates LOD threshold

In order to facilitate the present discussion, these three QTLs responsible for variation in developmental process among Jagger × 2174 RILs are referred to as the *VRN-A1* locus, the *PPD-D1* locus, and the *VRN-D3* locus.

Multiplex effects of single QTLs

The *VRN-A1* locus explained 21.5% of the phenotypic variation for initial stem length, 18.1% of the phenotypic variation for heading date, and 17.4% of the phenotypic variation for physiological maturity (Table 1). Thus the *VRN-A1* locus was functional from stem elongation to maturity of wheat.

The *PPD-D1* locus accounted for only 6.7% of the phenotypic variation at stem elongation, indicating this locus was functional immediately before or at initiation of stem elongation. In contrast, contributions of the *PPD-D1* locus ranged from 20.1% at physiological maturity to 29.7% at heading (Table 1). While the *PPD-D1* locus played multiple roles in regulating reproductive development, its effect was easily detected at the heading stage and beyond.

Table 1 R^2 (%) value and additive effect of markers for three genes *VRN-A1*, *PPD-D1* and *VRN-D3* in their QTLs

Trait[a]		*VRN-A1*		*PPD-D1*		*VRN-D3*	
		R^2 (%)	Additive	R^2 (%)	Additive	R^2 (%)	Additive
STE	2006[b]	26.2	−0.59	6.6	0.29	5.2	−0.27
	2007	28.9	0.83	5	−0.34	0	0
	2008	9.4	0.41	8.4	−0.39	0	0
	Mean	21.5	0.22	6.7	−0.15	1.7	−0.09
HD	2006	17.3	−1.3	32.6	1.55	16.6	−1.13
	2007	18.9	−1.3	34.2	1.74	14.2	−1.15
	2008	18.1	−0.77	22.4	0.88	13	−0.68
	Mean	18.1	−1.12	29.7	1.39	14.6	−0.99
PM	2006	24.6	−0.53	32.8	0.66	22.5	−0.56
	2007	16.4	−0.66	7.5	1.3	29.5	−1.16
	2008	11.3	−0.83	20.1	0.9	9.5	−0.82
	Mean	17.4	−0.67	20.1	0.95	20.5	−0.99

[a] STE denotes initial internode length at stem elongation, HD denotes heading date, and PM denotes physiological maturity. Heading date was scored using 1 April as day 1. Physiological maturity was scored using 1 May as day 1

[b] A dormancy-release scale of 1 (earliest) to 5 (latest) was scored as a proxy for STE in 2006 (Chen et al. 2009)

No significant effect was detected for the *VRN-D3* locus on initial stem length scored in 2007 and 2008, but 5.2% of the phenotypic variation was detected in 2006 (Table 1). Contributions of the *VRN-D3* locus were readily detected later in reproductive development, both at heading (14.6% of the phenotypic variation) and at physiological maturity (20.5%).

The preceding discussion certainly pointed to the likelihood that each developmental stage was regulated by multiple genes (Fig. 2). Variation in initiation of stem elongation was predominantly influenced by the *VRN-A1* locus, with relatively minor influence by the *PPD-D1* locus, and no significant influence by the *VRN-D3* locus. Variation in heading date and physiological maturity was influenced by all of these three loci. A developmental consequence of the overlapping effects of these three loci is that any single developmental trait is likely regulated by multiple loci. Further, statistical analysis showed no significant interactions among *VRN-A1*, *PPD-D1*, and *VRN-D3* for their effects on any of these characterized traits; thus the magnitude of the effect of one locus on each developmental trait was independent of the effect of another locus.

Allele combinations among the three QTLs

The contribution of the various gene markers to each phenotype was assessed by comparing their sums of squares, given the lack of epistatic interactions. Table 2 shows phenotypic means for the three traits, classified by genotypes for the three QTLs in the order of *VRN-A1*, *PPD-D1*, and *VRN-D3*. The Jagger allele was designated A and the 2174 allele was designated B.

Six alleles and eight genotypes produced phenotypes predominately intermediate to the two parental lines. The genotype expected to initiate stem elongation the earliest should be the recombinant (A_B_X), containing the Jagger *VRN-A1* allele and the 2174 *PPD-D1* allele. Similarly, the genotype expected to initiate stem elongation the latest should be the recombinant (B_A_X or B_B_X), with the 2174 *VRN-A1* allele and either the Jagger or 2174 *PPD-D1* allele. *VRN-D3* had no significant effect on stem elongation, whereas *VRN-A1* and *PPD-D1* determined phenotypes for stem elongation, which was consistent with the result on phenotypic variation by QTL analysis.

Table 2 Genetic effects of various gene combinations on developmental processes

Trait	Genotype[a]	Mean[b]	Significance[c]	Relative timing
Stem elongation	A_B_X	4.6	a	Earliest
	A_A_X	3.8	b	
	B_B_X	3.3	bc	
	B_A_X	3.0	c	Latest
Heading date	A_B_A	21.5	a	Earliest
	A_B_B	22.4	a	
	B_B_A	22.6	a	
	A_A_A	23.6	ab	
	B_B_B	24.7	bc	
	A_A_B	25.9	cd	
	B_A_A	26.3	cd	
	B_A_B	27.6	d	Latest
Physiological maturity	A_B_A	28.5	a	Earliest
	A_B_B	29.3	ab	
	B_A_A	29.3	ab	
	B_B_A	29.9	bc	
	A_A_A	30.0	bc	
	B_B_B	30.4	bc	
	A_A_B	30.9	c	
	B_A_B	32.2	d	Latest

[a] A genotypes for three genes is in the order of *VRN-A1*, *PPD-D1*, and *VRN-D3*. The Jagger allele was designated A and the 2174 allele was designated B. X denotes A or B, because *VRN-D3* had no effects on initial internode lengths at stem elongation

[b] Means of initial internode lengths (cM) at stem elongation for 2 years 2007 and 2008 was compared, because this trait was characterized in 2006 using winter dormancy release as a scale. Average of heading date (days) for 3 years was compared. Average of physiological maturity (days) for 2 years 2007 and 2008 was compared, because physiological maturity in 2006 was characterized in a different scale. Heading date was scored using 1 April as day 1. Physiological maturity was scored using 1 May as day 1

[c] A significance for comparing mean difference is at $P < 0.05$ level

The phenotypic difference between genotypes with the earliest heading date (A_B_A) and the latest heading date (B_A_B) was 6.1 days, whereas the largest difference for physiological maturity was 3.7 days for the same two genotypes (Table 2). Thus the promoting effect of *VRN-D3* on physiological maturity exceeded that on heading date. It might be expected that as maturity is approached, synchrony between shoot development and the environment will increase to ensure ultimate formation of grain.

Table 3 Genotypes of *VRN-A1*, *PPD-D1*, and *VRN-D3* in 18 winter wheat cultivars applied in the southern Great Plains

Genotype[a]	Cultivar
A_B_A	N/A
A_B_B	N/A
B_B_A	Custer, Jagalene, Lakin
A_A_A	Jagger
B_B_B	2174, Deliver, Endurance, Ok102
A_A_B	Overley
B_A_A	Above, Cutter, Intrada, OK Bullet, TAM 110
B_A_B	Guymon, Okfield, TAM 111, Trego

[a] A genotypes for three genes is in the order of *VRN-A1*, *PPD-D1*, and *VRN-D3*. The Jagger allele was designated A and the 2174 allele was designated B

Tri-locus genotypes of wheat cultivars

Genotypes of 18 commercially available winter wheat genotypes, including Jagger and 2174, were described in Table 3. No A_B_X genotypes for early stem elongation were found, indicating that these two genotypes were inadvertently selected against in the region. Association analysis of 18 winter wheat cultivars and the developmental process using cumulative thermal units (Edwards et al. 2007) showed that the *VRN-A1* locus had a significant effect on stem elongation in the previous study (Chen et al. 2009), but also had strong genetic effects on heading date ($Pr<0.05$). No significant effect of *PPD-D1* or *VRN-D3* was detected on the stem elongation or heading date stages in the 18 cultivars analyzed in this study.

Discussion

When two parental lines with diverse genetic backgrounds are used to generate random inbred lines, segregation in the phenotypes of agricultural interest is complicated by the fact that more than one gene contributes to the phenotype. Particularly in the case that the variation is slight—for example, maturity time with a few days difference in hexaploid wheat carrying triplicated genes—it will be extremely difficult to map genes that are responsible for segregation of this trait. In this study, we found that variation in developmental process in the winter wheat population was controlled by three major QTLs that were tightly associated with *VRN-A1*, *PPD-D1*, and *VRN-D3* loci.

All of these three loci had a durable effect on developmental process. The *VRN-A1* locus had stable effects from stem elongation to physiological maturity. The effect of the *PPD-D1* locus was minor at stem elongation but increased at heading date and maturity indicating that this locus started to have effect later than the *VRN-A1* locus. The *VRN-D3* locus was not detected at stem elongation but reached the maximum effect on physiological maturity scored in this study. It was unexpected that the effect of the *VRN-D3* locus associated with physiological maturity but not with stem elongation, because orthologous wheat *VRN-B3* was reported to have a critical role in determination of the developmental transition timing (Yan et al. 2006). It is likely that *VRN-D3* had a different mechanism from *VRN-B3* in regulating developmental process if *VRN-D3* was responsible for the QTL mapped in this study.

The marker for *VRN-D3* was developed in this study based on sequences of orthologues of *VRN-B3* in wheat and *VRN-H3* in barley, both of which were cloned in parallel experiments and were identified as the *FT* gene in temperate cereal crops (Yan et al. 2006). In spring wheat cultivar Hope, the dominant *Vrn-B3* allele was associated with the insertion of a retroelement in its promoter region, whereas in spring barley, mutations in the dominant *Vrn-H3* allele occurred in the first intron of this gene (Yan et al. 2006). Variation in the non-coding intronic region in *VRN-A3* (=*FT-A*) and *VRN-D3* (=*FT-D*) was also tightly associated with heading date in a large collection of diverse germplasm (Bonnin et al. 2008). Specific primers for *VRN-D3* were designed for $(G)_{3or4}$ polymorphism in exon 3, where Jagger had 4(G) but 2174 had 3(G). This polymorphism was also reported in many genetic materials (Bonnin et al. 2008). The loss of one nucleotide in the variety 2174 has resulted in a frame shift involving 81 amino acids. Hence, this mutated *VRN-D3* might be a non-functional allele, if it was indeed responsible for its corresponding QTL. Otherwise, a novel gene regulating heading date and physiological maturity of winter wheat is located at the *VRN-D3* locus.

The marker for *VRN-A1* was developed based on a difference in sequence in the coding region that was found to cause a mutation in the amino acid sequence of *VRN-A1* between the two cultivars (Chen et al. 2009). When winter and spring wheat are compared, allelic variation at *VRN1* relies on natural mutations at its promoter or intron one region (Fu et al. 2005; Yan et al. 2003). This gene has been extensively studied in recent studies (Danyluk et al. 2003; Dubcovsky et al. 2006; Murai et al. 2003; Trevaskis et al. 2003, 2006; von Zitzewitz et al. 2005). *VRN1* has been shown to have multiple mechanisms in regulation of flowering time in wheat (Pidal et al. 2009). If *VRN-A1* was indeed responsible for the QTL on chromosome 5AL, it could have different mechanisms in regulating the developmental process in winter wheat (as opposed to spring wheat). Otherwise, a novel gene regulating developmental process of winter wheat is located at the *VRN-A1* locus.

Conversely, the regulatory mechanism of the genes in the photoperiod pathway may be relatively conserved (Dubcovsky et al. 2006). The marker for *PPD-D1* was cited from the published information (Snape et al. 2001; Beales et al. 2007). Jagger is sensitive to photoperiod, whereas 2174 carries a mutated *PPD-D1* gene (i.e. *PPD-D1a* allele) that is insensitive to photoperiod (Chen et al. 2009).

Selection has already been exerted in extending the vegetative phase while maintaining time to maturity. The 2174 *VRN-A1* allele has been incorporated into several contemporary winter wheat cultivars to possibly avoid precocious stem elongation where frost or freeze events frequently occur in early spring (Chen et al. 2009). However, The 2174 *PPD-D1a* allele promoting development should be selected so that heading date is not excessively delayed due to the delayed stem elongation (Wang et al. 2009). The Jagger *VRN-D3* allele promoting development should also be selected for the same purpose in order to ensure early plant maturity in wheat growing areas where drought and hot weather frequently occur. Although no tight association between the phenotype including stem elongation and heading date and the genotype of *PPD-D1* or *VRN-D3* was found in cultivars analyzed using the previous phenotypic data (Edwards et al. 2007), a significant genetic effect of a single *VRN-D3* locus was detected in previous studies (Wang et al. 2009; Bonnin et al. 2008). These contradicting results could be explained due to the complexity of multiple genes involved in developmental processes in hexaploid wheat cultivars. Recombination of the three QTL genotypes could be used to explain phenotypes of a specific cultivar. For example, Trego which was extremely late in

reproductive development across years and locations (Edwards et al. 2007) has an expected B_A_B genotype consisting of 2174 *VRN-A1* and *VRN-D3* alleles and the Jagger *PPD-D1* allele, each with a repressing effect on development.

Many QTLs for a single trait have been reported, but one QTL may appear in one population but not in another one, because of various genetic backgrounds of the parental lines used in different experiments (Yan 2009). When three traits scored at specific developmental stages were simultaneously mapped in the same population in this study, it has provided valuable information for variation in a developmental phase between two successive stages. The maximum grain yield of a given cultivar is obtained by optimizing the combination of genes determining each developmental phase (McMaster 2009). The numbers of tillers per plant and spikelets per spike are affected by duration of the phase immediately preceding stem elongation; the number of kernels per spikelet is mainly regulated by duration of the phase from stem elongation to heading or flowering time; and, kernel weight is largely determined by the duration of post-flowering phases (Whitechurch and Slafer 2001). These patterns offer insight into the genetic potential that may be used to manipulate various phases of reproductive development for different objectives, given a myriad of these developmental genes found in nature. Simulation models of wheat have increasingly incorporated these developmental concepts to varying degrees to increase yields in a broad range of latitudes and environmental conditions. Simulated scenarios of variation in flowering time of 29 cultivars from 34 geographical areas worldwide could be explained according to genes for vernalization requirement and photoperiod sensitivity (White et al. 2008).

The tight association between each of the three QTLs and one of these flowering time genes has suggested that each of these flowering genes naturally and intuitively constituted one candidate gene for one QTL. However, these hypotheses could not be validated in this present study because of the limited size of RIL population that was used for mapping only. Specific RILs can be selected to develop backcross populations in which only one locus of the three QTLs is heterozygous while the other two are fixed for the same allele; therefore each of these backcross population can be used to clone each of the three QTLs in future cloning projects.

We concluded that the earliness or lateness of a developmental stage of winter wheat was controlled by more than one gene; moreover, the effect of a gene was detected at different developmental stages. Therefore, integration of different loci, their alleles, and duration of their effects altogether regulated the timing of each developmental stage, and thus various developmental phases. Improved understanding of the genetic basis of variation in the developmental phase will be particularly important to production of winter wheat worldwide.

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