Theor Appl Genet (2010) 121:717–730
DOI 10.1007/s00122-010-1343-4

ORIGINAL PAPER

Genome mapping of kernel characteristics in hard red spring wheat breeding lines

Toi J. Tsilo · Gary A. Hareland · Senay Simsek · Shiaoman Chao · James A. Anderson

Received: 5 December 2009 / Accepted: 3 April 2010 / Published online: 28 April 2010

Abstract Kernel characteristics, particularly kernel weight, kernel size, and grain protein content, are important components of grain yield and quality in wheat. Development of high performing wheat cultivars, with high grain yield and quality, is a major focus in wheat breeding programs worldwide. Here, we report chromosome regions harboring genes that influence kernel weight, kernel diameter, kernel size distribution, grain protein content, and grain yield in hard red spring wheat breeding lines adapted to the Upper Midwest region of the United States. A genetic linkage map composed of 531 SSR and DArT marker loci spanned a distance of 2,505 cM, covering all 21 chromosomes of wheat. Stable QTL clusters influencing kernel weight, kernel diameter, and kernel size distribution were identified on chromosomes 2A, 5B, and 7A. Phenotypic variation explained by individual QTL at these clusters varied from 5 to 20% depending on the trait. A QTL region on chromosome 2B confers an undesirable pleiotropic effect or a repulsion linkage between grain yield (LOD = 6.7; R^2 = 18%) and grain protein content (LOD = 6.2; R^2 = 13.3%). However, several grain protein and grain yield QTL independent of each other were also identified. Because some of the QTL identified in this study were consistent across environments, DNA markers will provide an opportunity for increasing the frequency of desirable alleles through marker-assisted selection.

Communicated by X. Xia.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-010-1343-4) contains supplementary material, which is available to authorized users.

T. J. Tsilo (✉) · J. A. Anderson
Department of Agronomy and Plant Genetics,
University of Minnesota, 411 Borlaug Hall, St. Paul,
MN 55108, USA
e-mail: tsilo001@umn.edu

J. A. Anderson
e-mail: ander319@umn.edu

G. A. Hareland
Wheat Quality Laboratory, United State
Department of Agriculture-Agricultural Research Service,
Fargo, ND 58105, USA

S. Simsek
Department of Plant Sciences, North Dakota State University,
Fargo, ND 58105, USA

S. Chao
Biosciences Research Laboratory,
United State Department of Agriculture-Agricultural
Research Service, Fargo, ND 58105, USA

Introduction

Development of high yielding varieties with good end-use quality is a major focus in wheat breeding programs. Grain yield is one of the major determinants of a farmer's income, while grain protein content is very important for bread-making quality. Although grain yield and grain protein content are often negatively associated (Kamra 1971; Bhatia 1975; Loffler and Busch 1982), researchers have also reported wheat cultivars with high grain yield and high grain protein content (Stuber et al. 1962; Johnson et al. 1973). These traits are inherited as a complex group of many components, which are also influenced by genotype × environment (G × E) interactions (Busch et al. 1969; Robert et al. 2001).

A comparison of wheat grain yield and yield component selection has shown that selection for kernel weight could result in an increase in grain yield (Alexander et al. 1984). Similar results were reported by Baril (1992). Several other

researchers have also reported that kernel weight and size are important because of their relationships with milling quality; for example, an increase in flour yield resulted from an increase in kernel weight (Wiersma et al. 2001) or kernel size (Marshall et al. 1984; Berman et al. 1996). However, the improvement of kernel weight and size alone has generally been found to have no benefits on grain yield; this is mainly due to the compensational effect among yield components (Kiesselbach and Sprague 1926). For a breeding program, it would be laborious, time-consuming, and costly to measure all yield components, and it is often not practical to select for all these components at the early stages of a breeding population. Therefore, breeders evaluate total grain yield, and are continuously releasing new varieties with increased yields.

With the advent of molecular markers, several researchers have used quantitative trait locus/loci (QTL) analysis to study the genetic control of yield components, including kernel weight and kernel morphology (Campbell et al. 1999; Prasad et al. 1999; Varshney et al. 2000; Zanetti et al. 2001; Groos et al. 2003; McCartney et al. 2005; Sun et al. 2009). Although these studies evaluated thousand-kernel weight, the QTL detected varied depending on the germplasm used. Different QTL were detected in a diverse germplasm when different methods were used to assess kernel size and shape (Giura and Saulescu 1996; Campbell et al. 1999; Dholakia et al. 2003; Breseghello and Sorrells 2007; Sun et al. 2009). Studies on kernel size have reported that kernel length and width are influenced by independent QTL. QTL reported in these studies represented only a set of QTL alleles that segregated in the germplasm studied. Considering the fact that wheat germplasm varies with different market classes, QTL studies for wheat kernel characteristics in all market classes are needed to provide good coverage of QTL alleles, including validation of QTL in different genetic backgrounds. Another important phenotypic assessment of kernel characteristics that has not been used in QTL analysis is kernel size distribution. This is an important trait because the uniformity of kernel size or its distribution allows for a more efficient milling process and quality control (Gaines et al. 1997; Yoon et al. 2002). Uniform kernels are ground more evenly during milling, which leads to high flour extraction rate and low ash content (Yoon et al. 2002).

In this paper, we report QTL influencing kernel weight, kernel diameter, and kernel size distribution in a recombinant inbred line population developed from hard red wheat breeding lines adapted to the Upper Midwest region of the USA using multiple evaluation methods. We also discuss the relationships of the percentage of large kernels, medium kernels, and small kernels with kernel weight and diameter.

Materials and methods

Grain materials and agronomic traits

A population of 139 recombinant inbred lines (RILs) was developed from a cross between two hard red spring wheat lines, MN98550 ('Bacup'/'McVey') and MN99394 (SD3236/SBF0402) (Tsilo et al. 2010a). The reason these lines were used to create a population was that both parents differed in their water absorption and dough mixing strength. The 139 RILs ($F_{6:8}$) were sown during spring 2006 at three Minnesota locations, St. Paul, Morris and Crookston, with two replications per line. Three check varieties and two parents were also included with eight replications in each location. The check varieties were 'Alsen' (Frohberg et al. 2006), 'Verde' (Busch et al. 1996), and 'Oklee' (Anderson et al. 2005). Plants were treated with fungicides to reduce fungal damage. Each experimental line was planted using a yield trial plot size of 2.6 m^2 with seven rows, and plots were laid out in a randomized complete block design. Heading date (Hd) was recorded as the number of days from planting to the date when 50% of the spikes had emerged from the flag leaf sheath. Plant height (Ht) was measured as the distance (cm) from the soil surface to the tip of the spike (excluding awns). The grain was harvested using a combine harvester and seed was thoroughly cleaned, and all non-wheat materials were removed before grain yield (Gyld) evaluation. Grain from two replicates was combined to generate one representative sample per line and four representative replicates per control.

Grain samples were sent to the USDA-ARS Wheat Quality Laboratory, Fargo, North Dakota for quality evaluation. The following kernel characteristics were evaluated: test weight (Twt), grain protein content (Gpc), kernel weight, kernel size, and kernel size distribution. Grain yield was measured as the mass of the total grain harvested per plot expressed as g m^{-2}. Test weight was measured as the mass of grain per unit volume (kg hl^{-1}) (AACC Method 55-10). Grain protein content was measured based on 12% moisture using near-infrared reflectance (AACC Method 46-30). Kernel weight was measured using two methods: (1) 1,000-kernel weight (Tkw) as the weight of one thousand seeds of a grain sample that was free of foreign materials and broken kernels (g) (Federal Grain Inspection Service, FGIS Method) and (2) single-kernel weight (Skw) as the average weight of 300 kernels determined using the Single Kernel Characterization System (SKCS) (SKCS 4100, Perten Instruments, Springfield, IL, USA) (AACC Method 55-31.01). Kernel size was measured using single-kernel diameter (Skdiam) as the average kernel diameter of 300 kernels determined with SKCS. All SKCS characteristics were measured in the Wheat Quality Laboratory at

North Dakota State University, Fargo. Kernel size distribution was measured using the sieving method (Ro-tap sieve shaker according to USDA/ARS Wheat Quality Lab). Three measurements were obtained based on the distribution of kernel size, which are the percentage of large kernels (Lgk), medium kernels (Medk), and small kernels (Smk).

Statistical analysis

Analysis of variance was conducted for all traits measured using SAS statistical software package version 9.1 (SAS Institute Inc. Cary NC, USA), assuming all factors as random. For Hd, Ht, and Gyld, lines were replicated per environment, and phenotypic values were averaged across replications, whereas phenotypic values for other kernel characteristics were derived from bulked replications. For Twt, Gpc, Skw, Skdiam, Tkw, Lgk, Medk, and Smk, the main effects of RILs and environment were tested for significance using the G × E mean square (MS_{ge}) as an error term. The G × E interaction was tested for significance using the error mean square (MS_e) estimated from the check genotypes that were replicated within environments, as described in an augmented design by Federer (1961). Variance components from PROC GLM output of SAS were used to estimate the broad-sense heritability (h_B^2) for each trait on a RIL mean basis (Fehr 1987). $h_B^2 = \sigma_g^2/[\sigma_g^2 + (\sigma_{ge}^2/e) + (\sigma_e^2/re)]$, where σ_g^2 is the genotypic variance $= (MS_{genotype} - MS_{ge})/re$, σ_{ge}^2 is the G × E interaction variance $= (MS_{ge} - MS_e)/r$, σ_e^2 is the error variance $= MS_e$, r is number of replications, and e is number of environments. The relationships of all traits measured were assessed by Pearson correlation coefficients using PROC CORR of SAS.

Molecular marker screening

Leaf tissue harvested from young plant material (parental lines and 139 RILs) was frozen and ground in liquid nitrogen. DNA was extracted from 20–30 seedlings per line using the protocol described by Riede and Anderson (1996) and modified by Liu et al. (2006). Two parents (MN99394 and MN98550) were screened for polymorphism at the USDA-ARS Small Grain Genotyping Center, Fargo, ND, using 877 simple sequence repeat (SSR) markers (GWM, GDM, BARC, CFA, CFD, WMC, and GPW) that covered the entire genome of wheat (Röder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002; Guyomarc'h et al. 2002; Somers et al. 2004; Sourdille et al. 2004; Song et al. 2005; http://wheat.pw.usda.gov/ggpages/SSR/WMC). Two-hundred forty-nine polymorphic markers were used to genotype the RILs. A 10-μL PCR reaction containing 1× PCR buffer (New England Biolabs, Inc. Beverly, MA, USA), 0.125 mM dNTPs, 0.4 pmol forward primer, 0.3 pmol reverse primer, 3.0 pmol of M13 primer labeled with one of the four fluorescent dyes (6-FAM, VIC, NED, and PET), 0.05 unit/μL *Taq* DNA polymerase (NEB), and ~75 ng genomic DNA was used to perform all PCR reactions. The PCR mixture was initially denatured at 94°C for 10 min, followed by 40 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, with a final extension step of 72°C for 5 min and 4°C indefinitely. The PCR thermal cycling was performed using the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). The PCR products amplified with four different dyes (6-FAM, VIC, NED, and PET) were multiplexed to a final volume of 10 μL, including 0.14 μL GeneScan-500 LIZ® size standard (Applied Biosystems) and 6.86 μL Hi-Di™ Formamide (Applied Biosystems). Mixed PCR products were denatured at 94°C for 5 min and chilled on ice. Amplified PCR products were separated by capillary electrophoresis using the ABI 3130xl Genetic Analyzer (Applied Biosystems). Separated fragments were analyzed using GeneMapper software v3.7 (Applied Biosystems) following descriptions in the user manuals. Genomic DNA of parental lines and 92 random RILs were sent to Diversity Arrays Technology Pty Ltd. (http://www.diversityarrays.com) for DArT marker genotyping. DNA was extracted from 20–30 plants per RIL following the protocol described by DArT (http://www.diversityarrays.com/pub/DArT_DNA_isolation.pdf). The marker names were described by Akbari et al. (2006). For example, wPt, tPt, and rPt, where the 'Pt' stands for *Pst*I and *Taq*I restriction enzymes used to generate clones; the 'w' is designated if the clone is derived from wheat library, and 't' from triticale library, or 'r' from rye library. The version 2.0 wheat DArT array of 5,137 clones was used for genotyping RILs. In addition, the array included some clones from a new library built from purified chromosome 3B. DArT hybridization, image analysis, and polymorphism scoring followed protocols previously described by Akbari et al. (2006).

Map construction and QTL analysis

Segregating SSR and DArT markers were scored for each individual of the RIL population with reference to parental genotypes. The SSR marker loci were scored for polymorphism as either homozygous or heterozygous loci. All missing, nonparental, and heterozygous marker alleles were treated as missing data during the map construction and QTL mapping. Genetic linkage maps were constructed using Mapmaker/Exp. version 3.0b software (Lander et al. 1987). Recombination frequencies were converted to centiMorgan (cM) values using the Kosambi mapping function (Kosambi 1944). The GROUP command with a LOD threshold of 3.0 and a distance of 37 cM was used to assign

markers to a linkage group. Marker loci in each linkage group were ordered based on repetitive use of the THREE POINT, ORDER, and TRY commands followed by RIPPLE command of MAPMAKER. The first best order was used, and linkage groups were assigned to known chromosomes by comparing marker names with previously published consensus maps of wheat (Somers et al. 2004) and the known map location of DArT markers, as provided by the Diversity Arrays Technology Pty Ltd.

The QTL analyses for individual environments were performed using the composite-interval mapping (CIM) method with the software application Windows QTL Cartographer V2.5 (Wang et al. 2005). The initial CIM step was run by SrMapQtl for QTL discovery using stepwise regression, which was followed by a final CIM step performed by ZMapQtl with a walk speed of 2 cM. Data for each experiment and for each trait were analyzed separately. Three hundred permutations were performed with a significance level of 0.05; however, to avoid obvious Type II error, a putative QTL was declared when the logarithm of odds (LOD) score was greater or equal to a LOD score of 2.5 in at least two environments. For each trait, the total phenotypic variation explained by all the QTL in the model was estimated using multiple regression analysis (PROC REG) in SAS. The QTL were named following the nomenclature described in the Cataloque of Gene Symbols for Wheat (http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/).

Results

Phenotypic variation

All traits showed approximately normal distributions (Fig. 1), suggesting polygenic inheritance. Mean values, ranges, and standard deviations for all traits are summarized in Table 1 together with mean values for MN99394 and MN98550. For example, Skw evaluated based on an average of 300 kernels showed a mean of 29.6 and a standard deviation of 3.0 with a minimum of 22.6 and maximum range of 37.1 for samples averaged across all three growing locations. The parental lines had similar trait values in all three environments. All traits showed

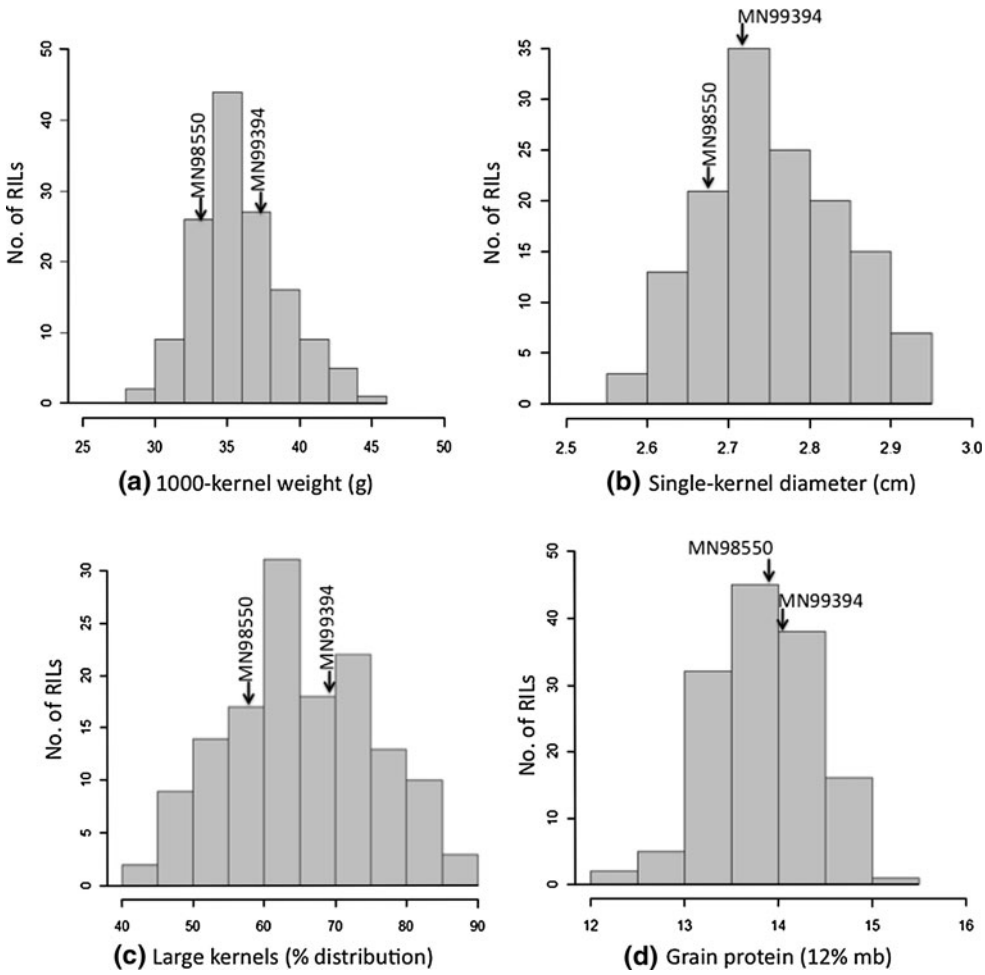

Fig. 1 Histogram patterns of 139 RILs for 3-location mean values of **a** kernel weight (1,000-kernel weight), **b** kernel size (single-kernel diameter), **c** kernel size distribution (% of larger kernels), and **d** grain protein content. Parental means are marked with *arrows*

Table 1 Mean values, standard deviations, and range of all traits evaluated in three environments in 2006

Trait	Environment	RIL population ($n = 139$)				Parental lines	
		Mean	Min	Max	SD	MN99394	MN98550
Hd	All	52.0	47.5	56.5	2.3	52.5	51.8
	Crookston	46.0	41.5	50.5	2.0	45.6	46.8
	Morris	57.3	54.0	61.0	1.9	57.6	56.9
	St. Paul	52.9	47.0	58.5	3.4	54.3	51.9
Ht	All	84.3	69.9	96.6	5.5	79.7	83.9
	Crookston	90.1	77.0	105.5	5.6	82.9	90.0
	Morris	79.6	61.0	97.8	6.9	75.6	78.7
	St. Paul	83.2	68.0	93.0	5.4	80.5	83.0
Gyld	All	630	513	727	44	607	649
	Crookston	673	391	823	64	661	702
	Morris	634	419	799	65	607	644
	St. Paul	582	442	705	46	554	601
Twt	All	81.1	78.7	82.9	0.9	80.8	81.0
	Crookston	81.2	76.5	84.7	1.4	81.0	81.0
	Morris	82.3	79.7	84.3	0.8	82.0	82.2
	St. Paul	79.9	77.3	82.3	1.1	79.5	79.7
Gpc	All	13.9	12.4	15.5	0.6	14.1	13.9
	Crookston	13.3	11.8	14.9	0.6	13.6	13.5
	Morris	14.3	12.4	16.0	0.7	14.6	14.3
	St. Paul	14.0	12.1	15.8	0.7	14.1	13.9
Skw (mg)	All	29.6	22.6	37.1	3.0	31.9	30.9
	Crookston	29.6	22.6	37.2	3.0	28.8	27.9
	Morris	34.1	27.2	40.1	2.5	32.9	33.1
	St. Paul	33.9	28.6	41.5	2.6	34.0	31.6
Skdiam (mm)	All	2.8	2.6	3.0	0.1	2.7	2.7
	Crookston	2.7	2.4	2.9	0.1	2.6	2.6
	Morris	2.8	2.6	3.0	0.1	2.8	2.8
	St. Paul	2.8	2.6	3.1	0.1	2.8	2.7
Tkw (g)	All	35.9	29.3	44.1	3.0	36.2	34.3
	Crookston	32.9	24.1	40.5	3.5	32.3	31.3
	Morris	37.6	29.5	46.1	3.2	38.3	36.2
	St. Paul	37.1	29.9	46.3	3.1	38.0	35.5
Lgk (%)	All	65.6	43.3	86.3	10.1	67.9	57.8
	Crookston	50.5	20.0	83.0	14.6	49.8	39.8
	Morris	72.3	51.0	91.0	9.8	74.5	64.8
	St. Paul	73.9	50.0	91.0	8.8	79.5	68.8
Medk (%)	All	28.4	11.3	44.3	7.9	26.7	34.6
	Crookston	39.9	15.0	61.0	10.6	41.3	48.0
	Morris	23.6	8.0	40.0	7.9	21.5	29.8
	St. Paul	21.8	7.0	40.0	7.2	17.3	26.0
Smk (%)	All	6.0	1.7	13.0	2.5	5.4	7.7
	Crookston	9.6	2.0	23.0	4.7	9.0	12.3
	Morris	4.1	1.0	13.0	2.1	4.0	5.5
	St. Paul	4.3	2.0	10.0	1.7	3.3	5.3

Hd heading date (days from planting); *Ht* plant height (cm); *Gyld* grain yield (g m^{-2}); *Twt* test weight (kg hl^{-1}); *Gpc* grain protein content (%); *Skw* single-kernel weight determined based on 300 kernels using single kernel characterization system (SKCS); *Skdiam* single-kernel diameter determined based on SKCS, *Tkw* 1,000-kernel weight; *Lgk* % of large kernels; *Medk* % of medium kernels; *Smk* % of small kernels

transgressive segregation including those for which parental values were similar (Table 1; Fig. 1). For all traits analyzed, a significant variation was due to effects of genotype (G) and environment (E) (Table 2), and to some extent a G × E interaction. Relative to genotype main effects, the magnitude of G × E interaction effects was often small. For all traits analyzed, the broad-sense heritability estimates ranged from 0.61 to 0.91 (Table 2).

Correlations among all traits measured are summarized in Table 3. Hd was significantly correlated with several kernel characteristics, suggesting that late heading plants apparently had a lesser capacity for grain filling than early plants and were low in test weight, grain protein content, kernel weight, and size. Gyld was negatively correlated with Gpc ($r = -0.41$ at $P \leq 0.001$). Even though when averaged across three growing locations, Gyld was not significantly correlated with kernel weight, diameter, and kernel size distribution parameters, the correlations were significant in two of the three locations (Supplementary Table 1). Test weight was significantly correlated with kernel weight, kernel diameter, and kernel size distribution, suggesting that heavier and larger kernels had a higher test weight. Single-kernel weight (Skw) showed a highly significant correlation ($r = 0.94$ at $P \leq 0.001$) with 1,000-kernel weight (Tkw). Single-kernel diameter (Skdiam) was highly positively correlated with the percentage of larger kernels, Lgk ($r = 0.93$ at $P \leq 0.001$), and negatively correlated with the percentage of medium and small kernels ($r \cong -0.90$ at $P \leq 0.001$), suggesting that kernel diameter and kernel size distribution have at least some gene(s) in common. There were no undesirable negative relationships among kernel weight, kernel size, percentage of large kernels, and protein observed (Table 3), suggesting that an increase in one of these traits will not adversely impede progress on other traits. Kernel uniformity should be increased by increasing the percentage of large kernels rather than to increase medium and small kernels.

Map construction

The initial genetic linkage map for this population included 803 marker loci. After removal of co-segregating markers, the final genetic map consisted of 531 SSR and DArT marker loci. The stem rust resistance gene *Sr6*, originally mapped by Tsilo et al. (2009), also mapped on the same chromosome region in this genetic map. The markers umn19 and umn26 for high molecular weight glutenin genes *Glu-1A* and *Glu-1D*, respectively (Liu et al. 2008), were also mapped in this population. The total genetic map length spanned a distance of 2,505 cM spread over 47 linkage groups, and covered all 21 wheat chromosomes plus one linkage group that was not assigned to a chromosome. Most wheat chromosomes had good marker coverage with the exception of 1D, which contained only three markers (Supplementary Fig. 1S). Most of the markers on the consensus map of chromosome 1D were

Table 2 Mean squares and heritabilities for agronomic traits and kernel characteristics of the recombinant inbred line population ($n = 139$) evaluated in three environments in 2006

	Mean Square				h_B^2 [b]
Trait	Genotype	Environment	G × E	Error[a]	
Hd	32.1***	9,062.0***	2.8***	0.78	0.91
Ht	183.4***	7,956.2***	17.4 [NS]	15.60	0.91
Gyld	11,749***	572,966***	4,548**	3,417	0.61
Twt	1.30***	121.35***	0.425*	0.27	0.67
Gpc	0.91***	35.07***	0.242**	0.13	0.73
Skw	16.70***	898.31***	2.65**	1.18	0.84
Skdiam	0.02***	1.31***	0.004**	0.002	0.80
Tkw	26.77***	939.67***	2.67**	1.44	0.90
Lgk	303.7***	23,782.8***	41.23**	17.01	0.86
Medk	185.45***	13,833.4***	20.91**	10.99	0.89
Smk	18.06***	1,353.4***	5.56***	1.25	0.69

Hd heading date; *Ht* plant height; *Gyld* grain yield; *Twt* test weight; *Gpc* grain protein content; *Skw* single-kernel weight determined based on 300 kernels using single kernel characterization system (SKCS); *Skdiam* single-kernel diameter determined based on SKCS; *Tkw* 1,000-kernel weight; *Lgk* % of large kernels; *Medk* % of medium kernels; *Smk* % of small kernels; *E* environment; *G* genotype

*, **, *** Significance at $P < 0.05$, 0.01, and 0.001, respectively; *NS* not significant at $P < 0.05$

[a] Except for Hd, Ht, and Gyld, the error mean squares for other traits were estimated from the check genotypes that were replicated within environments, as described in an augmented design by Federer (1961)

[b] Broad-sense heritability on an entry-mean basis

Table 3 Phenotypic correlation coefficients among traits based on trait values averaged across three environments in 2006

Traits[a]	Hd	Ht	Gyld	Twt	Gpc	Skw	Skdiam	Tkw	Lgk	Medk	Smk
Hd	1										
Ht	0.64***	1									
Gyld	0.05 ^{NS}	−0.02 ^{NS}	1								
Twt	−0.48***	−0.12 ^{NS}	0.12 ^{NS}	1							
Gpc	−0.25**	0.11 ^{NS}	−0.41***	0.12 ^{NS}	1						
Skw	−0.34***	0.01 ^{NS}	0.16$^{(0.06)}$	0.29***	0.19*	1					
Skdiam	−0.33***	−0.03 ^{NS}	0.11 ^{NS}	0.35***	0.25**	**0.93****	1				
Tkw	−0.34***	−0.01 ^{NS}	0.15$^{(0.07)}$	0.28***	0.13 ^{NS}	**0.94****	**0.90****	1			
Lgk	−0.31***	−0.03 ^{NS}	0.11 ^{NS}	0.32***	0.15$^{(0.08)}$	**0.88****	**0.93****	**0.92****	1		
Medk	0.29***	0.01 ^{NS}	−0.10 ^{NS}	−0.29***	−0.12 ^{NS}	**−0.86****	**−0.91****	**−0.91****	**−0.99****	1	
Smk	0.37***	0.10 ^{NS}	−0.12 ^{NS}	−0.36***	−0.22**	**−0.84****	**−0.89****	**−0.84****	**−0.92****	**0.87****	1

Values in bold are strong correlations among kernel characteristics

*, **, *** Significance at $P < 0.05$, 0.01, and 0.001, respectively; *NS* not significant at $P < 0.05$

[a] Traits were defined in Table 1

monomorphic in this population, meaning that the two parents shared most of the marker alleles on chromosome 1D. The final genetic map was used for QTL mapping and analysis.

QTL analysis for kernel weight, kernel size, and size distribution

The QTL were detected using composite interval mapping with the trait analysis in each of the three environments. QTL were also detected using the trait mean across three environments as presented in Supplementary Table 2. In this section, however, only QTL detected in at least two of the three environments are presented. QTL clusters were detected for kernel weight, kernel diameter, and kernel size distribution (Table 4; Fig. 2). The most important QTL or QTL cluster was located on chromosome 7A between *XwPt9824* and *XwPt4553* (Table 4; Fig. 2). This QTL explained 8–21% of the total variation in kernel weight (Skw and Tkw), kernel diameter, and kernel size distribution (Lgk, Medk, and Smk), and was detected in several environments (Table 4). A second QTL or QTL cluster was located on chromosome 5B between *Xgwm234* and *XwPt9006* (Table 4; Fig. 2). This QTL explained 5–16% of the total variation in kernel weight (Skw and Tkw), kernel diameter, and kernel size distribution (Lgk, Medk, Smk), and was detected in all environments for kernel weight and kernel size distribution. The third QTL or QTL cluster was located on 2A between *Xgwm339* and *Xbarc311* (Table 4; Fig. 2). This QTL was detected in at least two environments for kernel weight, kernel diameter, and kernel size distribution. The QTL on 6B (*QTkw.mna-6B*) was detected at least in two environments and accounted for up to 12% of the variation in kernel weight (Table 4; Fig. 2). The QTL on 5A (*QSkdiam.mna-5A*) was detected in two environments and accounted up to 13% of the variation in kernel diameter. For kernel weight, diameter, and size distribution, the total phenotypic variation explained by all the QTL ranged from 26.5 to 44.1% (Supplementary Table 3).

QTL analysis for grain protein content and grain yield

For grain protein content, each of the three QTL located on chromosomes 2B, 5A, and 6D were detected only in two of the three environments (Table 5; Fig. 2). The QTL *QGpc.mna-6D* explained 5.9–8.9% of the total variation in protein content (Table 5). A second QTL on 5A (*QGpc.mna-5A*) explained 6.5–11.1% of the variation. Alleles with increasing effects from *QGpc.mna-6D* and *QGpc.mna-5A* were contributed by MN98550. The third QTL on chromosome 2B (*QGpc.mna-2B*) had R^2 of 5.9 and 16.8% with LOD scores of 2.7 and 7.7, respectively, in the two environments in which it was detected (Table 5; Fig. 2). Alleles for *QGcp.mna-2B* were contributed by MN99394. Two grain yield QTL (*QGyld.mna-1B* and *QGyld.mna-2B*) were detected in two of the three locations (Table 5). The Gyld increasing effect from both QTL was contributed by alleles from MN98550. Interestingly, MN98550 was the same parent that contributed alleles with increasing effects on Gpc from two of the three Gpc QTL, as mentioned above. The *QGyld.mna-1B* QTL explained up to 14% of the total variation in Gyld. *QGyld.mna-2B* explained about 8% of the variation in Gyld and also coincided with the grain protein content QTL *QGpc.mna-2B* (Table 5). Other QTL not declared in at least two

Table 4 Summary of QTL identified for kernel weight, kernel diameter, and kernel size distribution using 139 recombinant inbred lines grown in three environments in 2006

Chr[a]	Marker interval/peak	Env.	Skw[b]			Tkw			Skdiam			Lgk			Medk			Smk		
			LOD	R^2	Add	LOD	R^2	Add	LOD	R^2	Add	LOD	R^2	Add	LOD	R^2	Add	LOD	R^2	Add
7A	*XwPt9824–XwPt4553*	Cr	4.2	13.3	1.10	3.2	8.6	1.04	2.6	8.3	0.03	2.6	7.8	4.10	2.6	8.6	−2.63			
		Mo	6.3	16.9	1.08	7.5	20.8	1.44	3.4	10.6	0.03	4.2	11.7	3.38	4.0	11.4	−2.70	3.4	9.5	−0.65
		St	3.9	10.2	0.83	7.9	19.8	1.40	3.2	7.6	0.03	5.7	15.6	3.51	6.0	16.6	−2.98	3.4	9.5	−0.54
5B	*Xgwm234–XwPt9006*	Cr	3.7	11.6	1.11	3.9	10.5	1.15	3.6	12.1	0.04	5.5	16.3	5.99	5.2	14.8	−4.12	4.0	12.6	−1.69
		Mo	*1.7*	*5.0*	*0.58*	3.3	8.5	0.93				3.0	7.1	2.50	3.2	8.2	−2.29	*2.4*	*6.3*	*−1.26*
		St	6.1	15.6	1.04	*2.3*	*5.5*	*0.75*	6.1	14.7	0.04	3.4	7.3	2.41	3.1	6.7	−1.90	3.1	6.8	−0.46
2A	*Xgwm339–Xbarc311*	Cr										3.2	7.8	−4.25	2.7	6.4	2.76			
		Mo	3.4	7.7	−0.74	4.3	8.9	−0.98	3.6	7.6	−0.03	*2.4*	*4.7*	*−2.20*				3.9	8.4	0.63
		St	4.5	9.4	−0.83	4.9	10.2	−1.05	5.1	10.3	−0.03	5.3	9.9	−2.88	4.6	8.7	2.22	7.1	14.1	0.67
6B	*XwPt0052*	Cr				2.9	7.7	−0.98							*2.0*	*5.6*	*2.50*			
		Mo				4.8	12.4	−1.13				*3.1*	*7.9*	*−2.75*	*3.5*	*9.0*	*2.38*			
		St	*4.4*	*13.3*	*−0.97*	*2.2*	*5.4*	*−0.73*	*3.9*	*9.7*	*−0.03*	*2.2*	*5.0*	*−1.99*	*2.1*	*4.9*	*1.61*			
5A	*Xbarc337b–Xcfa2250*	Mo							6.2	13.4	0.03									
		St							4.1	8.1	0.03									

Values in italics were QTL which were not detected, detected in one location with one method or not declared in a particular location because the LOD scores were little less than a threshold of 2.5

[a] Chromosomes were QTL for each trait were detected depending on the environment (Env.)

[b] Traits were defined in Table 1; the LOD score and percent phenotypic variation ($R^2 \times 100$) explained for by each of the QTL are provided along with the additive (add) allele effects for the loci. Positive additive allele effects indicate that the QTL alleles were contributed by MN99394, and negative indicates that the QTL alleles were contributed by MN98550. Three environments: *Cr* Crookston, *Mo* Morris, and *St* St. Paul

environments, but were declared based on trait means across three environments, were summarized in Supplementary Table 2. All QTL explained a total phenotypic variation of 40.9 and 41.3% in Gpc and Gyld, respectively (Supplementary Table 3).

QTL analysis for other traits

No consistent QTL were detected for Twt in this population. For heading date, two QTL (*QHd.mna-7B* and *QHd.mna-2D*) were detected in two locations, and each explained up to 10% of the variation in heading date (Table 5; Fig. 2). No heading date QTL was detectable at the Crookston location. For both heading date QTL, the effect of lateness was contributed by alleles from MN99394. For plant height, two QTL (*QHt.mna-5B* and *QHt.mna-4B*) were detected in two locations and *QHt.mna-7B* was detected in all three locations (Table 5; Fig. 2), indicating that these QTL were relatively stable. The *QHt.mna-7B* QTL coincided with *QHd.mna-7B*. Both parents contributed tallness alleles (Table 5). Other QTL not declared in at least two environments but declared based on trait mean across three environments were summarized in Supplementary Tables 2 and 3.

Discussion

Phenotypic variation and correlations of kernel characteristics

Parents of the mapping population used in this experiment were advanced breeding lines and were selected for a variety of agronomic and end-use quality traits. In this experiment, alleles from both parents contributed to the increase in kernel weight, size, and protein. Percentages of medium and small kernel size were negatively correlated with test weight, kernel weight (Skw and Tkw), and kernel diameter, whereas the percentage of large kernel size was positively correlated with test weight, kernel weight (Skw and Tkw), and kernel diameter, indicating that an increase in Lgk is desirable. Previous research has shown that an increase in kernel weight (Wiersma et al. 2001) or kernel size (Marshall et al. 1984; Berman et al. 1996) also increased flour yield. Considering that Medk and Smk have undesirable relationship with other traits, kernel uniformity should be increased by increasing the percentage of large kernels. During milling, bran is separated from the endosperm and when there is variation in kernel size distribution, small kernels pass through the roller mills unground, thereby requiring additional processing. The presence of small kernels and the requirement of additional processing lead to a low flour extraction rate and high ash content (Yoon et al. 2002). It has also been reported previously that the amount of bran that is not completely removed from the endosperm increases mineral composition or the ash content of the flour, and also decreases the brightness of the flour (Kent-Jones and Amos 1967). In another study, we also found that the presence of small kernels was negatively correlated with the brightness of the flour (Tsilo et al. 2010b). Kernel size distribution is heritable, and the estimates of heritability are high for the percentage of large kernel (86%) compared to the percentage of small kernels (69%) (Table 2). From our current results, we observed desirable correlations between kernel weight (Skw and Tkw) and kernel size (Skdiam) ($r \geq 0.90$ at $P \leq 0.001$), between kernel weight (Skw and Tkw) and the percentage of large kernels ($r \geq 0.88$ at $P \leq 0.001$), and between kernel diameter and the percentage of large kernels ($r = 0.93$ at $P \leq 0.001$). Although previous studies reported moderate correlations between kernel weight, length, and width with $r = 0.51$–0.68 at $P = 0.01$ (Dholakia et al. 2003) and $r = 0.21$–0.75 at $P = 0.01$ (Sun et al. 2009), our results were in agreement with Lee et al. (2006), who reported strong correlation ($r = 0.83$) between kernel weight and size. Studies have shown that kernel weight was positively correlated with grain yield (Baril 1992) and kernel growth rate (Rasyad and Van Sanford 1992); however, in this study, 3-location mean values of kernel weight (Skw and Tkw) were less correlated with grain yield (Table 3) compared to correlations obtained in the two individual locations Crookston and St. Paul (Supplementary Table 1S).

QTL analysis for kernel weight, kernel diameter, and size distribution

In order for a QTL to be useful for marker-assisted selection, it should be expressed in most environments and germplasm where individuals segregating for the traits will be tested. For this reason, we declared only QTL that were detected in more than one environment (Table 4). The QTL detected based on the trait mean of three environments also showed the same QTL that were stable in several environments (Supplementary Table 2).

For kernel weight, the *Xgwm339-Xbarc311* region on chromosome 2A was previously reported as a QTL region for kernel weight and grain yield in spring wheat germplasm (McCartney et al. 2005), but was not in the same region as the one environment QTL reported by Sun et al. (2009). In our study, this QTL peaks at *Xgwm249* and amplified an allele fragment of 181 bp on the parent MN99394 and 166 bp on MN98550. Campbell et al. (1999) identified four QTL for kernel weight on chromosomes 1A, 1B, 3B, and 7A in a cross of soft × hard winter wheat. Varshney et al. (2000) used monosomic

analysis and only QTL on 1A, 2B, and 7A had alleles conferring high kernel weight. Dholakia et al. (2003) identified QTL on 2B and 2D. Groos et al. (2003) reported QTL for kernel weight on nine chromosomes with only QTL on 2B, 2D, 5B, 6A, and 7A being somewhat stable across environments. Sun et al. (2009) identified stable QTL on 5D and 6A. From all the studies reported so far, a common QTL for kernel weight was on chromosome 7A. However, in our study we found that this QTL was either pleiotropic or closely linked to a QTL for kernel diameter and kernel size distribution, results that were not reported previously. We also mapped a QTL on chromosome 5B within the region of *Xgwm234-XwPt9006*. Groos et al. (2003) also detected a QTL for kernel weight on this chromosome, but it was located more than ~60 cM from the region we detected. The kernel weight QTL on chromosome 6B has not been previously reported. This QTL explained up to 13% of the total variation in kernel weight, and was detectable in all three environments using either Skw or Tkw. Based on the trait mean across three environments, six QTL for kernel weight on chromosomes 1B, 2A, 2D, 5B, 6B, and 7A were detected (Supplementary Table 2).

Several QTL were previously reported for kernel width and length on different chromosomes; for example, Campbell et al. (1999) reported QTL on chromosomes 1A, 2A, 2B, 2DL, and 3DL. Breseghello and Sorrells (2007) reported QTL on 1B, 2D, and 5B. Sun et al. (2009) reported QTL on chromosomes 4A and 6A that were detected in two out of four environments. The 6A QTL for kernel width co-localized with a QTL for kernel weight (Sun et al. 2009). In our study, four stable QTL were identified on 2A, 5A, 5B, and 7A for kernel diameter. Three of these QTL (2A, 5B, and 7A) were in the same regions as QTL for kernel weight as discussed above. The fourth kernel size QTL (*QSkdiam.mna-5A*) was in a region independent of kernel weight and other kernel characteristics. The kernel size QTL on 2A and 7A have not been reported previously. Moreover, no QTL have previously been reported using the kernel size distribution method. In this study, we showed that the percentage of large, medium, and small kernels were influenced by QTL on chromosome 2A, 5B, and 7A, and these QTL co-localized on the same region with the QTL for kernel weight and diameter. A QTL on 6B was not declared because the LOD scores (2.0–2.2) were less than the threshold of 2.5 (Table 4).

QTL analysis for grain protein and grain yield

For grain protein, QTL have been identified previously on several chromosomes, suggesting that this trait is controlled by many loci. Even in studies in which parental lines had small differences in Gpc, QTL were still detected. For example, Mares and Campbell (2001) identified three QTL on chromosomes 1B (LOD score 5.07), 2B (LOD score 7.09), and 5B (LOD score 3.75). Prasad et al. (1999) identified one QTL on chromosome 2DL explaining 18% of the phenotypic variation in Gpc. Groos et al. (2003) reported QTL on chromosomes 2A, 3A, 4D, and 7D, each explaining 10% of the phenotypic variation in Gpc. Zanetti et al. (2001) identified a QTL on 5A that explained 25% of the phenotypic variation in Gpc using a population derived from bread-wheat and spelt. However, in our population, the 5A QTL explained up to 11% of the phenotypic variation among bread-wheat lines. A Gpc QTL on 6D was located near the markers *Xcfd5*, *Xcfd45*, and *Xwmc773* at the distal region of the long arm of chromosome 6D, which is a different region from the location of the storage protein locus known to be on the short arm of 6D based on the reports of Payne et al. (1982) and Nelson et al. (2006).

The strong negative relationship between Gyld and Gpc in this study ($r = -41$; $P < 0.001$) was observed based on trait values averaged across three environments (Table 3). This negative correlation hinders genetic gains on these traits (Kamra 1971; Bhatia 1975; Loffler and Busch 1982). However, extensive efforts to identify all Gpc QTL alleles could help to diminish the negative association between Gyld and Gpc by identifying the Gpc QTL that are stable and independent of the effects of Gyld. A significant outcome in this study was that the two Gpc QTL, *QGpc.mna-5A* and *QGpc.mna-6D*, were independent of Gyld and other yield components such as kernel weight and size. Based on the trait values averaged across three environments (Supplementary Table 2), four QTL for Gpc on 1A (LOD = 3.3; $R^2 = 8\%$), 2D (LOD = 2.6; $R^2 = 7.8\%$), 5A (LOD = 4.5; $R^2 = 10.3\%$), and 6D (LOD = 5.9; $R^2 = 13.1\%$) were independent of Gyld. Other QTL for Gyld that were independent of Gpc were identified on chromosomes 3D (LOD = 3.1; $R^2 = 6.7\%$), 5B (LOD = 4.2; $R^2 = 16\%$), 6B (LOD = 2.7; $R^2 = 5.9\%$), and 6D (LOD = 2.7; $R^2 = 5.9\%$). Only one QTL on 2B confers undesirable pleiotropic effect or a repulsion linkage between Gyld (LOD = 6.7; $R^2 = 18\%$) and Gpc

Fig. 2 Stable quantitative trait loci associated with kernel weight, kernel diameter, kernel size distribution, grain yield (*Gyld*), grain protein content (*Gpc*), plant height (*Ht*), and heading date (*Hd*). The approximate QTL location for each trait is based on the mean of three environments and is indicated by the *intersection of vertical bar* (confidence interval based on the LOD scores above 2.5) and *horizontal bar* (indicates the maximum LOD score, and the maximum magnitude of the LOD score at any environment is indicated by the *thickness of the bar*). Detailed information about each QTL is given in Tables 4 and 5, and Supplementary Table 2. Map distances were determined based on Haldane mapping function and are given on the *left*. Chromosome names with *decimal numbers* indicate the linkage group in which QTL were detected ▶

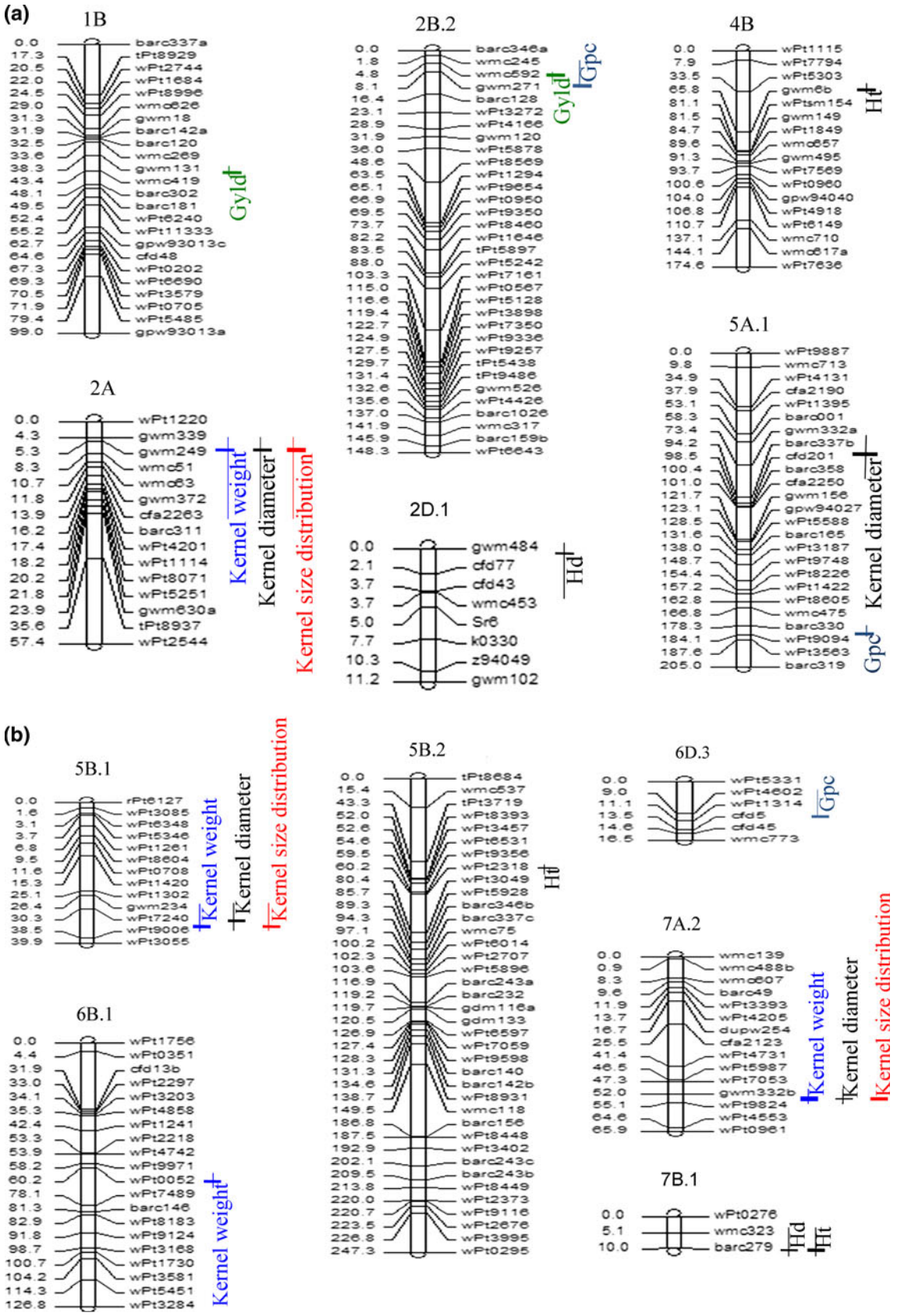
(a)
1B
0.0 barc337a
17.3 tPt8929
20.5 wPt2744
22.0 wPt1684
24.5 wPt8996
29.0 wmc626
31.3 gwm18
31.9 barc142a
32.5 barc120
33.6 wmc269
38.3 gwm131
43.4 wmc419
48.1 barc302
49.5 barc181
52.4 wPt6240
55.2 wPt11333
62.7 gpw93013c
64.6 cfd48
67.3 wPt0202
69.3 wPt6690
70.5 wPt3579
71.9 wPt0705
79.4 wPt5485
99.0 gpw93013a
Gyld
2A
0.0 wPt1220
4.3 gwm339
5.3 gwm249
8.3 wmc51
10.7 wmc63
11.8 gwm372
13.9 cfa2263
16.2 barc311
17.4 wPt4201
18.2 wPt1114
20.2 wPt8071
21.8 wPt5251
23.9 gwm630a
35.6 tPt8937
57.4 wPt2544
Kernel weight
Kernel diameter
Kernel size distribution
2B.2
0.0 barc346a
1.8 wmc245
4.8 wmc592
8.1 gwm271
16.4 barc128
23.1 wPt3272
28.9 wPt4166
31.9 gwm120
36.0 wPt5878
48.6 wPt8569
63.5 wPt1294
65.1 wPt9654
66.9 wPt0950
69.5 wPt9350
73.7 wPt8460
82.2 wPt1646
83.5 tPt5897
88.0 wPt5242
103.3 wPt7161
115.0 wPt0567
116.6 wPt5128
119.4 wPt3898
122.7 wPt7350
124.9 wPt9336
127.5 wPt9257
129.7 tPt5438
131.4 tPt9486
132.6 gwm526
135.6 wPt4426
137.0 barc1026
141.9 wmc317
145.9 barc159b
148.3 wPt6643
Gyld
Gpc
2D.1
0.0 gwm484
2.1 cfd77
3.7 cfd43
3.7 wmc453
5.0 Sr6
7.7 k0330
10.3 z94049
11.2 gwm102
Hd
4B
0.0 wPt1115
7.9 wPt7794
33.5 wPt5303
65.8 gwm6b
81.1 wPtsm154
81.5 gwm149
84.7 wPt1849
89.6 wmc657
91.3 gwm495
93.7 wPt7569
100.6 wPt0960
104.0 gpw94040
106.8 wPt4918
110.7 wPt6149
137.1 wmc710
144.1 wmc617a
174.6 wPt7636
Ht
5A.1
0.0 wPt9887
9.8 wmc713
34.9 wPt4131
37.9 cfa2190
53.1 wPt1395
58.3 barc001
73.4 gwm332a
94.2 barc337b
98.5 cfd201
100.4 barc358
101.0 cfa2250
121.7 gwm156
123.1 gpw94027
128.5 wPt5588
131.6 barc165
138.0 wPt3187
148.7 wPt9748
154.4 wPt8226
157.2 wPt1422
162.8 wPt8605
166.8 wmc475
178.3 barc330
184.1 wPt9094
187.6 wPt3563
205.0 barc319
Gpc
Kernel diameter
(b)
5B.1
0.0 rPt6127
1.6 wPt3085
3.1 wPt6348
3.7 wPt5346
6.8 wPt1261
9.5 wPt8604
11.6 wPt0708
15.3 wPt1420
25.1 wPt1302
26.4 gwm234
30.3 wPt7240
38.5 wPt9006
39.9 wPt3055
Kernel weight
Kernel diameter
Kernel size distribution
6B.1
0.0 wPt1756
4.4 wPt0351
31.9 cfd13b
33.0 wPt2297
34.1 wPt3203
35.3 wPt4858
42.4 wPt1241
53.3 wPt2218
53.9 wPt4742
58.2 wPt9971
60.2 wPt0052
78.1 wPt7489
81.3 barc146
82.9 wPt8183
91.8 wPt9124
98.7 wPt3168
100.7 wPt1730
104.2 wPt3581
114.3 wPt5451
126.8 wPt3284
Kernel weight
5B.2
0.0 tPt8684
15.4 wmc537
43.3 tPt3719
52.0 wPt8393
52.6 wPt3457
54.6 wPt6531
59.5 wPt9356
60.2 wPt2318
80.4 wPt3049
85.7 wPt5928
89.3 barc346b
94.3 barc337c
97.1 wmc75
100.2 wPt6014
102.3 wPt2707
103.6 wPt5896
116.9 barc243a
119.2 barc232
119.7 gdm116a
120.5 gdm133
126.9 wPt6597
127.4 wPt7059
128.3 wPt9598
131.3 barc140
134.6 barc142b
138.7 wPt8931
149.5 wmc118
186.8 barc156
187.5 wPt8448
192.9 wPt3402
202.1 barc243c
209.5 barc243b
213.8 wPt8449
220.0 wPt2373
220.7 wPt9116
223.5 wPt2676
226.8 wPt3995
247.3 wPt0295
Ht
6D.3
0.0 wPt5331
9.0 wPt4602
11.1 wPt1314
13.5 cfd5
14.6 cfd45
16.5 wmc773
Gpc
7A.2
0.0 wmc139
0.9 wmc488b
8.3 wmc607
9.6 barc49
11.9 wPt3393
13.7 wPt4205
16.7 dupw254
25.5 cfa2123
41.4 wPt4731
46.5 wPt5987
47.3 wPt7053
52.0 gwm332b
55.1 wPt9824
64.6 wPt4553
65.9 wPt0961
Kernel weight
Kernel diameter
Kernel size distribution
7B.1
0.0 wPt0276
5.1 wmc323
10.0 barc279
Hd
Ht

Table 5 Summary of QTL identified for grain protein content, grain yield, heading date, and plant height using 139 recombinant inbred lines grown in three environments

Trait[a]	QTL	Env.	Marker interval/peak	LOD	R^2	Add[b]
Gpc	*QGpc.mna-6D*	Cr	*Xcfd5*	4.3	8.9	−0.20
		Mo		*2.2*	*4.5*	*−0.15*
		St		2.6	5.9	−0.17
	QGpc.mna-5A	Mo	*Xbarc330–XwPt9094*	4.4	11.1	−0.24
		St		2.5	6.5	−0.18
	QGpc.mna-2B	Cr	Xwmc245–*Xgwm271*	7.7	16.8	0.28
		Mo		2.7	5.9	0.17
Gyld	*QGyld.mna-1B*	Cr	*Xgwm131–Xwmc419*	4.4	14.1	−25.06
		St		2.7	5.7	−11.31
	QGyld.mna-2B	Mo	*Xwmc592–Xgwm271*	2.9	8.3	−18.80
		St		4.0	8.5	−14.04
Hd	*QHd.mna-7B*	Mo	*Xwmc323*	2.9	7.8	0.54
		St		3.6	10.5	1.11
	QHd.mna-2D	Cr	*Xgwm484*	*2.1*	*5.6*	*0.48*
		Mo		4.0	10.4	0.63
		St		3.4	8.6	1.01
Ht	*QHt.mna-7B*	Cr	*Xwmc323*	4.9	13.3	2.10
		Mo		3.0	7.8	1.98
		St		6.0	15.0	2.12
	QHt.mna-5B	Cr	*XwPt2318*	3.0	12.6	2.02
		St		2.8	9.0	1.64
	QHt.mna-4B	Mo	*Xgwm6b*	3.8	12.2	−2.46
		St		5.4	15.4	−2.16

Values in italics were QTL not declared because the LOD scores were less below a threshold of 2.5

[a] Traits were defined in Table 1

[b] In each environment (Env.), the LOD score and percent phenotypic variation ($R^2 \times 100$) explained by each of the QTL are provided along with the additive (add) allele effects for the loci. Positive additive allele effects indicate that the QTL alleles were contributed by MN99394, and negative effects indicate that the QTL alleles were contributed by MN98550. Three environments: *Cr* Crookston, *Mo* Morris, and *St* St. Paul

(LOD = 6.2; $R^2 = 13.3\%$). The joint effects of all these QTL explained 40.9 and 41.3% of the variation in Gpc and Gyld, respectively.

In summary, we identified stable QTL clusters influencing kernel weight, kernel diameter, and kernel size distribution on chromosomes 2A, 5B, and 7A. Molecular markers that would be useful for these QTL clusters are *Xgwm249* for 2A, *Xgwm234* for 5B, and *Xgwm332* for 7A. Phenotypic variation explained by individual QTL at these clusters varied from 5 to 20% depending on the trait. Increase in kernel size uniformity is mostly favorable when increasing the percentage of large kernels. For grain yield and protein content, a QTL region on chromosome 2B confers an undesirable pleiotropic effect or a repulsion linkage between grain yield ($R^2 = 18\%$) and grain protein content ($R^2 = 13.3\%$). In this study, several grain protein and grain yield QTL independent of each other were also identified. Because the QTL identified in this study were consistent, DNA markers linked to these QTL could be useful in increasing the frequency of desirable alleles during early generations of breeding. These DNA markers would benefit the development of cultivars that combine high yield and high grain protein content.

Acknowledgments The authors gratefully acknowledge financial support from the Minnesota Annual Conference of the United Methodist Church, the Compton International fellowship, National Research Foundation of South Africa, Department of Science and Technology of South Africa, Agricultural Research Council of South Africa, the United State Department of Agriculture-Agricultural Research Service, and the USDA Cooperative Research, Education and Extension Service, Coordinated Agricultural Project grant number 2006-55606-16629. The authors would like to thank the University of Minnesota wheat breeding scientists, Gary Linkert and Catherine Springer, and the USDA/ARS personnel, Dale Hanson, Dehdra Puhr, and Jadene Wear, for their technical assistance.

References

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet 113:1409–1420

Alexander WL, Smith EL, Dhanasobhan C (1984) A comparison of yield and yield component selection in winter wheat. Euphytica 33:953–961

Anderson JA, Busch RH, McVey DV, Kolmer JA, Linkert GL, Wiersma JV, Dill-Macky R, Wiersma JJ, Hareland GA (2005) Registration of 'Oklee' wheat. Crop Sci 45:784–785

Baril C (1992) Factor regression for interpreting genotype-environment interaction in bread-wheat trials. Theor Appl Genet 83:1022–1026

Berman M, Bason ML, Ellison F, Peden G, Wrigley CW (1996) Image analysis of whole grains to screen for flour-milling yield in wheat breeding. Cereal Chem 73:323–327

Bhatia CR (1975) Criteria for early generation selection in wheat breeding programmes or improving protein productivity. Euphytica 24:789–794

Breseghello F, Sorrells ME (2007) QTL analysis of kernel size and shape in two hexaploid wheat mapping populations. Field Crops Res 101:172–179

Busch RH, Shuey WC, Frohberg RC (1969) Response of hard red spring wheat (*Triticum aestivum* L.) to environments in relation to six quality characteristics. Crop Sci 9:813–881

Busch RH, McVey DV, Linkert GL, Wiersma JV, Warner DO, Wilcoxson RD, Hareland GA, Edwards I, Schmidt H (1996) Registration of 'Verde' wheat. Crop Sci 36:1418

Campbell KG, Bergmem CJ, Gualberto DG, Anderson JA, Giroux MJ, Hareland G, Fulcher RG, Sorrells ME, Finney PL (1999) Quantitative trait loci associated with kernel traits in a soft by hard wheat cross. Crop Sci 39:1184–1195

Dholakia BB, Ammiraju JSS, Singh H, Lagu MD, Röder MS, Rao VS, Dhaliwal HS, Ranjekar PK, Gupta VS, Weber WE (2003) Molecular marker analysis of kernel size and shape in bread wheat. Plant Breed 122:392–395

Federer WT (1961) Augmented designs with one-way elimination of heterogeneity. Biometrics 17:447–473

Fehr WR (1987) Principles of cultivar development. MacMillan, New York

Frohberg RC, Stack RW, Olson T, Miller JD, Mergoum M (2006) Registration of 'Alsen' wheat. Crop Sci 46:2311–2312

Gaines CS, Finney PL, Andrews LC (1997) Influence of kernel size and shriveling on soft wheat milling and baking quality. Cereal Chem 74:700–704

Giura A, Saulescu NN (1996) Chromosomal location of genes controlling grain size in a large grained selection of wheat (*Triticum aestivum* L.). Euphytica 89:77–80

Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor Appl Genet 106:1032–1040

Gupta K, Balyan S, Edwards J, Isaac P, Korzun V, Röder M, Gautier MF, Joudrier P, Schlatter R, Dubcovsky J, De La Pena C, Khairallah M, Penner G, Hayden J, Sharp P, Keller B, Wang C, Hardouin P, Jack P, Leroy P (2002) Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor Appl Genet 105:413–422

Guyomarc'h H, Sourdille P, Charmet G, Edwards KJ, Bernad M (2002) Characterisation of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. Theor Appl Genet 104:1164–1172

Johnson VA, Dreier AF, Grabouski PH (1973) Yield and protein responses to nitrogen fertilizer of two winter wheat varieties differing in inherent protein content of their grain. Agron J 65:259–263

Kamra OP (1971) Genetic modification of seed protein quality in cereals and legumes. Z Pflanzenzüchtg 65:293–306

Kent-Jones DW, Amos AJ (1967) Modern cereal chemistry. Food trade Press Ltd, London

Kiesselbach TA, Sprague HB (1926) Relationship of the development of the wheat spike to environmental factors. J Am Soc Agron 18:40–60

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lee K-M, Shroyer JP, Herrman TJ, Lingenfelser J (2006) Blending hard white wheat to improve grain yield and end-use performances. Crop Sci 46:1124–1129

Liu S, Zhang X, Pumphrey MO, Stack RW, Gill BS, Anderson JA (2006) Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Funct Integr Genomics 6:83–89

Liu S, Chao S, Anderson JA (2008) New DNA markers for high molecular weight glutenin subunits in wheat. Theor Appl Genet 118:177–183

Loffler CM, Busch RH (1982) Selection for grain protein, grain yield, and nitrogen partitioning efficiency in hard red spring wheat. Crop Sci 22:591–595

Mares DJ, Campbell AW (2001) Mapping components of flour and noodle colour in Australian wheat. Aust J Agric Res 52:1297–1309

Marshall DR, Ellison FW, Mares DJ (1984) Effects of grain shape and size on milling yields in wheat. 1. Theoretical-analysis based on simple geo-metric-models. Aust J Agric Res 35:619–630

McCartney CA, Somers DJ, Humphreys DJ, Lukow O (2005) Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL 4452 9 AC 'Domain'. Genome 48:870–883

Nelson JC, Andreescu C, Breseghello F, Finney PL, Gualberto DG, Bergman CJ, Peña RJ, Perretant MR, Leroy P, Qualset CO, Sorrells ME (2006) Quantitative trait locus analysis of wheat quality traits. Euphytica 149:145–159

Payne PI, Holt LM, Lawrence GJ, Law CN (1982) The genetics of gliadin and glutenin, the major storage proteins of the wheat endosperm. Qual Plant Plant Foods Hum Nutr 31:229–241

Pestsova E, Ganal MW, Röder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Prasad M, Varshney RK, Kumar A, Balyan HS, Sharma PC, Edwards KJ, Singh H, Dhaliwal HS, Roy JK, Gupta PK (1999) A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. Theor Appl Genet 99:341–345

Rasyad A, Van Sanford DA (1992) Genetic and maternal variances and covariances of kernel growth traits in winter wheat. Crop Sci 32:1139–1143

Riede CR, Anderson JA (1996) Linkage of RFLP markers to an aluminum tolerance gene in wheat. Crop Sci 36:905–909

Robert N, Hennequet C, Bérard P (2001) Dry matter and nitrogen accumulation in wheat kernel: genetic variation in rate and duration of grain filling. J Genet Breed 55:297–306

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi L, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Integr Genomics 4:12–25

Stuber CW, Johnson VA, Schmidt JW (1962) Grain protein content and its relationship to other plant and seed characters in the parents and progeny of a cross *Triticum aestivum* L. Crop Sci 2:506–508

Sun X-Y, Wu K, Zhao Y, Kong F-M, Han G-Z, Jiang H-M, Huang X-J, Li R-J, Wang H-G, Li S-S (2009) QTL analysis of kernel shape and weight using recombinant inbred lines in wheat. Euphytica 165:615–624

Tsilo TJ, Chao S, Jin Y, Anderson JA (2009) Identification and validation of SSR markers linked to the stem rust resistance gene *Sr6* on the short arm of 2D in wheat. Theor Appl Genet 118:515–524

Tsilo TJ, Ohm JB, Hareland GA, Anderson JA (2010a) Association of size exclusion HPLC of endosperm proteins with dough mixing and bread-making characteristics in a recombinant inbred population of hard red spring wheat. Cereal Chem 87:104–111

Tsilo TJ, Hareland GA, Chao S, Anderson JA (2010b) Genetic mapping and QTL analysis of flour color and milling yield related traits using recombinant inbred lines in hard red spring wheat. Crop Sci (in press)

Varshney RK, Prasad M, Roy JK, Kumar N, Harjit-Singh DhaliwalHS, Balyan HS, Gupta PK (2000) Identification of eight chromosomes and a microsatellite marker on 1AS associated with QTLs for grain weight in bread wheat. Theor Appl Genet 100:1290–1294

Wang S, Basten CJ, Zeng ZB (2005) Windows QTL Cartographer 2.5. North Carolina State University, Raleigh

Wiersma JJ, Busch RH, Fulcher GG, Hareland G (2001) Recurrent selection for kernel weight in spring wheat. Crop Sci 41:999–1005

Yoon BS, Brorsen BW, Lyford CP (2002) Value of increasing kernel uniformity. J Agric Resour Econ 27:481–494

Zanetti S, Winzeler M, Feuillet C, Keller B, Messmer M (2001) Genetic analysis of bread-making quality in wheat and spelt. Plant Breeding 120:13–19