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ORIGINAL PAPER

Impact of the D genome and quantitative trait loci on quantitative traits in a spring durum by spring bread wheat cross

J. R. Kalous[1] · J. M. Martin[1] · J. D. Sherman[1] · H.-Y. Heo[1] · N. K. Blake[1] · S. P. Lanning[1] · J. L. A. Eckhoff[1,2] · S. Chao[3] · E. Akhunov[4] · L. E. Talbert[1]

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Abstract

Key message **The impact of the D genome and QTL in the A and B genomes on agronomic performance of hexaploid wheat and tetraploid durum was determined using novel recombinant inbred line populations derived from interploid crosses.**

Abstract Genetic differences between common hexaploid (6X) bread wheat (*Triticum aestivum*, $2n = 6x = 42$, genome, AABBDD) and tetraploid (4X) durum wheat (*T. turgidum* subsp. durum, $2n = 4x = 28$, genome, AABB) may exist due to effects of the D genome and allelic differences at loci in the A and B genomes. Previous work allowed identification of a 6X by 4X cross combination that resulted in a large number of fertile recombinant progeny at both ploidy levels. In this study, interspecific recombinant inbred line populations at both 4X and 6X ploidy with 88 and 117 individuals, respectively, were developed from a cross between Choteau spring wheat (6X) and Mountrail durum wheat (4X). The presence of the D genome in the 6X population resulted in increased yield, tiller number, kernel weight, and kernel size, as well as a decrease in stem solidness, test weight and seed per spike. Similar results were found with a second RIL population containing 152 lines from 18 additional 6X by 4X crosses. Several QTL for agronomic and quality traits were identified in both the 4X and 6X populations. Although negatively impacted by the lack of the D genome, kernel weight in Mountrail (4X) was higher than Choteau (6X) due to positive alleles from Mountrail on chromosomes 3B and 7A. These and other favorable alleles may be useful for introgression between ploidy levels.

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✉ L. E. Talbert
usslt@montana.edu; ltalbert@montana.edu

[1] Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717, USA

[2] Department of Research Centers, Eastern Agricultural Research Center, Box 393, Sidney, MT 59270, USA

[3] Cereal Crops Research Unit, United States Department of Agriculture-Agricultural Research Service, Fargo, ND 58102, USA

[4] Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506, USA

Introduction

Hexaploid (6X) bread wheat (*Triticum aestivum,* $2n = 6x = 42$, genome AABBDD) is an allopolyploid, containing three unique and complete sets of chromosomes, the A, B, and D genomes. The events that lead to hexaploid wheat's speciation involved two interspecific hybridization events between wild ancestors. Tetraploid *T. turgidum*, or emmer wheat, arose from a cross between *T. urartu* (A genome donor) and a species related to *Aegilops speltoides* (B genome donor). Tetraploid durum wheat (*T. turgidum* subsp. *durum*) is the primary cultivated tetraploid wheat. Hybridization between tetraploid emmer wheat and the D genome donor *Ae. tauschii* (Feuillet et al. 2008; Kilian et al. 2010) gave rise to the hexaploid species *T. aestivum*. *T. aestivum* subsp. *aestivum* is the primary cultivated form of this species.

Polyploidy offers advantages and disadvantages for the success of a species. The presence of multiple genomes offers potential for heterosis to be fixed within an individual. Multiple sets of genes can offer additional opportunities for improving traits of interest and importance. Multiple copies of the same gene allow for the masking

of negative recessive alleles by positive dominant alleles (Comai 2005). However, polyploidization also causes genetic separation between the new polyploid and its ancestors, reducing the amount of available genetic diversity (Haudry et al. 2007). Hybrids between related species with different ploidy levels tend to produce a high frequency of sterile progeny (Lanning et al. 2008) that isolates the common bread wheat gene pool from even a close relative, such as durum wheat.

Even with complications of infertility between bread wheat and its relatives, breeders have successfully introgressed novel alleles for qualitative traits. For example, the tetraploid wheat, Iumillo is one of the sources of stem rust (*Puccinia graminis* f. sp. *tritici*) resistance found in the historically important bread wheat cultivar, Thatcher (Sharma and Gill 1983; Kolmer et al. 1991). Hessian fly [*Mayetiola destructor* (Say)] resistance has also been transferred from tetraploid *T. turgidum* to hexaploid wheat (Sharma and Gill 1983). A gene for high grain protein has been transferred to bread wheat from *T. turgidum* supsp. *dicoccoides* (Mesfin et al. 1999). Breeders have also looked to synthetic wheats (*T. turgidum L. s.lat. X T. tauschii*; $2n = 6x = 42$, AABBDD) as a source for expanding the available wheat gene pool (Mujeeb-Kazi et al. 1996). However, introgression can also fail. Bai and Knott (1992) showed that leaf rust (*P. recondita* f. sp. *tritici*) and stem rust resistance derived from *T. turgidum* subsp. *dicoccoides* were successfully expressed in a *T. turgidum* subsp. *durum* background but suppressed by D genome chromosomes in the *T. aestivum* background.

Despite progress exploiting qualitative genes in an interspecific cross, little success has been reported for quantitative gene movement from a tetraploid to hexaploid background. Direct crosses between cultivated durum wheat and bread wheat have an advantage over synthetic wheats containing the *Ae. tauschii* D genome in that progeny are fixed for major domestication traits, and thus direct evaluation in yield trials are possible. Sterility in interspecific hybrids has been a major obstacle in the development of a sufficient number of progeny for analysis of quantitative traits. Lanning et al. (2008) looked for combinations of 6X/4X crosses that gave fertile progeny by crossing ten spring-type bread wheat varieties with each of three spring-type durum varieties. Hybrids derived from the interspecific crosses were inbred to the F_5 generation. Offspring survival rates were evaluated after every generation of the inbreeding cycle and significant differences in rates were observed between the ten hexaploid parents. Spring wheat Choteau (6X) and the durum wheat Mountrail (4X) were found to produce a sufficient amount of viable seed to develop recombinant inbred line (RIL) populations at both 4X and 6X ploidy levels. Most of the progeny in advanced generations were euploid (Lanning et al. 2008), as was observed in another set of 6X/4X crosses (Martin et al. 2011). The development of 4X and 6X RIL populations from crosses of 4X and 6X parents gives the opportunity to determine the effect of the D genome on quantitative traits in a wheat population, including epistatic interactions. Analysis of the RIL populations also provides the opportunity to identify positive alleles unique to durum wheat which may not be present in hexaploid wheat germplasm. Such alleles would not be detected in traditional QTL analysis based on intraspecific crosses.

Desirable agronomic traits are similar for both bread wheat and durum wheat. Grain yield, determined by yield components including number of spikes per unit area, number of seed per spike, and kernel size, is a primary target for breeders of both crops. The genetic separation of the two species provides the possibility that different favorable alleles may have been selected in each species throughout the 10,000 years of selection for adapted types. In areas of the northern Great Plains, resistance to the wheat stem sawfly (*Cephus cinctus* Norton) is a critical characteristic. The primary means of control is pith-filled, or solid stems, that inhibit larval development controlled largely by a QTL on chromosome 3B (Cook et al. 2004). Desirable end-use properties of bread and durum wheat both require high grain protein; yet strong gluten is also required for making high-quality bread in hexaploid bread wheat.

In this study, we developed RIL populations at both 6X and 4X ploidy levels to investigate the impact of durum alleles in a hexaploid background and bread wheat alleles in a tetraploid background for quantitative traits important to the wheat industry. The impact of the D genome on phenotype and its interaction with important QTL was assessed. These results provide insight into the evolution and domestication of wheat, and may help identify novel QTL for use in wheat improvement.

Methods and materials

Genetic material

RIL derivation, by single seed descent, and ploidy determination is described in Lanning et al. (2008). In short, single seed descent was conducted from F_2 to F_5 generations. The F_5 plants were assayed by polymerase chain reaction (PCR) using primer pairs specific for each of the D genome chromosomes. Amplification for all seven pairs indicated the plant was hexaploid, while lack of amplification for all of the primer sets indicated the plant was tetraploid. Chromosome counts were conducted on a subset of the plants to verify accuracy of the PCR results (Lanning

et al. 2008). Aneuploids were absent from the final population. Seed from each F_5 plant was increased to F_8 by planting and harvesting in bulk to provide seed for field plots. Table 1 shows the number of progeny developed from each of the nineteen 6X/4X crosses. The primary genetic materials were 6X and 4X RIL populations derived from a cross between Choteau (6X) and Mountrail (4X). The Choteau/Mountrail cross was found to have the least amount of attrition through generation advancement (Lanning et al. 2008). However, multiple pollinations were required to raise the progeny numbers to a suitable level for bi-parental quantitative trait loci (QTL) mapping. There was a mixture of hexaploid and tetraploid lines, with 205 individuals in all. Many tetraploid lines were discarded during the seed increase due to a dwarf growth habit which rendered them unsuitable for yield-testing in the field. Subsequent testing showed that the shortness was due to the presence of the Choteau allele for semidwarf habit at *Rht-B1* (McIntosh et al. 2003).

Table 1 The number of hexaploid and tetraploid lines tested per cross

Cross	6X lines	4X lines	Total lines
Choteau/Mountrail	117	88	205
Bread/Durum population			
Choteau/AC Avonlea	13	2	15
Choteau/Monroe	5	9	14
MT9565/AC Avonlea	2	11	13
MT9565/Monroe	3	14	17
MT9565/Mountrail	5	9	14
Bobwhite/Monroe	2	2	4
Chinese Spring/AC Avonlea	4	0	4
Chinese Spring/Monroe	3	1	4
Chinese Spring/Mountrail	8	0	8
Ernest/AC Avonlea	6	7	13
Ernest/Monroe	2	8	10
Hank/AC Avonlea	3	5	8
Hank/Monroe	3	2	5
Hank/Mountrail	1	9	10
Len/AC Avonlea	0	5	5
Len/Monroe	0	2	2
Len/Mountrail	0	5	5
McNeal/Monroe	0	1	1
Totals	60	92	152

Recombinant inbred lines (RILs) from the eighteen additional crosses were combined to form a phenotypic confirmation population referred to as the Bread/Durum population. The combined number of RILs in the Bread/Durum confirmation population totaled 152 lines, with 60 hexaploids and 92 tetraploids. Selection was again performed in the derivation of the tetraploid RIL eliminating 4X lines that were extremely short and poorly adapted to dryland conditions.

Field trial and experimental design analysis

The Choteau/Mountrail population was grown in four environments, Bozeman MT and Sidney MT in 2012 and 2013. Phenotypic data collected for each population are shown in Table 2. Grain trait measurements for the 2013 Sidney MT environment are missing due to a hail storm that destroyed plots shortly before harvest. Stem solidness data were not collected in the Bread/Durum population in 2013 because only the Choteau parent had the major gene for stem solidness (Cook et al. 2004) that produced solid-stemmed

Table 2 Year and location of phenotypic data collected on the Choteau/Mountrail and Bread/Durum populations

	Choteau/Mountrail				Bread/Durum	
	2012		2013		2012	2013
	Bozeman	Sidney	Bozeman	Sidney	Bozeman	Bozeman
Mature stem solidness	X	X	X	X	X	
Heading date	X	X	X	X	X	X
Plant height	X	X	X	X	X	X
Productive tiller number	X		X			
Yield	X	X	X		X	
Test weight	X	X	X			
Grain protein	X	X	X		X	X
Kernel hardness	X	X	X		X	X
Kernel weight	X	X	X		X	X
Kernel diameter	X	X	X		X	X
Sedimentation value	X	X	X			
Seeds per spike	X	X	X	X		

offspring. Thus, the solid stem phenotype was not well represented in the population.

The Choteau/Mountrail population and the Bread/Durum population were grown separately in augmented randomized complete block designs (Wolfinger et al. 1997). The Choteau/Mountrail experiment contained six checks replicated within five blocks in both years. The Bread/Durum population was grown with six checks replicated within four blocks in 2012 and nine checks replicated within four blocks in 2013. The same six checks were grown in both environments. Three additional checks were added in 2013 to represent more of the parental genotypes used during population development.

Phenotyping

Mature stem solidness average measurements were based on the amount of pith present in stem internode cross sections for all available internodes. The cross sections were scored on a 1–5 scale, with a hollow stem receiving a score of one and a solid stem receiving a score of five. Mature stem solidness was measured during the grain fill period prior to senescence with five internodes evaluated per stem and an overall score was calculated as an average of five stems.

Heading date for each entry was recorded as the day after January 1 when 50 % of the spikes within a plot had emerged from the flag leaf sheath. Plant height was evaluated by measuring the distance, in centimeters, from the soil surface to the average height of two or three main tillers, excluding the awns. Two measurements per plot were taken at random and averaged together for a final plant height for each plot.

Productive tiller number (PTN) was calculated based on the number of tillers with fertile spikes within a 30 cm span. Test weight was measured using a Fairbanks grain weight scale. Grain protein content analysis was performed on whole grain using a Foss Infratec 1241 Grain Analyzer (Foss Analytical AB) in the Montana State University Cereal Quality Lab, Bozeman, MT. Kernel hardness, kernel weight, and kernel diameter were analyzed using the Single Kernel Characterization System 4100 (Perten Instruments). Sedimentation values, which are an estimate of gluten strength where higher numbers indicate greater strength, were measured using a modified protocol described in Pinckney et al. (1957). Seeds per spike were calculated as an average of five heads randomly pulled from each plot, hand threshed, and hand counted.

Statistical analysis

Statistical analyses of agronomic and end-use quality data were conducted using SAS, v9.3 (SAS Institute Inc. 2010). PROC MIXED was used to calculate BLUP estimates for both the Choteau/Mountrail and Bread/Durum populations. PROC GLM was used to calculate least significant differences among the checks in the Choteau/Mountrail population. The hexaploid RIL mean was compared to the tetraploid mean using a *t* test for the Choteau/Mountrail and Bread/Durum populations.

Genetic mapping and QTL analysis

The Choteau/Mountrail population was genotyped with the iSelect 90K wheat SNP (single nucleotide polymorphism) array (Wang et al. 2014). The SNP markers were manually scored in the software GenomeStudio (Illumina). Fourteen polymorphic simple sequence repeat (SSR) or microsatellite markers were also placed on the A and B genome chromosomes. All microsatellite markers were assayed using the LI-COR DNA analysis system (LI-COR Biosciences). Markers for *RHT-B1* and *VRN-B1* (McIntosh et al. 2003) were screened using agarose gel electrophoresis as described by Blake et al. (2009).

Linkage mapping was carried out in the statistical software R (R Development Core Team 2014) using the R package R/qtl (Broman et al. 2003). The unique marker genotypes scored on the 6X and 4X lines were combined for map construction. Markers with more than 20 % missing data were dropped from the linkage analysis. Markers with segregation ratios significantly different from expected Mendelian segregation ratios were identified, and ultimately left in the final linkage map. The function findDupMarkers was used to identify co-segregating markers. The marker with the least amount of missing data was kept for linkage mapping and all other co-segregating markers were removed. Linkage groups were formed using the function formLinkageGroups with a maximum recombination frequency set at 0.2 and a minimum logarithm of the odds (LOD) set at five. The orderMarkers function was used to provide an initial marker order on each linkage group. Each linkage group was then visually assessed using the plotRF function. The ripple function was used to determine alternate orders of markers and the compareorder function was used for determining the most appropriate marker order, based on $\log_{10}$ likelihood, comparing the original marker order to an alternate marker order. PlotRF was used to visually assess the final marker order of each linkage group. Linkage groups were identified by chromosome based on the fourteen previously mapped SSR markers as well as previous wheat mapping data (Cavanagh et al. 2013; Wang et al. 2014). Recombination distances were determined based on the Kosambi mapping function (Kosambi 1944). The final step in map construction involved using the calc.errorlod function to identify potential genotyping errors due to double crossovers. Genotype data points with error LOD scores above four were replaced with missing data.

One- and two-dimensional interval mapping was implemented with Haley–Knott regression (Haley and Knott 1992) in R/qtl (Broman et al. 2003). A two-dimensional two-QTL

Table 3 Yield and yield component summary of the Choteau/Mountrail RIL population

	Parents			RIL population				
	Mean		LSD	6X ($n = 117$)		4X ($n = 88$)		6X vs. 4X
	Choteau	Mountrail		Mean	Range	Mean	Range	*T* test
Yield (kg ha^{-1})	3575	3379	388	2804	2165–3475	2399	1775–3017	**
PTN (Spike m^{-1})	142.7	124.0	23.6	135.9	91.7–188.3	124.1	81.7–168.3	**
Test weight (kg m^{-3})	748.9	757.1	10.0	724.5	658.8–747.6	734.1	706.6–759.3	**
Kernel weight (mg)	30.0	36.0	1.2*	32.9	29.1–38.4	30.4	26.8–34. 2	**
Kernel diameter (mm)	2.73	2.88	0.05*	2.79	2.62–3.00	2.70	2.53–2.87	**
Seeds per spike	34.9	38.0	2.3*	31.8	27.0–36.2	33.8	25.2–43.3	**

PTN Productive tiller number

*, ** Significant at $P < 0.05$ and 0.01, respectively

Table 4 Yield and yield component trait summary of the Bread/Durum population

	Parents			RIL population				
	Mean		*T* test	6X ($n = 61$)		4X ($n = 91$)		6X vs. 4X
	6X ($n = 6$)	4X ($n = 3$)		Mean	Range	Mean	Range	*T* test
Yield (kg ha^{-1})	3389	3518	NS	2436	1521–3410	2112	1489–2968	**
Kernel weight (mg)	30.2	39.5	**	32.2	28.2–35.6	32.9	29.1–38.6	*
Kernel diameter (mm)	2.71	2.95	*	2.76	2.62–2.94	2.78	2.65–2.99	*

NS not significant

*, ** Significant at $P < 0.05$ and 0.01, respectively

scan was conducted to identify all relevant QTL as well as important interacting QTL, specifically QTL acting in repulsion (Sen and Churchill 2001). Once a QTL model was established for the trait in question, QTL positions were refined based on the QTL present in the model. QTL significance was determined by a permutation test with 1000 replicates specific to the two-dimensional two-QTL scan to establish appropriate LOD cutoffs. The LOD cutoffs corresponded to a $P < 0.05$. An additional identifier differentiating 6X and 4X RILs was included in the search for QTL by including the ploidy identifier as a covariate during the QTL mapping analysis. The QTL models were visually investigated with the functions effectplot and plotPXG. The effectplot function shows the phenotypic means for each genotype of a specific marker in question. The function plotPXG displayed phenotypic means for the haplotype groups of each QTL model.

Results

Phenotypic summary

Table 3 summarizes the mean and range for yield and yield-related traits in the Choteau/Mountrail population. There were no significant differences found between the parents Choteau and Mountrail for yield, PTN, and test weight based on a *t* test. However, tetraploid Mountrail had greater kernel weight and greater kernel diameter. A *t* test showed significant differences between the 6X and 4X RIL means for yield, PTN, test weight, kernel weight, kernel diameter, and seeds per spike. The hexaploids yielded more, had more tillers, greater kernel weight, and greater kernel diameter. The tetraploids had greater test weight and more seeds per spike.

Table 4 summarizes the means and ranges for yield and yield component traits in the Bread/Durum population. A *t* test conducted on the parental 6X and 4X lines showed that the 4X parents, on average, had significantly greater kernel weight ($P < 0.01$) and greater kernel diameter ($P < 0.05$), but were not significantly different for yield. The 6X and 4X RILs differed significantly for yield, test weight, and kernel diameter. The 4X RIL had greater kernel weight by 0.7 mg and greater kernel diameter by 0.02 mm, but the 6X RIL were higher yielding by 324 kg ha^{-1}. The difference between kernel weight and kernel diameter between the two RIL ploidy groups was much less than that observed for the 4X and 6X parents.

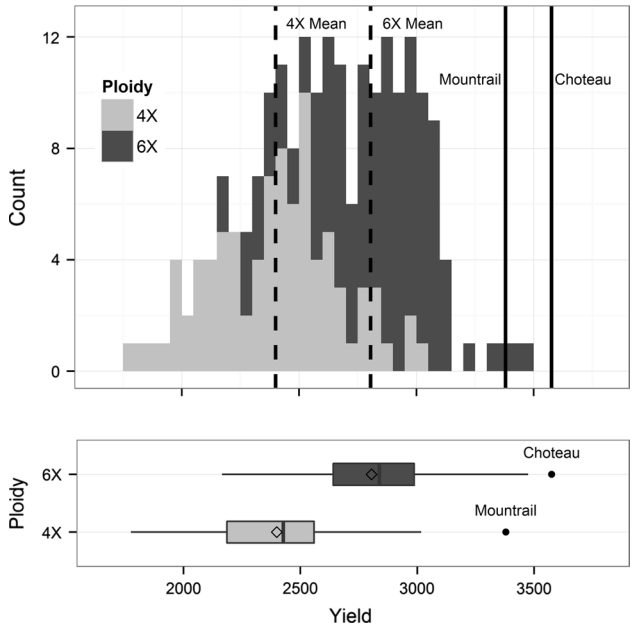

Fig. 1 Histogram and boxplot showing the distribution of yield for the Choteau/Mountrail 6X and 4X RIL relative to the mean yield of Choteau (6X) and Mountrail (4X)

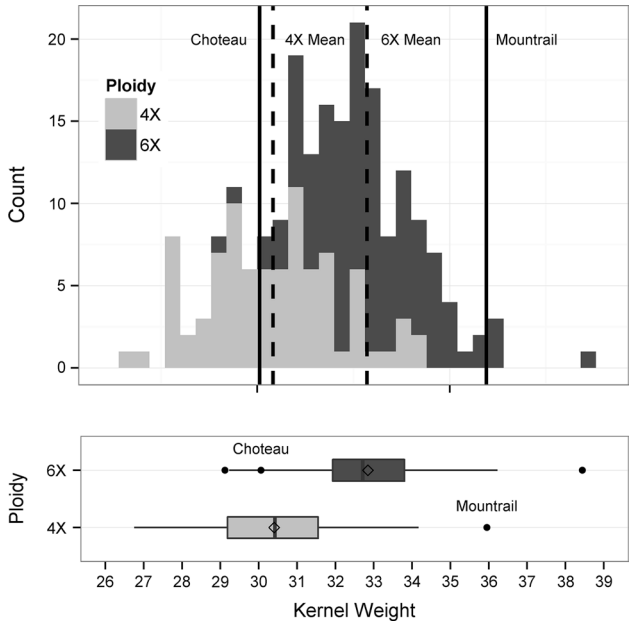

Fig. 2 Histogram and boxplot showing the distribution of kernel weight for the Choteau/Mountrail 6X and 4X RIL relative to the mean yield of Choteau (6X) and Mountrail (4X)

Comparison of RIL means with parental means (Tables 3, 4; Fig. 1) shows that the RIL were consistently lower yielding than the parents. For instance, yield of Choteau was 3575 vs. 2804 kg ha^{-1} for the 6X RIL mean (Table 3). Yield of Mountrail was 3379 vs. 2399 kg ha^{-1} for the 4X RIL. A similar trend was observed in the Bread/Durum population. The 6X RIL showed improvement in kernel weight over that of the 6X parents in both the Choteau/Mountrail and Bread/Durum populations (Tables 3, 4). Figure 2 depicts the distribution of kernel weight for RIL and parents in the Choteau/Mountrail population. Kernel weight of Choteau was 30.0 mg compared to a mean of 33.0 mg in the 6X RIL. The yield components impacted negatively in the RIL were test weight and number of seeds per spike (Table 3), where the RIL population means were both lower than the parents.

Table 5 shows the performance of the parents, Choteau and Mountrail, and the 6X and 4X RIL for several agronomic and end-use quality traits. The parents were significantly different for mature stem solidness, heading date, plant height, kernel hardness, and sedimentation value. At maturity, stem solidness was greater in Choteau. Choteau also had a greater sedimentation value. Mountrail was later heading, taller in stature, and had harder kernels. No significant differences were observed for grain protein content. The 6X and 4X RIL showed significant differences for all agronomic traits except grain protein. The 4X RIL population was more solid at maturity, even though the 6X parent was more solid than the 4X parent. The 4X RIL also had harder kernels. The 6X RIL population was earlier heading, taller and had greater sedimentation values.

The Bread/Durum population (Table 6) showed significant differences between 4X and 6X RIL with respect to heading date, plant height, and kernel hardness. Though non-significant the 4X RIL were again more solid than the 6X RIL. The 4X lines were also later heading, shorter, and had harder kernels.

Linkage map and QTL summary

The Choteau/Mountrail population was genotyped with SNP and SSR markers. Fourteen linkage groups were constructed and used for subsequent QTL analyses. Chromosome identification was determined based on previously mapped SSR markers. A total of 81,587 SNP markers were screened on the Choteau/Mountrail population. Of this total, 11,568 markers were found to be polymorphic among the 6X lines and 11,102 markers were polymorphic among the 4X lines. The 6X lines had 8882 co-segregating markers leaving 2686 unique marker genotypes. The 4X lines had 9192 co-segregating markers with 1910 unique marker genotypes. Initially, a map was constructed with 2269 unique SNP and SSR markers with a total map size of 3183.2 cM and an average inter-marker spacing of 1.4 cM. To reduce computation time required for later QTL analyses markers were removed that were less than 1 cM apart. The resulting final map included 995 genetic markers with an overall map length of 3178.7 cM. Average inter-marker spacing was 3.2 cM. The linkage map is summarized in Table 7. Linkage map construction indicates that the A and

Table 5 Phenotypic summary of agronomic traits in the Choteau/Mountrail RIL population

	Parents			RIL population				
	Mean		LSD	6X ($n = 117$)		4X ($n = 88$)		6X vs. 4X
	Choteau	Mountrail		Mean	Range	Mean	Range	*T* test
Mature Stem Solidness[a]	4.5	2.1	0.3*	2.7	1.5–4.4	3.5	1.7–4.7	**
Heading date	183.2	186.8	0.4*	185.5	182.6–188.2	186.1	183.2–188.9	**
Plant height (cm)	74.6	84.6	2.9*	83.8	68.6–97.1	67.5	51.4–83.4	**
Grain protein (%)	15.6	15.3	0.4	16.6	14.9–18.7	16.7	15.4–18.3	NS
Kernel hardness (skcs)	72.4	85.7	2.5*	69.6	57.6–84.8	89.8	79.5–97.4	**
Sedimentation value	3.7	2.2	0.2*	3.5	2.2–5.0	2.1	1.6–2.8	**

NS not significant

*, ** Significant at $P < 0.05$ and 0.01, respectively

[a] Mature stem solidness is measured on a 1–5 scale with 1 = hollow and 5 = solid, heading date is days from January 1

Table 6 Phenotypic summary of agronomic traits in the Bread/Durum population

	Parents			RIL population				
	Mean		*T* test	6X ($n = 61$)		4X ($n = 91$)		6X vs. 4X
	6X ($n = 6$)	4X ($n = 3$)		Mean	Range	Mean	Range	*T* test
Mature Stem Solidness[a]	3.2	1.9	NS	2.2	1.4–4.1	2.3	1.4–4.1	NS
Heading date	186.3	188.9	NS	189.4	184.4–195.3	190.5	184.8–196.9	**
Plant height (cm)	70.9	78.3	NS	73.6	58.8–89.9	69.0	41.4–87.3	**
Grain protein (%)	15.7	16.0	NS	16.8	15.6–19.2	17.0	15.2–18.9	NS
Kernel hardness (skcs)	79.6	87.8	NS	67.7	36.1–92.7	93.4	81.7–105.7	**

NS not significant

** Significant at $P < 0.01$

[a] Mature stem solidness is measured on a 1–5 scale with 1 = hollow and 5 = solid, heading date is days from January 1

Table 7 Summary of the Choteau/Mountrail genetic map

Choteau/Mountrail linkage map			
Chromosome	# of markers	Chromosome length	Average marker spacing
1A	58	202.2	3.5
1B	81	200.5	2.5
2A	73	273.8	3.8
2B	69	170.4	2.5
3A	73	275.1	3.8
3B	90	233.8	2.6
4A	69	209.0	3.1
4B	56	209.7	3.8
5A	71	222.2	3.2
5B	82	250.3	3.1
6A	46	161.2	3.6
6B	73	211.1	2.9
7A	82	357.3	4.4
7B	72	202.1	2.8
Total	995	3178.7	3.2

B genomes of the Choteau/Mountrail RIL were comprised of a mixture of Choteau and Mountrail alleles. The complete Choteau D genome was inherited intact in the hexaploid RILs and all D genome chromosomes were missing in the tetraploid RILs. Thus, the D genome was inherited as single genetic entity.

We identified QTL models responsible for explaining a portion of the variation for all twelve phenotypic traits evaluated. Table 8 lists the QTL that had a significant impact on yield and traits related to yield. The marker name, chromosome, and position closest to the identified QTL are reported, as well as associated LOD scores, R^2 values, and phenotypic means for the Choteau and Mountrail allele, at each QTL, in the 6X and 4X backgrounds.

Three QTL were identified controlling a portion of the variance associated with yield, on chromosomes 1A, 2B, and 7A. A strong interaction was also observed between the 2B and 7A QTL. The D genome increased yield and had the largest impact on yield as indicated by the high R^2 value for the effect of ploidy. The Choteau allele gave higher yields for QYld.mst-1A and QYld.mst-7A, with similar effects in

Table 8 Summary of the QTL identified for yield and yield-related traits in a Choteau/Mountrail RIL population

	Source	Marker	Chromosome [position (cM)]	LOD[a]	R^2 (%)[b]	Choteau allele mean (standard error)[c]		Mountrail allele mean (standard error)[c]	
						4X	6X	4X	6X
Yield	QYld.mst-1A	IWA3254	1A (52.4)	5.0	6.1	2479 (41.03)	2867 (32.96)	2335 (36.99)	2734 (34.98)
	QYld.mst-2B	IWB29332	2B (152.3)	6.1	7.7	2341 (43.72)	2783 (36.99)	2442 (36.99)	2821 (32.96)
	QYld.mst-7A	IWB34840	7A (340.7)	5.6	6.9	2409 (37.67)	2806 (39.01)	2386 (43.05)	2803 (32.28)
	Ploidy			22.6	34.2				
	QYld.mst-2B: QYld.mst-7A			5.4	6.7				
PTN	QTn.mst-5B	IWB69502	5B (170.0)	3.3	6.4	119.8 (2.70)	130.3 (2.37)	128.2 (2.67)	141.1 (2.30)
	Ploidy			4.5	9.0				
TW	QTw.mst-7B	IWB39660	7B (144.8)	3.1	5.8	737.6 (1.67)	727.4 (1.54)	730.1 (1.80)	722.0 (1.42)
	Ploidy			6.3	12.2				
KW	QGw.mst-3B	IWA6375	3B (126.8)	4.9	6.1	30.0 (0.25)	32.4 (0.20)	30.7 (0.22)	33.3 (0.20)
	QGw.mst-7A	IWA3562	7A (284.2)	5.2	6.5	30.0 (0.24)	32.4 (0.20)	30.8 (0.23)	33.3 (0.21)
	Ploidy			25.0	39.8				
KD	QSd.mst-4B	IWB72203	4B (36.6)	7.0	9.6	2.66 (0.02)	2.76 (0.01)	2.71 (0.01)	2.82 (0.01)
	QSd.mst-5A	IWB9138	5A (61.5)	5.0	6.8	2.70 (0.01)	2.80 (0.01)	2.68 (0.01)	2.77 (0.01)
	Ploidy			19.1	30.4				
Sd/Sp	QKps.mst-2A	IWB72154	2A (142.5)	4.6	8.8	32.3 (0.42)	31.4 (0.37)	35.3 (0.41)	32.3 (0.36)
	Ploidy			5.4	10.3				

PTN Productive tiller number, *TW* test weight, *KW* kernel weight, *KD* seed diameter and *Sd/Sp* seeds per spike

[a] *LOD* log10 likelihood ratio, [b] R^2 refers to the percent of phenotypic variance explained, [c] units for means are yield (kg ha^{-1}), PTN (spikes m^{-1}), TW (kg m^{-3}), KW (mg), KD (mm)

the 6X and 4X backgrounds. The Mountrail allele at QYld.mst-2B increased yield and the effect was greater in the 4X RIL. One QTL was identified on chromosome 5B associated with PTN (QTn.mst-5B). Mountrail contributed the allele for increasing the number of productive tillers. The magnitude of Mountrail's allelic effect was similar in both ploidy backgrounds. The D genome presence increased the number of productive tillers as well. A QTL linked to test weight (QTw.mst-7B) was identified on chromosome 7B with the Choteau parent contributing the positive allele. The presence of the D genome was associated with decreased test weight. Two QTL were associated with kernel weight (QGw.mst-3B, QGw.mst-7A) on chromosome 3B and 7A. For both QTL, Mountrail contributed the positive alleles increasing kernel weight. The D genome in the 6X RIL also increased kernel weight. Figure 3 shows the kernel weight distribution of haplotypes for ploidy, QGw.mst-3B, and QGw.mst-7A. The lowest kernel weight was observed in lines missing the D genome and containing the hexaploid QTL alleles, while the greatest kernel weight was observed in lines containing the D genome and possessing the tetraploid QTL alleles (Fig. 3). Two QTL on chromosomes 4B and 5A were identified linked to kernel diameter (QSd.mst-4B, QSd.mst-5A) (Table 8). The Choteau allele at QSd.mst-5A increased kernel diameter and the Mountrail allele increased kernel diameter at Qsd.mst-4B. Ploidy also impacted kernel diameter with the presence of the D genome giving greater kernel diameter. Mountrail contributed an allele for a QTL on chromosome 2A that increased the number of seeds per spike (QKps.mst-5A). This effect was greater in the 4X RIL. The D genome negatively affected seeds per spike leading to greater means overall in the 4X RIL. There was no interaction between the QTL alleles and the presence of the D genome (ploidy) for any of the traits presented in Table 8.

Results summarized in Table 9 show the impact of alleles from Choteau and Mountrail on several agronomic traits. A QTL on chromosome 3B impacted mature stem solidness (QSs.mst-3B) (Table 9). The Choteau allele increased stem solidness (Table 9). The absence of the D genome caused stem solidness to be greater in the 4X RIL. QTL on chromosomes 3A, 5A, and 7B were identified as controlling some of the variation observed in heading date. Mountrail alleles at both QHd.mst-5A and QHd.mst-7B caused later heading. The Choteau allele for QHd.mst-3A caused slightly later heading in both 6X and 4X RIL. The D genome had a large effect on plant height as did the QTL on chromosome 4B (QHt.mst-4B). Hexaploid individuals were almost 20 cm taller than tetraploid individuals. The Mountrail allele for QHt.mst-4B increased plant height by almost 10 cm in both the 6X and 4X backgrounds.

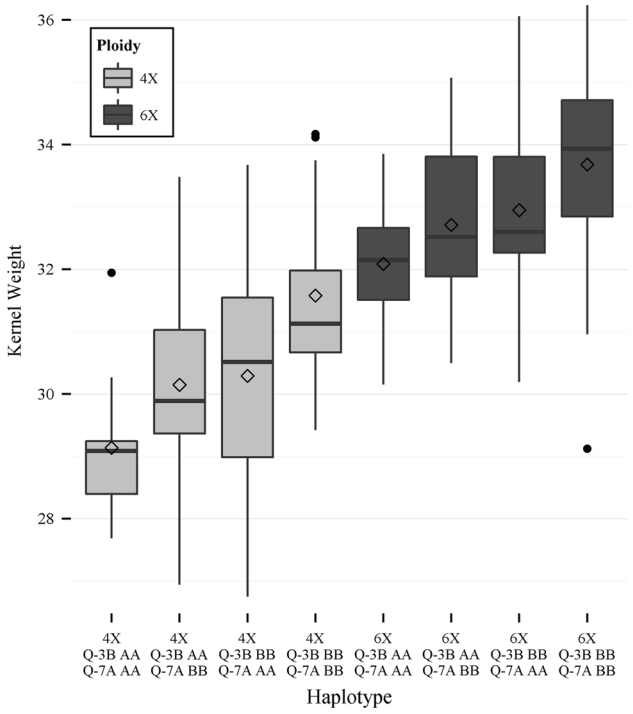

Fig. 3 Boxplots of haplotypes for the QTL, QGw.mst-3B and QGw.mst-7A controlling kernel weight in the 6X and 4X Choteau/Mountrail RIL. *X*-axis boxplot labels indicate the ploidy and either the Choteau allele (AA) or Mountrail allele (BB) for QGw.mst-3B and QGw.mst-7A, abbreviated Q-3B and Q-7A, respectively

Significant segregation distortion was observed on 4B spanning roughly 15 cM (data not shown). Mountrail contributed alleles for two QTL that raised the percent grain protein content on chromosome 4A (QGpc.mst-4A) and 4B (QGpc.mst-4B). The D genome was the major factor in determining kernel hardness with 4X RIL showing much harder kernels. However, a QTL on 4A (QHa.mst-4A) was observed with Choteau contributing the allele for increased kernel hardness. Four QTL were identified as controlling a large portion of variation in sedimentation value. Choteau alleles at QSev.mst-1A, QSev.mst-3A, and QSev.mst-4B on chromosomes 1A, 3A, and 4B, respectively, resulted in a higher sedimentation value. The Mountrail allele increased the sedimentation value at QSev.mst-1B on chromosome 1B. The D genome had the greatest impact increasing sedimentation values. QSev.mst-1A and QSev.mst-1B are located closely to *Glu-A1* and *Glu-B1* genes, controlling glutenin proteins reported in the GrainGenes database (http://www.wheat.pw.usda.gov/GG2/index.shtml). QSev.mst-4B, on 4B, was located near the *Rht-B1* locus, which controls plant height.

Discussion

Durum and bread wheat differ by the presence of the seven chromosome pairs of the D genome in bread wheat. The

Table 9 Summary of the QTL identified for agronomic traits in a Choteau/Mountrail RIL population

	Source	Marker	Chromosome (position)	LOD[a]	R^2 (%)[b]	Choteau allele mean (standard error)[c]		Mountrail allele mean (standard error)[c]	
						4X	6X	4X	6X
Mature stem solidness	QSs.mst-3B	IWB58481	3B (230.9)	81.7	71.8	4.3 (0.06)	3.7 (0.05)	2.7 (0.05)	1.9 (0.05)
	Ploidy			28.0	12.0				
Heading date	QHd.mst-3A	IWA4296	3A (167.7)	4.8	6.9	186.5 (0.19)	185.8 (0.16)	185.8 (0.16)	185.3 (0.15)
	QHd.mst-5A	VrnA	5A (142.8)	11.6	18.1	185.8 (0.17)	184.9 (0.15)	186.4 (0.16)	186.1 (0.14)
	QHd.mst-7B	IWB6455	7B (17.5)	11.6	18.0	185.5 (0.32)	185.0 (0.15)	186.2 (0.13)	186.0 (0.15)
Plant height	QHt.mst-4B	Rht-B1	4B (44.5)	21.1	15.7	59.0 (1.55)	78.3 (0.76)	68.9 (0.61)	87.8 (0.65)
	Ploidy			59.4	72.3				
Grain protein	QGpc.mst-4A	IWB20212	4A (67.5)	3.6	7.0	16.6 (0.10)	16.4 (0.08)	16.8 (0.10)	16.9 (0.09)
	QGpc.mst-4B	IWB51614	4B (51.1)	4.5	8.9	16.4 (0.17)	16.4 (0.09)	16.8 (0.08)	16.8 (0.08)
Kernel Hardness	QHa.mst-4A	IWB6369	4A (89.2)	3.6	1.1	90.8 (0.54)	70.9 (0.53)	88.6 (0.61)	68.6 (0.47)
	Ploidy			90.4	82.9				
Sedimentation value	QSev.mst-1A	IWB44038	1A (94.5)	4.6	1.6	2.2 (0.07)	3.6 (0.05)	2.1 (0.05)	3.4 (0.05)
	QSev.mst-1B	IWB47979	1B (22.1)	12.5	4.7	2.0 (0.05)	3.3 (0.05)	2.2 (0.05)	3.7 (0.04)
	QSev.mst-3A	IWB51852	3A (238.2)	3.6	1.2	2.1 (0.06)	3.6 (0.05)	2.1 (0.06)	3.4 (0.05)
	QSev.mst-4B	IWB73001	4B (33.2)	4.7	1.6	2.2 (0.08)	3.7 (0.05)	2.1 (0.05)	3.4 (0.05)
	Ploidy			74.8	62.9				

[a] LOD = log10 likelihood ratio, R^2 = percent phenotypic variance explained, [b] R^2 refers to the percent of phenotypic variance explained, [c] mature stem solidness was scored on a 1–5 scale, with 1 = hollow, and 5 = solid, heading date is days from January 1, plant height (cm), grain protein is a percent, and kernel hardness is SKCS units

crops also differ for several phenotypic traits. In particular, durum wheat tends to have larger kernels (Lacerenza et al. 2008; Trethowan et al. 2001), and has increased resistance to the wheat stem sawfly relative to bread wheat (Platt and Farstad 1946). Durum wheat also tends to have harder kernels due to the absence of the hardness (*Ha*) locus on chromosome 5D (Law et al. 1978). Bread wheat typically has stronger gluten, primarily due to the presence of the Dx5 and Dy10 glutenin alleles on chromosome 1D (Branlard and Dardevet 1985; Payne 1987; Blechl et al. 2007). Alleles on the D genome chromosomes interact with alleles on the A and B genome chromosomes to determine performance of hexaploid bread wheat. The Choteau/Mountrail cross which resulted in a high number of 4X and 6X progeny lines, allowed development of RIL that differed for the presence of the D genome, but contained a mixture of 4X and 6X alleles in the A and B genomes. These RIL provide the opportunity to identify favorable QTL for transfer between ploidy levels, and to evaluate the impact of the D genome on several agronomic and quality traits.

One observation from this study is an overall lack of vigor in both populations of 6X and 4X RIL compared to parental means as measured by grain yield (Fig. 1). This may be an indication of the presence of positive gene clusters acting together within the bread and durum wheat gene pools. The interspecific cross results in a breakdown of the positive epistatic interactions. Similar hypotheses have been invoked to explain decreased vigor in other species, whereby allelic combinations that co-evolve to achieve greater fitness are separated upon wide crossing (Edmands 1999). Levy and Feldman (2002) and Udall and Wendel (2006) reviewed polyploidy literature and suggested that polyploidization may be followed by alterations at the gene expression level (genetic diploidization) where homeologs take on novel roles, through sub- or neo-functionalization. This may also explain why there was stronger negative impact of recombining the 4X and 6X genes observed in the 4X RIL. Compensation by the intact D genome resulted in a smaller negative effect for the 6X RIL.

A full map with 2269 markers was initially developed from the SNP genotypic data. However, once we experienced the massive amount of computational time associated with numerous markers the decision was made to reduce marker density while maintaining good marker coverage across the wheat genome. The reduced map, containing 995 markers was used in the final QTL analyses, drastically reducing the necessary computational time. Overall map length decreased by roughly 5 cM, from 3183.2 to 3178.7 cM. Russo et al. (2014) reported a similar map size for a durum by *T. dicoccum* population (2879.3 cM). Wang et al. (2014) noted an increase in genetic map distances developed from high-density genotyping data over that of low-density genotyping data by roughly 30 %. The authors reasoned that the increased number of markers caused a increase in the number of minor genotyping errors resulting in greater overall map lengths.

The D genome was inherited completely, from the 6X parent Choteau, in the 6X RIL which kept us from identifying specific QTL on the D genome. However, by including an arbitrary marker denoting 4X and 6X RIL we were still able to estimate important D genome impacts on the A and B genomes as well as the individual phenotypes. The D genome had a major impact on most yield and yield-related traits measured in the RIL populations. In many cases, these differences were reflected in means for the 4X and 6X parents. For instance, the presence of the D genome resulted in greater tiller number in the 6X RIL compared to the 4X RIL. Choteau (6X) had greater tiller number than Mountrail (4X). Seeds per spike was greater in the tetraploid parent Mountrail, and also greater in the 4X RIL population than the 6X RIL population. However, in some cases the relationship between the parental means was opposite to that of the RIL means for the same ploidy level. Kernel weight is an example of this relationship. A positive correlation between ploidy and kernel weight has been observed in *T. aestivum*, *Plantago media*, and *Dactylis glomerata* (Halloran and Pennell 1982; Van Dijk and Van Delden 1990; Bretagnolle et al. 1995). However, in the present study, Mountrail and the other durum wheat parents had greater kernel weight than the hexaploid parents, as has been seen in other studies (Lacerenza et al. 2008; Trethowan et al. 2001). This suggests that either the impact of the D genome on kernel weight was negative, or that there were alleles with strong positive effects in the 4X parents. In fact, the D genome had a positive effect on kernel weight in the Choteau/Mountrail 6X RIL even though the hexaploid parent had lower kernel weight. The QTL analysis shows that this can be explained by alleles from durum wheat on chromosomes 3B and 7A that resulted in greater kernel weight.

Kernel diameter was highly correlated with kernel weight in the Choteau/Mountrail population ($r^2 = 0.89$, P value <0.01; data not shown). Other studies have shown similar amounts of correlation between kernel diameter and kernel weight (Breseghello and Sorrells 2007; Dholakia et al. 2003). However, we identified different QTL for each of these traits. Kernel weight QTL were on chromosomes 3B and 7A with positive alleles from durum wheat in both cases. Li et al. (2007) previously reported kernel weight QTL identified in a winter wheat RIL population, on chromosome 3B. Tsilo et al. (2010) identified kernel weight QTL on chromosome 7A in a hard red spring wheat RIL population. Campbell et al. (1999) also identified kernel weight QTL on chromosome 3B but in a soft by hard white wheat RIL population. Kernel diameter QTL were on 4B and 5A. QTL controlling kernel width have previously

been identified on chromosomes 4B and 5A (Campbell et al. 1999; Ramya et al. 2010; Tsilo et al. 2010). In our experiment, a positive allele from durum wheat on 4B co-segregated with the allele for plant height at *Rht-B1*, while spring wheat contributed the positive allele for the 5A QTL. The different QTL identified for these traits are somewhat artifactual, in that the QTL controlling kernel diameter had LOD scores approaching significance for kernel weight (data not shown), which makes sense in that kernel weight is a factor of kernel diameter. Conversely, the QTL controlling kernel weight were not observed as controlling kernel diameter possibly because kernel weight is a consequence of kernel density resulting from overall kernel diameter as well as kernel length.

Stem solidness is an important trait for areas of the northern Great Plains of North America because a solid stem imparts resistance to the wheat stem sawfly (Kemp 1934; Talbert et al. 2014). Choteau is a widely grown variety offering sawfly tolerance via solid stem (Lanning et al. 2004). A major QTL for solid stems is on chromosome 3B (Cook et al. 2004). The Choteau allele for this QTL resulted in very solid-stemmed RIL in our populations regardless of ploidy. The results of this study indicate that there may be alleles reducing solid stems located on the D genome. This conclusion is supported by the fact that the 4X RIL had a significantly higher solid stem mean than the 6X RIL, even though the main source of stem solidness originates from the 3B QTL present in Choteau. This result may help explain the increased resistance to the wheat stem sawfly typically observed in durum wheat (Platt and Farstad 1946).

QHt.mst-4B and QHd.mst-5A were associated with plant height and heading date, respectively. This result was expected since the markers associated with these QTL are actually known to tag the causal gene mutation. QHt.mst-4B is linked to *RHT-B1* as verified by screening the population with the perfect markers for alleles RHT-B1a and RHT-B1b (McIntosh et al. 2003). QHd.mst-5A is linked to the *Vrn-A1* locus controlling a major vernalization gene (McIntosh et al. 2003).

Kernel hardness is an important trait for determining end-use quality in bread and durum wheat. Durum wheat typically has harder kernels than bread wheat due to the absence of the puroindolines encoded by the Hardness (*Ha*) gene on chromosome 5D (Giroux and Morris 1998). The impact of the D genome was seen in both the Choteau/Mountrail and Bread/Durum populations, as 4X lines had significantly harder kernels. A QTL identified on chromosome 4A (QHa.mst-4A) appears to also impact kernel hardness with the positive allele originating in Choteau. The Choteau allelic effect was similar in both 4X and 6X backgrounds. Groos et al. (2004) also identified QTL on chromosome 4A impacting kernel hardness in a hard bread wheat RIL population. The D genome also contains favorable alleles for gluten strength on chromosome 1D (Branlard and Dardevet 1985), which was reflected in higher sedimentation values for 6X lines in the Choteau/Mountrail population. The positive D genome effect was reflected in the QTL results with an associated R^2 value of 62.9 (Table 9). Of the four QTL identified as impacting sedimentation value, QSev.mst-1B on chromosome 1B was the only QTL where Mountrail contributed the positive allele.

Conclusion

Identification of interspecific fertility between Choteau and Mountrail made the development of an interspecific RIL population possible. This RIL population composed of 4X and 6X RIL allowed for testing the effects of alleles from durum wheat in a bread wheat background, and vice versa. This population also allowed for testing the impact that the D genome has on the A and B genome in a yield trial setting. The 6X and 4X RIL had low grain yield relative to the parents, perhaps due to the breakup of positive epistatic gene interactions that co-evolved independently at each ploidy. The 6X RIL yielded more than the 4X RIL, likely due in large part to the presence of an intact D genome. Yield was positively impacted by the D genome, as was PTN, kernel weight, kernel diameter, and sedimentation value. Although recombination of 4X and 6X alleles was negative overall as indicated by poorer performance of the recombinant RIL, several positive alleles were identified. Specifically, alleles from Mountrail increasing kernel weight were identified on chromosomes 3B and 7A that could be incorporated into bread wheat germplasm. An allele from Choteau increasing test weight, identified on chromosome 7A could be incorporated into durum germplasm. These alleles may have potential to improve modern bread and durum wheat cultivars.

Author contribution statement J.R.K.—responsible for collecting and analyzing the data, prepared the manuscript. J.M.M.—provided expertise and assistance with the statistical analysis, helped edit the manuscript. J.D.S—provided expertise and assistance with linkage mapping and QTL analysis, helped edit the manuscript. H.-Y.H.—helped with collection of the phenotypic data. N.K.B.—assisted with the collection of the genotypic and phenotypic data, assisted in the population development, helped edit the manuscript. S.P.L—assisted with the collection of phenotypic data, assisted in the population development, helped edit the manuscript. J.L.A.E.—managed the field trial in Sidney, MT and collected phenotypic data. L.E.T.—responsible for the idea of the experiment, developed the population, helped in the collection of the data, prepared

the manuscript. S.C.—provided expertise in the collection of the genotypic data and marker analysis, helped edit the manuscript. E.A.—provided expertise and helped edit the manuscript.

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Conflict of interest The authors declare that they have no conflict of interest.

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