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ORIGINAL PAPER

Genetic loci associated with stem elongation and winter dormancy release in wheat

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Abstract In winter wheat (*Triticum aestivum* L.), the stem begins to elongate after the vernalization requirement is satisfied during winter and when favorable temperature and photoperiod conditions are attained in spring. In this study, we precisely measured elongation of the first extended internode on 96 recombinant inbred lines of a population that was generated from a cross between two winter wheat cultivars, Jagger (early stem elongation) and 2174 (late stem elongation). We mapped a major locus for stem elongation to the region where *VRN-A1* resides in chromosome 5A. Visible assessment of winter dormancy release was concomitantly associated with this locus. *VRN1* was previously cloned based on variation in vernalization requirement between spring wheat carrying a dominant *Vrn-1* allele and winter wheat carrying a recessive *vrn-1* allele. Both of two winter wheat cultivars in this study carry a recessive *vrn-A1* allele; therefore, our results suggest that either *VRN-A1* might invoke a new regulatory mechanism or a new gene residing close to *VRN-A1* plays a regulatory role in winter wheat development. Phenotypic expression of the *vrn-A1a* allele of Jagger was more sensitive to the year of measurement of stem elongation than that of the *vrn-A1b* allele of 2174. In addition to *QSte.osu.5A*, several loci were also found to have minor effects on initial stem elongation of winter wheat. Seventeen of nineteen locally adapted cultivars in the southern Great Plaints contained the *vrn-A1b* allele. Hence, breeders in this area have inadvertently selected this allele, contributing to later stem elongation and more conducive developmental patterns for grain production.

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Introduction

Wheat is traditionally harvested strictly as a grain crop, but in some areas of the world, winter wheat is utilized for forage and grain production in the same crop season, as in a dual-purpose management system (Carver et al. 2001; Manupeerapan et al. 1992). In the southern Great Plains of the USA, approximately 3.2 million hectares of wheat are managed annually for dual-purpose production (Pinchak et al. 1996). In the dual-purpose system, wheat is planted from August to September, grazed by cattle (*Bos taurus* L.) from mid-October to early March, and harvested for grain in June (Edwards et al. 2007). This management system requires a longer vegetative growth phase, during which more plant tissue is used for cattle grazing, compared with traditional cultivation for grain-only production (Redmon et al. 1996).

The life cycle of wheat from sowing to maturity is marked by several critical physiological and morphological stages, including seedling emergence, stem elongation or jointing, heading, flowering, and maturity (Gonzalez et al. 2002; Hay and Kirby 1991). When sown in fall, winter wheat is exposed to a period of low temperature to accelerate the transition from vegetative to reproductive development, a phenomena known as vernalization (Chouard 1960). This developmental mechanism ensures that winter wheat does not enter the reproductive phase, and therefore its stem does not elongate, prior to the time that potentially damaging cold temperatures could terminate reproductive development. Hence proper timing of stem elongation is

needed to avoid late-winter freeze and early-spring frost injuries. Wheat can tolerate temperatures as low as −10°C before stem elongation, but following stem elongation, a −4°C temperature may result in severe injury to wheat florets (Fowler et al. 2001). A later stem elongation stage also is desirable when wheat is used as a winter forage resource in the dual-purpose system (Redmon et al. 1996); however, an earlier stem elongation stage is desirable for lengthening the reproductive phase to increase the number of fertile florets (Goncharov 2003).

Field-grown winter wheat usually has a total of 13–14 leaves or nodes on the main stem, of which the lower 7–8 internodes do not extend, but the upper 5–6 internodes extend in sequence to form a hollow stem, which can be identified above the root system and below the developing head (McMaster 2005). When the first extended internode elongates and reaches approximately 1.5 cm, which is also called the first-hollow-stem (FHS) stage (Edwards et al. 2007), cattle should be removed from wheat pasture in a dual-purpose production system; otherwise, continued grazing could impair spike production (Khalil et al. 2002). Therefore, precocious stem elongation is an undesirable trait for a dual-purpose wheat cultivar.

The stem begins to elongate prior to winter dormancy release ("returning green" in Asia) and jointing, the stage at which enlarged nodes and extended internodal tissues form a crown that is detectable above the soil surface (Penrose et al. 2003; Whitechurch et al. 2007). At the jointing stage, the plant starts to produce terminal spikelets and should be at Zadoks stage 31 according to the scales developed for the *Triticeae* (Haun 1973; Zadoks et al. 1974). At this point, the plant apex has completed the transition from vegetative to reproductive development. Vernalization accelerates the developmental transition of winter wheat, but cold temperature also keeps winter wheat in a dormant state during the cold winter season (Chouard 1960). Winter dormancy will be released when favorable temperature and long-day conditions are attained in the following spring.

As an easily observable trait, heading date or flowering time has been used to represent this developmental transition in numerous studies on the effects of genetic loci and environmental stimuli in plants. For example, flowering time is usually scored to describe responses to vernalization (Simpson and Dean 2002). However, stem elongation should more precisely represent the timing of developmental transition than heading date or flowering time, as it occurs immediately following the transition but prior to heading. In addition, the phenotypic range in heading date is smaller than the phenotypic range in FHS stage (Edwards et al. 2007); thus FHS stage may facilitate more precise mapping of the vernalization effects in winter wheat. A recent study showed a positive and linear relationship between dates of FHS stage and heading for a large sample of winter wheat cultivars in the field for several years (Edwards et al. 2007). However, the genetic linkage between stem elongation and heading date may not be absolute in genetic populations with no prior selection for one or both events. As many genetic and environmental factors are involved in wheat development, no tight association was detected between these two phenological events (Kirby et al. 1999).

Genes controlling the development and growth of wheat are believed to function in three pathways: vernalization, photoperiod, and earliness per se (Laurie et al. 1995; Snape et al. 2001; Dubcovsky et al. 2006; Valárik et al. 2006). For winter wheat that is planted in fall and experiences a winter season, vernalization plays a critical role in transition of the switch from vegetative primordia to spikelet primordia production (i.e., the floral initiation stage) (Dubcovsky et al. 1998; Flood and Halloran 1986; Griffiths et al. 2003; Law 1966; Law and Wolfe 1966; Pugsley 1971, 1972). When any vernalization requirement in a winter wheat cultivar has been satisfied, its developmental rate will be mainly affected by genes in the photoperiod pathway, as it occurs in spring wheat (Jamieson et al. 1998; Snape et al. 2001; Beales et al. 2007).

The present study aims to identify genetic loci responsible for stem elongation in a population of recombinant inbred lines (RILs) produced by a cross between two winter wheat cultivars, Jagger (early stem elongation) and 2174 (late stem elongation). The molecular markers for mapped quantitative trait loci (QTLs) were then used to investigate their association with observed differences among contemporary winter wheat cultivars.

Materials and methods

Two locally adapted cultivars, Jagger and 2174, showed a significant difference of 13 days in date of FHS stage averaged across several years in the southern Great Plains (Edwards et al. 2007). Among years, stem elongation in Jagger started from February 10–20, whereas stem elongation in 2174 started from February 15–25 (J. Edwards, 2008 personal communication). Thus Jagger initiated stem elongation almost two weeks earlier than 2174 and with greater inconsistency among environments.

Jagger and 2174 were crossed to generate a population of RILs by single-seed descent from the F_2 generation. A set of 96 $F_{6:8}$ RILs with their parental lines was first evaluated at the Agronomy Res. Stn. in Stillwater, OK (latitude: 36°N, longitude: 97°W) USA in 2006. Winter dormancy release was visually scored on 16 March 2006 according to a rating scale used routinely in the Oklahoma State University breeding program: 1 = very early, canopy higher than 25 cm with advanced leaf expansion; 2 = early, canopy

between 15–25 cm; 3 = intermediate, canopy between 10–15 cm; 4 = late, canopy less than 10 cm; and 5 = very late, prostrate, non-elevated canopy with no apparent sign of leaf expansion. This approach provides an indirect indicator of winter dormancy release without actually measuring stem elongation.

After finding a major locus associated with winter dormancy release based on the visual scale, initial stem length of each $F_{6:8}$ RIL was measured at the North Central Res. Stn. near Lahoma, OK on 21 March 2007. The same set of RILs $F_{6:8}$ were tested at Stillwater in 2008, where the initial stem length was not measured until 26 March, as reproductive development was slightly delayed by a longer winter season. Approximately 20 plants were removed from the field plot for each line, from which 10 random plants were separated to measure hollow-stem length. The main stem of each plant was separated and removed from the roots just above the unextended internode section. The distance between the base and the end of the hollow stem was measured.

A total of 52 hard winter wheat cultivars or advanced lines were evaluated for their date of FHS stage during a 7-year period (1999–2005) in a previous study (Edwards et al. 2007). Nineteen cultivars of this collection, including Jagger and 2174, were characterized for molecular markers for a major QTL identified in this study.

A total of 1,500 SSR markers was used to identify DNA polymorphisms for the two parental lines on 6–9% acrylamide/bisacrylamide gel (19:1). Polymorphic markers were used to map 96 single-plant selections from each of the original $F_{6:8}$ RILs of the population. Previous PCR markers for *VRN-A1* were used to identify allelic variation of Jagger and 2174, in the promoter using primers VRN1AF (5′-GAAAGGAAAAATTCTGCTCG-3′) and VRN1AR (5′-TGCACCTTCCCCCGCCCCAT-3′) (Yan et al. 2004a), in intron 1 for deletion using primers Intr1/A/F2 (5′-AGCCTCCACGGTTTGAAAGTAA-3′) and Intr1/A/R3 (5′-AAGTAAGACAACACGAATGTGAGA-3′) and for insertion primers Intr1/C/F (5′-GCACTCCTAACCCACTAACC-3′) and Intr1/AB/R (5′-TCATCCATCATCAAGGCAAA-3′) (Fu et al. 2005). A new PCR marker specific to genome A was developed for a single nucleotide polymorphism (SNP) in exon 4 of *VRN-A1* between the two parental lines, based on the forward primer sequence 5′-CAACTTGTTTGGGACTAAAGGC-3′ from intron 2 and the reverse primer sequence 5′-CTGCAACTCCTTGAGATTCAAAG-3′ from intron 6. PCR was performed for 40 cycles (90°C for 30 s, 55°C for 30 s, and 72°C for 60 s per cycle) followed by a 10-min final extension at 72°C. PCR products were digested with *Dpn* II and run on a 1% agarose gel.

A PCR marker was developed to distinguish between varieties of sensitivity and insensitivity to photoperiod in wheat (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 *PPD-D1* from Jagger and 2174, and polymorphic genomic fragments of *PPD-D1* allowed it to be mapped in the study population.

Genetic linkage groups were first constructed using MapMaker 3.0 (Whitehead Institute for Biomedical Research, Cambridge, MA). WinQTLCart 2.5 (North Carolina State University, Raleigh) was then used to conduct analyses using interval mapping (IM), and composite interval mapping (CIM). The IM models 5 and 6 were used with a 10-cm window size and 2-cm walk speed. A QTL was declared when logarithm of the odds (LOD) score exceeded the threshold calculated using a permutation level at 300 and significance level at 0.05. Centimorgan values were calculated based on the Kosambi mapping function. The PROC GLM function of SAS Version 9 (SAS Institute, Raleigh, NC) was also used to test the effects of a single marker and to test interactions between the gene or SSR markers and years.

Results

Phenotypic variation in stem elongation and winter dormancy release

The 96 $F_{6:8}$ RILs in 2007 showed clear segregation for initial stem length, varying from 1.44 to 8.25 cm (Fig. 1a), indicating that stems of all tested RILs had elongated. This quantitative measurement of reproductive development provided a relatively wide window of trait expression for mapping in the Jagger × 2174 population. In addition, this single-point measurement revealed variation in stem elongation that differentiated the population of RILs by up to 2 weeks in developmental time. In 2008, the same set of 96 $F_{6:8}$ RILs again also showed clear segregation for initial stem length (Fig. 1b), but the phenotypic range was compressed between 1.19 and 6.87 cm, consistent with a longer winter season.

The mean stem length of the tested RILs was not distributed necessarily in a bimodal manner (Fig. 1a, b). Furthermore, the respective stem lengths of Jagger and 2174 were 4.5 and 2.1 cm in 2007 and 3.3 and 3.0 cm in 2008. The restricted range of parent stem lengths relative to the RIL population indicated that gene dispersion existed between these parents and that recombination among multiple regulatory loci may have caused some RILs to elongate earlier or later than the two parental lines.

The distribution pattern for visual assessment of winter dormancy release paralleled the quantitative measurement of stem elongation (data not shown). As a more empirical trait, winter dormancy release is a convenient way to

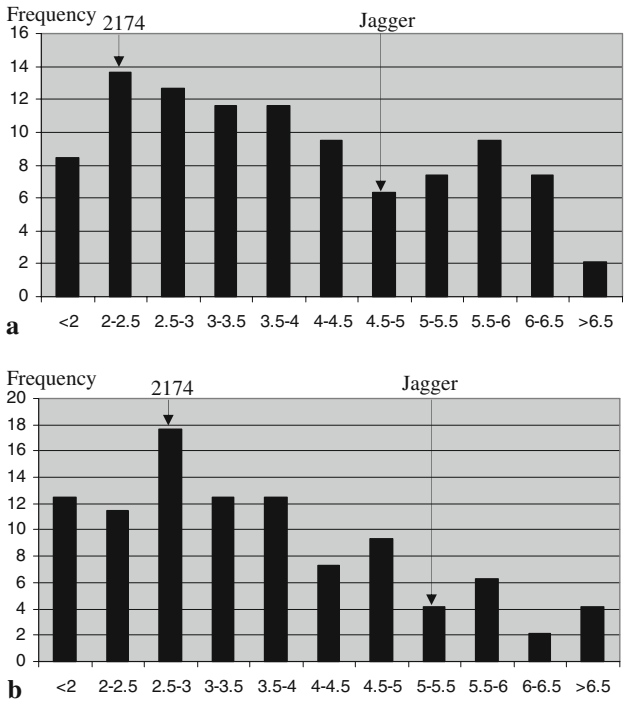

Fig. 1 Frequency distribution for stem elongation of 96 RILs from a cross between Jagger and 2174 winter wheat. The initial stem length of 10 plants of each line was measured on 21 March 2007 (**a**) and 26 March 2008 (**b**) when the main stem of all *lines* in the population had elongated. The mean stem lengths (cm) were grouped, and the values of two *parental lines* were assigned to a given group that is indicated by an *arrow*

describe jointing in breeding populations, a critical developmental stage in wheat production. Among the 96 RILs, winter dormancy release evaluated in 2006 and stem elongation measured in 2007 were phenotypically associated ($r = 0.49$, $P < 0.05$), although these two phenological descriptors were scored in different locations and different years. The association between winter dormancy release and stem elongation simultaneously measured in 2008 was also significant ($r = 0.55$, $P < 0.01$). The significant correlations between stem elongation and winter dormancy release of RILs in the population indicated that these two traits were likely controlled by a common gene(s).

Genetic loci responsible for stem elongation

A total of 246 polymorphic markers screened from 1,500 SSR markers was used to map stem elongation in the segregating population. Four genomic regions on chromosomes 5A, 1B, 2D, and 6A were found to have significant effects on stem elongation.

Nine SSR markers forming a linkage group mapped to the long arm of chromosome 5A (Fig. 2a). To determine if *VRN-1* on chromosome 5A (*VRN-A1*) was linked with this group, two PCR markers for variation in the promoter region (Yan et al. 2004a) or intron one (Fu et al. 2005) of *VRN-A1* were used to test for polymorphism between Jagger and 2174; however, no difference was detected (Fig. 3a, b). The amplified promoter and intron 1 regions of the *VRN-A1* gene from the two parental lines were also sequenced and confirmed to have no mutation sites present in these critical regulatory sites (data not shown).

These results were not surprising, because the previous PCR markers for *VRN-A1* were developed to distinguish *VRN-A1* alleles between spring wheat carrying a dominant *Vrn-A1* allele and winter wheat carrying a recessive *vrn-A1* allele. In this study, both winter wheat cultivars should carry a recessive *vrn-A1* allele. Further sequencing of the *VRN-A1* gene showed that a point mutation occurred in exon 4 of *VRN-A1*. This point mutation resulted in a polymorphism between Jagger and 2174 that can be distinguished by the restriction enzyme *Dpn* II (Fig. 3c). Therefore, a PCR marker specific for this polymorphic site in the *VRN-A1* gene was developed and mapped in the Jagger × 2174 population. *VRN-A1* was found to reside in the linkage group, flanked by *Xcfa2141* and *Xbarc151*, in a range of approximately 30 cm (Fig. 2a).

The linkage group containing *VRN-A1* that was associated with variation in stem elongation in 2007 (STE07) mapped to chromosome 5A and is referred to as *QSte.osu-5A*. *VRN-A1* exhibited the most significant association with STE07 (Fig. 2a). An apparent second peak was centered on marker *Xbarc319* in this locus, but it dissipated with CIM analysis, which statistically adjusts for the genotypes at marker loci other than those immediately flanking the candidate QTL.

A second locus associated with stem elongation STE07 was mapped to chromosome 1B, and this locus is thus referred to as *QSte.osu-1B* (Fig. 2b). Eight SSR markers resided in this group. The QTL peak was centered on markers *Xcfa2129* and *Xcfd48* and flanked by two SSR markers *Xcfd20* and *Xwmc134* covering approximately 18 cm, and all of them were located on the long arm of chromosome 1B in a high-density SSR map (Somers et al. 2004).

Using the PCR marker for *PPD-D1* (Beales et al. 2007), Jagger showed a 414-bp band that represents an allele sensitive to long days, whereas 2174 showed a 288-bp band that represents an allele insensitive to long days (data not shown). *QSte.osu-2D* including *PPD-D1* on chromosome 2D was found associated with variation in the initial stem length, but the LOD values for this association were lower than the threshold value (Figure not shown). *PPD-D1* had effects on the initial stem length, explaining 5 and 8.4% of the total phenotypic variation in 2007 and 2008 respectively.

A minor locus found near *Xwms427* on chromosome 6A (QSte.osu-6A) was also associated with variation in the initial stem length but just failed to meet the threshold LOD values (data not shown).

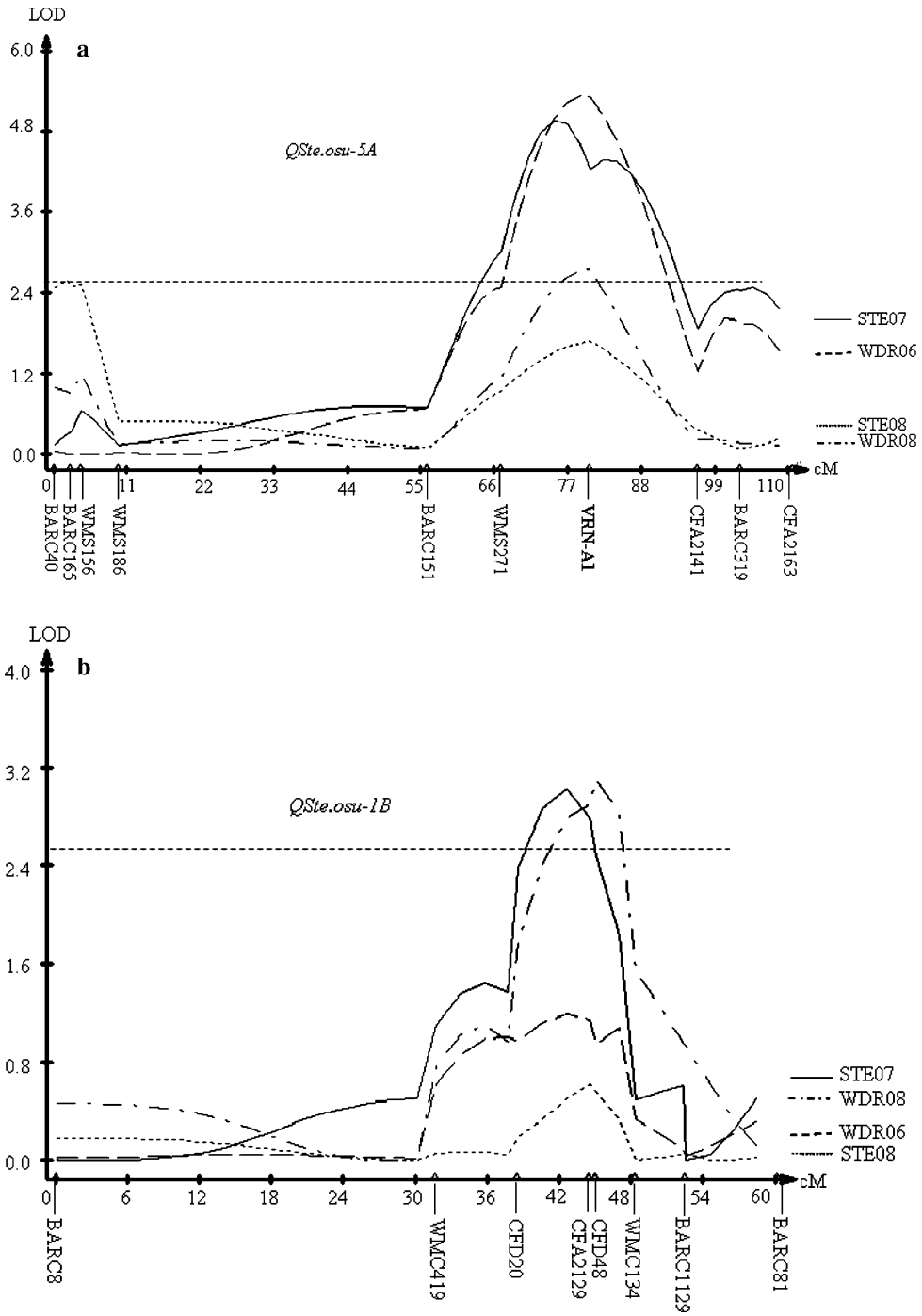

Fig. 2 Genetic loci associated with stem elongation of 96 RILs from a cross between Jagger and 2174 winter wheat. **a** *QSte.osu-5A*. This QTL map was constructed using Internal Mapping program. **b** *QSte.osu-1B.* This QTL map was constructed using Composite Internal Mapping to decrease disruption from those markers immediately flanking the QTL. STE07 and STE08 denote initial stem elongation measured in 2007 and 2008 respectively. WDR06 and WDR08 denote winter dormancy release assessed in 2006 and 2008 respectively

All four QTLs for stem elongation had a significant effect on this trait in 2007. *QSte.osu-5A* (*VRN-A1*), *QSte.osu-1B* (*Xcfa2129*), *QSte.osu-2D* (*PPD-D1*), and *QSte.osu-6A* (*Xwms427*) respectively explained 28.9, 15.5, 6.7, and 5.8% of the phenotypic variation in stem elongation (Table 1).

Interactions between *QSte.osu-5A* and years

In order to confirm the main role of *QSte.osu-5A* in regulation of stem elongation in winter wheat, we repeated experiments with same set of RILs in 2008 at a different location in north-central Oklahoma. Even though results from different years and locations were compared, initial stem length showed significant correlations between 2007 and 2008 ($r = 0.47$, $P < 0.01$), indicating a great validity to the repeatability of QTL effects. On the average between 2007 and 2008, a single marker on each of QTLs showed significant effects on initial stem length (Table 1). It is noteworthy that the Jagger allele for the QTLs on 5A (*VRN-A1*), 1B (*Xcfa2129*), and 6A (*Xwms427*) promoted stem

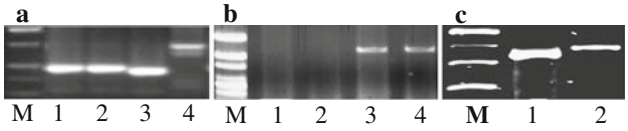

Fig. 3 Development of a *VRN-A1* marker in winter wheat. **a** Alleles for *VRN-A1* promoter were identified using primers VRN-A1F and VRN-A1R, a recessive *vrn-A1* allele in Jagger (*lane 1*) and 2,174 (*lane 2*), a dominant *Vrn-A1b* allele in Marquis (PI 94548) (*lane 3*) and *Vrn-A1a* in Triple Dirk D line (*lane 4*). **b** Alleles for *VRN-A1* intron one were identified, the deletion in intron one is absent in Jagger (1) and 2,174 (2), and the insertion in intron one is present in Jagger (3) and 2,174 (4). **c** The PCR marker for *VRN-A1* distinguishes between the two *parental lines* using specific primers for PCR following *Dpn* II digestion: Jagger (1), 2,174 (2)

Table 1 The phenotypic variation explained by single marker on the initial stem length (cm) of the 96 Jagger × 2174 RILs averaged over years 2007 and 2008

	R^2 (%)	Jagger allele	2174 allele	Difference	*Pr*
VRN-A1	19.2	4.13	3.31	0.82	0.0001
Xcfa2129	10.1	3.99	3.37	0.62	0.0028
PPD-D1	6.7	3.44	4.02	−0.68	0.0067
Xwms427	5.8	4.01	3.51	0.50	0.0185

elongation, whereas the Jagger allele for the locus on 2D (*PPD-D1*) had an opposite effect (Table 1). Statistical analysis revealed no significant inter-locus interactions (data not shown), indicating each QTL exerted an independent effect on stem elongation.

The LOD value of *QSte.osu-5A* on STE08 was decreased in 2008 relative to 2007 (Fig. 2a). The contribution of *QSte.osu-5A* to variation in stem elongation decreased from 28.9% in 2007 to 9.4% in 2008. Interestingly, a new QTL on chromosome 5A was detected in the same linkage group as *VRN-A1* (Fig. 2a). This locus centered on *Xwms156*, which had a significant effect on initial stem length in 2008, explaining 13.5% of the phenotypic variation. Further analysis showed no significant interactions between *Xwms156* and *VRN-A1* on the *QSte.osu-5A* locus.

A statistical result showed that not only the *VRN-A1* locus had a highly significant effect on the initial stem length but also the interaction between the *VRN-A1* locus and years was significant ($P < 0.05$), explaining variable magnitude of the *VRN-A1* locus effect between years (Table 2). The effect of the *vrn-A1a* allele of Jagger on initial stem length was less stable over years than the *vrn-A1* allele of 2174. The initial stem length of all RILs carrying the *vrn-A1b* allele did not change significantly between years (3.23 cm in 2007 vs. 3.37 cm in 2008), whereas the RILs carrying the *vrn-A1a* allele showed a significant

Table 2 The interaction between *VRN-A1* and year on the initial stem length in the 96 Jagger × 2174 RILs in years 2007 and 2008

Source	*Df*	SS	MS	*F*	*Pr*
Year	1	26.4567	26.4567	14.66	0.0002
VRN-A1	1	3.6090	3.6090	2.00	0.1590
VRN-A1 × Year	1	10.3836	10.3836	5.75	0.0174
Error	185	333.8178	1.8044		

difference between years, (4.53 cm in 2007 vs. 3.74 cm in 2008). This pattern is not unique from that seen among the parental cultivars, 2174 and Jagger, when evaluated for timing of first-hollow-stem stage, as Jagger will often show wide fluctuation in FHS stage among years (Edwards et al. 2007). In 2008, it had the longer length of winter season. The extended low temperature might have resulted in a decrease of the difference in the stem elongation stage between the two alleles of Jagger and 2174.

Genetic loci responsible for winter dormancy release

Winter dormancy release was simultaneously analyzed with the stem length, which provided validity to QTLs controlling stem elongation as a quantitative trait. Winter dormancy release showed significant correlations between 2006 and 2008 ($r = 0.53$, $P < 0.01$). Winter dormancy release assessed in 2006 (WDR06) coincidently mapped in *QSte.osu-5A* and showed a perfect match with the LOD peak for stem elongation (Fig. 2a). *QSte.osu-5A* also had greatest effect on winter dormancy release among QTLs detected in this population. Though these two traits were measured in different environments and different years separated by two weeks in calendar date, they were obviously controlled by the same major gene on *QSte.osu-5A* locus. Winter dormancy release assessed in 2008 (WDR08) was also mapped associated with *QSte.osu-1B* and explained 13.8% phenotypic variation (Fig. 2b). Only a small effect was observed on WDR06 at this locus.

Association of *VRN-A1* and stem elongation among winter wheat varieties

A total of 19 locally adapted winter wheat cultivars, including Jagger and 2174, were genotyped using the *VRN-A1* marker developed in this study and analyzed with previous FHS data using cumulative thermal units (Edwards et al. 2007). Seventeen of those cultivars (Above, Cutter, Custer, Deliver, Endurance, Fannin, Guymon, Intrada, Jagalene, Lakin, Ok102, OK Bullet, Okfield, TAM 110, TAM 111, and Trego) carried the same *vrn-A1b* allele as 2174. Only one cultivar, Overley, carried the same *vrn-A1a* allele as Jagger. A significant difference in cumulative thermal units

required for arrival at FHS stage was detected between these two alleles ($P < 0.05$). No significant difference was detected in stem elongation among cultivars for the SSR markers on other QTLs.

Discussion

This marks the first report of a QTL mapped in association with stem elongation and winter dormancy release for a population generated from two winter wheat cultivars. Chouard (1960) discussed the common effect of cold temperature on three traits—vernalization, breaking of winter dormancy, and releasing of brachyblasty that was termed for "the shortening of internodes" and expressed by a "rosette" configuration—although these three phenomena can be differentiated based on their responses to other external stimuli such photoperiod and gibberellin. In that context, it was not completely unexpected that both stem elongation and winter dominancy release were mapped in association with a vernalization gene. Indeed, the RILs of the population were winter types and were tested in the field under natural winter conditions. However, *VRN-A1* was previously cloned based on variation in vernalization requirement between a spring wheat (no vernalization requirement) carrying a dominant *Vrn-1* allele and a winter wheat (requires vernalization) carrying a recessive *vrn-1* allele (Yan et al. 2003). The genetic mechanism(s) differentiated by spring versus winter types might not apply to variation between two winter wheat cultivars, since all winter cultivars should have a recessive allele at all three *VRN1* loci (Fu et al. 2005; Halloran 1967; Law et al. 1994; Pugsley 1971, 1972; Yan et al. 2004a). A dominant *Vrn-A1* allele was produced from mutations in the promoter or first-intron regions of a wild recessive *vrn1* allele (Dubcovsky et al. 2006; Fu et al. 2005; Yan et al. 2004a). No difference in these regulatory regions was found between Jagger and 2174, supporting our previous hypothesis. Thus it is reasonable to postulate that a novel genetic mechanism controls variation in apical development in winter cultivars by *VRN-A1* if it was a genetic determinant in this locus. Alterations in the effects of *vrn-A1* for one allele but not for the other between years, probably related to various lengths of the winter season, support our hypothesis that *VRN-A1* is involved in regulation of the developmental process in winter wheat. Further experiments need to be performed under controlled conditions in a greenhouse.

The wheat *VRN-A1* gene is an orthologue of the *Arabidopsis* meristem-identity gene *APETALA1* (*AP1*), which encodes a MADS-box protein and is responsible for the transition from vegetative to reproductive apices (Mandel et al. 1992). *AP1* in Arabidopsis influences flowering time but was not reported to be regulated by vernalization (Mandel et al. 1992). The function of *VRN1* and its interaction with other flowering genes has been extensively studied in wheat and other cereals (Danyluk et al. 2003; Dubcovsky et al. 2006; Murai et al. 2003; Trevaskis et al. 2003, 2006; von Zitzewitz et al. 2005; Yan et al. 2006; Yan et al. 2004b, 2005). More recent studies have indicated that *VRN-1* might play a pleiotropic role in wheat development. Segregation of heading date in a population generated from a cross between two spring wheat cultivars was associated with *VRN-A1* under field and controlled greenhouse conditions (Kuchel et al. 2006). Ectopic expression of the wheat *VRN1* in Arabidopsis not only promoted flowering but also altered development of floral organs, demonstrating pleiotropic effects of *VRN1* in plants (Adam et al. 2007). In transgenic wheat, RNA interference of *VRN1* by transforming a segment including the 3′-specific region of this gene delayed apical transition to the reproductive stage only by 2–3 weeks (Loukoianov et al. 2005), but a deletion in the promoter and MADS-box of this gene resulted in the plant's inability to shift from vegetative to the reproductive phase (Shitsukawa et al. 2007). All of these studies on *VRN1* function point to the possibility that *VRN1* could be regulated by different mechanisms. How the difference in developmental progression among winter wheat cultivars is regulated by *VRN-A1* remains to be more precisely investigated.

Alternatively, a new gene tightly linked to *VRN-A1* may be involved in the regulation of reproductive development in winter wheat, although it has not been reported that any locus close to *VRN-A1* regulates flowering time in cereal species. An additional locus *QEet.ocs-5A.2* located on the long arm of chromosome 5A has been reported responsible for heading date segregation in a population of wheat substitution lines, but *QEet.ocs-5A.2* is different from *VRN-A1* because it is contained in the same locus as *Xwms156*, which is close to the centromere of chromosome 5A (Kato et al. 2002) and was also observed effects in our experiment in 2008 (Fig. 2a). Further work will be conducted to backcross isogenic lines with the parental lines to create a population that will be used for fine mapping of the *QSte.osu-5A* locus.

QSte.osu-1B could be in a homoelogous region in the long arm of genome A that was reported to have a QTL for heading date (Kuchel et al. 2006). They have a common marker *Xcfa2129* that was located in a similar genomic location based on a high density of linkage maps (Somers et al. 2004). These comparative map studies indicate the presence of a QTL affecting wheat reproductive development on the long arm of chromosome 1B in a region close to the centromere. It was not reported that any gene resides in this region to regulate developmental process in cereal plants.

The *PPD-D1a* allele contains a 2-kb deletion upstream from its coding region that caused its mis-expression and a

perfect PCR marker for *PPD-D1,* based on this deletion, is developed (Beales et al. 2007). We used this marker to map *PPD-D1* in our population. Wheat varieties carrying the *PPD-D1a* allele are insensitive to photoperiod and flower earlier compared with those varieties sensitive to photoperiod (Beales et al. 2007). Jagger is photoperiod sensitive and 2174 insensitive based on the proposed perfect marker for *PPD-D1*. In the Jagger by 2174 population, *PPD-D1* exerted only a minor effect (6.7%) on stem elongation. *PPD-D1* had a large effect on heading date in spring wheat cultivars grown at high latitudes (Snape et al. 2001; Turner et al. 2005; Beales et al. 2007), and 41% of the variation for heading date in a winter wheat population grown at low latitudes, as this study, was also attributed to *PPD-D1* (Xu et al. 2005). We attribute this inconsistency to the different traits (heading date vs. stem elongation) being measured or from differences in the genetic background.

QSte.osu-6A was not found in any collinear region in cereals in previous studies. However, it was reported that chromosome 6A carries a gene controlling heading date by reciprocal sets of chromosome substitution lines between two winter wheat cultivars Cheyenne and Wichita (Zemetra and Morris 1984).

In this study, a significant correlation between stem elongation and heading date was observed, but no further analyses were conducted on heading date because other loci were found to affect heading date. This study focused on mapping of genetic loci responsible for stem elongation in winter wheat and on developing a molecular marker for selecting delayed stem elongation suitable for dual-purpose wheat production. Most winter wheat cultivars released in the southern Great Plains carry the *vrn-A1b* allele, demonstrating that wheat breeders in this region have indirectly incorporated this allele into local wheat cultivars. For example, Jagalene was derived from the cross of Jagger and Abilene, but the later stem elongation of Jagalene is consistent with a *VRN-A1* allele different from Jagger.

In summary, both stem elongation and winter dormancy release were mapped to a major locus including *VRN-A1* and modified by other minor loci in a segregating population generated from two winter wheat cultivars. Although the gene at each QTL is not yet known, genome-wide molecular markers found in this study will be useful to select for an extended vegetative phase for production of more forage biomass in a dual-purpose (graze-plus-grain) management system. These molecular markers can also be manipulated to avoid late-winter freeze damage and to regulate components of grain yield in production of winter wheat.

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