MAPPING OF QUANTITATIVE TRAITS IMPORTANT TO DUAL-PURPOSE MANAGEMENT OF WINTER WHEAT

By

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MAPPING OF QUANTITATIVE TRAITS

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OF WINTER WHEAT

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TABLE OF CONTENTS

Chapter Page

I. GENETIC LOCI IN THE PHOTOPERIOD PATHWAY INTERACTIVELY MODULATE REPRODUCTIVE DEVELOPMENT OF WINTER WHEAT

ABSTRACT....2
INTRODUCTION....3
MATERIALS AND METHODS....5
Plant materials....5
Phenotypic data collection....5
Linkage map construction and QTL discovery....6
RESULTS....8
Segregation of initial stem length and heading date....8
Construction of a linkage map....9
QTLs for initial stem length and heading date....10
Interactive effects of the three QTLs....12
DISCUSSION....13
REFERENCES....17

II. QUANTITATIVE TRAIT LOCI CONTRIBUTING TO HIGH TEMPERATURE SEED DORMANCY IN WINTER WHEAT TARGETED FOR EARLY SEEDING

ABSTRACT....27
INTRODUCTION....28
MATERIALS AND METHODS....30
Plant materials....30
Seed germination test....31
Linkage map construction....31
Single marker analysis and QTL detection....32
Statistical analysis....32
RESULTS....33
Seed dormancy at high and warm temperature....33
Single marker analysis....34
Linkage map and QTL discovery....34
Combined effects and QTL x environment interaction....35
Relationship of grain color with high temperature dormancy....36
DISCUSSION....36
REFERENCES....41

LIST OF TABLES

CHAPTER I

Table Page

1. Phenotypic correlation between initial stem elongation (STE) and/or heading date (HD) in years 2005, 2006, and 2007……..………....23

2. QTLs for initial stem elongation (STE) and heading date (HD), their LOD scores, the phenotypic variation explained, and means for each allele class……………………………………………23

CHAPTER II

1. Simple statistics of seed dormancy at high and warm temperatures in years 2006-2007....…………………….…………………………...46

2. Phenotypic correlation of seed dormancy at high and warm temperatures in years 2006 and 2007.........…………..……………..…………46

3. Analysis of variance of genetic and environmental factors affecting high temperature seed dormancy...………..…………………….……………46

4. Association of markers with seed dormancy at high and warm temperatures detected by single-marker correlation analysis…...…….………47

5. Description of the putative QTLs affecting high temperature seed dormancy ……………………………………………………………….47

6. Relationship of seed color with high temperature seed dormancy……..…..47

LIST OF FIGURES

CHAPTER I

Figure Page

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……………………….……….……..24

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………………….……...24

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……….……......25

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…………………….…………………………………..…...25

CHAPTER II

1. Frequency distribution of high temperature seed dormancy in two seed producing environments across two test years. Low germination rate signifies stronger dormancy. Parents are indicated. ‘Intrada’, open triangle; ‘Cimarron’, bold triangle. See Table 1 for the meaning of code for each test…...48

2. LOD profiles of the genetic loci for warm and high temperature seed dormancy. QTLs on chromosomes 3A and 4A are only detected at high temperature regime. LOD curves at warm temperature are presented for comparisons. Note that LOD scores at the vertical axes are in different scales. Markers are shown on horizontal axes at the

bottom of each panel. a). Chromosome 3A, 24 ºC; b). Chromosome 3A, 35/27 ºC; c). Chromosome 4A, 24 ºC; d). Chromosome 4A, 35/27 ºC…………………………………………………………………..48

3. QTL × QTL, QTL × environment interaction analysis in QTLnetwork. Blue ball means the 4A QTL effect consists of additive and additive × environment effect; red ball means the 3A QTL effect has no additive × environment interaction. The absence of connection line between QTLs indicates no QTL × QTL interaction……………………………………………………...…...49

CHAPTER I

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 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 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 involved in photoperiod response.

Key words: photoperiod gene, vernalization genes, dual purpose wheat, first hollow stem, genetic mapping.

Introduction

Responses to temperature and photoperiod are two 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; Snape et al. 2001b). 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 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 U.S. southern Great Plains are a winter wheat type that may require exposure for two to six weeks to minimum air temperatures of 2 to 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; Wang et al. 1995b). 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 represent 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. 1995), 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 in the same year.

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 three years from 2005 through 2007. All 115 RILs and both parents were planted in a replicates-in-sets experimental design, with a plot size of 1.3 m x 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 one week around September 20 of each year. The initial internode length was measured during March 1-10, depending on when all of the RILs had produced 1.5 cm hollow stem. Heading dates were recorded during the middle of April. The daylength at stem elongation and heading time in the experimental sites was about 11.5 and 13 hours 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 et al. (2000), and Chen et al. (2009). Approximately 20 plants of each line from the field plot were sampled and separated. Ten largest 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.

Linkage map construction and QTL discovery 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, 258 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-bp 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 x Cimarron population.

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 data 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 to 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.

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. Means were 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 x 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 three 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 screen polymorphisms between two parental lines, and 241 polymorphic markers (25.8%) were used for mapped 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 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 Chi-square test 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 and Cimarron. Sequencing results indicated PCR products were from a single-gene copy. After digestion with restriction enzyme *Rsa* I, polymorphic bands were observed between Intrada (269 bp + 215 bp) and Cimarron (269 bp + 119 bp + 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 to 25% of the phenotypic variation in initial stem length with significant LOD values of 2.75 (2005), 4.93 (2006), and 1.36 (2007), and (Table 2). This QTL also explained 8 to

26% of the phenotypic variation in heading date, with significant LOD values of 2.33 (2007) to 5.22 (2006).

A second QTL was located on the short arm of chromosome 2D, based on an assembled group of 10 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 for deletion) and Cimarron (288-bp band for insertion) 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 years 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 to 38% of the phenotypic variation in heading date, constituting the largest effect on this trait among all of three QTLs (Table 2).

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 three QTLs, explaining 12 to 26% of the phenotypic variation. It also accounted for 10 to 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 three 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 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 ($Pr>0.05$).

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.

Various combinations of different alleles with different effects, as well as gene interactions, determined various stem elongation and heading date phenotypes in the Intrada x 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 extreme 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 elongation, whereas the extreme 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 (Roder et al. 1998; Somers et al. 2004). On the other hand, the two parents 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 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 x 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 x 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 and Cimarron. Therefore, variation in initial stem length and heading date in the Intrada x 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 (Lauria 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 U.S. Central Plains (Chen et al. 2009).

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 x 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 x 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 x winter populations (BG213 x *H. spontaneum* and BG213 x ‘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 x 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 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 1966b; 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 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 establish variation in reproductive development in winter wheat. The genetic effects of all three QTLs on the developmental process 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, that is, 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. Initiation of stem elongation might be achieved without greatly delaying heading date on the basis of the three QTLs identified thus far. The intermediate

phenotypes of both 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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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

Note: 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.

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**

Note: significance level for comparing mean difference, * <0.05; ** <0.01.

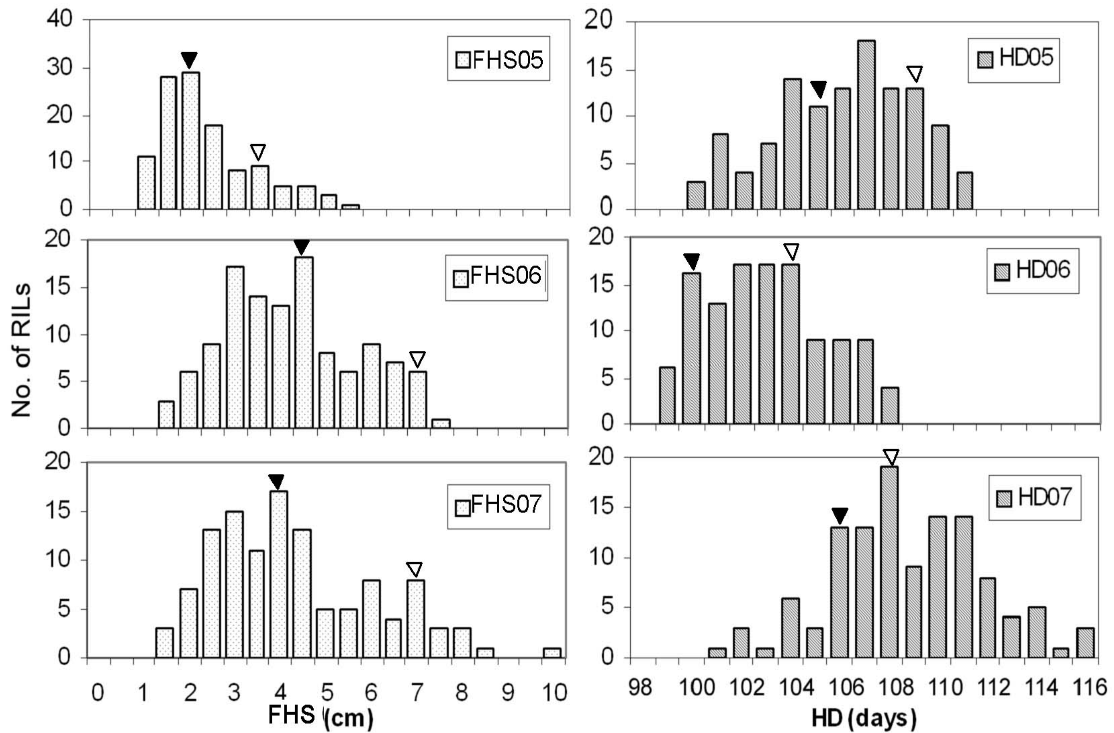

Fig. 1 Frequency distributions for initial stem elongation (STE) and heading date (HD) in the RIL population of Intrada/Cimarron evaluated in 3-year trials. Open triangle indicates the parent Intrada; bold triangle indicates Cimarron.

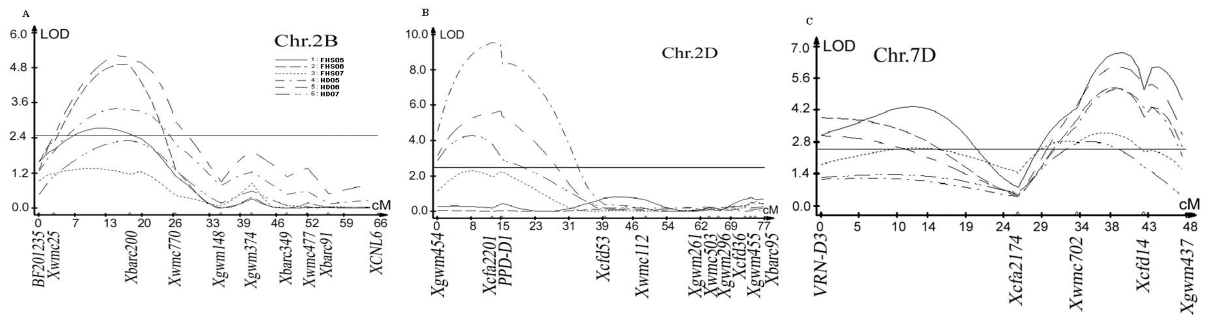

Fig. 2 Location of three QTLs for initial stem elongation (STE) and heading date (HD) suggested by interval mapping of WinQTLCart 2.5. a) QTL on chromosome 2B; b) QTL on chromosome on 2D, and c). QTL on chromosome 7D.

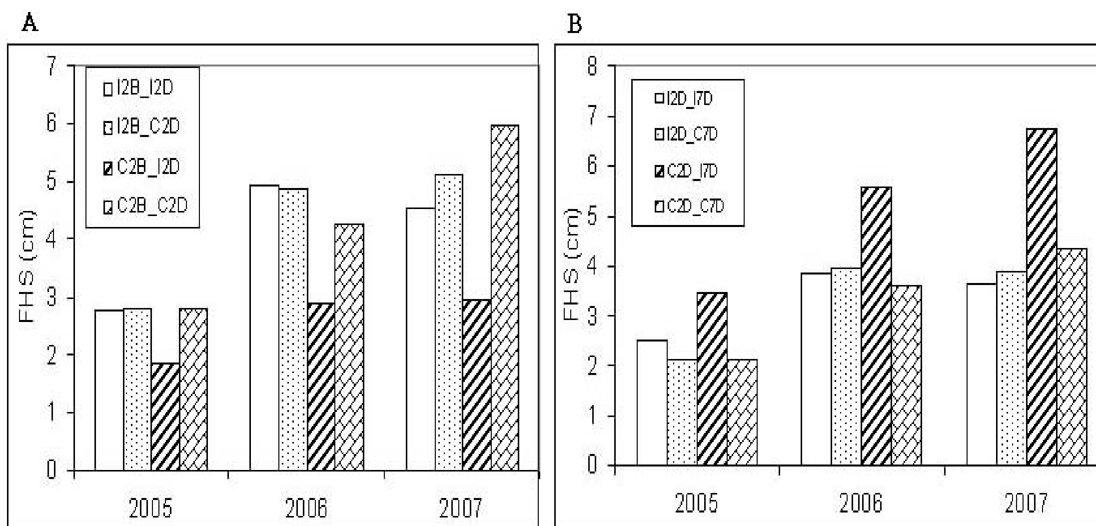

Fig. 3 Mean initial stem elongation of marker genotypes to show two-QTL interactions. a) Interactions between *Xbarc200* and *PPD-D1* QTLs; b) Interactions between *PPD-D1* and *Xcfd14* QTLs.

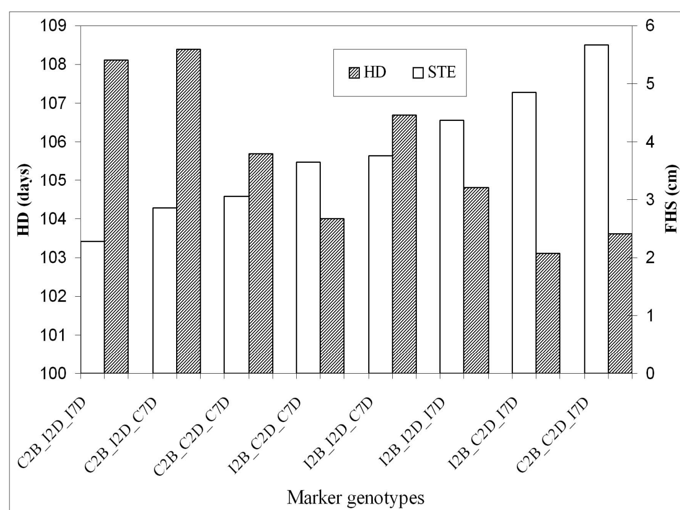

Fig. 4 Mean initial stem elongation (STE) and heading date (HD) of eight genotypes classified by the closest marker for each QTL. In the symbol of genotypes, the allele is denoted by the first letter of parent plus chromosome name. For instance, I2B and C2B represent the Chromosome 2B QTL alleles from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

CHAPTER II

QUANTITATIVE TRAIT LOCI CONTRIBUTING TO HIGH TEMPERATURE SEED DORMANCY IN WINTER WHEAT TARGETED FOR EARLY SEEDING

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Abstract

Winter wheat is usually planted in early September in the southern Great Plains to allow ample biomass accumulation for cattle (*Bos taurus* L.) grazing. As a negative consequence of the early sowing date, prompt stand establishment may be averted by high temperature seed dormancy (HTSD), a phenomenon condition that is poorly understood at the genetic level. In this study, 94 recombinant inbred lines ('Intrada' x 'Cimarron') segregating for HTSD expression were used to map QTLs controlling HTSD. Seed samples were collected in harvest years 2006 and 2007 from both the field and greenhouse and stored at room temperature until late August. Germination rates were investigated in growth chambers at two temperature regimes, a high temperature of 35/27ºC day/night, and a warm temperature of 24ºC. At 24ºC, all RILs showed germination rates exceeding 93%, indicating that warm temperature seed dormancy (WTSD) was essentially undetectable at the test time. At 35/27ºC, two major QTLs were detected on chromosomes 3A and 4A, matching QTLs previously known to control WTSD. *QHtsd.osu-3A* was tightly linked with SSR marker *Xbarc310*, explaining 8 to 23% of the phenotypic variance. *QHtsd.osu-4A* was tightly linked with marker *XDuPw4*, accounting for 3 to 62% of the phenotypic variance. *QHtsd.osu-3A* was stably expressed, whereas *QHtsd.osu-4A* showed significant interaction with environment. Our results did not show significant association between brancoat color and HTSD.

Keywords: seed dormancy, high temperature stress, wheat, genetic mapping

Introduction

Retention of seed dormancy in plants prevents viable seed from untimely germination under hostile environmental conditions (Copeland 2001; Simpson 1990). Prolonged retention, however, may prevent germination even in the presence of favorable environmental conditions. The level of seed dormancy increases until physiological maturity and declines gradually throughout the post-harvest period. The acquisition, maintenance, and release of seed dormancy differ among genotypes and strongly depend on ambient conditions such as temperature (Bewley 1997; Gubler et al. 2005; Koornneef et al. 2002). In wheat, seed dormancy is given attention largely due to starch damage caused by pre-harvest sprouting (PHS). In regions where free moisture occurs on ripened grain before harvest, PHS leads to poor bread baking quality and reduces the economic value of wheat grain (Wu and Carver 1999).

Seed dormancy bears economic impact in the southern Great Plains of the USA for another reason— high-temperature sensitivity at germination when wheat is seeded early for forage-plus-grain production. In this region dual-purpose management dominates winter wheat production. About 3 M hectares of winter wheat are grazed annually during the winter months, followed by grain harvest in spring. Wheat vegetative tissue provides high-quality forage to local livestock enterprises, but forage yield decreases sharply with planting date (Edwards et al. 2009). A 20-day delay of planting date in September can lead to a 68% reduction in forage yield. Therefore early planting is the most effective way to increase forage availability (Hossain et al. 2003).

Hot soil conditions frequently occur throughout September in the southern Great Plains. For example, five out of the last 15 years at Altus, Oklahoma produced at least three consecutive days with maximum soil temperatures at 5-cm depth exceeding 35ºC. Even the minimum soil temperature frequently exceeded the optimal range for wheat germination (Data source: Mesonet, 1994-2008). Many local wheat cultivars exhibit poor germination under such conditions and are rated annually for this characteristic (often termed high temperature germination sensitivity) (Edwards, 2008). Although seed germination may commence as soil temperature decreases, non-uniform or delayed emergence may compromise early forage production and adequate wheat pasture establishment. In contrast, the same cultivars may have the capacity to germinate

uniformly under normal planting conditions in October (Krenzer et al. 2002). This phenomenon has been termed high-temperature seed dormancy (George 1967; Walker-Simmons 1988) in wheat, or thermoinhibition (Argyris et al. 2008; Tamura et al. 2006) in Arabidopsis (*Arabidopsis thaliana*) and lettuce (*Lactuca sativa*), or thermodormancy (Tilsner, 1985) in oat (*Avena fatua*). Hereafter, we adopt the term high-temperature seed dormancy (HTSD). Separate from this term is the level of seed dormancy measured in the context of PHS tolerance (ca. 20±5ºC), called warm temperature seed dormancy (WTSD).

Temperature is the most important environmental factor that influences dormancy expression immediately following imbibition. For genotypes that have essentially lost dormancy, high temperature typically elevates germination in the range of 5 to 30ºC, with an optimum temperature around 20ºC. For genotypes still having dormancy, extremely high temperature inhibits germination. Greater sensitivity to temperature means that lower temperature is required to achieve complete germination (George, 1967; Mares, 1984). Temperature in the range of 15 to 20ºC is the most effective in differentiating genotypic levels of seed dormancy (George, 1967; Mares, 1984; Nyachiro et al. 2002). Dormancy is released gradually during dry storage. Stronger dormancy requires longer storage time to be removed. Moreover, dormancy at 30ºC lasts longer than that at 20ºC. Even after one year storage, it still exists to some extent in some cultivars (George 1967).

Recent genetic studies of seed dormancy have focused on PHS tolerance in wheat, with more than 20 genetic loci reported. Typically, red-grained genotypes have stronger seed dormancy than white-grained ones. Inhibitive substances in red bran enhance seed dormancy along with other genetic factors (Wu and Carver, 1999; Flintham 2000). Three homoeologous genes on the long arms of group 3 chromosomes quantitatively determine grain color (Flintham 2000; Himi et al. 2002). Wheat orthologues of the maize (*Zea mays*) *viviparous* (*Vp-1*) gene have been found on the long arms of chromosomes 3A, 3B, and 3D, respectively. They are 30 cM apart from genes controlling grain color (Bailey et al. 1999; McKibbin et al. 2002; Yang et al. 2007). Besides these two classes of major genes on group 3 chromosomes, QTLs for seed dormancy or PHS have been reported on 3A (Kulwal et al. 2004; Lohwasser et al. 2005; Mori et al. 2005), 3D (Imtiaz et al. 2008), 4A (Flintham et al. 2002; Imtiaz et al. 2008; Kato et al. 2001; Lohwasser et al. 2005;

Mori et al. 2005; Tan et al. 2006), 4B (Mori et al. 2005), 5B (Mares et al. 2005; Tan et al. 2006), 6B and 7D (Roy et al. 1999). In these studies, seed dormancy was assessed at warm germination temperature (15-24C) at harvest maturity. None is known if and how these genes or QTLs continue to act during the post-harvest period.

In dual-purpose management of winter wheat, HTSD is currently addressed in a passive way. Generally, genotypes with stronger dormancy at 20ºC have lower germinability at 30ºC; however, this relationship is not always true among tested genotypes (Edwards, 2005; George, 1967; Mares, 1984). Prediction of HTSD directly by the performance at warm temperature is therefore inappropriate. Genetic mechanisms underlying seed dormancy at high and warm temperatures may differ in some aspects. Each year this classification is examined on contemporary cultivars from late August through early October to provide information to farmers. High-temperature insensitive wheat cultivars are recommended in the case of early planting to avoid poor stand establishment (Edwards 2008; Krenzer 2003).

Breeding pressure for HTSD has not been applied for various reasons. Soil temperature conditions immediately following planting may be erratic. Field simulation of heat stress by advancing planting date may compromise selection for other traits critical to forage and grain production. Moreover, confounding effects under field conditions may bias phenotypic selection in the field, such as environmental conditions during seed production, soil crusting, fugal infections, sowing depth, and short coleoptile length. Characterization of HTSD by genetic mapping strategy needed to clarify its relationship with WTSD and ideally enable breeding efforts for both high PHS tolerance and low HTSD for the long run. In this study, a population of recombinant inbred lines (RILs) developed from two winter wheat cultivars was used to identify QTLs controlling HTSD.

Materials and methods

Plant materials: A population of 94 F7-derived recombinant inbred lines (RILs) was developed from the cross between 'Intrada' (Carver et al. 2003) and 'Cimarron' by the single seed descent method. Cimarron and Intrada are both hard winter wheat cultivars released by Oklahoma State University in 1990 and 2000, respectively. Intrada (Rio

Blanco/TAM 200) is white-grained, while Cimarron (Payne*2/CO725052) is red-grained. Intrada and Cimarron have medium and high HTSD, respectively (Edwards, 2008; Krenzer, 2002).

Seed germination tests: Seeds were produced in both the field and greenhouse in harvest years 2006 and 2007. Harvest dates varied from June 5 to June 7 across years and environments, and the seeds were stored at room temperature for after-ripening until the end of August, or approximately the time local farmers commence planting for a dual-purpose wheat system. One-year old seeds of both parents were additionally used as controls to indicate the intensity of inhibition due to high temperature.

One hundred intact seeds of each RIL or parent were blotted onto the lower half of a sheet of pre-wetted germination paper (20 × 35cm). Two narrow paper slips (2.5cm wide) were placed by the seed sample. Then the paper was double-folded to maintain moisture. Twenty sets of seed samples were partially overlapped and stacked on a germination tray. In the germination chamber, the trays were rotated once per day to ensure uniformity of temperature treatment.

Germination tests were conducted at two temperature regimes, 35/27ºC and 24/24ºC. Each test was coded as in Table 1. For example, FLD06H indicates seed samples harvested from the field in 2006 were evaluated at the high temperature regime (35/27ºC). In 2007, the test at 24ºC was not repeated for the greenhouse samples because of low seed supply. Sprouted seeds were counted 5 days after imbibition, with sprouting indicated by protrusion of either the shoot or radical. A lower germination rate signified stronger seed dormancy. Kernel color was determined visually.

Linkage map construction: Methods used to construct the linkage map for the Intrada/Cimarron population were previously described by Wang et al. (2009). Prior to completing this study, the genetic map already consisted of 238 SSR, 1 SNP, and 2 gene markers covering 15 wheat chromosomes. Some markers were not yet linked or assigned to small linkage groups. After the putative QTLs responsible for HTSD had been identified, SNP markers in the vicinity were chosen from the Wheat SNP database to increase map density in the target regions. One such SNP marker, BF474615-4A, was mapped. Its primer sequences were 5'-TGA TGG ATG CTT GAG ACA GC-3' and 5'-AAA AAA AAC CTC ATA AGA ATG AAA CAT T-3'. In addition, Zhang et al. (2008)

designed an EST-derived marker zxq118 which effectively differentiated PHS tolerance among 19 white wheat germplasms and co-located with the 4AL QTL present in many Australian PHS-resistant germplasms. The P5/P6 primers 5'-CTG ACT GAT ATA CGG CAA TC-3' and 5'-ATG TGA TTG GTT GAT CAA GCG-3' were tested in this study. This marker showed an allelic difference between Intrada and Cimarron and was thus mapped.

Single marker analysis and QTL detection: As reported by Wang et al. (2009), Intrada and Cimarron are southern Plains-adapted wheat cultivars that might share a considerable portion of common ancestry. Low polymorphisms were noted in some chromosomal regions. Composite interval mapping (Zeng 1993) in WinQTLCart v2.5 (NCSU, Cary, NC) was used to detect QTLs responsible for the target trait. Walking speed was 1-2 cM, and a QTL was declared when its LOD score exceeded the default value of 2.5.

Statistical analysis: Seed dormancy is greatly influenced by environmental factors. As for HTSD, pre-harvest and post-harvest environmental conditions may modify its expression. Therefore, the seed-producing environment and the seed germination environment drew dual attention in this study at both genotype and QTL levels.

At the genotype level, phenotypic correlation coefficients were calculated for each pair of tests (harvest years 2006 and 2007 in the field and greenhouse) using the SAS procedure CORR (SAS Institute, Cary, NC). Then the genetic correlation was calculated by the formula, $\sigma_{ij} / \sqrt{(\sigma^2_{gi} * \sigma^2_{gj})}$, where σ_{ij} is the covariance after removing the environmental effect attributed to genotype x replication interactions (experimental error covariance) within the field or greenhouse environments, σ^2_{gi} and σ^2_{gj} are the genetic variances, i and j denote a pair of variables compared (Imtiaz et al. 2008). Broad-sense heritability was based on the formula, $h^2=\sigma^2_g/(\sigma^2_g+\sigma^2_e)$, where σ^2_g and σ^2_e represent the variance components due to genotype and error, respectively (Hallauer and Mirranda, 1981). The respective variance components were estimated by the SAS procedure VARCOMP. Mean squares were determined by the GLM procedure in SAS.

At the QTL level, QTL × QTL interactions and the combined effect of QTLs in each test were analyzed by multiple interval mapping (Kao et al. 1999) in WinQTLCart v2.5. The position of each QTL was represented by the closest marker to its center.

Further, the presence of epistatic interaction and QTL × environment interaction was determined by QTLnetwork v2.0 (Yang et al. 2008).

Results

Seed dormancy at high and warm temperature

Across all experiments, the level of seed dormancy was investigated 78 to 98 days after harvest. For the same seed lot, tests at the 24ºC germination treatment were carried out about 6 days later than at 35/27 ºC (Table 1). The mean germination rate at 24ºC exceeded 93% for all tests, showing little variation among RILs. Only a limited number of RILs showed germination rates below 90% for the seed samples harvested from the field, partly because of moldy or few unsprouted seeds. Obviously, WTSD was nearly undetectable at test time. At the 35/27ºC temperature regime, however, the mean germination rate among the 94 RILs was 83.8% (FLD06H) and 77.0% (GH06H) in 2006, and 70.3% (FLD07H) and 69.5% (GH07H) in 2007. High temperature had an inhibitory effect on the germination of some RILs, indicating the expression of HTSD. Differences in seed dormancy between parental lines were inconsistent between harvest years. The parent Cimarron produced a lower germination rate than Intrada in 2006 (FLD06H and GH06H) but higher germination in 2007 (FLD07H). In previous investigations, germination of Cimarron was classified to be more temperature-sensitive than Intrada (Edwards, 2008; Krenzer, 2002).

Some RILs had stronger HTSD or were more thermosensitive, while other RILs exhibited higher germinability than both parents. This implied that transgressive segregation occurred in the population. On the other hand, the continuous distribution of germination among the RILs indicated that multiple genetic loci may be involved in the control of germination at high temperature (Fig. 1).

As a whole, the RILs showed stronger HTSD in 2007 based on mean germination rates and frequency of phenotypes at the lower end of the distribution (Table 1, Fig.1). Expression of HTSD among the 94 RILs was not consistent between harvest years but was relatively consistent between two tests of each year (Table 2). The tests FLD06H and GH06H in 2006 were moderately correlated (r=0.37, Pr<0.0001). The two tests in 2007 were highly consistent (r=0.78, Pr<0.0001). However, test GH06H test was not correlated

with GH07H (P>0.05) even though the temperature and water settings in the greenhouse were identical between two years. The FLD06H and FLD07H tests were moderately correlated (r=0.47, Pr<0.0001). The genetic correlation between years was only 0.19 (Pr<0.01). Apparently, HTSD was influenced by environmental factors greatly. On the other hand, the pair of tests at high and warm temperatures using the same seed source were poorly associated except for the pair of GH06H and GH06W (r=0.32, Pr<0.0001).

In this study, the environmental factors could be separated into effects of year and site-specific conditions. The former roughly included post-harvest factors such as seed storage conditions, duration of storage before testing, and pre-harvest environmental conditions; the latter also included pre-harvest environmental effects, but those intrinsic to field versus greenhouse. Mean squares were compared to determine the relative influences of two classes of environmental factors (Table 3). Main effects due to genotype and the two classes of environmental factors were all significant (Pr<0.0001). The mean square for year effects was about 7 and 13 times that for site-specific conditions and genotypes, respectively. Moreover, the interaction effect of genotype × year was significant (Pr<0.0001), whereas that of genotype × seed-producing conditions was negligible (Pr>0.05).

Single marker analysis

A total of 243 polymorphic markers were mapped for the Intrada/Cimarron population. By single marker correlation analysis, 11 markers showed exceptional association with HTSD (Pr<0.01) in at least one test at high or warm temperature. These markers were assigned to chromosomes 3A, 4A, and 2D, respectively (Table 4). The chromosomal locations of the mapped markers were consistent with those given in the Sommers et al. (2004) SSR consensus map. No other markers showed significant correlation regardless if they were linked or unlinked.

Linkage map and QTL discovery

Composite interval mapping was run on the constructed 15 linkage groups for both HTSD and WTSD. Two major QTLs were identified for HTSD on chromosomes 3A and 4A. Other than markers *Xgwm455* and *Xbarc95* on chromosome 2D, there were minor LOD peaks with scores of about 2.0. We did not claim it a QTL. No QTLs were found for WTSD (Fig. 2a, 2c).

The first QTL resided on the short arm of chromosome 3A, designated *QHtsd.osu-3A*, having *Xbarc310* as the closest SSR marker (Fig. 2b). About 68 SSR markers previously reported on chromosome 3A were screened. Eventually, 14 of them were mapped on chromosome 3A in our population. Nine linked markers on the long arm (not shown in the figure) formed a separate group because of severe segregation distortion. To increase the map density, we checked 13 SNP markers that had been assigned to deletion bins of 3AS. Unfortunately, none of them showed an allelic difference between the parents (data not shown). *QHtsd.osu-3A* accounted for 8 to 23% of the total phenotypic variance, with LOD scores ranging from 2.2 to 9.2 among the four tests; LOD scores of GH06H (LOD=2.3) and GH07H (LOD=2.2) were close to the threshold (2.5). The parent Cimarron carried the allele promoting HTSD at this locus (Table 5).

The second QTL was found on the long arm of chromosome 4A (*QHtsd.osu-4A*), and tightly linked with three closely linked markers *Xbarc170*, *Xzxq118*, and *XDuPw4* (Fig. 2d). Sixty-nine SSR markers were screened and nine were mapped on this chromosome. Sixteen SNP markers were considered, but only the marker BF474615-4A exhibited polymorphic between the parents. Its PCR amplicons had a T for Intrada instead of a C for Cimarron. After digestion with the enzyme TaqI, Intrada produced two bands (322+451bp), whereas Cimarron showed three bands (124+197+450bp). It was mapped outside of the QTL peak, but indicated that this QTL was proximal to the deletion bin 4AL13-0.59-0.66. The direct amplicons of marker *Xzxq118* was 504 bp for Intrada and 341 bp for Cimarron. According to Zhang et al. (2008), wheat breeding lines having smaller bands were PHS-resistant. In this study, RILs producing the 341-bp band, or Cimarron allele, were more thermosensitive.This QTL explained a very large proportion of the total phenotypic variance in 2007, reaching 41% and 62% for tests FLD07H and GH07H, respectively. Cimarron contributed the allele promoting HTSD. Conversely, this QTL was significant (LOD=3.4) only for test GH06H in 2006, with a R^2 of 14%. Regarding the test FLD06H, the LOD score was 0.5 and R^2 was 3%. RILs carrying the Intrada allele had lower germination rate.

Combined effects and QTL × environment interaction

By means of MIM in WinQTLCart, both the combined effect and interaction effect were analyzed. *QHtsd.osu-3A* and *QHtsd.osu-4A* were represented by markers Xbarc310 and Xzxq118, respectively. Two loci combined accounted for 33, 29, 71, 71% of the total phenotypic variances in tests FLD06H, GH06H, FLD07H, GH07H, respectively. All digenic interactions were not significant. As previously mentioned, environmental factors influenced HTSD greatly. For comparison, the broad-sense heritability based upon RILs was 28% and 74% in years 2006 and 2007, respectively. Given the current experimental precision, nearly all genetic variation within the Intrada/Cimarron population appeared to be explained by just two QTLs.

QTL × QTL and QTL × environment interactions were further analyzed in QTLnetwork 2.0 (Fig.4). No QTL × QTL epistatic interaction was detected, consistent with the conclusion derived from MIM analysis. *QHtsd.osu-3A* showed non-significant interaction with environmental factors. However, another QTL on chromosome 4A showed a very strong interaction effect, owing to the variable HTSD over two years.

Relationship of grain color with high temperature seed dormancy

The 94 Intrada/Cimarron RILs considered in this study included 72 red-grained and 22 white-grained lines. A Chi-square test confirmed that two genes were involved in the segregation of grain color ($Pr<0.01$). Red-grained lines showed a little stronger HTSD than white-grained lines (Table 6). However, the difference between two color classes was not significant in tests GH06H, FLD07H, and GH07H ($Pr>0.05$). Grain color genes were not accurately mapped in this study. The linkage map we constructed for Intrada/Cimarron population covered the long arms of chromosomes 3A and 3B with considerably high density. No polymorphic markers were linked on the long arm of chromosome 3D. We conducted composite interval mapping for HTSD and did not detect any QTL in these regions (figure not presented).

Discussion

Two major QTLs on chromosome arms 3AS and 4AL were found to be responsible for HTSD in the Intrada/Cimarron population. By means of alignment with the QTLs reported for WTSD or PHS tolerance, many markers are found to be coincident in the corresponding regions. *QHtsd.osu-3A* was located in the interval of *Xbarc321-*

Xbarc310-Xwmc488. In this region, Mori et al. (2005) reported a major QTL for seed dormancy (warm temperature) or PHS tolerance, attributable to a highly dormant Japanese wheat cultivar 'Zenkoujikomugi'. Liu et al. (2008) found a major PHS QTL associated with marker *Xbarc321* in the US HWW wheat cultivar 'Rio Blanco', a chief resistance donor widely used in US HWW wheat breeding programs.

QHtsd.osu-4A on the long arm of chromosome 4A resides in a large interval containing markers *Xgwm637, Xbarc170, Xzxq118, XDuPw4*, and *Xwmc513*. The markers BF474615-4A and *Xzxq118* confirmed that this QTL is located near or in the deletion bin 4AL13-0.59-0.66 (http://probes.pw.usda.gov:8080/snpworld/Map; Zhang et al. 2008). The sequence of EST-SSR marker *XDuPw4* is highly homologous to the gene PM-19 encoding an ABA-induced protein in wheat (Eujayl et al. 2002). Ranford et al. (2002) indicated that expression of PM-19 was associated with dormancy of barley embryos. In the same region, a QTL for WTSD or PHS was identified in five wheat populations derived from red- or white-grained parents of various origins; by map alignment, this QTL was centered on the marker Xbarc170 (Mares et al. 2005). Zhang et al. (2008) developed a very close PCR marker *Xzxq118* for this locus that could effectively differentiate the dormant allele in Australian white wheat germplasms. *Xbarc170* and *Xzxq118* are also close to the center of the QTL we detected on 4AL. In addition, coincident markers *Xwmc770, Xbarc170*, and *Xbarc190* could be found at a 4AL QTL in Chinese landrace 'Tutoumai A' (Chen et al. 2007).

The coincidence of chromosomal locations and markers suggests that identical genes both at 3AS and 4AL loci might contribute to HTSD as well as WTSD at harvest maturity. Physiological and molecular studies in wheat and other plants strongly support the interrelationship between HTSD and WTSD. The acquisition, maintenance, and release of seed dormancy rely on internal plant hormones. Abscisic acid (ABA) inhibits germination while gibberellic acid (GA) has an opposite effect. The balance between these two hormones largely regulates the onset of germination (Bewley 1997; Gubler et al. 2005; Koornneef et al. 2002). High temperature acts on the expression of seed dormancy by altering the levels of, or response to, plant hormones. In Arabidopsis, Toh et al. (2008) reported that *ABA1/ZEP, NCED2, NCED5*, and *NCED9* genes were up-regulated by high temperature, leading to increased ABA and germination inhibition. The

GA signaling genes *SPINDLY* (*SPY*) and *RGL2* were suppressed and GA levels were low at high temperature. By comparing two accessions 'Salinas' (thermosensitive) and UC96US23 (insensitive) in lettuce, Argyris et al. (2008) indicated that high temperature and light differentially influenced the expression of many genes involved in ABA, GA, and ethylene pathways. ABA-related genes were up-regulated while GA- and ethylene-related genes were down-regulated by high temperature. Hence ABA content increased in 'Salinas' seed and germination was inhibited at high temperature. As for wheat, Walker-Simmons et al. (1988) reported that wheat embryos isolated from dormant seeds showed little HTSD. However the responsiveness to exogenous ABA could be elevated by 100 times at 30ºC than at 15ºC.

In this study, the two QTLs for HTSD were not detected in three tests for WTSD. There are two possible explanations for the expression of HTSD but not WTSD. Reactivation of genes at both loci may account for variation of HTSD in the Intrada/Cimarron population. Studies in Arabidopsis and lettuce reveal that ABA-related genes are more highly expressed under high-temperature conditions while GA and ethylene-related genes are more highly repressed. The elevated ABA levels result in thermoinhibition (Argyris et al. 2008; Toh et al. 2008). However, the temperature sensitivity of wheat seeds declines gradually during after-ripening. Seeds of dormant cultivars lose part or most of their HTSD after one year of storage (George 1967). We also used one- and two-year-old seeds of both parents as a control treatment in the high temperature tests. One-year seeds exhibited HTSD (ca. 50% population-average germination rate), whereas two-year seed had no remaining HTSD.

Reactivation of seed dormancy genes may still not completely explain the expression of HTSD. Environmental factors during grain filling can alter the level of WTSD at harvest (Biddulph et al. 2005; Reddy et al. 1985). During dry storage, seeds still undergo complex biosynthesis and catabolism in preparation for germination (Gubler et al. 2005; McCarty, 1995; Walker-Simmons et al. 1987). We observed both the seed-producing environmental effect and year effect were notably larger than the effect of genotype, suggesting that the metabolism of dormancy-related substances before and after harvest could influence HTSD in addition to the reactivation of dormancy governing genes.

Measurement of HTSD occurred 78 to 98 days after harvest, a time point relevant to plant-back for early-seeded production systems. At the same time we examined WTSD at 24ºC, which is close to the upper limit of temperature regimes used in previous studies on PHS. Few seeds were observed dormant at that temperature. Thus our results produced no correlation between HTSD (35/27ºC) and WTSD (24ºC). It is not unreasonable to hypothesize that tests conducted closer to harvest maturity may show association between HTSD and WTSD, but our purpose here was to examine these traits in the context of plant-back germinability. Further experiments addressing both pre- and post-harvest dormancy and the role of plant hormones could provide more mechanistic insight into HTSD.

Interactions of QTL × QTL, QTL × environment were evaluated for both QTLs. QTL × QTL interaction was not indicated by our results. Only *QHtsd.osu-4A* on chromosome arm 4AL produced a significant QTL x environment interaction. The relatively strong expression of this QTL in 2007 coincided with high phenotypic variation for HTSD that year. Mori et al. (2005) also indicated that *QPhs.ocs-3A.1* from Zenkoujikomugi was more stably expressed than the other two QTLs on chromosome arms 4AS and 4BL. It was consistently detected at both 15ºC and 20ºC. In a secondary experiment we investigated HTSD at constant 35ºC in years 2005 (two runs) and 2006 in the Intrada/Cimarron population. Germination was severely suppressed with average germination rates of 7.1%, 20.2%, and 15.9%, respectively; yet, *QHtsd.osu-3A* was detected in all tests, with LOD scores exceeding 2.5. *QHtsd.osu-4A* could not be detected at this temperature regime (LOD <1.0; data not presented).

At both loci, Cimarron donated alleles that increased high-temperature sensitivity except for one test involving the *QHtsd.osu-4A* locus (GH06H). However, Cimarron itself showed a higher germination rate than the mean performance of RILs bearing both Cimarron alleles. This aberration occurred with the parents of another mapping population Jagger/2174 (data not shown). Most of the genetic variation was explained in the Intrada/Cimarron population in year 2007 by the two loci combined. There is little probability that a major QTL in the nuclear genome has not been found. In Arabidopsis, Tamura et al. (2006) noted that maternal effects controlled expression of thermoinhibition

in the mutant TRW71-1. The F1 seeds of Ws×TRW71-1 showed 0% germination, while the reciprocal cross TRW71-1×Ws showed 74% germination at 32ºC.

Intrada was the first hard white wheat cultivar released by the Oklahoma Agric. Exp. Stn., showing moderate tolerance to PHS (Carver et al, 2003). One of its two parents, 'Rio Blanco', carries a QTL on 3AS for PHS tolerance (Liu et al. 2008) in the same chromosomal region found in the Intrada/Cimarron population. Unexpectedly, Intrada produced negligible contribution to HTSD in this study. Only in the GH06H test did its allele at the 4AL locus increase high-temperature sensitivity. Therefore, Intrada might have not inherited the 3AS allele from 'Rio Blanco'. Argyris et al. (2008) reported that only a small increase in temperature was needed to alter germination phenotypes between two accessions of lettuce. Both 'Salinas' and UC96US23 exhibited complete germination in the temperature range of 20 to 30ºC. At 33ºC, only UC96US23 germinated. Whether or not Intrada has alleles at 3AS and 4AL loci promoting dormancy at a lower temperature regime than 35/27ºC remains to be verified.

It is well known that red wheat cultivars on average have higher PHS tolerance than white cultivars (Groos et al. 2002; Wu and Carver, 1999). Himi et al. (2002) suggested that grain color genes enhanced seed dormancy by increasing the sensitivity of embryos to ABA. Grain color in the Intrada/Cimarron RIL population segregated according to a di-genic model (data not shown). Mean germination rates of red-grained lines versus white-grained lines were generally not significant. Molecular markers have been reported for the grain color genes on chromosome arms 3AL and 3BL (Groos et al. 2002; Kulwal et al. 2005). The regions bearing markers *Xwmc169* and *Xwmc388* on 3AL, and *Xbarc164* and *Xwmc418* on 3BL, in the Intrada/Cimarron population co-located with the reported regions by alignment with the wheat SSR consensus map (Somers et al. 2004). However, no QTL in the vicinity was associated with HTSD, implying that grain color genes were not important in the regulation of HTSD in Intrada/Cimarron population. Therefore, not all components affecting WTSD at harvest maturity are active at high temperature.

At least 20 QTLs have been reported for WTSD or PHS tolerance in wheat. Some of them are located in different regions of the same chromosomes. This fact underscores the genetic and mechanistic complexity of seed dormancy. HTSD should be more

complex in principle because more intrinsic and environmental factors are involved. This study is an initial attempt to explore the genetic loci involved in the regulation of HTSD. It appears that not all genetic components for WTSD are of equivalent importance to HTSD. This may allow the opportunity to complement or align breeding efforts on WTSD and HTSD. Stronger WTSD (for PHS tolerance) but weaker HTSD (less high temperature germination sensitivity at early planting in hot soils) is a reasonable breeding target. A number of mapping populations have been created for further study of WTSD and PHS tolerance, including potential maternal effects and allelic differences in high temperature response. Characterization of them at both high and warm temperatures may help reveal wheat seed dormancy in more depth.

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Table 1 Simple statistics of seed dormancy at high and warm temperatures in years 2006-2007

Test§	Days after harvest	Seed producing environment	Temperature regime (C)	Germination rate (%)		
				Mean	Intrada	Cimarron
FLD06H	86	Field	35/27	83.8±9.3	90.3	81.3
GH06H	91	Greenhouse	35/27	77.0±15.8	91.5	73.5
FLD06W	93	Field	24	96.1±2.2	95.0	97.6
GH06W	98	Greenhouse	24	98.3±2.2	99.5	97.0
FLD07H	78	Field	35/27	70.3±21.2	82.7	86.7
GH07H	85	Greenhouse	35/27	69.5±29.3	92.0	96.0
FLD07W	89	Field	24	93.4±4.9	84.6	97.6
GH07W	92	Greenhouse	24	98.9±1.2¶	-	-

§ Each test code consists of 3 parts: seed source, year, evaluation temperature. FLD = field, GH=greenhouse; H=high temperature, W=warm temperature.

¶ Mean germination rate based on 19 RILs with low germination rate at high temperature regime.

Table 2 Phenotypic correlation of seed dormancy at high and warm temperatures in years 2006 and 2007

	FLD06H	GH06H	FLD06W	GH06W	FLD07H	GH07H	FLD07W
FLD06H§	1	0.37**	-0.02	0.18	0.47**	0.37**	-0.15
GH06H		1	-0.28**	0.32**	0.07	-0.06	-0.12
FLD06W			1	-0.22*	0.04	0.13	0.24*
GH06W				1	0.11	0.00	-0.08
FLD07H					1	0.78**	-0.12
GH07H						1	-0.10
FLD07W							1

*, ** denote the significance level of 0.05 and 0.01, respectively.

§ Test codes are as in Table 1.

Table 3 Analysis of variance of genetic and environmental factors affecting high temperature seed dormancy

Source	DF	MS	F	Pr
Year§	1	10013	58.81	<0.0001
Location¶	1	1421	8.35	0.0048
Year * location	1	918	5.39	0.0224
Genotype	93	764	4.48	<0.0001
Genotype * year	93	561	3.30	<0.0001
Genotype * location	93	115	0.67	0.9702
Residual	93	170		

§Year: compounding effects of seed storage condition and test time, representing post-harvest conditions

¶Location: effect of seed-producing environments (field and greenhouse), representing pre-harvest conditions

Table 4 Association of markers with seed dormancy at high and warm temperatures detected by single-marker correlation analysis

Marker	Chromosome	Probability						
		FLD06H[§]	GH06H	FLD06W	GH06W	FLD07H	GH07H	FLD07W
Gwm296	2D	0.0877	0.5650	0.1047	0.4084	0.0001	0.0001	0.9414
Gwm455	2D	0.2386	0.6504	0.2347	0.4529	0.0011	0.0005	0.8387
Barc310	3A	<0.0001	0.0031	0.1309	0.1081	<0.0001	0.0010	0.4499
Ksm47	3A	0.0023	0.0556	0.6262	0.0869	<0.0001	0.0309	0.1411
Barc321	3A	0.0079	0.1094	0.1855	0.1007	0.0003	0.0644	0.0754
Gwm2	3A	0.2146	0.6602	0.3631	0.3305	0.0068	0.0086	0.9664
Gwm637	4A	0.2734	0.0060	0.2581	0.1916	0.0038	0.0049	0.7384
Barc170	4A	0.7592	0.0036	0.0202	0.3715	<0.0001	<0.0001	0.4611
duPw4	4A	0.1822	0.0002	0.0217	0.6342	<0.0001	<0.0001	0.8898
Zxq118	4A	0.5292	0.0005	0.0717	0.7306	<0.0001	<0.0001	0.6623
Wmc513	4A	0.1457	0.2290	0.0059	0.9048	<0.0001	<0.0001	0.8647
BF474615	4A	0.0250	0.9611	0.0115	0.6903	<0.0001	0.0002	0.7236
Barc190	4A	0.6516	0.3778	0.0007	0.2046	0.0006	0.0385	0.0193
Wmc757	4A	0.7096	0.0524	0.0113	0.1717	0.0024	0.0788	0.0647

§ Test codes are as in Table 1.

Table 5 Description of the putative QTLs affecting high temperature seed dormancy

Locus	Marker	Test	LOD	R^2	Additive effect
QHtsd.osu-3A	*Xbarc310*	FLD06H[§]	4.1	17	4.5
		GH06H	2.3	8	5.9
		FLD07H	9.2	23	10.8
		GH07H	2.2	8	5.0
QHtsd.osu-4A	*Xzxq118*	FLD06H	0.5	3	2.3
	/XDuPw4	GH06H	3.4	14	-8.8
		FLD07H	12.7	41	17.2
		GH07H	14.6	62	27.6

§ Test codes are as in Table 1.

Table 6 Relationship of seed color with high temperature seed dormancy.

Grain color	Germination rate at 35/27C (%)			
	FLD06H[§]	GH06H	FLD07H	GH07H
White	88.3	80.4	74.7	73.9
Red	82.4	75.9	68.9	68.2
Difference	5.9**	4.5ns	5.8ns	5.7ns

** Significant at the level of 0.01.

§ Test codes are as in Table 1.

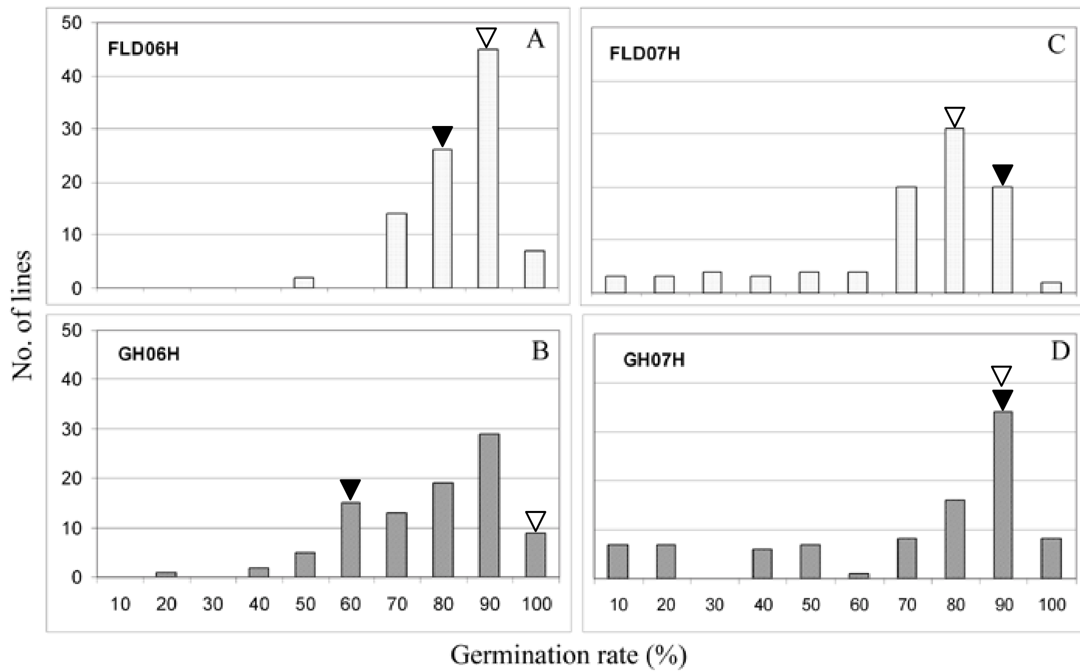

Fig. 1 Frequency distribution of high temperature seed dormancy in two seed producing environments across two test years. Low germination rate signifies stronger dormancy. Parents are indicated. 'Intrada', open triangle; 'Cimarron', bold triangle. See Table 1 for the meaning of code for each test.

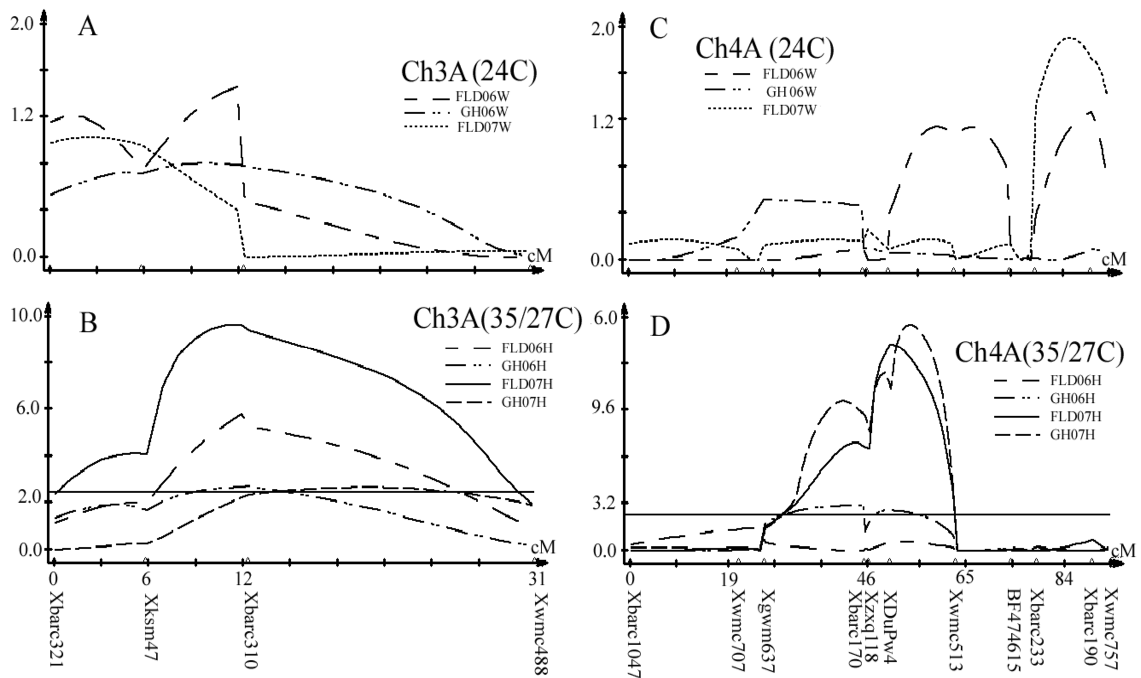

Fig.2 LOD profiles of the genetic loci for warm and high temperature seed dormancy. QTLs on chromosomes 3A and 4A are only detected at high temperature regime. LOD curves at warm temperature are presented for comparisons. Note that LOD scores at the

vertical axes are in different scales. Markers are shown on horizontal axes at the bottom of each panel. a). Chromosome 3A, 24 ℃; b). Chromosome 3A, 35/27 ℃; c). Chromosome 4A, 24 ℃; d). Chromosome 4A, 35/27 ℃.

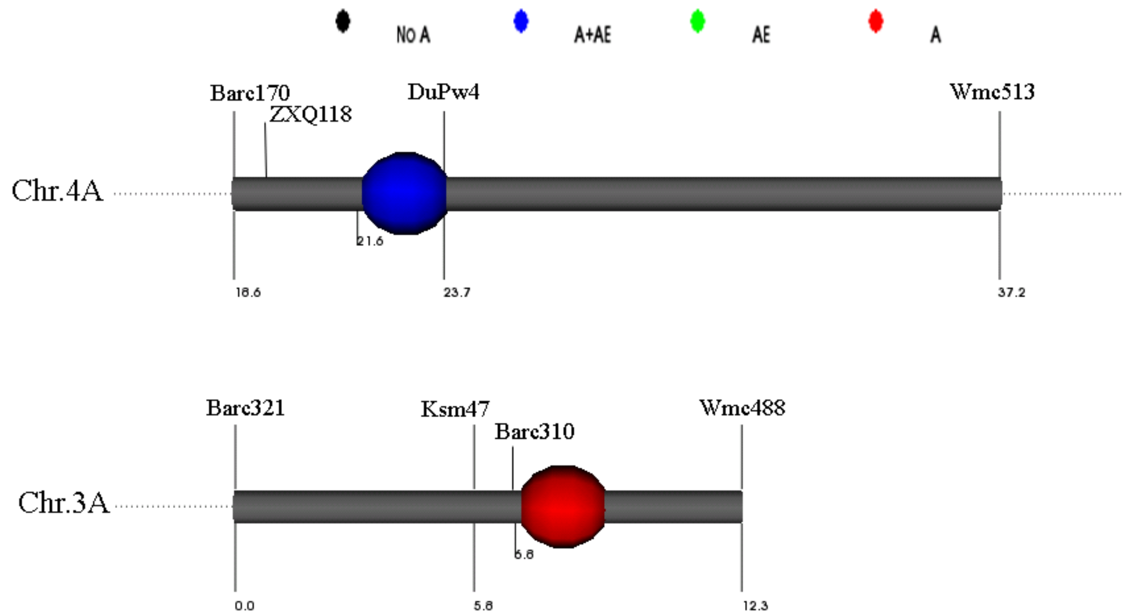

Fig. 3 QTL × QTL, QTL × environment interaction analysis in QTLnetwork. Blue ball means the 4A QTL effect consists of additive and additive × environment effect; red ball means the 3A QTL effect has no additive × environment interaction. The absence of connection line between QTLs indicates no QTL × QTL interaction.

VITA

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Doctor of Philosophy

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Title of Study: MAPPING OF QUANTITATIVE TRAITS IMPORTANT TO DUAL-PURPOSE MANAGEMENT OF WINTER WHEAT

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Scope and Method of Study:

In the southern Great Plains, winter wheat is widely used for forage-plus-grain production. Knowledge of the genetic control of traits important to dual-purpose management would facilitate breeding efforts leading to better adapted cultivars. Linkage mapping was conducted on 94 recombinant inbred lines derived from two locally adapted cultivars, ‘Intrada’ and ‘Cimarron’. Single-marker analysis and interval mapping methods were applied to identify putative QTLs. Traits including initial stem elongation, heading date, and high-temperature seed dormancy, were investigated in the field at Stillwater, Oklahoma from 2004 to 2007.

Findings and Conclusions:

A linkage map was completed with 243 SSR, SNP, or gene markers, covering 15 wheat chromosomes and uncharacterized small linkage groups. Three QTLs in the photoperiod pathway interactively regulated both initial stem elongation and heading. The Intrada *Xbarc200* and *Xcfd14* alleles and the Cimarron *PPD-D1* allele accelerated stem elongation and heading stages; conversely, the Cimarron *Xbarc200* and *Xcfd14* alleles and the Intrada PPD-D1 allele delayed stem elongation and heading stages. Regarding high-temperature seed dormancy, two major QTLs were detected on chromosomes 3AS and 4AL, matching QTLs previously known to control warm-temperature seed dormancy. The Cimarron *Xbarc310* and *Xzxq118* alleles promoted sensitivity to high temperature. Grain color had no significant association with expression of high-temperature seed dormancy. Our results showed that initial stem elongation vs. heading, or high vs. warm temperature seed dormancy, were interrelated by common genetic loci. Careful selection of alleles is required to enhance the adaptation of winter wheat to dual-purpose management systems.

ADVISER’S APPROVAL: Brett F. Carver