IMPACT OF THE D GENOME AND QUANTITATIVE TRAIT LOCI ON QUANTITATIVE TRAITS IN A SPRING BREAD WHEAT BY SPRING DURUM WHEAT CROSS

by

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ABSTRACT

Desirable agronomic traits are similar for common hexaploid (6X) bread wheat (*Triticum aestivum*, 2n = 6x = 42, genome, AABBDD) and tetraploid (4X) durum wheat (*T. turgidum durum*, 2n = 4x = 28, genome, AABB). However, bread and durum wheat are genetically isolated from each other due to an unequal number of genomes that cause sterility when crossed. Previous work allowed identification of a 6X and 4X parent that when crossed resulted in a large number of 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). Lines within each population contained a mixture of alleles from each parent for loci in the A and B genomes. The presence of the D genome in the 6X population resulted in increased yield, tiller number, and seed size. The D genome also resulted in a decrease in stem solidness, lower test weight and fewer seed per spike. Similar results were found with a second RIL population containing 152 lines from 18 additional 6X by 4X crosses. Several additional QTL for agronomic and quality traits were identified in both the 4X and 6X populations. Positive durum alleles increasing kernel weight in hexaploids, on chromosomes 3B and 7A may be useful for introgression by bread wheat breeders.

CHAPTER 1

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 led 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*, 2n = 4x = 28) 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. a*estivum* 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. The sterility is due to the formation of pentaploid offspring that fail to navigate meiosis properly and produce inviable aneuploid seed upon maturation.

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 resistance found in the historically important bread wheat cultivar, Thatcher (Sharma and Gill 1983, Kolmer et al. 1991). Hessian fly 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 and stem rust resistance, derived from *T. turgidum* subsp. *dicoccoides* was successfully expressed in a *T. turgidum* subsp. *durum* background but suppressed by D genome chromosomes in the *T. aestivum* background.

Despite progress exploiting simply inherited genes in an interspecific cross, little success has been reported for quantitative gene movement from a tetraploid to hexaploid background. Direct crosses between cultivated and 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. 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 the yield components including number of spikes per unit area, number of seeds per spike, and seed 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), currently existing in bread wheat germplasm. 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 on 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.

CHAPTER 2

LITERATURE REVIEW

Wheat Domestication and Evolution

Plant domestication is the process by which a wild plant species is selectively bred to suit the needs of humans. Various traits are altered during this process including but not limited to grain/fruit yield, growth habit, photoperiod sensitivity, reduced seed dispersal, seed dormancy, and a decrease in undesirable compounds improving flavor (Doebley et al. 2006). The selection of a free-threshing spike and a non-brittle rachis are arguably the most important traits allowing for the development of common bread wheat (Kilian 2010). *T. aestivum* (2n = 6x = 42) came about through a cycle of domestication and spontaneous interspecific hybridization. *T. urartu* (2n = 2x = 14, genome AA) hybridized with a close relative of *Ae. speltoides* (2n = 2x = 14, genome BB), forming wild emmer wheat (*T. dicoccoides*, 2n = 4x = 28, genome AABB). Roughly 10,000 years ago wild emmer was domesticated forming *T. dicoccum* (2n = 4x = 28, genome AABB). About 9,000 years ago cultivated emmer hybridized with *Ae. tauschii* (2n = 2x =14, genome DD) forming spelt wheat (2n = 6x = 42, genome AABBDD) (Kihara 1944, McFadden and Sears 1946, Peng et al. 2011). The cultivated emmer wheat and spelt were further modified through selection resulting in free-threshing durum wheat (*T. turgidum* ssp. *durum*, 2n = 4x = 28, genome AABB) and bread wheat (*T. aestivum*, genome AABBDD), respectively.

Dvorak et al. (1988) compared the abundance and fragment length of repeated nucleotide sequences among accessions of *T. aestivum* (6X), *T. turgidum* (4X) ssp. *dicoccoides* and ssp. *durum*, *T. timopheevii* (4X), *T. urartu* (2X), *T. monococcum* (2X) ssp. *aegilopoides* and ssp. *monococcum*, *Ae. speltoides* (2X) ssp. *aucheri* and ssp. *lingustica,* and *Ae. tauschii* (2X) ssp. *strangulata* and ssp. *meyerii*. Their results showed that *T. m.* ssp. *aegilopoides* and *T. m.* ssp. *monococcum* genomes were comparable. *T. urartu* did not share the same genome with *T. m.* ssp. *aegilopoides* and *T. m.* ssp. *monococcum*. The A genome of *T. turg.* ssp. *dicoccoides, T. turg.* ssp. *durum, T. timopheevii*, and *T. aestivum* was inherited from a diploid wheat more similar to *T. urartu* and not *T. monococcum*. Dvorak et al. (1993) again supported previous work that *T. urartu* was the A genome donor in *T. turgidum, T. timopheevii* and *T. aestivum*. Examination of *T. zhukovskyi* (4X) showed that one A genome originated in *T. urartu* and the other A genome came from *T. monococcum*.

Through hybridization techniques and cytology McFadden and Sears (1946) showed that the D genome donor of *T. aestivum* is *Ae. tauschii*. Dvorak et al. (1998) analyzed the *Nor3* locus encoding rRNA, the glutenin locus *Glu1* and 53 single-copy restriction fragment length polymorphisms in several accessions of *T. aestivum* ssp. *aestivum*, ssp. *yunnanense*, ssp. *tibetanum*, ssp. *petropavlovskyi*, ssp. *macha*, ssp. *spelta*, ssp. *vavilovii*, *Ae. tauschii* ssp. *tauschii* and ssp. *strangulata*. All of the *T. aestivum* subspecies shared an identical relationship to that of *Ae. tauschii*. The authors further concluded that modern hexaploid wheat regardless of subspecies originated from a common D-genome genepool and argued for multiple hybridizations between tetraploid

wheat and *Ae. tauschii* forming fertile hexaploid amphiploids. Talbert et al. (1998) found evidence for at least two hybridization events forming hexaploid wheat after investigating differences in nucleotide sequence for a D genome specific locus in several *T. aestivum* and *Ae. tauschii* accessions.

Matsuoka and Nasuda (2004) provided evidence that *T. turgidum* ssp. *durum* was the female progenitor of hexaploid bread wheat, by hybridizing the durum cultivar Langdon with an *Ae. tauschii* accession. Langdon was specifically chosen because of the presence of a gene for meiotic restitution contained within it's genome. Prior to this study, direct hybridization between *T. turgidum* ssp. *durum* and *Ae. tauschii* was found to have high sterility rates in the absence of embryo rescue techniques (Tanaka 1961; Fukuda and Sakamoto 1992). Other studies point to *T. turgidum* ssp. *carthlicum* as being the female tetraploid progenitor of bread wheat because of the high fertility rates observed between ssp. *carthlicum* and *Ae. tauschii* (Kihara et al. 1965; Fukuda and Sakamoto 1992). Matsuoka and Nasuda (2004) argue however, that *T. turgidum* ssp. *carthlicum* is a descendent of *T. aestivum* due to the morphological similarity between the two species.

Huang et al. (2002) studied the genes *Acc-1*, a plastid acetyl-CoA carboxylase and *Pgk-1*, a plastid 3-phosphoglycerate kinase. Based on phylogenetic relationships between a wide selection of diploid, tetraploid, and hexaploid wheat and wheat relatives, the authors were able to conclude that *T. urartu* was the A genome donor to wheat tetraploids, supporting previous work and *Ae. taushii* was the D genome donor in *T. aestivum* also in support of established conclusions. The authors also found common loci

in the S genome of *Ae. speltoides* and the G genome of *T. timopheevii*. However, common genomic material was not observed between *Aegilops* and the B genome of *T. turgidum* leaving the B genome donor still unknown.

Polyploidy

Polyploidy is the state in which more than two complete sets of chromosomes exist within an individual. Bread wheat is an example of an allopolyploid, in that it contains three genomes derived from distinct species. Hexaploid wheat is denoted as 2n = 6x = 42. There are 42 chromosomes, with seven pairs of chromosomes making up each of the A, B, and D genomes (AABBDD). Although genetically similar, the three genomes arose from three different species and so are genetically diverse, therefore, similar chromosomes from different genomes are called homeologous instead of homologous and this type of polyploid is referred to as allopolyploid. Also, homologous chromosome pairing typically does not occur between the different genomes. *Solanum tuberosum* or potato is an example of an autotetraploid, designated as 2n = 4x = 48, genome AAAA with twelve sets of chromosomes per genome. In autopolyploids the sets of chromosomes have the same origin and are of the same type meaning that all sets for the same chromosomes can pair and undergo recombination. Typically autopolyploidy is the result of a doubling in chromosome number rather than interspecific hybridization as is the case with allopolyploids. In addition to bread wheat and potato several other crop species show evidence of polyploidy including maize, sugar cane, coffee, cotton and tobacco (Otto and Whitton 2000). In fact, polyploidization may have been an important

part of domestication because it tends to increase seed size. Otto and Whitton (2000) also summarize evidence of polyploidization among several animal species, including insects, fish, amphibians, reptiles, and mammals, although the authors note that the prevalence of polyploidy in animals is considerably less than in plant species.

Comai (2005) provides a thorough review on the pros and cons associated with polyploidy. Among the advantages described the author notes the fixation of heterosis within a polyploid. Heterozygosity is maxed in the F_1 generation and slowly decays by half with each successive round of inbreeding. But in polyploids intergenomic recombination can be suppressed ensuring the maintenance of heterosis even after several rounds of inbreeding. A second described point in favor of polyploidy is genetic redundancy. When multiple copies of alleles exist for a single gene there is a greater probability of hiding a deleterious recessive allele. In addition to the masking of negative alleles, gene redundancy offers a greater opportunity for potentially positive mutations to occur that would otherwise render a diploid infertile or unfit when compared to non-mutant peers. Comai (2005) also describes several disadvantages to polyploidy. Arguably the biggest issue is with homoeologous chromosome pairing during meiosis. In a diploid organism typically two homologous chromosomes pair during meiosis forming a bivalent. In a polyploid however, there is an increased chance of forming multivalent chromosome pairs during meiosis, specifically in the case of an autopolyploid where an individual contains multiple sets of the same genome. For example, trivalent pairs can occur leaving one gamete with three homologous chromosomes and the alternative

gamete with only one homologous chromosome. These faulty gametes when fused with their counterparts result in aneuploid individuals that are oftentimes sterile.

Liu et al. (1998) studied the genomic restructuring that occurs in polyploids after interspecific hybridization. In this study, the researchers hybridized various *Triticum* and *Aestivum* diploids and polyploids and observed the changes in low-copy coding DNA sequences. The authors observed the genetic loss of parental DNA fragments and the emergence of novel DNA fragments, with both genetic alterations occurring independently and together. Genetic changes appeared to occur randomly as well as non-randomly suggesting that certain alterations were necessary to ensure even pairing among homologous chromosomes. DNA methylation and cytoplasm-nuclear interactions were thought to be possible causes for the overall genetic changes.

Bento et al. (2011) studied genomic restructuring following polyploidization in hexaploid and octoploid triticale. They found a differential loss in genomic material between wheat and rye DNA. With respect to wheat, repetitive DNA sequences showed the greatest loss, followed by low-copy DNA, and finally coding sequences. Rye genomic material however, was lost at a relatively even rate regardless of the type of DNA sequence. The authors conclude that this difference could potentially be due to the fact that wheat is itself a polyploid and therefore better prepared for genomic perturbations on such a large scale. The researchers were also able to show that larger genomes underwent greater genomic modifications than their smaller genome counterparts.

Schranz and Osborn (2000) experimented with resynthesized *Brassica napus* from *B. rapa* and *B. oleracea*. The *B. napus* polyploids were then advanced via single plant descent for six generations and evaluated for days to flowering (DTF). Interestingly, novel flowering time variation was observed among the reconstituted *B. napus* lineages. Also, the novel DTF variation was showed to be highly heritable among the lineages.

Liu et al. (2001) looked at genomic changes in resynthesized cotton (*Gossypium*) species. Utilizing amplified fragment length polymorphisms (AFLPs), the authors compared differences in banding patterns among the parental species and nine allopolyploid progeny. Contrary to the studies discussed above, very little genomic restructuring was observed in the allopolyploid progeny. Also, novel DNA methylation changes were not observed based on the utilization of methylation-sensitive AFLP analysis. The authors do note that the plants under evaluation were only advanced two generations beyond chromosome doubling whereas the *Brassica* and wheat studies involved progenies up to and beyond the sixth generation.

Linkage Mapping

Linkage mapping is the process by which Mendelian markers and/or genetic markers are ordered along individual chromosomes, based on the amount of recombination occurring between markers for a particular mapping population. Multiple crossing schemes exist for the geneticist to choose from when developing a mapping population. More mapping populations exist with more complicated crossing schemes

than those discussed below; this is simply a snapshot of some of the possibilities. Development of a recombinant inbred line (RIL) population begins with the selection of two individuals with genetic diversity particularly differing for a phenotypic trait of interest. The two parental genotypes are then hybridized forming a number of F_1 hybrid progeny exhibiting 100% heterozygosity at each genomic locus. In self-pollinating organisms, the F_1 individual is then inbred for several generations until an acceptable level of homozygosity is achieved. Heterozygosity is reduced by half after each generation of inbreeding due to the segregation of heterozygous loci into 25% homozygous recessive, 50% heterozygous, and 25% homozygous dominant. An F_2 mapping population is similar to an RIL population in that two parents are initially crossed forming F_1 hybrids. However, the F_1 individuals are either intercrossed or self-pollinated to form a collection of F_2 individuals. Simply inherited genes in an F_2 population should be segregating in a 1:2:1 ratio of homozygous recessive to heterozygous to homozygous dominant. Another potential mapping population is called a doubled haploid (DH) population. Individual F_1 plant gametes are subjected to a chromosome doubling technique forming a completely homozygous individual. RIL and doubled haploid populations are immortal and can be tested in multiple environments over a number of years.

A second aspect to consider when undertaking a linkage mapping project is the type of genetic marker to use. Several factors need to be considered when selecting a genetic marker platform. Several genetic marker options exist each with their own advantages and disadvantages. Microsatellite or simple sequence repeat (SSR) markers

are a mainstay in genetic map construction (Brondani et al. 2002; Diwan et al. 2000; Liu et al. 2006; Ramsay et al. 2000; Song et al. 2004; Yu et al. 2000). SSR markers are desirable due to their ability to distinguish between heterozygous and homozygous genotypes. They are also highly reproducible across laboratories. However, the initial cost of developing primer sets can be expensive and the inherent multiallelic nature can pose problems when trying to compare across variable genotypes. The value of single nucleotide polymorphism (SNP) markers is starting to be realized over other marker platforms because of their high-throughput ability bringing down cost per data point (Hiremath et al. 2012; Syvänen 2005; Wang et al. 2014; Zhang et al. 2013). SNPs are also the most common form of genetic polymorphism (Khlestkina and Salina 2006).

The actual development of genetic linkage maps is relatively straightforward. The initial step is to group markers together into linkage groups that are likely on the same chromosome. This can be done based on pair-wise recombination fractions and likelihood ratio test statistics, also known as logarithm of odds or LOD scores between marker pairs. Recombination fractions or recombination frequencies are calculated as the number of recombined gametes between two loci divided by the total number of gametes. A non-recombinant individual would have the same alleles as one of the parental lines for two markers, whereas a recombinant would have a different allele at one of the two marker loci when compared to one of the parental genotypes. LOD score calculations are more complicated than recombination frequencies however they are still based on the number of recombinants and non-recombinants observed between two genetic loci. In

general LOD scores are equal to the $\log_{10}$ of the probability that two loci are linked divided by the probability that two loci are unlinked (Xu 2013).

$$LOD\ Score = \log_{10} \frac{Probability\ that\ two\ loci\ are\ linked}{Probability\ that\ two\ loci\ are\ unlinked}$$

LOD scores of three or greater are typically used as a general guideline for evidence of linkage between markers because a LOD of three means that the likelihood of linkage is 1,000 times greater than the likelihood of no linkage (Xu 2013). Once markers are distributed among linkage groups appropriate marker ordering analyses are employed till the best marker order relevant to the mapping population is established.

Haldane (1919) was the first paper to suggest a method for transforming observable recombination fractions into more interpretable map distances. The Haldane mapping function is based on a Poisson model and assumes that there is no crossover interference occurring along the chromosome (Speed 2005). An alternative to the Haldane mapping function is the Kosambi mapping function (Kosambi 1943). The Kosambi mapping function assumes greater crossover interference as the density of genetic markers increases (Jannink 2005). Alternative mapping functions that are variations on the Haldane and Kosambi mapping functions exist but as to which is the most appropriate is dependent on marker density and more so on the species under study.

As the number of markers available for many different species continues to increase into the tens of thousands one important issue arises involving the increase in genotype calling errors. Lincoln and Lander (1992) note that even a conservative

genotypic error rate of 3.0% can lead to a significant proportion of spurious crossovers. Fortunately, Lincoln and Lander (1992) propose a routine method for the detection of potential genotyping errors present within linkage data. The authors start by considering genotypic data as a phenotype rather than a genotype. Essentially, the authors are assuming that the genotypic data is a very close representation of the unobservable actual genotype. Put another way, genotypic data, free of errors, would be completely penetrant. Next a penetrance function is specified based on a predetermined error rate, followed by the construction of the most likely linkage map dependent on the penetrance function. Finally, error LOD scores for each genotype are calculated by taking the log base 10 of the probability that the observed genotype is not equal to the unobservable true genotype, divided by the probability that the observed genotype is equal to the true genotype.

Segregation distortion is another issue when building a genetic linkage map. Segregation distortion arises when genotypic frequencies for specific genetic loci deviate from expected Mendelian segregation ratios. Hackett and Broadfoot (2003) looked at the effects of segregation distortion on genetic map construction in a simulation study. The simulation study consisted of a doubled haploid population with three chromosomes and ten evenly spaced markers per chromosome. One marker on each of the three chromosomes was selected to impact progeny fertility resulting in regions of distorted ratios. The authors concluded that when the number of individuals in the population is high enough (~150 individuals) segregation distortion has little effect on marker order and chromosome length. The authors do concede that these results do not apply to the

situation where more than one locus is involved in offspring viability. Lu et al. (2002) looked at segregation distortion effects in four mapping populations of maize. The authors of this study found the prevalence of segregation distortion to vary ranging between 19% and 36%. Eighteen chromosomal regions were identified as showing segregation distortion. The results supported evidence that map distances are unaffected when only a single factor causing distortion is present within a given region. If however, two linked gametophytic factors are present map distances are skewed due to biased recombination fractions.

QTL Mapping

The process of identifying the underlying regions of genomic information that are in control of a phenotypic evaluation of interest is the goal of quantitative trait loci (QTL) mapping. Scientists scour a linkage map in search of portions that impact a trait of concern or interest. Analysis of variance (ANOVA) or marker regression is the simplest method for linking a QTL and phenotype via a genetic marker. Soller et al. (1976) first proposed marker regression after experimenting with a simulated F_2 backcross population. Essentially, the mechanics of marker regression are to split a population into groups based on a single marker genotype and then compare the genotypic groupings with the associated phenotypic mean values. Ideally, clear separation of phenotypic mean values based on the genotypic groupings would be observed. ANOVA is best suited for situations where the phenotype is qualitative, in that phenotypic variation is discreet rather than continuous. The clear benefit for working with marker regression is

its simplistic nature as well as the fact that a genetic linkage map is not absolutely necessary for testing for the presence of QTL impacting a trait of interest. However, you need dense marker coverage to ensure that there is little recombination between the best markers identified and the QTL under study enabling accurate estimates of the QTL effect. The best case scenario would be for the marker to actually tag the causal mutation in the gene responsible for affecting the trait of interest. Power of detection is also heavily dependent on marker coverage and directly correlated, but more markers brings up the issue of multiple testing which is associated with an increase in Type I error or increase in false positives.

Lander and Botsetin (1989) proposed interval mapping (IM) as a way of overcoming the drawbacks of marker regression which are the underestimation of QTL effects, poor estimation of QTL genetic location, and an overwhelmingly large number of progeny required for testing. As discussed above the underestimation of a QTL effect is due to the possibility of recombination between the marker and the QTL. Since it is difficult to distinguish between a QTL with small effect and a QTL where markers are more distant, it is difficult to be certain about the location of a QTL. The author's solution to overcoming the pitfalls of marker regression involve the usage of LOD scores as well as evaluating flanking markers that span an interval of genomic space hopefully containing a QTL. Interval mapping involves estimating the probability of a genotype at a given QTL based on the genotypes of the nearest flanking markers. Marker regression provides discreet estimations of a QTL while interval mapping connects each marker and provides a curved representation for the probability of a QTL location. QTL location is

better understood with interval mapping because interval mapping provides evidence for a QTL that may be located between markers. A better understanding of QTL location also provides a better estimation of the effect a QTL has on a phenotype. An issue not discussed is the fact that an individual missing a genotype data point cannot be tested for association with a phenotype, this issue is resolved with interval mapping. If a line lacks a genotype then the next closest flanking marker can be used in the LOD score estimation. However, one disadvantage of interval mapping is the increase in complexity and therefore computational time over ANOVA.

To address the increase in computational effort associated with interval mapping Haley and Knott (1992) developed a method that uses multiple regression of flanking markers rather than maximum-likelihood estimation. The authors were able to generate results that were comparable to the alternate maximum-likelihood estimates.

A critical assumption made with marker regression and interval mapping is the assumption that there is only one QTL affecting a trait. Composite interval mapping (CIM) described by Jansen (1993) utilizes interval mapping but also includes a selection of covariates. The covariates are other markers found in the linkage group. These markers are essentially placeholders for potential QTL markers. Their inclusion helps to reduce residual variation and allow for a more refined interval scan across the genome. Unfortunately the problem with CIM is deciding which markers and how many to include as covariates to provide a more accurate scan. This is an issue that has yet to be addressed. Multiple interval mapping (MIM) described by Kao et al. (1999) is similar to composite interval mapping in that putative QTL are considered simultaneously while

searching for other QTL, but it differs in that MIM uses genomic intervals rather than individual markers as covariates. MIM also does not use the assumption that only a single QTL is involved when searching for marker-trait associations.

During the process of searching for QTL the question arises of how much evidence is necessary to declare a QTL real or not. Lander and Botstein (1989) used simulated datasets to try and elucidate the appropriate threshold values so as to reduce Type I error to an acceptable level. The author's results showed that the type of cross involved, the number of individuals evaluated, marker density, and genome size were all important factors to consider when setting appropriate LOD score thresholds. In general a LOD score between two and three is suggestive of a QTL and a LOD score greater than three provides strong evidence for the location of a QTL. An alternative to fixed thresholds is permutation testing. Churchill and Doerge (1994) describe the implementation of estimating population specific thresholds for the significance of a QTL based on permutation testing. Basically to carry out a permutation test for QTL mapping the researcher randomizes the phenotypic data while keeping the genotypic data intact and then performs the relevant QTL mapping method (ANOVA, IM, CIM, MIM), after which the researcher selects the largest attained LOD score. The process of shuffling the phenotype data and finding the maximum LOD score is repeated 1,000 plus times. This pool of simulated LOD scores is then used for selecting an appropriate LOD score threshold. Permutation testing has the advantage over fixed thresholds in that it is specifically tailored for the dataset in question.

CHAPTER 3

METHODS AND MATERIALS

Phenotyping

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). 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 of all seven pairs indicated the plant was hexaploid, while lack of amplification for all of the primer sets indicated the plant was tetraploid. Aneuploids were absent from the final population and believed to be lost due to infertility in earlier generations. Seed from each F_5 plant was increased to F_8 to provide seed for field plots. 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. 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 performed in the derivation of the tetraploid RIL in that a large percentage of the 4X lines were extremely short and poorly adapted to dryland conditions. These lines were discarded as their height prohibited harvest in the dry conditions encountered in Montana. Subsequent testing showed that the shortness was due to the presence of the Choteau mutant allele for semi-dwarf habit at *Rht-B1* (McIntosh et al. 2003). We also looked at allelic frequencies in two additional sets of lines. The first set consisted of elite spring wheat cultivars collected from major spring wheat breeding programs, in North America referred to as the Spring Wheat Association Mapping Panel, on the T3 database (http://triticeaetoolbox.org/wheat/). The second set of lines also collected from the T3 database consisted of wheat landrace genotypes, collected worldwide.

The Choteau/Mountrail population was grown in Bozeman MT and Sidney MT in 2012 and 2013 for four locations. Phenotypic data collected for each population are shown in Table 2. Grain trait measurements for the 2013 Sidney MT location, are missing due to a hail storm that destroyed plots shortly before harvest. Stem solidness data was not collected on 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 offspring. Given the small number of solid-stemmed lines we felt the phenotype was not accurately represented in the population.

The Choteau/Mountrail population and the Bread/Durum population were grown separately in augmented randomized complete block designs. The Choteau/Mountrail experiment contained the same 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.

Juvenile stem solidness and 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. Juvenile stem solidness was taken when the majority of the population showed 2-3 visible internodes. Again, final solid stem scores were based on an average of the visible internodes.

Heading date for each entry was recorded as the day after Jan. 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 (Tecator 1241, Foss Analytical AB, Höganäs, Sweden) in the Montana State University Cereal Quality Lab, Bozeman, MT. Kernel hardness, weight, and diameter were analyzed using the Single Kernel Characterization System 4100 (Perten Instruments). Sedimentation values, which are an estimate of gluten strength where higher numbers equate to 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 analyses were conducted using SAS, v9.3 (© 2002-2010 by SAS Institute Inc., Cary, NC). 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. The chi-squared test of independence was used to determine if allele frequencies differed between the elite and landrace germplasm.

Genotyping 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). 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 8,882 co-segregating markers leaving 2,686 unique marker genotypes. The 4X lines had 9,192 co-segregating markers with 1,910 unique marker genotypes. Also, fourteen polymorphic simple sequence repeat (SSR) or microsatellite markers were placed on the A and B genome chromosomes. All microsatellite markers were assayed using the LI-COR DNA analysis system (LI-COR Biosciences, Lincoln,NE). Markers for *RHT-B1* and *VRN-A1* (McIntosh et al. 2003) were also screened using agarose gel electrophoresis as described by Blake et al. (2009).

The unique marker genotypes scored on the 6X and 4X lines were combined for map construction. 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). 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. 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 again 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 (Cavanaugh et al. 2013; Wang et al. 2014). Recombination distances were determined based on the Kosambi mapping function (Kosambi 1943).

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 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 1,000 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. This was done 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.

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**

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
JSS	X	X	X			
MSS	X	X	X	X	X	
Heading Date	X	X	X	X	X	X
Plant Height	X	X	X	X	X	X
PTN	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		

[a] JSS = Juvenile Stem Solidness, MSS = Mature Stem Solidness, PTN = Productive Tiller Number

CHAPTER 4

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, productive tiller number, and test weight based on a t-test. However, tetraploid Mountrail had a higher 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, heavier kernel weight, and a larger kernel diameter. The tetraploids had higher 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 heavier kernel weight ($P < 0.01$) and larger 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 heavier kernel weight by 0.68 mg and larger kernel diameter by 0.02 mm, but the 6X RIL were higher yielding by 324.19 kg/ha. 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.

Comparison of RIL means with parental means (Tables 3 and 4; Figure 1) shows that the RIL were consistently lower yielding than the parents. For instance, yield of Choteau was 3574.87 kg ha^{-1} versus 2804.07 kg ha^{-1} for the 6X RIL mean (Table 3). Yield of Mountrail was 3379.14 kg ha^{-1} versus 2399.16 kg ha^{-1} for the 4X RIL. A similar trend was observed for 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 and 4). Figure 2 depicts the distribution of kernel weight for RIL and parents in the Choteau/Mountrail population. Kernel weight of Choteau was 30.06 mg compared to 32.85 mg of 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 populations 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 juvenile and mature stem solidness, heading date, plant height, kernel hardness, and sedimentation value. Choteau was more solid in young and mature growth stages. Choteau also had a higher 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 protein. The 4X RIL were more solid during early and late growth stages, although the 6X parent was more solid than the 4X parent. The 4X RIL also had harder kernels. The 6X RIL were earlier heading, had taller growth habit and higher sedimentation values.

The Bread/Durum population (Table 6) showed significant differences between 4X and 6X lines 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. An initial set of 2,269 markers was mapped, spanning 3,183.2 centiMorgans (cM) with an average inter-marker spacing of 1.4 cM. Marker coverage was reduced by removing markers that were within one centiMorgan of each other, bringing the total number of markers in the map down to 995. The removal of markers reduced the overall map length by 4.5 cM to 3,178.7 cM. Average inter-marker spacing increased to 3.2 cM. The full and reduced maps are summarized in Table 7. Linkage map construction indicates that the A and B genomes of the Choteau/Mountrail RIL were comprised of a mixture of Choteau and Mountrail alleles.

We identified QTL models responsible for explaining a portion of the variation for all thirteen 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 and R^2

values. Table 9 summarizes the impact of 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 had the largest impact on yield as indicated by the high LOD value for the effect of ploidy. The Choteau allele caused higher yields for QYld.mst-1A and QYld.mst-7A, with similar effects in the 6X and 4X backgrounds. The Mountrail allele boosted yield for QYld.mst-2B and the effect was greater in the 4X RIL. One QTL was identified on chromosome 5B associated with productive tiller number (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 caused an increase in 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 found in lines missing the D genome and containing the hexaploid QTL alleles, while the heaviest kernels were found in lines containing the D genome and possessing the tetraploid QTL alleles (Figure 3). Two QTL on chromosomes 4B and 5A were identified

linked to kernel diameter (QSd.mst-4B, QSd.mst-5A) (Tables 8 and 9). The Choteau allele at QSd.mst-5A increased kernel diameter and the Mountrail allele increased kernel diameter at Qsd.mst-4B. Ploidy impacted kernel diameter with the presence of the D genome causing increased diameter. Mountrail contributed an allele for a QTL on chromosome 2A that increaseed 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 higher means overall in the 4X RILs. There was no interaction between the QTL alleles and the presence of the D genome (ploidy) for any of the traits presented in Tables 8 and 9.

Results summarized in Tables 10 and 11 show the impact of alleles from Choteau and Mountrail on several agronomic traits. A QTL on chromosome 3B impacted juvenile (QSs.mst-3B) and mature (QSs.mst-3B) stem solidness (Table 10). For both traits, the Choteau allele increased stem solidness (Table 11). A second QTL (QSs.mst-4A) on chromosome 4A was important for juvenile stem solidness but not mature stem solidness. The Choteau allele increased juvenile stem solidness. Interaction between ploidy and QSs.mst-3B was observed as the Mountrail allele performed differentially depending on the presence of the D genome. The 6X RIL with the allele had less solid stems than the 4X RIL with the allele. At plant maturity, stem solidness was greater in the 4X RIL with the positive allele for the QTL on chromosome 3B. QTL on chromosomes 3A, 5A, and 7B were identified as controlling some of the variation observed in heading date. The QTL on 5A (QHd.mst-5A) impacted 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. Significant segregation distortion was observed on 4B spanning roughly 15 cM. Mountrail contributed alleles for two QTL that boosted 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 close to *Glu-A1* and *Glu-B1* genes, controlling glutenin proteins reported in the GrainGenes database (http://wheat.pw.usda.gov/GG2/index.shtml). QSev.mst-4B, on 4B, was located near the *Rht-B1* locus, which controls plant height.

The relative frequencies of alleles for each QTL at its respective SNP within elite bread wheat germplasm and landrace material were determined (Table 12). The data for the set of elite bread wheat germplasm comes from the spring wheat association mapping panel. It and the data for the landrace material are reported on the T3 wheat database

(http://triticeaetoolbox.org/wheat/). Elite lines tended to have the favorable allele for most of the QTL. Exceptions include a favorable allele for kernel weight (QGw.mst-7A) for which the favorable allele was present in only 20% of the lines. Additionally, two alleles causing increased sedimentation values were present in a minority of the elite lines. One favorable allele for yield, QYld.mst-1A was present in higher frequency in the elite lines than in the landraces. Additionally, an allele for increased sedimentation at QSev.mst-4B was not observed in any of the landraces but was present in 29% of the elite lines.

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

	Choteau	Mountrail		6X (n = 117)		4X (n = 88)		6X vs. 4X
	Mean		LSD	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	**
TW (kg m^{-3})	748.9	757.1	10.0	724. 5	658.8 – 747.6	734.1	706.6 – 759.3	**
KW (mg)	30.0	36.0	1.2*	32.9	29.1 – 38.4	30.4	26.8 – 34. 2	**
KD (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	**

[a] Significance Level: *, ** = P < 0.05 and 0.01, respectively

[b] PTN = Productive Tiller Number, TW = Test Weight, KW = Kernel Weight, KD = Kernel Diameter

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

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

[a] Significance Level: *, ** = P < 0.05 and 0.01, respectively

[b] KW = Kernel Weight, [c] KD = Kernel Diameter

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

	Choteau	Mountrail		6X (n = 117)		4X (n = 88)		6X vs. 4X
	Mean		LSD	Mean	Range	Mean	Range	T-test
JSS	4.7	2.4	0.4*	3.1	1.8 – 4.5	3.7	2.2 – 4.6	**
MSS	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	**
SDSSV	3.7	2.2	0.2*	3.5	2.2 – 5.0	2.1	1.6 – 2.8	**

[a] Significance Level: *, ** = P < 0.05 and 0.01, respectively

[b] JSS = Juvenile Stem Solidness, MSS = Mature Stem Solidness, SDSSV = Sedimentation Value

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

	6X Parents	4X Parents		6X (n = 61)		4X (n = 91)		6X vs. 4X
	Mean		T-test	Mean	Range	Mean	Range	T-test
MSS	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	**

[a] Significance Level: * = P < 0.05 and 0.01, NS = Not Significant

[b] MSS = Mature Stem Solidness

Table 7. Summary of the full and reduced Choteau/Mountrail genetic maps.

Full Linkage Map				Reduced Linkage Map			
Chromosome	# of Markers	Chromosome Length	Average Marker Spacing	Chromosome	# of Markers	Chromosome Length	Average Marker Spacing
1A	132	203.0	1.5	1A	58	202.2	3.5
1B	220	201.5	0.9	1B	81	200.5	2.5
2A	161	273.8	1.7	2A	73	273.8	3.8
2B	184	170.9	0.9	2B	69	170.4	2.5
3A	127	275.4	2.2	3A	73	275.1	3.8
3B	212	234.1	1.1	3B	90	233.8	2.6
4A	130	209.0	1.6	4A	69	209.0	3.1
4B	117	209.7	1.8	4B	56	209.7	3.8
5A	161	223.2	1.4	5A	71	222.2	3.2
5B	176	250.3	1.4	5B	82	250.3	3.1
6A	111	161.2	1.5	6A	46	161.2	3.6
6B	203	211.6	1.0	6B	73	211.1	2.9
7A	182	357.6	2.0	7A	82	357.3	4.4
7B	153	202.1	1.3	7B	72	202.1	2.8
Total	2269	3183.2	1.4	**Total**	995	3178.7	3.2

Table 8. Summary of the QTL found with multiple interval mapping for yield and yield related traits.

	Source	Marker	Chromosome	Position	LOD	R^2 (%)
Yield	QYld.mst-1A	IWA3254	1A	52.4	5.0	6.1
	QYld.mst-2B	IWB29332	2B	152.3	6.1	7.7
	QYld.mst-7A	IWB34840	7A	340.7	5.6	6.9
	Ploidy				22.6	34.2
	QYld.mst-2B: QYld.mst-7A				5.4	6.7
Productive Tiller Number	QTn.mst-5B	IWB69502	5B	170.0	3.3	6.4
	Ploidy				4.5	9.0
Test Weight	QTw.mst-7B	IWB39660	7B	144.8	3.1	5.8
	Ploidy				6.3	12.2
Kernel Weight	QGw.mst-3B	IWA6375	3B	126.8	4.9	6.1
	QGw.mst-7A	IWA3562	7A	284.2	5.2	6.5
	Ploidy				25.0	39.8
Kernel Diameter	QSd.mst-4B	IWB72203	4B	36.6	7.0	9.6
	QSd.mst-5A	IWB9138	5A	61.5	5.0	6.8
	Ploidy				19.1	30.4
Seeds per Spike	QKps.mst-2A	IWB72154	2A	142.5	4.6	8.8
	Ploidy				5.4	10.3

[a] LOD = log10 likelihood ratio

[b] R^2 = Percent of phenotypic variance explained

Table 9. Summary of phenotypic means for yield and yield related QTL found in the Choteau/Mountrail population.

Trait	QTL	Ploidy	Choteau Allele Mean (Standard Error)	Mountrail Allele Mean (Standard Error)
Yield	QYld.mst-1A	6X	2867 (32.96)	2734 (34.98)
		4X	2479 (41.03)	2335 (36.99)
	QYld.mst-2B	6X	2783 (36.99)	2821 (32.96)
		4X	2341 (43.72)	2442 (36.99)
	QYld.mst-7A	6X	2806 (39.01)	2803 (32.28)
		4X	2409 (37.67)	2386 (43.05)
Productive Tiller Number	QTn.mst-5B	6X	130.3 (2.37)	141.1 (2.30)
		4X	119.8 (2.70)	128.2 (2.67)
Test Weight	QTw.mst-7B	6X	727.4 (1.54)	722.0 (1.42)
		4X	737.6 (1.67)	730.1 (1.80)
Kernel Weight	QGw.mst-3B	6X	32.4 (0.20)	33.3 (0.20)
		4X	30.0 (0.25)	30.7 (0.22)
	QGw.mst-7A	6X	32.4 (0.20)	33.3 (0.21)
		4X	30.0 (0.24)	30.8 (0.23)
Kernel Diameter	QSd.mst-4B	6X	2.76 (0.01)	2.82 (0.01)
		4X	2.66 (0.02)	2.71 (0.01)
	QSd.mst-5A	6X	2.80 (0.01)	2.77 (0.01)
		4X	2.70 (0.01)	2.68 (0.01)
Seeds per Spike	QKps.mst-2A	6X	31.4 (0.37)	32.3 (0.36)
		4X	32.3 (0.42)	35.3 (0.41)

[a] Units for effects are yield (kg ha^{-1}), productive tiller number (spikes m^{-1}), test weight (kg m^{-3}), kernel weight (mg), seed diameter (mm).

Table 10. QTL summary of agronomic traits in the Choteau/Mountrail population.

	Source	Marker	Chromosome	Position	LOD	R^2 (%)
Juvenile Stem Solidness	QSs.mst-3B	IWB58481	3B	230.9	69.8	71.0
	QSs.mst-4A	BARC343	4A	166.1	3.5	1.5
	Ploidy				24.5	13.6
	QSs.mst-3B:Ploidy				12.7	6.1
Mature Stem Solidness	QSs.mst-3B	IWB58481	3B	230.9	81.7	71.8
	Ploidy				28.0	12.0
Heading Date	QHd.mst-3A	IWA4296	3A	167.7	4.8	6.9
	QHd.mst-5A	VrnA	5A	142.8	11.6	18.1
	QHd.mst-7B	IWB6455	7B	17.5	11.6	18.0
Plant Height	QHt.mst-4B	Rht-B1	4B	44.5	21.1	15.7
	Ploidy				59.4	72.3
Grain Protein	QGpc.mst-4A	IWB20212	4A	67.5	3.6	7.0
	QGpc.mst-4B	IWB51614	4B	51.1	4.5	8.9
Kernel Hardness	QHa.mst-4A	IWB6369	4A	89.2	3.6	1.1
	Ploidy				90.4	82.9
Sedimentation Value	QSev.mst-1A	IWB44038	1A	94.5	4.6	1.6
	QSev.mst-1B	IWB47979	1B	22.1	12.5	4.7
	QSev.mst-3A	IWB51852	3A	238.2	3.6	1.2
	QSev.mst-4B	IWB73001	4B	33.2	4.7	1.6
	Ploidy				74.8	62.9

[a] LOD = log10 likelihood ratio

[b] R^2 = Percent of phenotypic variance explained

Table 11. Phenotypic means summary of QTL found associated with agronomic traits of importance in the Choteau/Mountrail population.

Trait	QTL	Ploidy	Choteau Allele Mean (Standard Error)	Mountrail Allele Mean (Standard Error)
Juvenile Stem Solidness	QSs.mst-3B	6X	4.2 (0.07)	2.3 (0.06)
		4X	4.2 (0.07)	3.3 (0.07)
	QSs.mst-4A	6X	3.2 (0.11)	3.0 (0.12)
		4X	3.8 (0.13)	3.6 (0.14)
Mature Stem Solidness	QSs.mst-3B	6X	3.7 (0.05)	1.9 (0.05)
		4X	4.3 (0.06)	2.7 (0.05)
Heading Date	QHd.mst-3A	6X	185.8 (0.16)	185.3 (0.15)
		4X	186.5 (0.19)	185.8 (0.16)
	QHd.mst-5A	6X	184.9 (0.15)	186.1 (0.14)
		4X	185.8 (0.17)	186.4 (0.16)
	QHd.mst-7B	6X	185.0 (0.15)	186.0 (0.15)
		4X	185.5 (0.32)	186.2 (0.13)
Plant Height	QHt.mst-4B	6X	78.3 (0.76)	87.8 (0.65)
		4X	59.0 (1.55)	68.9 (0.61)
Grain Protein	QGpc.mst-4A	6X	16.4 (0.08)	16.9 (0.09)
		4X	16.6 (0.10)	16.8 (0.10)
	QGpc.mst-4B	6X	16.4 (0.09)	16.8 (0.08)
		4X	16.4 (0.17)	16.8 (0.08)
Kernel Hardness	QHa.mst-4A	6X	70.9 (0.53)	68.6 (0.47)
		4X	90.8 (0.54)	88.6 (0.61)
Sedimentation Value	QSev.mst-1A	6X	3.6 (0.05)	3.4 (0.05)
		4X	2.2 (0.07)	2.1 (0.05)
	QSev.mst-1B	6X	3.3 (0.05)	3.7 (0.04)
		4X	2.0 (0.05)	2.2 (0.05)
	QSev.mst-3A	6X	3.6 (0.05)	3.4 (0.05)
		4X	2.1 (0.06)	2.1 (0.06)
	QSev.mst-4B	6X	3.7 (0.05)	3.4 (0.05)
		4X	2.2 (0.08)	2.1 (0.05)

[a] Juvenile and mature stem solidness were scored on a 1 to 5 scale, with 1 = hollow, and 5 = solid, heading date is days from Jan. 1, plant height (cm), and kernel hardness (skcs).

Table 12. Summary of allelic frequencies for QTL in a selection of hexaploid landraces and elite germplasm.

Trait	QTL	Frequency of Alleles in Elite 6X Lines			Frequency of Alleles in 6X Landraces			χ^2
		n	Choteau	Mountrail	n	Choteau	Mountrail	
Yield	QYld.mst-1A	93	***0.70***	0.30	27	***0.26***	0.74	15.1**
	QYld.mst-2B	242	0.85	***0.15***	41	0.63	***0.37***	9.2**
	QYld.mst-7A	243	***0.74***	0.26	41	***0.88***	0.12	2.9
PTN[a]	QTn.mst-5B	243	1.00	***0.00***	41	0.93	***0.07***	[c]18.0**
Test Weight	QTw.mst-7B	243	***0.74***	0.26	41	***0.73***	0.27	0.0
Kernel Weight	QGw.mst-3B	242	0.90	***0.10***	1659	0.36	***0.64***	253.3**
	QGw.mst-7A	91	0.20	***0.80***	27	0.19	***0.81***	0.0
Kernel Diameter	QSd.mst-4B	243	0.98	***0.02***	41	0.98	***0.02***	[c]0.1
	QSd.mst-5A	242	***0.80***	0.20	41	***0.80***	0.20	0.0
Seeds per Spike	QKps.mst-2A	243	1.00	***0.00***	41	1.00	***0.00***	[c]0.1
Stem Solidness	QSs.mst-3B	-	-	-	-	-	-	-
	QSs.mst-4A	-	-	-	-	-	-	-
Heading Date	QHd.mst-3A	237	***0.32***	0.68	1652	***0.49***	0.51	24.3**
	QHd.mst-5A	-	-	-	-	-	-	-
	QHd.mst-7B	241	0.87	***0.13***	41	0.83	***0.17***	0.2
Plant Height	QHt.mst-4B	-	-	-	-	-	-	-
Grain Protein	QGpc.mst-4A	241	0.67	***0.33***	41	0.98	***0.02***	14.4**
	QGpc.mst-4B	243	0.71	***0.29***	41	0.74	***0.26***	0.2
Kernel Hardness	QHa.mst-4A	243	***0.98***	0.02	41	***0.98***	0.02	[c]0.0
Sedimentation Value	QSev.mst-1A	242	***0.36***	0.64	41	***0.15***	0.85	6.5*
	QSev.mst-1B	243	0.52	***0.48***	41	0.22	***0.78***	11.7**
	QSev.mst-3A	-	-	-	-	-	-	-
	QSev.mst-4B	243	***0.29***	0.71	41	***0.00***	1.00	14.2**

The allele with a greater phenotype is indicated in italic and bold, [a] Productive Tiller Number,
[b] χ^2 Significance Level: *, ** = P < 0.05 and 0.01, respectively, [c]P-values were simulated due to low expected values

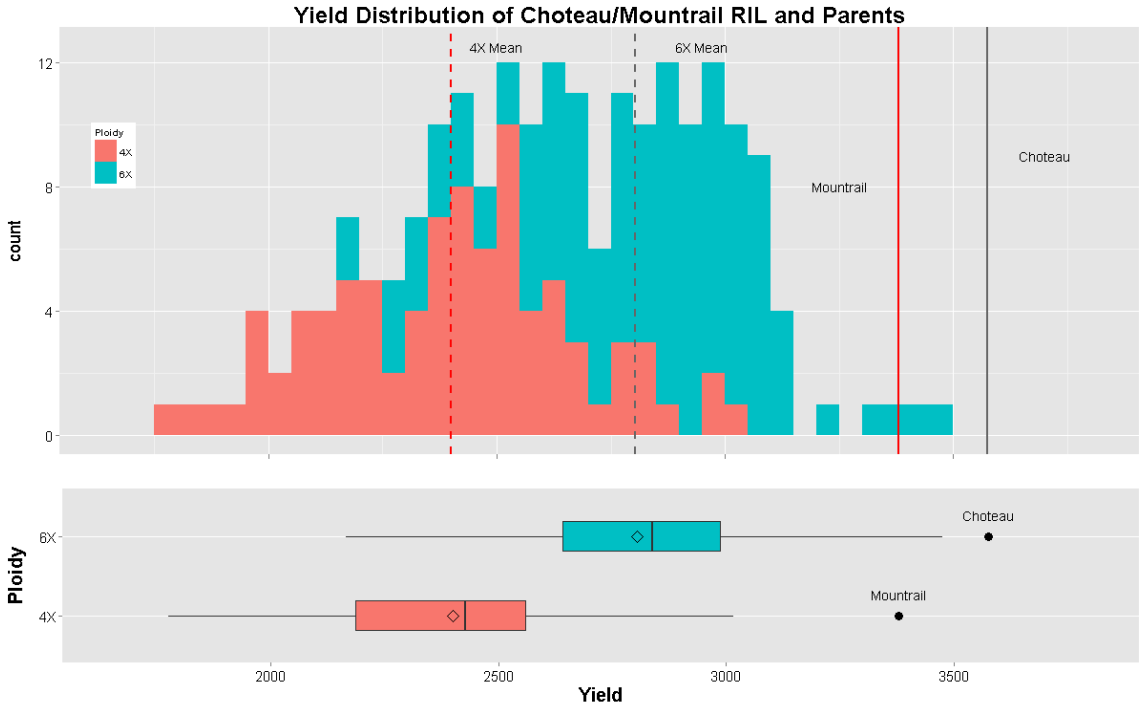

Figure 1. Histogram and boxplot showing the distribution of yield for the Choteau/Mountrail 6X and 4X RIL relative to the mean yield of Choteau (6X) and Mountrail (4X)

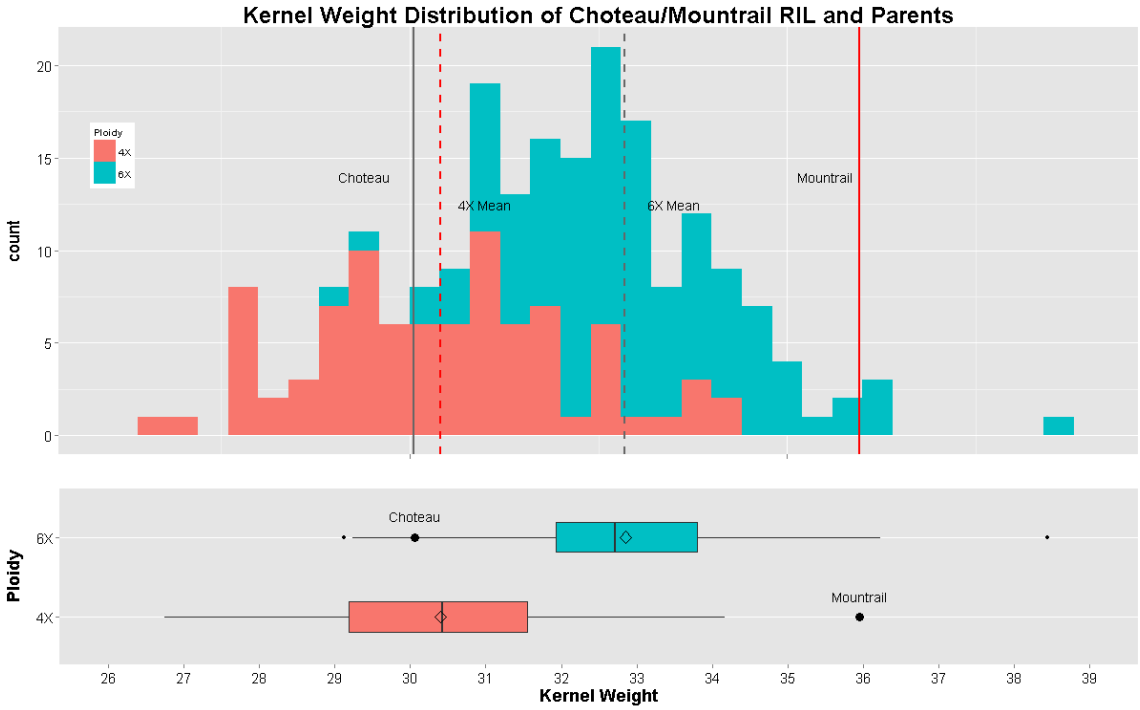

Figure 2. Histogram and boxplot showing the distribution of kernel weight for the Choteau/Mountrail 6X and 4X RIL relative to the mean yield of Choteau (6X) and Mountrail (4X).

Figure 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.

CHAPTER 5

DISCUSSION

Durum and bread wheat differ by the presence of the seven chromosome pairs of the D genome in bread wheat. The 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 seed due to 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. 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 combination, that resulted in a high number of 4X and 6X progeny lines, allowed development of RILs 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 (Figure 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 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 a 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 over 2,000 markers was initially developed from the SNP genotypic data. However, once we experienced the massive amount of computational time associated with this many markers the decision was made to reduce marker density while maintaining good marker coverage across the wheat genome. The reduced map, containing ~1,000 markers was used in the final QTL analysis drastically reducing the necessary computational time.

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 higher in the tetraploid parent Mountrail, and also higher in 4X RIL population than the 6X RIL population. However, in some cases the parental means were opposite 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 wheat, hoary plantain, and orchardgrass (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 heavier kernels than the hexaploid parents as has been seen in other studies (Lacarenza 2008; Trethowan 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 smaller kernel weight. The QTL analysis shows that this can be explained by alleles from durum wheat on chromosomes 3B and 7A that resulted in smaller kernel weight.

A possible advantage of the durum by spring wheat crosses is that it provides the possibility to identify novel QTL that may not be identified in durum by durum or bread wheat by bread wheat crosses. For instance, it may be that the favorable allele is either absent or rare in one of the species. Data reported on the T3 database (http://triticeaetoolbox.org/wheat/) showed that the positive allele for kernel weight at the 7A QTL was found in most of the elite germplasm. However, the 3B QTL was mostly absent from elite germplasm. Thus, this particular QTL allele would likely not be identified in 6X by 6X crosses. Positive alleles from Mountrail, such as those causing smaller kernel weight are candidates for introgression into hexaploid wheat.

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 Mountrail durum wheat in both cases. Kernel diameter QTL were on 4B and 5A. A positive allele from durum wheat on 4B co-segregated with the allele for increased plant height at *Rht-B1*, while spring wheat contributed the positive allele for the 5A QTL. The different QTL identified for these traits is 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). Choteau is a widely grown variety offering sawfly tolerance via solid stem (Lanning et al., 2004). A major quantitative trait locus for solid stems is on chromosome 3B (Cook et al. 2004). The Choteau allele for this QTL resulted in very solid-stemmed RILs in our populations regardless of ploidy. However, Table 10 shows that there is a significant QTL by ploidy interaction for juvenile stem solidness, as the Choteau allele had a greater effect in the 6X versus the 4X progeny (Table 11), possibly due to the fact that the Mountrail allele for QSs.mst-3B does not reduce stem solidness as much in the absence of the D genome. The results of this study indicate that there may be alleles reducing solid stem located on the D genome. This conclusion is further 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 increased hardness allele originating in Choteau. The Choteau allelic effect was similar in both 4X and 6X backgrounds. 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 both the Choteau/Mountrail and Bread/Durum populations. The positive D genome effect was reflected in the QTL results with an associated R^2 value of 62.86 (Table 10). Of the four

QTL identified as impacting sedimentation value, QSev.mst-1B on chromosome 1B was the only QTL where Mountrail was the positive allele.

CHAPTER 6

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 of 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, likely 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 from both Choteau and Mountrail were identified. Specifically, alleles from Mountrail increasing kernel weight were identified on chromosomes 3B and 7A that could be incorporated into bread wheat germplasm. Also, an allele from Choteau increasing test weight on chromosome 7A could be incorporated into durum germplasm. These alleles should be further investigated in additional hexaploid and tetraploid backgrounds to better understand their behavior with potential to improve modern bread and durum wheat cultivars.

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