# GENETIC DISSECTION OF GRAIN YIELD AND YIELD COMPONENT TRAITS IN HEXAPLOID SPRING WHEAT

by

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# ABSTRACT

Hexaploid wheat accounts for 30% of global grain production, ranking in the top three major food crop species along with maize and rice. Grain yield from hexaploid wheat is an important agronomic consideration for sustainable agriculture. As the human population continues to grow and the amount of farmable land decreases it is imperative that a focus be placed on improving grain yield performance. Grain yield is a quantitative trait and as such improved performance is largely influenced by genetic variation, environment and genotype x environment interactions. Due to the quantitative nature of grain yield the mechanisms of genetic control are largely unknown. The purpose of the presented research was to genetically dissect grain yield and yield component traits in hexaploid spring wheat grown in Montana in order to leverage new understanding to improve Montana germplasm and future breeding programs. This investigation included three research aims: (i) to determine the genetic impact of introgressed durum yield component alleles on hexaploid spring wheat agronomic and end-use quality performance (Chapters 2 and 3); (ii) investigate how resource availability as simulated by plant competition and seed density impacted yield component allele response at four yield component quantitative trait loci (Chapter 4); and (iii) to better understand the mechanism of genetic control of *QTn.mst-6B* a quantitative trait locus associated with tiller number through high-resolution mapping (Chapter 5). This research highlights the complexity of pleotropic interaction among yield component traits and variability associated with grain yield as impacted by environment and resources availability. Results from the three aims provide a detailed investigation of single quantitative trait loci for use as novel sources of cultivar improvement and increased genetic gain as well as, a better understanding of grain yield and yield component traits.

# CHAPTER ONE

## INTRODUCTION

Globally wheat (*Triticum aestivum* L., 2n = 6x = 42) is among the top three staple cereal crops along with maize (*Zea mays* L.) and rice (*Oryza sativa* L.) accounting for 761.9 million tonnes of annual global grain production (FAOSTAT, 2018). In the US 15.2 million ha of wheat are in production of which Montana is ranked the third largest production state for wheat area (USDA Small Grains 2020 Summary, 2020). Additionally, wheat is an important component of diet accounting for 20% of calories consumed by the average human (FAOSTAT, 2019). Further, wheat is a valuable food crop in the US and accounts for more than a 8.9 billion-dollar market value (USDA Crop Value 2019 Summary, 2019). Therefore, it is largely recognized that wheat is a staple in US agriculture and the need to produce secure and sustainable wheat is essential to support the global food demand.

Hexaploid wheat is an allopolyploid containing three complete copies of the A, B and D genomes. Hexaploid wheat was derived from two naturally occurring independent hybridization events about 10,000 years ago. The first hybridization occurred between *Triticum urartu* Thumanian ex Gandilyan (2n=2x=14), the A genome donor, and *Aegilops speltoides* Tausch (2n=2x=14), the B genome donor. This hybridization resulted in the development of tetraploid emmer wheat (*Triticum turgidum* L. spp. *dicoccoides*, 2n=4x=28). Further domestication of emmer wheat lead to the cultivation of durum wheat (*Triticum turgidum* L. ssp. *durum*, 2n=4x=28). The second hybridization event occurred between tetraploid emmer wheat with the A and B genomes to *Aegilops tauschii* Coss. (2n=2x=14) the D genome donor resulting in hexaploid wheat (Feuillet et al., 2007; Faris, 2014; Kabbaj et al., 2017). Introduction of wheat to

the US is considered to have occurred between 1860 and 1870 with introduced germplasm tracing center of origin back to the Middle East fertile crescent (Cox, 1991; Dubcovsky and Dvorak, 2007; Feuillet et al., 2007). Among the first introduced cultivars of hexaploid wheat from the fertile crescent included Turkey and Marquis which subsequently became the foundation of many US breeding programs, greatly limiting the genetic variation of the wheat germplasm (Cox, 1991). As such hexaploid wheat has undergone a genetic bottleneck resulting in a loss of genetic variation, in part due to the polyploidization events leading to formation of hexaploid wheat, as well as domestication and modern breeding (Cox, 1991; Haudry et al., 2007; Cavanagh et al., 2013).

Domestication of wheat through the selection of traits such as growth habit, grain hardness and kernel color has led to the classification of six primary market classes each determined by end-use and adaptability to growing region (Tilley et al., 2012). These market classes include hard red winter, hard-red spring, soft-red winter, soft-white, hard-white and durum wheat (US Wheat Association, 2016; Tilley et al., 2012). The hard-red spring market class accounts for the majority of wheat area grown in Montana with 1.5 million ha planted in 2020 (USDA Small Grains 2020 Summary, 2020). Milled flour from hard red spring wheat (HRSW) grain is used primarily for the baking of leavened bread (Zilić et al., 2011; Tilley et al., 2012; Jung and Seo, 2014). Key considerations in the improvement of HRSW is end-use quality and agronomic performance.

Characteristics important for improvement of end-use quality and agronomic performance are typically quantitative in nature. A key agronomic characteristic considered in the improvement of wheat is grain yield (GY). Grain yield in wheat results from interactions

among underlying yield component traits, often negatively correlated (Sreenivasulu and Schnurbusch, 2012; Slafer et al., 2014). Grain yield component traits include productive tiller number (PTN, also referred to as spikes per plant), seeds per spike (SPS), spikelet number per spike (SNS), and individual kernel weight (KWT) (Yoshida, 1972; Slafer, 2003; Xing and Zhang, 2010; Nadolska-Orczyk et al., 2017). Pleiotropic interactions between yield component traits largely limits GY improvement (Slafer, 2003; Gupta et al., 2006; Sadras, 2007). Improvement of GY is further complicated because GY and yield component traits are largely influenced by environment and genotype x environment interactions resulting in variable allelic performance across environments (Allard and Bradshaw, 1964; Reynolds et al., 2002; Kuchel et al., 2007b; El-Soda et al., 2014; Slafer et al., 2014; Mohammadi et al., 2015).

Environment is a key consideration in selecting for improved GY. Abiotic factors impacting GY can include temperature and moisture. Growing season temperature significantly impacts grain establishment and grain filling as this physiological time point corresponds to the hottest part of the season (Porter and Gawith, 1999; Gourdji et al., 2012). Optimum temperatures range between 18 to 22 degrees Celsius (Porter and Gawith, 1999; Lanning et al., 2010). Increase in temperature results in earlier senescence and a decrease in the grain filling period ultimately resulting in poor GY (Porter and Gawith, 1999). Lanning et al. (2010) evaluated experimental HRSW lines in Montana environments and associated a negative correlation in GY with increased temperatures in July due to a reduction in KWT. Gourdji et al. (2012) suggests that in order to improve GY germplasm must be developed that shows tolerance to heat stress. Available moisture is also key to increased GY (Lollato et al., 2019). Torrion and Stougaard (2017) investigated HRSW cultivars under several irrigation regimes and noted that a lack of moisture

during the grain filling period significantly reduced final GY. This reduction in GY is likely due to a lack of available moisture triggering the early onset of plant senescence (Talbert et al., 2001; Woo et al., 2013; Torrion and Stougaard, 2017). Increasing GY performance in relation to environment and genetic variation is a complex problem and is unlikely to be solved with a single strategy.

In the last decade improved phenotyping and genotyping methods have been used in an attempt to increase GY in HRSW (Tester and Langridge, 2010). Primary of these strategies is the utilization of wide crosses made to hexaploid wheat in order to introduce novel allele combinations (Trethowan et al., 2011; Li et al., 2018; Hao et al., 2019). Hao et al. (2019) investigated progeny from synthetic wheat crossed to hexaploid wheat and detected a 30% improvement in GY performance in progeny containing 17% of the synthetic wheat introgressions compared to locally adapted wheat lines. Association analysis and marker assisted selection (MAS) has also been a tool for improvement of GY in breeding populations specifically for the selection of low heritability traits that require costly phenotyping (Gupta et al., 2010; Tester and Langridge, 2010; Mwadzingeni et al., 2016). Building upon MAS the focus of large-scale genomic selection tools which allow for the accurate selection of low heritability yield component traits while reducing generation cycling time and phenotyping costs has also become a relevant strategy (Tester and Langridge, 2010; Juliana et al., 2019). The deployment of the before mentioned strategies have greatly improved the understanding of GY and increased germplasm performance. However, with a continually growing population and annual GY increases plateauing between 0.5% to 1.0% it is imperative that GY increases are continually improving to meet the growing demand (Dixon et al., 2009; Hawkesford et al., 2013; Tshikunde

et al., 2019). Hence, a more thorough genetic dissection of the control mechanisms of GY may help to exploit untapped genetic resources and enhance genetic progress.

The purpose of this body of research was to genetically dissect GY and yield component traits in hexaploid spring wheat grown in Montana. This investigation included three research aims: (i) to determine the genetic impact of introgressed durum yield component alleles on hexaploid spring wheat agronomic and end-use quality performance (Chapters 2 and 3); (ii) investigate how resource availability as simulated by plant competition and seed density impacted yield component allele response at four yield component QTL (Chapter 4); and (iii) to better understand the mechanism of genetic control of *QTn.mst-6B* a QTL associated with tiller number through high-resolution mapping (Chapter 5). This research highlights the complexity of pleotropic interaction among yield component traits and variability associated with GY as impacted by environment and resources availability. Results from the three aims provide a detailed investigation of single QTLs for use as novel sources of cultivar improvement and increased genetic gain as well as, a better understanding of GY and yield component traits.

# CHAPTER TWO

# IMPROVING HEXAPLOID SPRING WHEAT BY INTROGRESSION OF ALLELES FOR YIELD COMPONENT TRAITS FROM DURUM WHEAT

## Contribution of Authors and Co-Authors

Manuscript(s) in Chapter(s) 2

Author: Brittney H. Jones

Contributions: conceived study, experimental work including design, data collection, statistical analysis and manuscript writing and preparation

Co-Author: Nancy K. Blake

Contributions: development of SXD near-isogenic lines, field design, and phenotypic data collection

Co-Author: Hwa-Young Heo

Contributions: field design, phenotypic data collection, field harvest

Co-Author: Jay R. Kalous

Contributions: quantitative trait loci analysis for the identification of durum yield component loci

Co-Author: John M. Martin

Contributions: aided in the writing of statistical code and contributed to the analysis and interpretation of data

Co-Author: Jessica A. Torrion

Contributions: field design and phenotypic data collection

Co-Author: Luther E. Talbert

Contributions: initiated and coordinated the project, contributed to data analysis and final manuscript writing and preparation

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# Improving Hexaploid Spring Wheat by Introgression of Alleles for Yield Component Traits from Durum Wheat

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Abbreviations:
DAP, Days after planting
FLS, Flag leaf senescence
FHB, Fusarium head blight
GLDAH, Green leaf duration after heading
GY, Grain yield
HD, Heading date
HIF, Heterogeneous inbred family
HT, Height
KWT, Kernel weight
NIL, Near isogenic line
PCR, Polymerase chain reaction
PTN, Productive tiller number
QTL, Quantitative trait loci
RIL, Recombinant inbred line
SNS, Spikelet number per spike
SPS, Seeds per spike

## ABSTRACT

Hexaploid bread and tetraploid durum wheat have been cultivated in similar geographic areas for approximately 10,000 years. The crossing barrier caused by ploidy difference suggests that different favorable alleles for yield-related traits may have accumulated in the two crops. Previous work allowed identification of favorable alleles at six quantitative trait loci (QTL) from durum wheat in a recombinant inbred line (RIL) population from a cross of Mountrail durum and Choteau spring wheat. The purpose of this study was to determine the impact of six durum alleles at yield component QTL in several spring wheat backgrounds. Three spring wheat cultivars were crossed with six hexaploid lines derived from the original Choteau/Mountrail cross to generate RIL. Heterozygous RIL, containing both the durum and the bread wheat alleles, were identified for each of the QTL. The heterozygous RIL were used to develop near-isogenic lines (NIL) for the six introgressed QTLs. The NIL were grown in five environments under irrigated and rainfed conditions in Montana in 2017 and 2018. A durum allele QTL on chromosome 3B resulted in increased kernel weight in all five environments. The introgressed durum QTL alleles caused pleiotropic interactions among yield component traits. Environment and genetic background significantly impacted the stability of introgressed QTL on yield components for four of the six QTL. Results suggest that alleles from durum may be useful for yield improvement of hexaploid spring wheat. However, interrelationships of yield components, pleiotropic interactions, and environment will impact the value of durum wheat alleles in hexaploid wheat backgrounds.

## INTRODUCTION

Modern plant breeding has subjected crop species to a bottleneck effect resulting in the reduction of genetic variance and diversity (Shi and Lai, 2015; Gaut et al., 2018). Genome-wide sequence comparison in maize (*Zea mays*) (Clark et al., 2004; Hufford et al., 2012), rice (*Oryza sativa*) (Zhu et al., 2007; He et al., 2011; Huang et al., 2012), and soybean (*Glycine max*) (Lam et al., 2010; Zhou et al., 2015) indicate reduced genetic diversity of crop germplasm compared to wild progenitors. Maize landraces are estimated to have retained 83% of nucleotide diversity of wild progenitor Z. *parviglumus* (Hufford et al., 2012), while soybean is estimated to have retained only half the of nucleotide diversity of its progenitor *G. soja* (Zhou et al., 2015). Bread wheat has undergone a genetic bottle neck resulting in maintenance of approximately 69% of the genetic variation observed in ancestral species, while durum wheat retains 84% of ancestral variation (Haudry et al., 2007). The lack of diversity within crops has led to efforts identifying useful genes from related species for introgression into elite germplasm.

Genetic diversity within cultivated wheat has been impacted not only by domestication and modern breeding, but also due to the polyploidization event leading to the formation of hexaploid bread wheat. Bread wheat (*Triticum aestivum*, 2n=6x=42) is an allopolyploid containing complete copies of genomes A, B and D. The domestication history of hexaploid bread wheat included two independent allopolyploid hybridizations. The first hybridization event between A genome donor *Triticum urartu* (2n=2x=14) and a B genome donor related to *Aegilops speltoides* (2n=2x=14) led to the development of tetraploid emmer wheat (*Triticum turgidum* ssp. *dicoccoides*, 2n=2x=28). Further domestication of emmer wheat led to the development of commonly cultivated tetraploid durum wheat (*Triticum turgidum* ssp. *durum*, 2n=4x=28). A

second hybridization between tetraploid emmer wheat and the D genome donor *Aegilops tauschii* (2n=2x=14) gave rise to hexaploid wheat (*Triticum aestivum*) (Feuillet et al., 2007; Faris, 2014; Kabbaj et al., 2017).

While polyploidy has created barriers for crossing between wheat and its progenitors, the genome redundancy also creates opportunities for genetic improvement through trait introgression. Genes are found in triplicate; thus, loss of a specific chromosome segment is likely to be compensated by homoeologous copies of the gene (Lachowiec et al., 2016). As a result, replacement of chromosome segments in wheat with segments from wild relatives may have fewer deleterious side effects than with diploid crops. The introgression of resistance genes from wild relatives has improved the robustness of wheat in response to pests and disease. The wild grass relatives of wheat *Aegilops tauschii* and *Agropyron* have been used to confer resistance genes to wheat streak mosaic virus (Friebe et al., 1996). Several species from the Poaceae grass family including rye (*Secale cereale*) and *Dasypyrum villosum* have also been used to introduce genes conferring resistance to powdery mildew (Hao et al., 2018; Zhang et al., 2018a).

Durum wheat is an attractive source for introgression of useful genes into hexaploid wheat. The domestication bottleneck caused by formation of durum wheat is not exacerbated by crossing barriers with its progenitor emmer wheat (Kabbaj et al., 2017). Durum wheat and hexaploid wheat share two of three genomes, allowing for successful chromosome pairing and genetic exchange. However, the aneuploid nature of the hybrids leads to a high degree of sterility and causes challenges in the production of fertile progeny. Despite these challenges, useful genes for qualitative traits have been introduced into hexaploid wheat from tetraploid wheat. For example, the durum parent ‘Iumillo’ was used to transfer stem rust (*Puccinia graminis* f. sp.

*tritici*) resistance genes *Sr9g/Yr7* into hexaploid wheat and subsequently into the commonly used hexaploid cultivar 'Thatcher' (Sharma and Gill 1983). Resistance genes *H9* and *H10* to the Hessian fly (*Mayetiola destructor*) were transferred from *Triticum turgidum* into bread wheat (Sharma and Gill, 1983). A gene for high grain protein, an important characteristic for end-use quality in bread wheat, was introgressed from *Triticum turgidum* ssp. *dicoccoides* (Mesfin et al., 2000).

Desirable agronomic traits for both durum and bread wheat are similar in regard to yield and yield components. The ploidy differences suggest that favorable alleles for quantitative traits may have been derived separately in the two species during 10,000 years of cultivation in the same areas. A major challenge for utilization of alleles for quantitative traits from durum wheat is that identification of QTL requires large populations which are difficult to obtain in interspecific crosses (Collard et al., 2005; Bernardo 2008; Xu et al., 2017). Lanning et al., (2008) tested multiple cross combinations between durum and hard red spring wheat lines to identify a cross-combination that generated a high number of viable progeny. The cross combination of hexaploid spring wheat 'Choteau' (Lanning et al., 2004) (PI 633974) by tetraploid durum wheat 'Mountrail' (Elias and Miller, 2000) (PI 607540) was used to develop a population of recombinant inbred lines (RIL) with both tetraploid and hexaploid chromosome constitutions.

Kalous et al. (2015) used the Choteau/Mountrail cross to develop a population of 205 RIL consisting of 117 hexaploid lines and 88 tetraploid lines. A QTL analysis for yield and yield component traits was conducted over four environments. Kalous et al. (2015) identified six alleles from durum wheat which had a positive impact on yield or yield components in hexaploid

RIL. The QTL were identified across the A and B genomes. No QTL were identified on the D genome as this was inherited as a complete genetic entity from the hexaploid Choteau parent. Quantitative trait loci were designated *QGw.mst-3B*, *QGw.mst-7A*, and *QGw.mst-6B* for kernel weight, *QYld.mst-2B* for yield, *QKps.mst-2A* for seeds per spike, and *QTn.mst-5B* for productive tiller number.

A challenge for utilization of QTL alleles is the possibility of background effects whereby an allele from a particular donor parent may exhibit epistatic interaction with the recipient genome. This interaction has been classified as the QTL x genetic background interaction (Blanc et al., 2006; Bernardo, 2008; Jannink, 2008). Pumphrey et al. (2007) introduced the QTL allele *Fhb1* for Fusarium head blight (*Fusarium graminearum*) (FHB) resistance in wheat into nineteen near-isogenic line (NIL) pairs of various genetic backgrounds. In 15 of the 19 NIL pairs the resistance allele resulted in a reduction in FHB infection. However, in the remaining four NIL pairs FHB resistance was reduced by the introduction of the resistant allele, though none of the responses were significant. The change in allele effect demonstrates the potential for epistatic interactions resulting in unfavorable interactions with unknown genes in the recipient genome. Yield component traits are complex and often controlled by many QTL with minor effects with an associated increase in the QTL x genetic background interactions (Bernardo, 2008; Blanc et al., 2006)

Selection for yield components to improve yield is challenged by the inter-relationship among the components. Yield components include number of spikes per area or plant, number of seeds per spike, and kernel weight (Yoshida, 1972; Slafer, 2003; Xing and Zhang, 2010; Nadolska-Orczyk et al., 2017). Spikelet number per spike is often used as a more stable indicator

of number of seeds per spike (González et al., 2011; González-Navarro et al., 2015; Waddington et al., 1983). Yield component traits are often negatively correlated with one another, such that as one trait increases there is a consequential decrease in another yield component. Commonly observed negative correlations between yield components include grain weight to number of grains, number of grains per spike to number of spikes, and grains per spikelet to number of spikelets per spike (Gupta et al., 2006; Sadras et al., 2013; Slafer, 2003, 2014).

The objective of the present research was to introduce six alleles with positive effects on yield components from durum wheat into several hexaploid hard red spring lines. Near-isogenic lines containing either the durum or spring wheat allele at the respective QTL were developed in several backgrounds and tested in five environments. Results address the utility of alleles for quantitative traits from tetraploid durum wheat for the genetic improvement of hexaploid bread wheat.

# MATERIALS AND METHODS

## Plant Materials

A population consisting of NIL differing for durum and spring wheat alleles at QTL across the genome was used to assess the impact of durum alleles on yield-related traits in multiple spring wheat backgrounds. Quantitative trait loci included *QYld.mst-2B* for yield, *QKps.mst-2A* for seeds per spike, *QTn.mst-5B* for productive tiller number, *QGw.mst-3B*, *QGw.mst-7A*, and *QGw.mst-6B* for kernel weight (Table 1). Development of the NIL began with random selection of six hexaploid RIL from the Choteau/Mountrail population (Kalous et al., 2015). These were each crossed with elite spring wheat lines 'Vida' (PI 642366) (Lanning et al., 2006) and 'Duclair' (PI 660981) (Lanning et al., 2011) developed by Montana State University, and 'Berkut' developed by the International Maize and Wheat Improvement Center (CIMMYT). Near-isogenic lines were developed for all six of the QTL following the heterogeneous inbred family (HIF) strategy (Haley et al., 1994; Pumphrey et al., 2007). In short, $F_5$ RIL plants were developed by single seed descent and screened with markers associated with each QTL to identify heterozygous plants. Progeny homozygous for the durum allele and spring allele were selected. Homozygous plants were self-pollinated to generate NIL seed for field testing (Table 1). NILs were planted as pairs such that a NIL pair represents two lines of the same genetic background, distinguishable only by the durum or spring allele at the designated QTL.

## Experimental Design

The confirmation population was evaluated in five field season-years (environments) in randomized complete block designs consisting of three blocks per environment. A total of 82 entries were include in the confirmation population which included 72 NIL and 10 parental

check lines. The experiment was grown under irrigated and rainfed conditions at the Arthur H. Post Research Farm in Bozeman, MT (latitude 45.68°N, longitude 111.04°W, elevation 1469 m) in 2017 and 2018 and at the Northwestern Ag Research Center in Kalispell, MT (latitude 48.19°N, longitude 114.32°W, elevation 900 m) under rainfed conditions in 2018 (Table 2). Bozeman 2017 and 2018 irrigated and rainfed environments were planted 11 May 2017 and 4 May 2018. The Kalispell environment was planted 2 May 2018. The Bozeman location received 19.1 cm and 25.4 cm of precipitation during the 2017 and 2018 growing season (Apr-Jul) with an additional 15.2 cm and 16.5 cm added in the irrigated environments in 2017 and 2018, respectively. Irrigation was applied prior to heading. The Kalispell environment received 15.9 cm of precipitation in the 2018 growing season (Apr-Jul). Plots in Bozeman 2017 consisted of two rows spaced 30 cm apart x 3.0 m, whereas the 2018 plots in Bozeman were three rows spaced 30 cm apart x 4.9 m. Plots in Kalispell in 2018 were seven rows spaced 18 cm apart x 4.6 m. Seeding density for all trials was 269 seed $m^{-2}$ for rainfed trials and 323 seed $m^{-2}$ for irrigated trials. Bozeman 2017 and 2018 rainfed environments were harvested on 23 Aug. 2017 and 10 Sept. 2018. Additionally, Bozeman 2017 and 2018 irrigated environments were harvested on 29 Aug. 2017 and 19 Sept. 2018. Kalispell 2018 rainfed environment was harvested on 1 Sept. 2018.

## Phenotypic Data Collection

Phenotypic data collected in all experiments included phenological, morphological, and yield component traits (Table 2). Traits included heading date (HD), flag-leaf senescence (FLS), green leaf duration after heading (GLDAH), and plant height (HT). Yield and yield component

traits included productive tiller number (PTN), seeds per spike (SPS), kernel weight (KWT), spikelet number per spike (SNS), and grain yield (GY).

Heading date was determined as days after planting (DAP) when 50% of spikes had fully emerged from the boot. Flag leaf senescence was determined as DAP when 50% of flag leaves had become 100% chlorotic. Green leaf duration after heading was the number of days between HD and FLS. Plant height was measured from the base of the plant to the top of the spike, excluding the awns. Productive tiller number, defined as the number of tillers producing a fertile spike, was determined by counting a meter section of row near plant maturity. Seeds per spike was determined as average number of seeds from five randomly sampled spikes per plot. Kernel weight was characterized by taking 1000-seed weights per plot and converting to a single kernel weight. Spikelet number per spike was determined as the average number of fertile spikelets from five randomly sampled spikes per plot. Grain yield was determined from the raw grain weight of each plot.

### Genotypic Characterization of *Vrn-A1* and *Vrn-B1* Vernalization Loci

Pairs of near-isogenic lines for *QTn.mst-5B* were further genotyped in order to characterize alleles present at the *Vrn-A1* and *Vrn-B1* vernalization loci located on chromosomes 5A and 5B, respectively. Primer sets used for the amplification of allelic variation at *Vrn-A1* and *Vrn-B1* between NIL have been previously described (Fu et al., 2005; Yan et al., 2004; Zhang et al., 2008) and included primer pairs VRN1AF / VRN1-INT1R, Intr/B/F / Intr1/B/R3 and Intr/B/F / Intr1/B/R4. Polymerase chain reaction (PCR) conditions followed previously published protocols (Zhang et al., 2008). Amplified PCR products were separated and visualized for primer

pair VRN1AF / VRN1-INT1R on 2.5% agarose gel and additional primer pairs on 1% agarose gels.

## Statistical Analysis

Statistical analyses of all phenological and yield component traits used SAS software, version 9.4 (SAS Institute Inc. 2012). Data for each of the phenotypic traits sorted by QTL were analyzed via analysis of variance (ANOVA) using a model for a randomized complete block design for each environment, where environment was determined by year and rainfed versus irrigated. An analysis of variance was conducted using PROC GLM data combined over environments where the model included environment, replication within environment, NIL pair, allele and their interactions. All factors were considered fixed effects. PROC GLM was used to calculate least significant differences (LSD) among parental check means and environmental means.

## RESULTS

Near-isogenic lines varying for yield component QTL were grown in five environments including both rainfed and irrigated treatments. Table 2 shows climatological data for the five growing environments and average grain yield averaged over all lines in the confirmation population. A distinct advantage regarding resource availability was observed between the 2017 and 2018 environments. The highest amount of moisture was in Bozeman 2018 environments and the lowest was in Bozeman 2017 environments, representing a 1.3-fold increase. The increase in precipitation in the 2018 environments resulted in significantly increased average grain yield based on LSD pair-wise comparison of mean grain yield over environments (Table 2). The highest mean grain yield was in the Bozeman 2018 irrigated environment with a 2-fold increase compared to grain yield in the Bozeman 2017 rainfed environment The Kalispell 2018 rainfed environment had the highest mean grain yield compared to the Bozeman rainfed environments.

Spring wheat and durum wheat parental lines were included in experimental planting design as checks. Table 3 shows data averaged across five environments for yield, yield component traits and phenological characteristics for parental lines. Data not shown for RIL parental lines from the original Choteau/Mountrail cross. Cultivars demonstrated varying yield potential for PTN, KWT, SPS and SNS. Significant variability was also present between the cultivars for HD. Durum wheat is often characterized as having large spikes and large individual kernel weight. Mountrail, a tetraploid durum wheat, had the highest KWT in comparison with the hexaploid wheat parental check cultivars across all environments ($P < 0.05$) as well as a high number of SNS ($P < 0.05$). Vida, a hexaploid spring wheat, had the lowest number of SPS

compared to the other parental checks ($P < 0.05$). Hexaploid parents Vida, Duclair and Choteau demonstrated high PTN compared to hexaploid spring wheat cultivar Berkut and the tetraploid durum parent Mountrail ($P < 0.05$).

An allele from the durum cultivar Mountrail at *QGw.mst-3B* was shown by Kalous et al. (2015) to cause an increase in kernel weight relative to the alternative allele from spring wheat Choteau. Table 4 shows data averaged over five environments for yield component and phenological traits for three pairs of NIL varying for the spring and durum alleles at *QGw.mst-3B*. The allele from durum wheat was associated with a 1.8 mg increase in KWT relative to the allele from spring wheat ($P < 0.001$). Grain yield did not differ for NIL pairs with the alternative alleles ($P > 0.05$). However, the 2017 Bozeman irrigated environment resulted in a significant GY increase ($P < 0.05$) as well as an increase in KWT ($P < 0.001$) and number of SPS ($P < 0.05$) in association with the durum allele. Conversely, the 2018 Kalispell rainfed environment resulted in increased KWT ($P < 0.01$) and a decrease in SNS ($P < 0.05$) associated with the durum wheat allele (Table 4). Significant phenological differences between alleles included an earlier HD of 0.08 days ($P < 0.001$) for lines with the allele from durum wheat.

The positive association of the durum allele at *QGw.mst-3B* on KWT was significantly impacted by pair x allele and environment x allele interactions (Table 4). Only one of the three NIL pairs showed a significant increase in KWT associated with the durum allele ($P < 0.001$) (Supplemental Table 1). An environment x allele interaction was also observed for KWT, with three of the five environments showing a significant KWT increase associated with the allele from durum wheat (Table 4).

An allele from the durum wheat cultivar Mountrail at *QGw.mst-7A* was shown by Kalous et al. (2015) to cause an increase in kernel weight relative to the alternative allele from spring wheat. Table 5 shows data averaged over five environments for yield component and phenological traits for seven NIL pairs varying for alleles at *QGw.mst-7A*. The durum wheat and spring wheat NIL did not differ for KWT ($P > 0.05$). However, SNS was significantly decreased by 0.8 spikelets over all environments due to the durum allele relative to the spring wheat allele ($P < 0.001$).

The durum allele at *QGw.mst-7A* which was associated with decreased SNS was significantly impacted by environment and genetic background. There was a significant pair x allele interaction for SNS ($P < 0.05$) (Table 5). The durum allele impact was negative for SNS in all seven NIL pairs in all hexaploid backgrounds including Vida, Berkut and Duclair (Supplemental Table 1). A significant environment x allele interaction was observed for PTN and SNS. The environment x allele interaction for SNS was due to a magnitude change in the impact of the durum allele for SNS across the five environments ($P < 0.05$).

An allele from the durum cultivar Mountrail at *QGw.mst-6B* was shown by Kalous et al. (2015) to increase kernel weight relative to the alternative allele from spring wheat Choteau. The NIL pairs did not differ for durum and spring alleles for any yield component traits. Flag leaf senescence was significantly earlier by 0.4 days for the durum allele relative to the spring wheat allele ($P < 0.05$). There was no significant difference between the durum allele and spring allele for other phenological characteristics ($P > 0.05$). Supplemental Table 2 shows data averaged over five environments for yield component and phenological traits for seven NIL pairs varying for alleles at *QGw.mst-6B.*An allele from the durum cultivar Mountrail for *QTn.mst-5B* was

shown by Kalous et al. (2015) to increase productive tiller number relative to the spring wheat Choteau allele. Table 6 shows data averaged over five environments for yield component and phenological traits for six NIL pairs varying for spring wheat or durum wheat alleles *at QTn.mst-5B.* The allele from durum wheat was associated with a 19.8 PTN $m^{-1}$ reduction relative to the spring wheat allele ($P < 0.001$). Grain yield was also significantly decreased by 609.6 kg $ha^{-1}$ in association with the durum allele ($P <0.001$). Additionally, the durum allele was associated with a significant increase in KWT ($P < 0.0001$) and SNS ($P < 0.0001$) relative to the spring wheat allele. Significant differences associated with the durum allele were observed for phenological characteristics including a postponement of HD by 3.5 days and later FLS by 3.1 days relative to the spring wheat allele.

The impact of the durum allele for *QTn.mst-5B* which decreased PTN and postponed maturity was significantly influenced by the environment. Significant environment x allele interactions due to magnitude changes were seen for HD ($P < 0.05$) (Table 6). The durum allele resulted in a significantly later HD in all five of the environments tested. Spikelet number per spike was also impacted by environment x allele interaction, resulting from a magnitude change across environments. Additional pair x allele interactions were observed for all agronomic characteristics excluding GLDAH, SPS and KWT ($P > 0.05$) such that three NIL pairs out of six showed a significant reduction in PTN and postponement in HD and FLS due to the durum allele (Supplemental Table 1). The physical proximity of the *QTn.mst-5B* (peak marker at 5.9 Mb region) is near the *Vrn-B1* (5.7 Mb region) locus on chromosome 5B (IWGSC RefSeq v1.0 5B pseudomolecule; IWGSC, 2018.). NIL pairs for *QTn.mst-5B* differed for alleles at *Vrn-A1*.Two

of the three NIL pairs at *QTn.mst-5B* had the spring allele at *Vrn-A1* while one pair had the winter allele.

An allele from the durum cultivar Mountrail at *QKps.mst-2A* was shown by Kalous et al. (2015) to increase seeds per spike relative to the spring wheat cultivar Choteau. There were no significant differences between the durum and spring wheat alleles for the NIL for agronomic characteristics ($P > 0.05$) Additionally, environment and genetic background had little impact on agronomic characteristics at *QKps.mst-2A.* Supplemental table 3 shows data averaged over five environments for yield component and phenological traits for seven NIL pairs varying for alleles at *QKps.mst-2A.* An allele from the durum cultivar Mountrail at *QYld.mst-2B* was shown by Kalous et al. (2015) to increase grain yield relative to the spring wheat cultivar Choteau. Table 7 shows the data averaged over five environments for yield component and phenological traits for six NIL pairs varying for alleles at *QYld.mst-2B.* There was no difference in GY between the durum and spring alleles ($P >0.05$). However, there was a significant decrease in KWT of 1 mg associated with the durum allele relative to the spring wheat allele ($P < 0.001$). Additionally, the durum allele was associated with a significant increase of 0.2 SNS relative to the spring wheat allele ($P < 0.05$). Significant differences were observed for phenological characteristics including earlier HD by 0.5 days ($P < 0.01$) and increased GLDAH by 0.7 days ($P < 0.01$) associated with the durum allele.

The durum allele at *QYld.mst-2B* did not show significant environment x allele interaction for agronomic traits, suggesting stability of the durum allele across multiple environments. Significant pair x allele interactions were observed for KWT and SNS differences at *QYld.mst-2B* (Table 7). Only two of the six NIL pairs showed a significant decrease in KWT

in association with the durum allele (Supplemental Table 1). Additionally, only two NIL pairs showed a significant increase in SNS in association with the durum allele relative to the spring wheat allele (Supplemental Table 1).

## DISCUSSION

Durum wheat and bread wheat have been grown in similar agricultural environments for approximately 10,000 years. The genetic separation of the species due to ploidy differences suggests that favorable alleles at different loci for yield-related traits may have been selected. Kalous et al. (2015) identified several favorable alleles from durum wheat at QTL for yield and yield components in a RIL population derived from a tetraploid durum wheat by hexaploid spring wheat cross. The present study used a NIL population to test the impact of the durum allele in several spring wheat backgrounds and in several environments.

The NIL population showed allele effects on yield and yield component traits matching those identified by Kalous et al. (2015) for two of the six QTL (*QGw.mst-3B* and *QTn.mst-5B*). Yield components not previously identified by Kalous et al. (2015) varied significantly between NILs for two of the six QTLs (*QGw.mst-7A* and *QYld.mst-2B*). Two of the QTLs identified by Kalous et al. (2015) showed no significant differences among the NIL with alternative alleles in the present study (*QGw.mst-6B* and *QKps.mst-2A*). Pleiotropic effects of the durum alleles on multiple yield traits were commonly observed, as well as significant interactions of alleles with environment and genetic background.

The durum allele at *QGw.mst-3B* was associated with an overall significant increase in KWT across all five environments, confirming the results of Kalous et al. (2015) (Table 4). Quantitative trait loci for KWT in durum wheat (Peng et al., 2003; Maccaferri et al., 2013; Patil et al., 2013) and spring wheat (Sun et al., 2008; Ramya et al., 2010; Li et al., 2015), based on intraspecific crosses, have been previously reported, though none were identified on

chromosome 3B. This study confirms the impact of an introduced allele from durum wheat on chromosome 3B for increased KWT in a hexaploid background.

One unexpected result from Kalous et al. (2015) was that the hexaploid lines from the durum wheat by bread wheat cross had larger kernels and kernel weight than the tetraploid lines derived from the same cross. Thus, the presence of the D genome resulted in higher kernel weight. However, durum wheat typically has higher kernel weight than does bread wheat. This study shows that Mountrail durum wheat had higher kernel weight than the bread wheat cultivars (Table 3). This suggests that durum wheat possesses alleles in the A and B genome for increased kernel weight. The results of this study show that one of these alleles is at *QGw.mst-3B*. Frequency of the durum haplotype at *QGw.mst-3B* was determined by genotype comparison of hexaploid spring wheat lines from the Spring Wheat AM Panel (deposited in The Triticeae Toolbox (T3); https://triticeaetoolbox.org/wheat/). Data suggest that the durum haplotype was present in only 7% of hexaploid wheat lines. Thus, *QGw.mst-3B* may represent a unique source for improved kernel weight in hexaploid wheat.

The allele from durum wheat at *QTn.mst-5B* was identified as increasing PTN relative to the spring wheat allele by Kalous et al. (2015). In the present study, the durum allele at *QTn.mst-5B* resulted in a significant negative impact on PTN based on mean over environments for three of six NIL pairs (Table 6 and Supplemental Table 1). In the original Choteau/Mountrail population, hexaploid RILs varying for the durum and spring allele produced a mean of 136 PTN $m^{-1}$ across five environments, a 10% increase compared with the tetraploid RILS derived from the same cross, suggesting that the D genome positively impacts PTN (Kalous et al., 2015). In this study the hexaploid parent Choteau produced 34% higher PTN than the tetraploid parent

Mountrail (Table 3). Vida demonstrated the highest number of PTN produced relative to Mountrail and Choteau with a 14 % increase compared to Choteau (Table 3). Reduction in PTN was observed for three of the six NIL pairs. All three of the impacted NILs were from a cross with Vida (Supplemental Table 1). One potential explanation for the reversal of the durum allele impact at *QTn.mst-5B* in the hexaploid background may be epistatic interaction with the other loci for PTN segregating in the Vida-derived NIL pairs. Additionally, the Vida-derived NIL pairs also differed for genotype at vernalization gene *Vrn-A1*, such that two NIL pairs had the spring allele and the third pair the winter allele. This difference however did not impact expression of the *QTn.mst-5B*, as the three NIL pairs that showed significant reduction in PTN for *QTn.mst-5B* varied for *Vrn-A1* genotype. Differences within NIL pair were observed for alleles at the *Vrn-B1* locus such that lines within a pair varied for the spring or winter type allele. It is therefore likely that with the physical proximity of the *QTn.mst-5B* peak and *Vrn-B1* gene may have resulted in the co-selection of segregating allele types in the NIL pairs at both loci.

The durum alleles at *QGw.mst-7A* and *QYld.mst-2B* impacted yield component traits other than the one initially identified by Kalous et al. (2015). Kalous et al. (2015) did not measure SNS in the Choteau/Mountrail RIL population therefore it is possible that had this trait been measured QTL for SNS may have been identified. This result is also suggestive of the pleiotropic nature of yield component traits in the current study. The durum allele at *QGw.mst-7A* for KWT was associated with a decrease in SNS rather than an increase in KWT (Table 5). Thus, interpretation of results from the present study with the NILs would likely prompt designation of the allele from hexaploid wheat as being the positive allele due to its impact on spikelet number. Kalous et al. (2015) defined the alternative allele as positive due to its impact on KWT. Additional QTL

associated with KWT (Su et al., 2016), and SNS (Faris et al., 2014; Zhang et al., 2018b) have been mapped to the long arm of chromosome 7A in a similar location to *QGw.mst-7A*. Results from this study show little pleiotropic interaction between the durum allele at *QGw.mst-7A* and additional yield component traits (Table 5). However, the recurrent identification of this co-located QTL effecting KWT and SNS suggests a strong negative pleiotropic interaction. The synonymous QTL to *QGw.mst-7A* identified by Zhang et al. (2018b) has undergone further characterization and positional cloning efforts resulting in the identification of four candidate genes (Kuzay et al., 2018). A plausible candidate gene is *WHEAT ORTHOLOG OF APO1 (WAPO1)*, which is the ortholog to *ABERRANT PANICLE ORGANIZATION 1 (APO1)* in rice and is associated with floral organ and panicle development (Kuzay et al., 2018).

An important issue for utilization of alleles for improving a specific yield component is the typically antagonistic nature of pleiotropic effects. The durum allele at *QYld.mst-2B* was associated with a decrease in kernel weight and increase in SNS (Table 7). Though there was no observed increase in GY in this study as also reported in Kalous et al. (2015), the decrease in KWT and increase in SNS shows an impact on yield components. In the present study, the negative relationship between these yield components resulted in no impact on grain yield. Increased grain yield requires that improvement in one yield component is not associated with a concomitant decrease in a different yield component. This necessity may explain the lack of allele impact on grain yield and the associated lack of allele x environment and allele x pair interactions.

The durum alleles at *QGw.mst-6B* (Supplemental Table 2) and *QKps.mst-2A* (Supplemental Table 3) showed no significant impact on the selected yield component trait as

identified by Kalous et al. (2015). The lack of significant impact associated with *QKps.mst-2A* may be explained by the small effect size associated with the QTL in the Kalous et al. (2015) study (Table 1). Additionally, the genetic backgrounds used in the present study had a higher percentage of hexaploid background than the population of Kalous et al. (2015). This may have led to loss of positive epistatic interactions between durum alleles. An impact of *QGw.mst-6B* was only identified in one of four environments in the original Kalous et al. (2015) study, also suggesting a potentially minor effect QTL.

Phenological traits may influence yield and yield components (Trethowan et al., 2001; Blake et al., 2009; González et al., 2011; Jobson et al., 2019). For instance, GLDAH has been associated with an increase in KWT in spring wheat (Talbert et al, 2001; Blake et al., 2007; Naruoka et al., 2012). Four the six introgressed durum QTLs including *QGw.mst-3B, QGw.mst-6B, QTn.mst-5B* and *QYld.mst-2B,* were pleiotropically associated with HD, FLS and GLDAH. Earlier heading was associated with the durum allele at *QGw.mst-3B,* potentially impacting and leading to an overall increase in KWT (Table 4). Green leaf duration after heading was increased in association with the durum allele at *QYld.mst-2B,* as well as a decrease in KWT and increase in SNS (Table 7). This result may be caused by allocation of resources to developing kernels for available spikelets per spike rather than increasing overall kernel weight. Heading date is impacted by photoperiod response controlled by the expression of *Ppd-A1*, *Ppd-B1* and *Ppd-D1* genes on chromosomes 2A, 2B and 2D respectively (Blake et al., 2015). The loci *QKps.mst-2A* and *QYld.mst-2B* located on group two chromosomes are physically mapped to the long arm of 2A and 2B, respectively, whereas both *Ppd-A1* and *Ppd-B1* are located on the short arms of the respective chromosomes (Beales et al., 2007). The large physical distance between

the QTL and *Ppd* genes suggests a lack of linkage between the loci. The *QTn.mst-5B* QTL had a large impact on HD and FLS. The durum allele appears to be linked to variation in the alleles located at the 5B *Vrn-B1* gene. Segregation of winter and spring alleles at *Vrn-B1* in the NILs may provide an explanation in the observed facultative winter type phenotypes resulting in later HD and FLS (Levy and Peterson, 1972; Blake et al., 2009). Results from this study suggest a strong interaction between yield component traits and plant phenology and an associated impact on overall germplasm improvement.

Resource availability largely impacts yield potential in field crops (Nasseer et al., 2016). Yield and yield component traits are often impacted by high environment by genotype interactions resulting in changes in relative genotype performance and stability across environments (Allard and Bradshaw, 1964; Kang et al., 2004; El-Soda et al., 2014). Environment x allele interactions were observed for four of the six QTL in the present study. The durum allele at *QGw.mst-3B* was associated with a highly significant environment x allele interaction for KWT (Table 4). However, the association of KWT with the durum allele was always positive, suggesting environmental resources influence the expression of the allele. *QTn.mst-5B* was also impacted by environment and genotype interactions such that SNS, HD and GLDAH all demonstrated significant environment x allele interactions (Table 6), resulting in various trait responses associated with the durum allele. Heading date was significantly different between NIL pairs for the durum and spring wheat alleles. However, the magnitude of difference varied between 2017 and 2018 environments (Table 6). The difference in HD may be due to temperature differences between the 2017 and 2018 environments, resulting in a facultative vernalization response in the cooler 2018 year. Notably, there was no observed environment x

allele interaction associated with *QYld.mst-2B* and *QKps.mst-2A*. The durum allele at *QKps.mst-2A* had no impact on agronomic characteristics and there was no corresponding environmental impact. However, the lack of environment x allele interaction associated with *QYld.mst-2B* suggests that the durum allele demonstrates a higher degree of stability. The primary impact of *QYld.mst-2B* may be on SNS which is less impacted by environmental variance due to predetermination of spikelet number during the early stages of plant physiological development (Waddington et al., 1983; González et al., 2011; González-Navarro et al., 2015). Therefore a potential explanation for the lack of environment x allele interaction observed at QYld.mst-2B may be due to the minimal environmental stimulus required to determine SNS early in plant development.

The genetic background also plays an important role in the stability and impact of introgressed alleles. Notably, all six of the introgressed durum yield component QTLs were associated with a significant genetic background by allele interaction (pair x allele). The NIL pairs in this study possessed one-fourth of their alleles from the donor durum wheat parent and three-fourths of alleles from hexaploid bread wheat. These results indicate that the overall impact of introgressed durum alleles is influenced by the selection of genetic background of recurrent line.

In conclusion, this study focused on the confirmation of six durum QTL alleles impacting yield component traits in different hexaploid genetic backgrounds tested in diverse environments. Durum alleles at *QGw.mst-3B* and *QYld.mst-2B* were associated with significant improvement in the spring wheat backgrounds, resulting in an increase of yield component traits over multiple genetic backgrounds and environments. Results from this study suggest that alleles

from durum wheat effecting grain yield may be useful for the improvement of hexaploid spring wheat. However, interrelationships of yield components, environment, and genetic background will determine the value of durum wheat alleles in hexaploid wheat backgrounds.

## SUPPLEMENTAL MATERIAL

*Supplemental materials can be found online at the Montana State University open access repository ScholarWorks*

Supplemental Table 1. Impact of alleles at six yield component quantitative trait loci over five environments and multiple pairs of near-isogenic lines.

Supplemental Table 2. *QGw.mst-6B* allele means by environment for phenological and yield component traits based on seven pairs of near-isogenic lines

Supplemental Table 3. *QKps.mst-2A* allele means by environment for phenological and yield component traits based on seven pairs of near-isogenic lines

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TABLES

**Table 1**. Quantitative trait loci (QTL) for yield and yield-related traits identified in the Choteau/Mountrail recombinant inbred line population for which near-isogenic lines (NIL) were developed.

| Trait | QTL | $R^2$(%)† | Marker | Chromosome | No. NIL Pairs | No. Lines | SW Background |
|---|---|---|---|---|---|---|---|
| Yield | *QYld.mst-2B* | 7.7 | IWB6888 | 2B | 6 | 12 | Berkut, Duclair, Vida |
| Seeds Per Spike | *QKps.mst-2A* | 8.8 | IWB72154 | 2A | 7 | 14 | Berkut, Duclair, Vida |
| Productive Tiller Number | *QTn.mst-5B* | 6.4 | IWB7213 | 5B | 6 | 12 | Berkut, Vida |
| Kernel Weight | *QGw.mst-3B* | 6.1 | IWA6375 | 3B | 3 | 6 | Berkut, Vida |
| | *QGw.mst-7A* | 6.5 | IWB29518 | 7A | 7 | 14 | Berkut, Duclair |
| | *QGw.mst-6B* | NS‡ | IWB53647 | 6B | 7 | 14 | Berkut, Duclair, Vida |

†$R^2$ refers to the percent of phenotypic variance explained
‡This QTL was only detected in one of four environments from Kalous et al. (2015)

**Table 2**. Temperature, precipitation and measured traits for five environments used to compare near-isogenic lines for yield component traits.

| Environments | Year | **Planting Date** | **Total Water† Received** | | **Mean Temperature** | **Harvest Date** | **Mean Grain Yield‡** |
|---|---|---|---|---|---|---|---|
| | | | Annual | Growing Season | Apr-Jul | | |
| | | | ----------*cm*---------- | | *°C* | | *kg ha$^{-1}$* |
| Bozeman, MT - rainfed | 2017 | 11 May | 43.8 | 19.1 | 13.9 | 24 Aug | 3344.7$^{E}$ |
| Bozeman, MT - irrigated | 2017 | 11 May | 59.0 | 34.3 | 13.9 | 29 Aug | 4273.3$^{D}$ |
| Bozeman, MT - rainfed | 2018 | 4 May | 54.0 | 25.4 | 13.3 | 10 Sept | 5610.8$^{C}$ |
| Bozeman, MT - irrigated | 2018 | 4 May | 70.5 | 41.9 | 13.3 | 19 Sept | 6702.6$^{A}$ |
| Kalispell, MT - rainfed | 2018 | 2 May | 43.5 | 15.9 | 13.9 | 1 Sept | 5835.5$^{B}$ |

†Total water received included precipitation and irrigation. Annual precipitation (Sept through Aug of the following year). Growing season precipitation (Apr through Jul).

‡Pair-wise comparison of grain yield using Least Significant Difference (LSD) = 147.91 kg ha$^{-1}$ means with the same letter are not significantly different

**Table 3.** Parental means averaged over five environments for phenological and yield component traits[†].

| | | Phenological Characteristic | | | Yield and Yield Components | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Background | Ploidy | HD | FLS | GLDAH | GY | PTN | KWT | SPS | SNS |
| | | ----------*d*---------- | | | *kg ha$^{-1}$* | *no. m$^{-1}$* | *mg* | ---------*no.*--------- | |
| Choteau | 6X | 55.5$^{C}$ | 92.5$^{A}$ | 36.9$^{A}$ | 5617.46$^{A}$ | 202.18$^{A}$ | 33.10$^{C}$ | 40.51$^{B}$ | 13.24$^{C}$ |
| Mountrail | 4X | 60.3$^{A}$ | 92.3$^{A}$ | 32.0$^{A}$ | 5952.10$^{A}$ | 150.70$^{B}$ | 42.11$^{A}$ | 42.94$^{B}$ | 14.22$^{B}$ |
| Duclair | 6X | 54.6$^{C}$ | 91.9$^{A}$ | 37.3$^{A}$ | 5807.52$^{A}$ | 193.82$^{A}$ | 34.34$^{BC}$ | 39.74$^{B}$ | 13.11$^{C}$ |
| Berkut | 6X | 61.4$^{A}$ | 95.0$^{A}$ | 33.6$^{A}$ | 5873.76$^{A}$ | 148.06$^{B}$ | 37.83$^{B}$ | 51.03$^{A}$ | 15.43$^{A}$ |
| Vida | 6X | 57.8$^{B}$ | 95.8$^{A}$ | 38.0$^{A}$ | 6550.23$^{A}$ | 229.46$^{A}$ | 34.26$^{BC}$ | 34.94$^{C}$ | 11.52$^{D}$ |
| LSD[‡] | | 1.5 | 11.1 | 7.9 | 1781.00 | 41.32 | 3.93 | 3.08 | 0.65 |

[†]PTN, productive tiller number; SPS, seeds per spike; KWT, single kernel weight; SNS, spikelet number per spike; GY, grain yield; HD, heading date (d after planting); FLS, flag leaf senescence (d after planting); GLDAH, green leaf duration after heading (d); HT, plant height

[‡]Least Significant Difference (LSD), means with the same letter are not significantly different

**Table 4.** *QGw.mst-3B* allele means by environment for phenological and yield component traits based on three pairs of near-isogenic lines[†].

| Environment | Allele | Yield and Yield Components | | | | | Phenological Characteristic | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GY | PTN | KWT | SPS | SNS | HD | FLS | GLDAH |
| | | *kg ha$^{-1}$* | *no. m$^{-1}$* | *mg* | *no.* | | *d* | | |
| 2017 Rainfed - Bozeman | Spring Wheat | 3192.5 | 140.1 | 32.6 | 36.2 | 12.8 | 57.6 | 83.4 | 25.9 |
| | Durum Wheat | 3289.3 | 138.2 | 34.4* | 36.7 | 12.5 | 56.9* | 82.0 | 25.1 |
| 2017 Irrigated - Bozeman | Spring Wheat | 3939.3 | 185.5 | 28.0 | 32.9 | 12.6 | 57.7 | 82.8 | 24.1 |
| | Durum Wheat | 4413.8* | 190.7 | 33.2**** | 36.0* | 12.6 | 58.0* | 82.8 | 24.8 |
| 2018 Rainfed - Kalispell | Spring Wheat | 5521.5 | 107.1 | 40.6 | 43.7 | 13.2 | 62.9 | 97.9 | 35.0 |
| | Durum Wheat | 5779.1 | 102.3 | 42.7** | 41.0 | 12.4* | 61.9*** | 97.6 | 35.7 |
| 2018 Rainfed - Bozeman | Spring Wheat | 5168.3 | 181.5 | 37.7 | 42.0 | 14.2 | 61.9 | 98.7 | 36.8 |
| | Durum Wheat | 5348.1 | 200.9 | 38.2 | 41.5 | 14.0 | 61.0* | 98.4 | 37.4 |
| 2018 Irrigated - Bozeman | Spring Wheat | 5751.9 | 222.2 | 42.9 | 43.4 | 13.9 | 61.6 | 105.9 | 44.3 |
| | Durum Wheat | 6655.7 | 225.9 | 42.5 | 42.7 | 13.9 | 60.6*** | 105.3 | 45.0 |
| Mean (overall) | Spring Wheat | 4914.7 | 167.3 | 36.4 | 39.7 | 13.3 | 60.5 | 93.7 | 33.2 |
| | Durum Wheat | 5097.2 | 171.6 | 38.2**** | 39.6 | 13.1 | 59.7**** | 93.3 | 33.6 |
| pair x allele | | NS | NS | **** | NS | NS | *** | NS | NS |
| environment x allele | | NS | NS | *** | NS | NS | NS | NS | NS |
| environment x pair x allele | | NS | NS | *** | NS | NS | ** | NS | NS |

[†]PTN, productive tiller number; SPS, seeds per spike; KWT, single kernel weight; SNS, spikelet number per spike; GY, grain yield; HD, heading date (d after planting); FLS, flag leaf senescence (d after planting); GLDAH, green leaf duration after heading (d); HT, plant height
*, **, ***, **** Spring wheat and durum wheat allele means differ at significance level of $P<0.05$, $P<0.01$, $P<0.001$, and $P<0.0001$, respectively.
NS = not significant

**Table 5.** *QGw.mst-7A* allele means by environment for phenological and yield component traits based on seven pairs of near-isogenic lines†.

| Environment | Allele | Yield and Yield Components | | | | | Phenological Characteristic | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GY | PTN | KWT | SPS | SNS | HD | FLS | GLDAH |
| | | *kg ha$^{-1}$* | *no. m$^{-1}$* | *mg* | *no.* | | *d* | | |
| 2017 Rainfed - Bozeman | Spring Wheat | 3105.6 | 126.0 | 35.9 | 35.9 | 12.8 | 53.7 | 80.3 | 26.6 |
| | Durum Wheat | 3113.5 | 125.4 | 35.8 | 36.5 | 12.1*** | 53.5 | 80.8 | 27.3 |
| 2017 Irrigated - Bozeman | Spring Wheat | 3994.5 | 166.1 | 32.5 | 36.8 | 13.4 | 54.7 | 81.7 | 27.0 |
| | Durum Wheat | 4158.6 | 161.5 | 32.9 | 34.7 | 12.0**** | 54.3 | 82.2 | 27.9 |
| 2018 Rainfed - Kalispell | Spring Wheat | 5756.6 | 105.8 | 43.5 | 38.7 | 11.9 | 57.0 | 94.8 | 37.8 |
| | Durum Wheat | 5618.7 | 102.3 | 45.1 | 37.3 | 11.5* | 56.9 | 94.9 | 38.0 |
| 2018 Rainfed - Bozeman | Spring Wheat | 4979.0 | 172.4 | 40.4 | 40.8 | 13.9 | 57.6 | 96.9 | 39.8 |
| | Durum Wheat | 5167.5 | 191.1* | 39.5 | 39.1 | 13.0*** | 57.6 | 96.7 | 39.1 |
| 2018 Irrigated - Bozeman | Spring Wheat | 5970.1 | 234.3 | 43.5 | 37.0 | 13.2 | 57.6 | 102.6 | 45.0 |
| | Durum Wheat | 5831.6 | 217.5* | 44.3 | 40.1* | 12.6** | 57.7 | 102.2 | 44.6 |
| Mean (overall) | Spring Wheat | 4761.2 | 160.9 | 39.1 | 37.8 | 13.0 | 56.1 | 91.3 | 35.2 |
| | Durum Wheat | 4778.0 | 159.6 | 39.5 | 37.6 | 12.2**** | 56.0 | 91.4 | 35.4 |
| pair x allele | | NS | NS | NS | NS | * | NS | NS | NS |
| environment x allele | | NS | * | NS | NS | * | NS | NS | NS |
| environment x pair x allele | | NS | NS | NS | NS | NS | NS | NS | NS |

†PTN, productive tiller number; SPS, seeds per spike; KWT, single kernel weight; SNS, spikelet number per spike; GY, grain yield; HD, heading date (d after planting); FLS, flag leaf senescence (d after planting); GLDAH, green leaf duration after heading (d); HT, plant height
*, **, ***, **** Spring wheat and durum wheat allele means differ at significance level of $P<0.05$, $P<0.01$, $P<0.001$, and $P<0.0001$, respectively.
NS = not significant

**Table 6.** *QTn.mst-5B* allele means by environment for phenological and yield component traits based on six pairs of near-isogenic lines[†].

| Environment | Allele | Yield and Yield Components | | | | | Phenological Characteristic | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GY | PTN | KWT[¥] | SPS[¥] | SNS[¥] | HD | FLS | GLDAH |
| | | *kg ha$^{-1}$* | *no. m$^{-1}$* | *mg* | --------*no.*-------- | | ----------*d*---------- | | |
| 2017 Rainfed - Bozeman | Spring Wheat | 3448.5 | 139.9 | 35.4 | 34.0 | 12.8 | 57.5 | 82.8 | 25.3 |
| | Durum Wheat | 2849.2*** | 132.2 | 37.3* | 35.5 | 14.2**** | 60.9**** | 87.2**** | 26.3 |
| 2017 Irrigated - Bozeman | Spring Wheat | 4399.2 | 195.3 | 31.7 | 36.6 | 13.1 | 58.4 | 84.2 | 25.8 |
| | Durum Wheat | 3573.6**** | 170.7** | 35.0*** | 36.7 | 14.4**** | 62.8**** | 86.4** | 23.6** |
| 2018 Rainfed - Kalispell | Spring Wheat | 6432.1 | 122.1 | 43.3 | 39.9 | 12.5 | 61.9 | 98.4 | 36.4 |
| | Durum Wheat | 6010.4* | 111.1 | 46.1** | 41.9 | 13.4** | 65.5**** | 102.2**** | 36.7 |
| 2018 Rainfed - Bozeman | Spring Wheat | 6281.9 | 214.1 | 38.8 | 43.8 | 14.7 | 61.2 | 99.9 | 38.7 |
| | Durum Wheat | 5865.2* | 186.3** | 39.7 | 40.8 | 14.1 | 64.4**** | 102.2** | 37.8 |
| 2018 Irrigated - Bozeman | Spring Wheat | 7592 | 257.6 | 42.2 | 41.7 | 13.9 | 61.2 | 108.6 | 47.4 |
| | Durum Wheat | 6807.5**** | 229.7** | 43.2 | 43.0 | 13.9 | 63.9**** | 111.8**** | 47.8 |
| Mean (overall) | Spring Wheat | 5630.8 | 185.8 | 38.3 | 39.2 | 13.4 | 60.0 | 94.8 | 34.7 |
| | Durum Wheat | 5021.2**** | 166.0**** | 40.2**** | 39.6 | 14.0**** | 63.5**** | 97.9**** | 34.4 |
| pair x allele | | *** | *** | NS | NS | **** | **** | **** | NS |
| environment x allele | | NS | NS | NS | NS | **** | * | NS | * |
| environment x pair x allele | | * | NS | NS | NS | *** | * | NS | NS |

[†]PTN, productive tiller number; SPS, seeds per spike; KWT, single kernel weight; SNS, spikelet number per spike; GY, grain yield; HD, heading date (d after planting); FLS, flag leaf senescence (d after planting); GLDAH, green leaf duration after heading (d); HT, plant height

[¥]Data only includes five out of six NIL pairs

*, **, ***, **** Spring wheat and durum wheat allele means differ at significance level of $P<0.05$, $P<0.01$, $P<0.001$, and $P<0.0001$, respectively.

NS = not significant

**Table 7.** *QYld.mst-2B* allele means by environment for phenological and yield component traits based on six pairs of near-isogenic lines†.

| Environment | Allele | Yield and Yield Components | | | | | Phenological Characteristic | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GY | PTN | KWT | SPS | SNS | HD | FLS | GLDAH |
| | | *kg ha$^{-1}$* | *no. m$^{-1}$* | *mg* | ------*no.*------- | | ----------*d*---------- | | |
| 2017 Rainfed - Bozeman | Spring Wheat | 3404.8 | 147.4 | 33.3 | 37.4 | 12.1 | 54.2 | 80.8 | 26.6 |
| | Durum Wheat | 3467.9 | 145.0 | 31.8* | 37.4 | 12.5 | 53.9 | 80.8 | 26.9 |
| 2017 Irrigated - Bozeman | Spring Wheat | 4488.4 | 203.3 | 30.7 | 34.7 | 12.1 | 55.3 | 82.7 | 27.4 |
| | Durum Wheat | 4540.6 | 187.2 | 30.1 | 36.8 | 12.6 | 54.7* | 82.6 | 27.8 |
| 2018 Rainfed - Kalispell | Spring Wheat | 5512.2 | 115 | 41.0 | 40.3 | 11.8 | 57.1 | 93.9 | 36.8 |
| | Durum Wheat | 5831.2 | 124.5 | 40.2 | 44.2* | 11.9 | 56.6 | 94.8* | 38.3** |
| 2018 Rainfed - Bozeman | Spring Wheat | 5222.1 | 196.5 | 36.1 | 41.7 | 13.6 | 57.8 | 96.8 | 39.1 |
| | Durum Wheat | 5203.6 | 209.4 | 34.8* | 42.1 | 13.7 | 57.4 | 96.9 | 39.6 |
| 2018 Irrigated - Bozeman | Spring Wheat | 6405.0 | 247.5 | 38.9 | 41.9 | 13.1 | 57.6 | 102.7 | 45.2 |
| | Durum Wheat | 6170.7 | 241.1 | 38.2 | 42.5 | 13.5 | 57.1 | 102.8 | 45.7 |
| Mean (overall) | Spring Wheat | 5006.5 | 181.9 | 36.0 | 39.2 | 12.6 | 56.4 | 91.4 | 35.0 |
| | Durum Wheat | 5022.8 | 181.4 | 35.0*** | 40.6 | 12.8* | 55.9** | 91.6 | 35.7** |
| pair x allele | | NS | NS | **** | NS | * | NS | NS | NS |
| environment x allele | | NS | NS | NS | NS | NS | NS | NS | NS |
| environment x pair x allele | | NS | NS | NS | NS | NS | NS | NS | NS |

†PTN, productive tiller number; SPS, seeds per spike; KWT, single kernel weight; SNS, spikelet number per spike; GY, grain yield; HD, heading date (days after planting); FLS, flag leaf senescence (days after planting); GLDAH, green leaf duration after heading (days from 1 Jan); HT, plant height

*, **, ***, **** Spring wheat and durum wheat allele means differ at significance level of $P<0.05$, $P<0.01$, $P<0.001$, and $P<0.0001$, respectively.

NS = not significant

# CHAPTER THREE

# IMPACT OF YIELD COMPONENT ALLELES FROM DURUM WHEAT ON END-USE QUALITY OF SPRING WHEAT

## Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: Brittney H. Jones

Contributions: conceived study, experimental work including design, data collection, statistical analysis and manuscript writing and preparation

Co-Author: Nancy K. Blake

Contributions: development of SXD near-isogenic lines, field design, and phenotypic data collection

Co-Author: Hwa-Young Heo

Contributions: field design, phenotypic data collection, field harvest

Co-Author: Jay R. Kalous

Contributions: quantitative trait loci analysis for the identification of durum yield component loci

Co-Author: John M. Martin

Contributions: aided in the writing of statistical code and contributed to the analysis and interpretation of data

Co-Author: Deanna L. Nash

Contributions: end-use quality and baking quality testing

Co-Author: Jessica A. Torrion

Contributions: field design and phenotypic data collection

Co-Author: Luther E. Talbert

Contributions: initiated and coordinated the project, contributed to data analysis and final manuscript writing and preparation

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# Impact of yield component alleles from durum wheat on end-use quality of spring wheat

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Abbreviations:
GPC, Grain protein content
GY, Grain yield
HIF, Heterogeneous inbred family
HMW-GS, High molecular weight glutenin subunits
HRSW, Hard red spring wheat
KWT, Kernel weight
LSD, Least significant difference
NIL, Near-isogenic line
PTN, Productive tiller number
QTL, Quantitative trait locus
RCBD, Randomized complete block design
RIL, Recombinant inbred line
SDS-PAGE, SDS polyacrylamide gel electrophoresis
SKCS, Single-kernel characterization system
SNS, Spikelet number per spike
SPS, Seeds per spike
SSD, Single seed descent

**Declarations:**

**Conflicts of interest/Competing interests**
On behalf of all authors, the corresponding author states that there is no conflict of interest

**Author contribution statement**
BJ contributed the majority of experimental work, NB, HH, JT contributed to field design and phenotypic data collection, JM aided in the writing of statistical code and contributed to the analysis and interpretation of data. LT initiated and coordinated the project, contributed to data analysis and final manuscript writing and preparation. All authors reviewed the manuscript and provided suggestions.

## ABSTRACT

Hard red spring wheat (HRSW) (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L. ssp. *durum*) are used for different end-use products. HRSW is used for bread baking while durum wheat is used for pasta production. This study investigated the impact of alleles from durum wheat for yield component traits on HRSW end-use quality traits. Near-isogenic lines for four quantitative trait loci, *QGw.mst-3B, QGw.mst-7A, QTn.mst-5B,* and *QYld.mst-2B* were evaluated in rainfed and irrigated environments. An allele from durum wheat at *QGw.mst-3B* for increased kernel weight (KWT) positively impacted end-use quality traits with associated increases in kernel protein and loaf volume across several environments. The durum allele associated with *QGw.mst-3B* presents a source of improved agronomic potential and end-use quality. The durum allele at *QTn.mst-5B* delayed heading date, impacting productive tiller number and KWT and additional end-use quality traits. The durum allele at *QYld.mst-2B* for GY showed both positive and negative effects on end-use quality. The durum allele for reduced spikelet number per spike at *QGw.mst-7A* had little to no impact on end-use quality. The alleles from durum wheat investigated in this study are potential sources of cultivar improvement however interactions between yield component and end-use quality traits may influence utilization.

**Keywords**: Durum Wheat, End-use Quality, Hexaploid Wheat, Introgression, Pleiotropic interaction, Yield component traits

## INTRODUCTION

Development of new wheat (*Triticum aestivum* L.) cultivars has two objectives. First, cultivars must have favorable genes for profitable production in targeted areas. Second, they must have genetic attributes necessary to produce desirable products for end-users. Hard red spring wheat (HRSW) is grown in the northern Great Plains of the US and Prairie Provinces of Canada. Most of the HRSW grain is used for making leavened bread both domestically and internationally (Zilić et al., 2011; Tilley, Chen, & Miller, 20122012; Jung & Seo, 2014). A challenge in wheat breeding is that there is often interaction between agronomic and end-use quality traits resulting in an often-negative impact on one or the other. Thus, the impact of genes introduced to increase agronomic productivity need to be assessed for possible impact on end-use quality.

Hexaploid bread wheat and tetraploid durum wheat (*Triticum turgidum* L. ssp. *durum)* have been grown in similar geographic regions for approximately 10,000 years (Faris, 2014). Selection targets for agronomic traits have been similar, especially regarding increased grain yield (GY) during hot and dry summer conditions. Hexaploid bread wheat (2n=6x=42) is an allopolyploid derived from two independent hybridization events leading to complete copies of the A, B and D genomes (Faris, 2014). The first hybridization event occurred between the A genome donor *Triticum urartu* Thumanian ex Gandilyan (2n=2x=14) and a B genome donor related to *Aegilops speltoides* Tausch (2n=2x=14), leading to the development of tetraploid emmer wheat (*Triticum turgidum* L. spp. *dicoccoides*, 2n=4x=28). Emmer wheat was further domesticated into cultivated durum wheat (*Triticum turgidum* L. ssp. *durum*, 2n=4x=28). A second hybridization event between tetraploid emmer wheat and the D genome donor *Aegilops*

*tauschii* Coss. (2n=2x=14) resulted in hexaploid wheat (*Triticum aestivum* L.) (Feuillet, Langridge, & Waugh, 2007; Faris, 2014; Kabbaj et al., 2017). The low amount of crossing between durum wheat and hexaploid bread wheat suggests the possibility that different sets of favorable genes may have been selected in each species.

Hexaploid wheat has undergone a genetic bottleneck resulting in the loss of genetic variation compared to ancestral progenitor species (Haudry et al., 2007; Cavanagh et al., 2013). Diversity for HRSW grown in the northern Great Plains is low due a limited number of cultivar introductions (Cox, 1991). Wide crosses back to progenitor species to reintroduce genetic variation and capitalize on favorable traits relating to GY and end use performance are potential sources for meeting future GY and end performance demands (Yumurtaci, 2014; Mammadov et al., 2018). Several desirable qualitative traits have been introgressed from durum wheat into HRSW for disease and pest resistance (Sharma & Gill 1983; Jung & Seo, 2014), and end-use quality (Mesfin et al., 2000). Introduction of alleles from durum wheat to hexaploid bread wheat with quantitative effects has been more difficult.

The main quantitative trait most often targeted by wheat breeders is GY. Grain yield is comprised of underlying yield component traits including productive tiller number (PTN, also referred to as spike number), spikelet number per spike (SNS), number of seeds per spike (SPS) and kernel weight (KWT) (Yoshida, 1972; Slafer, 2003; Xing & Zhang, 2010; Nadolska-Orczyk et al., 2017). The yield components are themselves quantitative traits influenced by many genes and the environment (Allard & Bradshaw, 1964; Kang et al., 2004; El-Soda et al., 2014). Wide crosses to progenitor species for improved GY performance have the potential to negatively impact end-use quality. Crosses between hexaploid wheat and rye (*Secale cereal* L.) for the

introgression of resistance genes located on the short arm of rye chromosome 1(1RS) provide an example (Jung & Seo, 2014). Wheat-rye translocations including 1BL/1RS and 1AL/1RS were introduced as a source for resistance to stem rust (*Puccinia graminis* f. sp. *tritici*), stripe rust (*Puccinia striiformis* f. sp. *tritici*), leaf rust (*Puccinia recondite* f. sp. *tritici*), and powdery mildew (*Erysiphe graminis* f. sp. *tritici*). The translocated sections of rye chromosome have been shown to have deleterious impacts on end-use quality by reducing loaf volume and dough development (Martin & Stewart, 1986). Loaf volume correlates with both total flour protein content and glutenin protein concentration. Glutenin concentration is determined by the expression of *Glu-1* and *Glu-3* loci located on the long arms of the group 1 chromosomes (1A, 1B, and 1D) (Galili & Feldman, 1983; Kuchel et al., 2006; Anjum et al., 2007). The reduction in bread-making quality as a result of the rye translocations 1AL/1RS and 1BL/1RS is due to the replacement of the hexaploid alleles at *Glu-A3* and *Glu-B3* genes with rye alleles resulting in decreased gluten concentration (Lee, Graybosch, & Peterson, 1995; Oak & Tamhankar, 2017).

While harboring genetic variation that confers improved GY performance, wide crosses with progenitor species have the potential to negatively impact end-use quality traits. Grain size or weight is typically positively correlated with yield of white flour in the milling process, due to an associated increase in kernel endosperm (Mares, Moss, & Ellison, 1986; Dziki & Laskowski, 2005; Baasandorj et al., 2015). Grain size or weight has also been correlated with baking quality. Goel et al. (2019) found a negative correlation between gluten strength and kernel size and KWT in a recombinant inbred line (RIL) population derived from two Indian wheat cultivars. Similar negative interactions between KWT and grain protein content (GPC) were also shown by Groos et al. (2003) in a HRSW RIL population and Blanco et al. (2012) in a durum wheat RIL

population. Additionally, loaf volume is typically negatively associated with increased kernel size and KWT and is related to a decrease in high molecular weight glutenin subunits (HMW-GS) content resulting in decreased loaf volume (Park et al., 2006; Shewry, 2009; Baasandorj et al., 2015). Sherman et al. (2014) identified multiple quantitative trait loci (QTL) for end-use quality traits in a HRSW population. Several of these QTL were coincident with QTL for kernel size and number of SPS. The impact of number of spikes and number of SPS on end-use quality is likely to be due to the impact of these traits on kernel size and KWT.

Both HRSW and durum wheat require high GPC ranging from 13% to 15% (Peña et al., 2002; Tilley et al., 2012). Hard red spring wheat flour is routinely used for baking breads due to the high gluten strength of the dough (Zilić et al., 2011; Tilley et al., 2012; Jung & Seo, 2014). Flour from an ideal HRSW cultivar should be high in protein, have high water absorption, longer time to maximum dough resistance, good mixing tolerance, and large loaf volume (Finney et al., 1987). Durum wheat classified by its hard kernel texture is milled into semolina used in pasta making (Finney et al., 1987). Lanning et al. (2003) evaluated bread quality in a population of 10 hexaploid and 14 tetraploid RIL from a bread wheat by durum wheat cross. The hexaploid lines had stronger gluten, increased water absorption, and higher loaf volume than the tetraploid lines, likely due to the presence of the glutenin alleles present on the D genome chromosomes.

An impediment for introgression of beneficial alleles from related species has been the development of sufficiently large populations in inter-species crosses to detect QTL. Lanning et al. (2008) demonstrated the ability to generate viable progeny through a cross between hexaploid HRSW ‘Choteau’ (PI 633974) (Lanning et al., 2004) and tetraploid durum wheat ‘Mountrail’(PI 607540) (Elias & Miller, 2000). The relative fertility of this cross allowed generation of RIL

with both hexaploid and tetraploid chromosome constitutions. Kalous et al. (2015) developed a population of 205 RIL consisting of 117 hexaploid lines and 88 tetraploid lines from the Choteau/Mountrail cross (Lanning et al., 2008). Several alleles from Mountrail durum wheat were positively associated with GY or yield component traits in hexaploid RIL from a QTL analysis performed on the Choteau/Mountrail RIL population (Kalous et al., 2015).

Near-isogenic lines (NIL) were developed for a subset of the QTL identified by Kalous et al. (2015). The QTL included *QGw.mst-3B* for KWT, *QGw.mst-7A* for SNS, *QYld.mst-2B* for GY, and *QTn.mst-5B* for PTN. Jones et al. (2019) confirmed the impact of the durum allele in hexaploid backgrounds on yield component traits for the four QTL. All of the QTL impacted more than one yield component trait. This may reflect the pleiotropic interactions typically identified between yield components (Gupta, Rustgi, & Kumar, 2006; Sadras & Rebetzke, 2013; Slafer, 2003; Slafer, Savin, & Sadras, 2014). Verification of the QTL impacts on yield components suggested potential value for GY enhancement in HRSW. However, a concern with the introgression of alleles from durum wheat is the possibility of deleterious impacts on hexaploid HRSW end-use quality.

The objective of the present research was to determine the impact of introgressed yield component alleles from durum wheat on HRSW end-use quality. In the present study, NILs tested by Jones et al. (2019) containing either the durum allele or hexaploid bread wheat allele for the respective QTL were grown in three environments and evaluated for key parameters associated with end-use quality. Results address the value of introgressed alleles from durum wheat for the genetic improvement of HRSW.

# MATERIALS AND METHODS

## Plant Materials

Near-isogenic lines contrasting for durum or spring wheat alleles for four QTL associated with yield component traits listed in Table 1, were evaluated for allele impact on end-use quality. In the present study two NIL pairs were used to evaluate end-use quality parameters in multiple HRSW backgrounds. Near-isogenic line pairs consisted of two NIL pair members distinguishable by the presence of the durum or spring allele at causal QTL as determined by marker analysis. Development of the NIL began with selection of hexaploid RIL from the Choteau HRSW/Mountrail durum wheat population for development of new RIL populations. Lines derived from this cross each had 50% bread wheat and 50% durum wheat genetic background for the A and B genomes (Kalous et al., 2015). Each of the selected RIL were crossed with elite HRSW lines 'Vida' (PI 642366) (Lanning et al., 2006), 'Duclair' (PI 660981) (Lanning et al., 2011) and 'Berkut' (developed by the International Maize and Wheat Improvement Center (CIMMYT)). Recombinant-inbred line populations were developed for all crosses by single seed descent (SSD) to the $F_5$ generation. Near-isogenic lines were developed for the QTL following the heterogeneous inbred family (HIF) strategy (Haley et al., 1994; Pumphrey, Bernardo, & Anderson, 2007). This entailed screening the $F_5$ RIL plants developed by SSD with markers, listed in Table 1, associated with QTL peaks to identify heterozygous plants. Markers were selected from the 90-K SNP array (Wang et al., 2014; Kalous et al., 2015). Homozygous $F_6$ progeny from the heterozygous $F_5$ plants were selected for the spring and durum wheat alleles, respectively. Homozygous $F_6$ plants were self-pollinated to generate NIL seed for field testing. The NIL are expected to contain approximately 75% of their genes on the A and B

genomes from the HRSW parents. The D genome contains 100% of its genes from the HRSW parents. In the case of *QYld.mst-2B* and *QGw.mst-7A* NIL pairs were derived in both Berkut and Duclair backgrounds however for *QTn.mst-5B* and *QGw.mst-3B* NIL pairs were derived in Vida and Berkut, respectively (Table 1).

## Experimental Design

Near-isogenic lines were evaluated in three environments in randomized complete block design (RCBD) consisting of three blocks per environment. Near-isogenic line pairs were grown in 2018 under irrigated and rainfed conditions at the Arthur H. Post Research Farm in Bozeman, MT (latitude 45.68°N, longitude 111.04°W, elevation 1469 m) and rainfed conditions at the Northwestern Ag Research Center in Kalispell, MT (latitude 48.19°N, longitude 114.32°W, elevation 900 m). Plots in Bozeman were three 4.9 m rows spaced 30 cm apart and Kalispell 2018 plots were seven 4.6 m rows spaced 15 cm apart. Seeding density was 269 seed $m^{-2}$ for rainfed trials and 323 seed $m^{-2}$ for irrigated trials. Climatological data for the 2018 Bozeman and Kalispell growing environments summarized in Jones et al. (2019). The present study consisted of two NIL pairs for each QTL and milling and baking quality was assessed across rainfed and irrigated replicated environments. Parental checks were evaluated in each of the three environments and included elite HRSW cultivars Choteau, Vida, Berkut, and Duclairas well as the durum cultivar Mountrail (Table 2).

## End-use Quality Analysis

Grain used for end-use quality analysis was harvested from all plots from three of the five environments where agronomic traits were measured and reported in Jones et al. (2019). Traits included kernel protein, kernel hardness, flour yield, flour protein, flour ash, mixing time, mixing

tolerance, water absorption and bake loaf volume. Methods approved by the AACC were used to analyze flour yield, flour protein, flour ash, mixing time, mixing tolerance, water absorption, bake loaf volume and crumb score. Kernel protein was measured using near-infrared transmittance (Infratech 1241 Grain Analyzer, Foss North America, Eden Prairie, MN) (AACC Method 39-10.01). Kernel hardness and kernel weight were determined on a single kernel basis using the single-kernel characterization system (SKCS) instrument (SKCS Model 4100 Perten Instruments, Springfield, IL) (AACC Method 55-31.01) from the Bozeman rainfed and irrigated environments. Kernel hardness was not measured in the 2018 Kalispell environment. Wheat samples were tempered to a 15% moisture before milling (AACC Method 26-10.02). Straight grade flour was obtained using a Brabender Quadromat Senior Mill (C.W. Brabender Instruments Inc., South Hackensack, NJ). Flour protein was determined by Near Infrared Reflectance using a Technicon InfraAnalyzer 400 (Technicon Industrial Systems, Tarrytown, NY) (AACC Method 39–11.01) at a 14% moisture basis. Whole wheat flour for flour ash testing was milled using Laboratory Mill 3100 (Perten). Dough rheology properties were measured using the mixograph (AACC Method 54–40.02). Bread-making properties were measured using a standard bake test methodology (AACC Method 10–09.01 and AACC Method 10-05.01).

### Characterization of High-Molecular-Weight Glutenin Proteins

High-molecular-weight glutenin proteins for homoeologous genes *Glu-A1*, *Glu-B1* and *Glu-D1* were characterized for each of the NIL pairs and parental elite HRSW and durum cultivars (Supplemental Table 1). Glutenin subunits were analyzed using SDS polyacrylamide gel electrophoresis (SDS-PAGE) (Payne, Holt, & Law, 1981). The HMW-GS were scored based on the classification of Payne & Lawrence (1983).

## Statistical Analysis

End-use quality performance traits were sorted by QTL and data analyzed separately for each QTL. An ANOVA was conducted using PROC GLM with SAS software, version 9.4 (SAS Institute Inc., 2012) for each environment and then combined over the three environments (Bozeman rainfed, Bozeman irrigated and Kalispell rainfed). The model included environment, replication within environment, NIL pair, allele and their interactions. All factors were considered fixed effects. Kernel weight data was taken from three of the five environments evaluated by Jones et al. (2019) and a subset of the NIL pairs per QTL and analyzed using the same statistical model. PROC GLM was used to calculate least significant difference (LSD) among parental check means averaged across the three environments.

# RESULTS

In the present study NIL with contrasting durum and spring wheat alleles for four QTL associated with yield component traits were grown in three Montana environments including both rainfed and irrigated treatments in 2018. Quantitative trait loci in the present study included *QGw.mst-3B* for KWT, *QGw.mst-7A* for SNS, *QTn.mst-5B* for PTN, and *QYld.mst-2B* for GY. Previous investigation of the four QTL by Jones et al. (2019) confirmed the impact of the durum alleles on yield component traits in hexaploid HRSW backgrounds.

Durum and HRSW elite parental checks were included in planting for all three of the 2018 environments. Means for all parental checks differed for end-use quality traits (Table 2). Relative to the hexaploid HRSW checks, the tetraploid durum check Mountrail had significantly lower flour yield, mixing time, water absorption and loaf volume. Mountrail also had the highest kernel hardness and flour ash. The HRSW parental line Berkut had the lowest kernel protein and flour protein compared to the additional parental checks. This reduced protein content may explain the low loaf volume relative to the other hexaploid HRSW checks.

Near-isogenic line pairs for each of the four QTL were characterized for HMW-GS to determine the allele profile inherited from the spring wheat or durum parent. Supplemental Table 1 shows characterized glutenin subunits for the *Glu-A1*, *Glu-B1* and *Glu-D1* loci for NIL pairs for the four QTL. Near-isogenic line pair members for *QGw.mst-7A*, *QTn.mst-5B* and *QYld.mst-2B* were identical for HMW-GS profiles. In one of the NIL pairs for *QGw.mst-3B* the individual members within a single pair (1C12) were different in profile for HMW-GS. Differences in HMW-GS between the individuals of the 1C12 NIL pair suggest that the heterozygous RIL selected in the derivation of NIL was segregating for the Mountrail durum and Choteau

hexaploid wheat alleles at the *Glu-A1* and *Glu-B1* loci (Supplemental Table 1). However, in the second pair of NIL (1D11) for *QGw.mst-3B* both members were identical for HMW-GS profile.

The durum allele at *QGw.mst-3B* was identified by Kalous et al. (2015) as causing increased KWT. Jones et al. (2019) showed that KWT was increased by 1.8 mg for NIL carrying the durum allele. Table 3 shows durum and spring allele means for two NIL pairs derived from a cross to Berkut (Choteau/Mountrail//Berkut). Using a subset of the KWT data from Jones et al. (2019) in the present study KWT was significantly ($P < .01$) increased in the Kalispell rainfed environment in association with the durum allele. The durum allele was associated with a 3.2 g $kg^{-1}$ increase ($P < .01$) in kernel protein and a 3.9 g $kg^{-1}$ increase ($P < .001$) in flour protein compared to the spring wheat allele when combined over all environments. Allele x pair interactions were significant for kernel protein ($P < .01$) and flour protein ($P < .05$) such that only a single NIL pair (1D11) accounted for significant allele differences for both measured traits (Supplemental Table 2). Members of this pair had identical HMW-GS profiles (Supplemental Table 1). Percent flour ash was increased by 0.2 g $kg^{-1}$ in association with the durum allele. Loaf volume was significantly increased in association with the durum allele by 43.8 $cm^3$ ($P < .001$) relative to the spring wheat allele. The allele x pair interaction was significant for flour ash ($P < .05$), and loaf volume ($P < .01$) such that the NIL pair 1D11 accounted for the majority of the differences observed between allele types (Supplemental Table 2). Further, a significant allele x environment interaction was observed for loaf volume ($P < .01$). This significant interaction likely resulted because the magnitude of the differences varied in environments with the largest increase in loaf volume from the durum allele compared to the spring wheat allele occurring in the 2018 Bozeman irrigated environment.

The durum allele at *QGw.mst-7A* was identified by Kalous et al. (2015) as causing increased KWT. Jones et al. (2019) did not observe a significant impact on KWT for *QGw.mst-7A* NIL pairs but observed a significant durum allele associated reduction of SNS by 0.8 relative to the spring wheat allele. Table 4 shows durum and spring wheat allele means for end-use quality traits for two NIL pairs derived from crosses with Berkut and Duclair (Choteau/Mountrail//Berkut or Duclair). The durum allele was associated with significant reduction in mixing tolerance by 0.3 units combined across environments ($P < .05$). The durum allele showed decreased water absorption (13.5 g $kg^{-1}$ $P < .05$) compared to the spring wheat allele when combined across environments. But the allelic difference was only statistically significant for the Bozeman rainfed environment ($P < .01$). The single NIL pair 1F4 in the Berkut background accounted for differences observed for water absorption between alleles (Supplemental Table 2). Alleles at *QGw.mst-7A* had no effect on additional end-use quality traits ($P > .05$). There was no observed allele x environment interaction for any end-use quality traits.

The durum allele at *QTn.mst-5B* was identified by Kalous et al. (2015) to increase PTN in a hexaploid background. Jones et al. (2019) also observed an impact of the durum allele on PTN. However, rather than increasing PTN, the durum allele was associated with a significant decrease of 19.8 productive tillers $m^{-1}$ relative to the spring wheat allele. Additionally, Jones et al. (2019) reported that the durum allele was associated with significant delay in heading and senescence along with a reduction in GY and increase in KWT. *QTn.mst-5B* is closely linked with the vernalization gene *Vrn-B1* on the long arm of chromosome 5B. Near-isogenic line pairs for *QTn.mst-5B* were fixed for the spring-type allele at *Vrn-A1* locus however at the *Vrn-B1* locus the lines within NIL pair 12C2 varied for the spring and winter allele. Such that the NIL

pair individual with the durum allele at *QTn.mst-5B* was fixed for the winter-type allele at *Vrn-B1*. Jones et al. (2019) suggested that the variation between lines of NIL pair 12C2 at the *Vrn-B1* locus likely pleiotropically impacted PTN and KWT differences associated with the durum allele. Table 5 shows durum and spring wheat allele means for end-use quality traits for two NIL pairs derived from a cross with Vida (Choteau/Mountrail//Vida). The allele from durum wheat was associated with a significant decrease of 1.9 hardness units ($P < .05$) combined across environments. Additionally, the durum allele significantly increased flour yield by 6.2 g $kg^{-1}$ ($P < .001$) relative to the spring wheat allele. A significant ($P < .05$) allele x environment interaction was observed for flour yield. This was because the magnitude of allelic differences varied across environments with the difference at Kalispell being greatest. Additionally, pair x environment interaction was significant ($P < .01$) for flour yield such that for a single NIL pair (12C2) the durum allele more consistently increased flour yield across environments (Supplemental Table 2). Flour ash was increased by 0.4 g $kg^{-1}$ ($P < .0001$) relative to the spring wheat allele combined across environments. Loaf volume was significantly reduced by the durum allele by 49.5 $cm^3$ ($P < .01$). An allele x pair interaction was observed for loaf volume, where only a single NIL (12A10) accounted for the differences observed between the durum and spring wheat alleles (Supplemental Table 2).

The durum allele at *QYld.mst-2B* was identified by Kalous et al. (2015) to increase GY in a hexaploid background. Jones et al. (2019) tested *QYld.mst-2B* NIL pairs and observed no significant durum allele impact on GY but rather a significant decrease of 1.0 mg in KWT relative to the spring wheat allele. Table 6 shows durum and spring allele means for end-use quality traits for two NIL pairs derived from crosses with Berkut and Duclair

(Choteau/Mountrail//Berkut or Duclair). Using a subset of the KWT data from Jones et al. (2019) in the present study KWT was significantly ($P < .01$) decreased in association with the durum allele when combined across environments. Kernel protein and flour protein were significantly increased in association with the durum allele by 3.1 g $kg^{-1}$ ($P < .01$) and 3.0 g $kg^{-1}$ ($P < .01$), respectively, when combined across environments. The durum allele increased flour ash by 0.1 g $kg^{-1}$ ($P < .05$) and decreased flour yield by 3.7 g $kg^{-1}$ relative to the spring wheat allele ($P < .0001$) when combined over environments. Mixing tolerance was decreased by 0.3 and mixing time was reduced by 0.5 minutes for the durum allele compared to the spring wheat allele. Significant allele x pair interactions were observed for flour yield and mixing time. This interaction can be explained in that the NIL pair (2B6) derived from the cross Choteau/Mountrail//Berkut showed a negative impact of the durum allele on flour yield and mixing time whereas the Choteau/Mountrail//Duclair derived NIL pair (20A_C11) which showed a flour yield increase associated with the durum allele in a single environment (Supplemental Table 2). Mixing time had a significant allele x environment interaction ($P < .05$) resulting from variation in magnitude of differences across environments.

## DISCUSSION

Introgression of QTL alleles from durum wheat may have undesirable consequences in HRWS for several reasons. Durum wheat and HRSW are used to produce different end-use products. Hard red spring wheat is milled into flour for the baking of breads whereas durum wheat is milled into semolina for pasta. Additionally, durum wheat and HRSW differ for several characteristics important in marketing and end-use quality. Durum wheat typically has larger kernel size and increased kernel hardness whereas HRSW has increased gluten strength for bread making (Lacerenza et al., 2008; Patil et al., 2013; Trethowan et al., 2001). Additionally, a potential issue with using alleles from durum to improve HRSW is that the introgressed allele or alleles at closely linked loci may have negative impacts on non-target traits particularly those associated with agronomic performance. The present study used NIL pairs for four QTL to test the impact of GY and yield component alleles from durum wheat on end-use quality characteristics in HRSW.

Markers for four QTL alleles from durum wheat previously shown to impact GY or yield components in HRSW were used to develop sets of NIL for each of the QTL used in the present study (Table 1). Comparison of QTL specific NIL pairs allowed for the determination of the impact on end-use quality of the QTL alleles in nearly identical genetic backgrounds. Results showed a diversity of effects for the introgressed alleles from durum wheat on HRSW end-use quality. The durum allele at *QGw.mst-3B* showed a positive impact on multiple end-use quality traits including increased kernel and flour protein (Table 3). Durum alleles at *QTn.mst-5B* (Table 5) and *QYld.mst-2B* (Table 6) showed both negative and positive impacts upon end-use quality traits measured. The durum allele at *QGw.mst-7A* showed a small but significant decrease in water absorption and mixing tolerance but no other quality traits were affected (Table 4). Jones

et al. (2019) showed the durum allele at *QGw.mst-7A* reduced SNS. These results would indicate that the durum allele a *QGw.mst-7A* is most likely not a useful source of improvement in HRSW. It is possible that the durum allele at *QGw.mst-3B* may present an option for HRSW yield component improvement without associated deleterious impacts on end-use quality traits.

The allele from durum wheat at *QGw.mst-3B* caused increased kernel protein and loaf volume (Table 3). Further, Jones et al. (2019) associated the durum allele with increased KWT measured from the same 2018 environments as the present study. Several studies have reported similar positive correlations between kernel protein and loaf volume in wheat (Sutton et al., 1992; Ohm, Chung, & Deyoe, 1998; Maghirang et al., 2006; Dowell et al., 2008). The positive increase in kernel protein and KWT in the present study is contradictory to the commonly observed negative interaction between these two traits (Ohm et al., 1998; Groos et al., 2003; Oury et al., 2003; Oury & Godin, 2007; Blanco et al., 2012; Goel et al., 2019). However, Oury & Godin (2007) showed the relationship between KWT and kernel protein may be impacted by environmental conditions, such that in some environments this negative correlation is weak and/or not detected. Sutton et al. (1992) suggested that an increase in KWT and kernel protein may be a result of an increase in gluten concentration accompanied with the increase in starch in a single kernel. The endosperm of the wheat kernel accounts for 70-80% of final dry weight (Brocklehurst, 1977; Chojecki, Gale, & Bayliss, 1986). Gluten proteins are distributed in the endosperm (Bailey et al., 2002; Tosi et al., 2011) and account for nearly 80% of total grain protein (Shewry, 2009). The NIL pair (1D11) at *QGw.mst-3B* that accounted for differences in kernel protein did not differ for HMW-GS (Supplemental Table 1). Therefore, the observed increase in kernel protein is unlikely to be associated with differences in HMW-GS. Environment did not significantly impact the increase in kernel protein associated with the

durum, suggesting a stable increase in kernel protein across several environments. Overall, the durum allele at *QGw.mst-3B* was associated with increased KWT, kernel protein and loaf volume. Therefore, it is possible that the durum allele may be a source of HRSW improvement.

The durum allele at *QTn.mst-5B* was associated with an increase in flour yield and a reduction in loaf volume (Table 5). Jones et al. (2019) showed that the durum allele at *QTn.mst-5B* was also associated with an increase in KWT. The *QTn.mst-5B* locus co-varies with linked alleles at the vernalization locus *Vrn-B1* (Jones et al., 2019), which likely contributed to reduced PTN and delayed heading for NIL containing the durum allele. Kernel weight and PTN typically share a negative pleiotropic relationship (Slafer et al., 2003; Nasseer et al., 2016; Xie, Mayes, & Sparkes, 2016; Jones et al., 2019). Thus, the durum allele at *QTn.mst-5B* for reduced PTN likely also resulted in increased KWT. Increased KWT has been positively associated with increased flour yield (Baasandorj et al., 2015; Jobson et al., 2018) as observed in this study. Further, increases in KWT and flour yield have been associated with reduction in kernel protein as observed in several studies (Mares et al., 1986; Ohm et al., 1998; Dowell et al., 2008; Bassandorj et al., 2015). Therefore, pleiotropic interaction driving variation in KWT and flour yield may have also influenced observed reduction in loaf volume associated with the durum allele at *QTn.mst-5B* (Table 5).

Data from the present study shows a significant increase associated with the durum allele at *QYld.mst-2B* for kernel protein and flour protein (Table 6). Flour yield was significantly reduced ($P < .01$) in association with the durum allele, but most notably in just one of the two genetic backgrounds (Supplemental Table 2). Additionally, the durum allele was associated with decreased KWT and increased SNS (Jones et al., 2019). The negative relationship between KWT and protein content in both kernel and flour demonstrates a pleiotropic interaction between these

traits. This interaction has been well documented in the literature (Ohm et al., 1998; Groos et al., 2003; Oury et al., 2003; Oury and Godin, 2007; Blanco et al., 2012; Goel et al., 2019). This pleiotropic interaction between KWT and protein may have impacted flour yield resulting in a decreased flour yield associated with the durum allele, relative to the spring wheat allele. Previous research has shown that smaller kernels are often associated with reduced flour yield and increased kernel and flour protein (Mares et al., 1986; Baasandorj et al., 2015). The reduction in flour yield and increase in kernel protein may be due to a reduction in the size of the kernel endosperm and increase of protein in the aleurone layer (Baasandorj et al., 2015).

## CONCLUSIONS

This study highlights the challenge in improving single quantitative traits that share pleiotropic relationships with additional traits through the introgression of novel alleles. Hard red spring wheat is grown for its high GY potential and end-use quality characteristics important for milling of white flour and the baking of breads. In the present study four durum wheat yield component QTL were investigated for the impacts on end-use quality characteristics in HRSW. Results suggest that yield component traits and end-use quality characteristics may be pleiotropically associated. Additionally, physiological characteristics that underlie GY and yield components are likely to also impact end-use quality as was observed for the durum allele at *QTn.mst-5B*. The durum allele for increased KWT at *QGw.mst-3B* resulted in improved end-use quality compared to the spring wheat allele. Therefore, the durum allele at *QGw.mst-3B* may be useful source for both GY and end-use quality improvement in HRSW. A potential avenue for further improvement of HRSW may be through the stacking of several of the QTL investigated in the present study in a single background to enhance end-use quality and GY performance. Results from the present study highlight the importance of QTL investigation of yield component traits

for associated impacts on end-use quality traits and the potential for HRSW improvement through the introgression of durum allele yield component QTL.

## SUPPLEMENTAL MATERIAL

*Supplemental materials can be found online at the Montana State University open access repository ScholarWorks*

Supplemental Table 1. High molecular weight glutenin subunit (HMW-GS) profile of near-isogenic lines (NIL) for yield component quantitative trait loci (QTL) and parental line checks

Supplemental Table 2. Impact on end-use quality of alleles at four yield component quantitative trait loci (QTL) over three 2018 environments

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# TABLES

**Table 1**. Quantitative trait loci (QTL) for yield and yield-related traits identified in the Choteau/Mountrail recombinant inbred line population for which near-isogenic lines were developed.

| Trait | QTL | $R^2$(%)[a] | Marker[b] | Chromosome | Spring Wheat Background |
|---|---|---|---|---|---|
| Grain Yield | *QYld.mst-2B* | 7.7 | IWB6888 | 2B | Berkut, Duclair |
| Productive Tiller Number | *QTn.mst-5B* | 6.4 | IWB7213 | 5B | Vida |
| Kernel Weight | *QGw.mst-3B* | 6.1 | IWA6375 | 3B | Berkut |
| Spikelet Number per Spike | *QGw.mst-7A* | 6.5 | IWB29518 | 7A | Berkut, Duclair |

[a]$R^2$ refers to the percent of phenotypic variance explained (Kalous et al., 2015)
[b]Markers selected from the 90-K SNP assay (Wang et al., 2014) and associated with QTL peaks by Kalous et al. 2015

**Table 2.** Parental means averaged over three environments for end-use quality traits

| Background | Ploidy | Kernel Protein *g kg$^{-1}$* | Kernel Hardness[a] | Flour Yield | Flour Protein | Flour Ash | Mixing Tolerance[b] | Mixing Time *min* | Water Absorption *g kg$^{-1}$* | Loaf Volume *cm$^3$* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ----------*g kg$^{-1}$*---------- | | | | | | |
| Choteau | 6X | 142.7$^{A}$ | 65.1$^{D}$ | 697.7$^{B}$ | 130.2$^{A}$ | 4.1$^{C}$ | 2.8$^{BC}$ | 3.0$^{C}$ | 684.4$^{A}$ | 1091.1$^{A}$ |
| Vida | 6X | 135.8$^{B}$ | 72.8$^{B}$ | 711.8$^{A}$ | 121.7$^{B}$ | 4.2$^{C}$ | 2.3$^{C}$ | 3.0$^{C}$ | 663.7$^{B}$ | 1045.0$^{B}$ |
| Berkut | 6X | 116.4$^{C}$ | 72.6$^{B}$ | 692.6$^{C}$ | 101.3$^{C}$ | 3.7$^{D}$ | 3.6$^{A}$ | 5.2$^{A}$ | 648.7$^{C}$ | 945.6$^{C}$ |
| Duclair | 6X | 136.0$^{B}$ | 68.0$^{C}$ | 693.6$^{BC}$ | 123.1$^{B}$ | 4.3$^{B}$ | 3.3$^{AB}$ | 3.9$^{B}$ | 673.4$^{AB}$ | 1098.9$^{A}$ |
| Mountrail | 4X | 131.1$^{D}$ | 80.6$^{A}$ | 657.9$^{D}$ | 124.3$^{B}$ | 5.5$^{A}$ | 2.7$^{BC}$ | 1.9$^{D}$ | 566.7$^{D}$ | 675.6$^{D}$ |
| LSD[c] | 6X | 2.588 | 2.383 | 4.4 | 3.2 | 0.1 | 0.7 | 0.4 | 13.6 | 38.6 |

[a] 25-34 = soft, 35-44 = medium soft, 45-64 = medium hard, 65-80 = hard;
[b] Measured on the mixograph, 1 = weak and 5 = strong;
[c] Means with same letter are not significantly different

**Table 3.** *QGw.mst-3B* end-use quality trait allele means for two pairs of near-isogenic lines grown in three 2018 replicated environments.

| Environment | Allele | Kernel Weight[a] *mg* | Kernel Protein *g kg$^{-1}$* | Kernel Hardness[b] | Flour Yield | Flour Protein | Flour Ash | Mixing Tolerance[c] | Mixing Time *min* | Water Absorption *g kg$^{-1}$* | Loaf Volume *cm$^3$* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | ----------*g kg$^{-1}$*---------- | | | | | | |
| 2018 Rainfed - Bozeman | Spring Wheat | 39.6 | 156.8 | 62.6 | 688.5 | 141.2 | 3.9 | 3.3 | 4.5 | 712.8 | 1142.5 |
| | Durum Wheat | 40.2 | 156.6 | 63.5 | 685.8 | 142.7 | 4.0** | 3.8 | 4.2 | 699.2 | 1169.2 |
| 2018 Irrigated - Bozeman | Spring Wheat | 45.6 | 149.5 | 57.8 | 690.3 | 136.3 | 3.8 | 2.8 | 4.2 | 737.8 | 1094.2 |
| | Durum Wheat | 45.2 | 153.3* | 55.0** | 696.0 | 140.7* | 3.9** | 2.5 | 3.8 | 745.7 | 1191.7**** |
| 2018 Rainfed - Kalispell | Spring Wheat | 39.7 | 115.3 | . | 704.0 | 104.3 | 4.0 | 4.7 | 5.5 | 661.8 | 982.5 |
| | Durum Wheat | 43.1** | 121.3* | . | 707.2 | 110.3** | 4.2*** | 4.7 | 5.2 | 679.8 | 990.0 |
| Mean (overall) | Spring Wheat | 41.7 | 140.6 | 60.2 | 694.3 | 127.3 | 3.9 | 3.6 | 4.7 | 704.2 | 1073.1 |
| | Durum Wheat | 42.8 | 143.8** | 59.2 | 696.3 | 131.2*** | 4.1**** | 3.7 | 4.4 | 708.2 | 1116.9*** |
| allele x pair | | NS† | ** | NS | NS | * | * | NS | NS | NS | ** |
| allele x environment | | * | NS | ** | * | NS | NS | NS | NS | NS | ** |
| pair x environment | | * | NS | NS | ** | NS | NS | NS | NS | NS | NS |
| allele x pair x environment | | * | NS | NS | NS | NS | NS | NS | NS | NS | NS |

[a]Kernel weight means determined using a subset of data from Jones et al. (2019);
[b] 25-34 = soft, 35-44 = medium soft, 45-64 = medium hard, 65-80 = hard, kernel hardness was not measured in the 2018 Kalispell Rainfed environment;
[c] Measured on the mixograph, 1 = weak and 5 = strong;
*, **, ***, **** Significant at the .05, .01, .001, and .0001, respectively;
†NS = not significant

**Table 4.** *QGw.mst-7A* end-use quality trait allele means for two pairs of near-isogenic lines grown in three 2018 replicated environments

| Environment | Allele | Kernel Weight[a] *mg* | Kernel Protein *g kg$^{-1}$* | Kernel Hardness[b] | Flour Yield | Flour Protein *g kg$^{-1}$* | Flour Ash | Mixing Tolerance[c] | Mixing Time *min* | Water Absorption *g kg$^{-1}$* | Loaf Volume *cm$^3$* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2018 Rainfed - Bozeman | Spring Wheat | 39.0 | 160.8 | 61.5 | 690.4 | 148.8 | 4.1 | 2.3 | 3.2 | 715.3 | 1167.7 |
| | Durum Wheat | 38.4 | 160.4 | 61.3 | 687.0 | 148.3 | 4.1 | 2.3 | 2.7 | 682.1** | 1172.1 |
| 2018 Irrigated - Bozeman | Spring Wheat | 42.9 | 158.7 | 55.1 | 704.8 | 147.0 | 4.0 | 1.8 | 2.7 | 708.5 | 1185.0 |
| | Durum Wheat | 43.1 | 158.7 | 53.7 | 701.0 | 146.5 | 4.0 | 1.5 | 2.6 | 708.5 | 1187.5 |
| 2018 Rainfed - Kalispell | Spring Wheat | 40.1 | 133.0 | . | 697.7 | 123.2 | 4.4 | 4.0 | 3.6 | 675.3 | 1058.3 |
| | Durum Wheat | 46.8 | 132.7 | . | 699.5 | 121.7 | 4.4 | 3.3** | 3.6 | 668.0 | 1060.8 |
| Mean (overall) | Spring Wheat | 40.7 | 150.8 | 58.3 | 697.6 | 139.6 | 4.2 | 2.7 | 3.2 | 699.7 | 1136.7 |
| | Durum Wheat | 42.8 | 150.6 | 57.5 | 695.8 | 138.8 | 4.2 | 2.4* | 3.0 | 686.2* | 1140.1 |
| allele x pair | | NS† | NS | ** | NS | NS | NS | NS | NS | NS | NS |
| allele x environment | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| pair x environment | | NS | *** | NS | NS | *** | NS | NS | NS | NS | ** |
| allele x pair x environment | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

[a]Kernel weight means determined using a subset of data from Jones et al. (2019);
[b] 25-34 = soft, 35-44 = medium soft, 45-64 = medium hard, 65-80 = hard, kernel hardness was not measured in the 2018 Kalispell Rainfed environment;
[c] Measured on the mixograph, 1 = weak and 5 = strong;
*, **, ***, **** Significant at the .05, .01, .001, and .0001, respectively;
†NS = not significant

**Table 5.** *QTn.mst-5B* end-use quality trait allele means for two pairs of near-isogenic lines grown in three 2018 replicated environments.

| Environment | Allele | Kernel Weight[a] *mg* | Kernel Protein *g kg$^{-1}$* | Kernel Hardness[b] | Flour Yield | Flour Protein *g kg$^{-1}$* | Flour Ash | Mixing Tolerance[c] | Mixing Time *min* | Water Absorption *g kg$^{-1}$* | Loaf Volume *cm$^3$* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2018 Rainfed - Bozeman | Spring Wheat | 40.0 | 152.8 | 58.9 | 706.3 | 140.5 | 4.7 | 1.5 | 2.4 | 676.2 | 1080.0 |
| | Durum Wheat | 41.0 | 157.7 | 56.0** | 716.6*** | 144.3 | 4.9 | 1.2 | 2.1 | 679.5 | 1032.5 |
| 2018 Irrigated - Bozeman | Spring Wheat | · | 149.7 | 56.3 | 712.5 | 136.7 | 4.7 | 1.2 | 2.2 | 686.8 | 1020.8 |
| | Durum Wheat | · | 150.0 | 55.5 | 712.8 | 135.7 | 5.2** | 0.8 | 2.2 | 674.3 | 944.2** |
| 2018 Rainfed - Kalispell | Spring Wheat | 44.8 | 124.8 | . | 719.0 | 115.2 | 4.8 | 2.5 | 3.1 | 655.2 | 919.2 |
| | Durum Wheat | 48.9** | 118.8 | . | 726.8** | 109.2* | 5.3** | 2.2 | 3.4 | 647.3 | 895.0 |
| Mean (overall) | Spring Wheat | · | 142.4 | 57.6 | 712.6 | 130.8 | 4.7 | 1.7 | 2.6 | 672.7 | 1006.7 |
| | Durum Wheat | · | 142.2 | 55.7* | 718.8*** | 129.7 | 5.1**** | 1.4 | 2.6 | 667.1 | 957.2** |
| allele x pair | | NS† | NS | NS | NS | NS | NS | NS | NS | NS | ** |
| allele x environment | | NS | * | NS | * | * | NS | NS | NS | NS | NS |
| pair x environment | | NS | NS | NS | ** | ** | NS | NS | NS | NS | NS |
| allele x pair x environment | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

[a]Kernel weight means determined using a subset of data from Jones et al. (2019), kernel weight was not measured in the 2018 Bozeman Irrigated environment;
[b] 25-34 = soft, 35-44 = medium soft, 45-64 = medium hard, 65-80 = hard, kernel hardness was not measured in the 2018 Kalispell Rainfed environment;
[c] Measured on the mixograph, 1 = weak and 5 = strong;
*, **, ***, **** Significant at the .05, .01, .001, and .0001, respectively;
[†]NS = not significant

**Table 6.** *QYld.mst-2B* end-use quality trait allele means for two pairs of near-isogenic lines grown in three 2018 replicated environments

| Environment | Allele | Kernel Weight *mg* | Kernel Protein *g kg$^{-1}$* | Kernel Hardness[a] | Flour Yield | Flour Protein *g kg$^{-1}$* | Flour Ash | Mixing Tolerance[b] | Mixing Time *min* | Water Absorption *g kg$^{-1}$* | Loaf Volume *cm$^3$* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2018 Rainfed - Bozeman | Spring Wheat | 36.4 | 149.3 | 68.0 | 683.5 | 136.7 | 4.1 | 2.0 | 3.1 | 691.3 | 1159.2 |
| | Durum Wheat | 33.2*** | 151.7 | 65.7 | 673.7**** | 139.7 | 4.3** | 1.7 | 2.9 | 699.0 | 1162.5 |
| 2018 Irrigated - Bozeman | Spring Wheat | 38.3 | 148.5 | 62.0 | 693.2 | 135.2 | 4.2 | 1.8 | 3.2 | 700.7 | 1156.7 |
| | Durum Wheat | 36.8 | 149.7 | 62.9 | 685.7*** | 136.3 | 4.2 | 1.5 | 3.0 | 716.2 | 1130.8 |
| 2018 Rainfed - Kalispell | Spring Wheat | 40.6 | 126.8 | . | 699.8 | 117.7 | 4.5 | 3.5 | 4.5 | 676.8 | 1050.8 |
| | Durum Wheat | 38.1** | 132.7** | . | 688.5**** | 122.5** | 4.6 | 3.0* | 3.3**** | 673.5 | 1078.3 |
| Mean (overall) | Spring Wheat | 38.4 | 141.6 | 65.0 | 692.2 | 129.8 | 4.3 | 2.4 | 3.6 | 689.6 | 1122.2 |
| | Durum Wheat | 36.1**** | 144.7** | 64.3 | 688.5**** | 132.8** | 4.4* | 2.1** | 3.1** | 696.2 | 1123.9 |
| allele x pair | | ** | NS | NS | **** | NS | NS | NS | ** | NS | * |
| allele x environment | | NS† | NS | NS | NS | NS | NS | NS | * | NS | NS |
| pair x environment | | * | NS | NS | **** | NS | NS | NS | * | * | NS |
| allele x pair x environment | | NS | * | NS | * | * | NS | ** | NS | NS | NS |

[a]Kernel weight means determined using a subset of data from Jones et al. (2019);
[b] 25-34 = soft, 35-44 = medium soft, 45-64 = medium hard, 65-80 = hard, kernel hardness was not measured in the 2018 Kalispell Rainfed environment;
[c] Measured on the mixograph, 1 = weak and 5 = strong;
*, **, ***, **** Significant at the .05, .01, .001, and .0001, respectively;
†NS = not significant

# CHAPTER FOUR

# ALLELIC RESPONSE OF YIELD COMPONENT TRAITS TO RESOURCE AVAILABILITY IN SPRING WHEAT

## Contribution of Authors and Co-Authors

Manuscript in Chapter 4

Author: Brittney H. Jones

Contributions: conceived study, experimental work including design, data collection, statistical analysis and manuscript writing and preparation

Co-Author: Nancy K. Blake

Contributions: development of near-isogenic line pairs, seed increase, phenotypic data collection

Co-Author: Hwa-Young Heo

Contributions: field design, phenotypic data collection, field harvest

Co-Author: John M. Martin

Contributions: aided in the writing of statistical code and contributed to the analysis and interpretation of data

Co-Author: Jessica A. Torrion

Contributions: field design and phenotypic data collection

Co-Author: Luther E. Talbert

Contributions: initiated and coordinated the project, contributed to data analysis and final manuscript writing and preparation

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# Allelic response of yield component traits to resource availability in spring wheat

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**Conflicts of interest/Competing interests**

On behalf of all authors, the corresponding author states that there is no conflict of interest

**Availability of data and material**

Data provided in supplementary files

**Code availability**

Not Applicable

**Author contribution statement**

BJ contributed the majority of experimental work, NB, HH, JT contributed to field design and phenotypic data collection, JM aided in the writing of statistical code and contributed to the analysis and interpretation of data. LT initiated and coordinated the project, contributed to data analysis and final manuscript writing and preparation. All authors reviewed the manuscript and provided suggestions.

**Abstract**

A greater understanding of grain yield (GY) and yield component traits in spring wheat may increase selection efficiency for improved GY in high and low yielding environments. The objective of this study was to determine allelic response of four yield component quantitative trait loci (QTL) to variable resource levels which were manipulated by varying intraspecific plant competition and seeding density. The four QTL investigated in this study had been previously identified as impacting specific yield components. They included *QTn.mst-6B* for productive tiller number (PTN), *WAPO-A1* for spikelet number per spike (SNS), and *QGw.mst-3B* and *TaGW2-A1* for kernel weight (KWT). Near-isogenic lines (NIL) for each of the four QTL were grown in multiple locations with three competition (border, no-border and space-planted) and two seeding densities (normal 216 seeds $m^{-2}$ and low 76 seeds $m^{-2}$). Allele response at *QTn.mst-6B* was driven by changes in resource availability whereas allele response at *WAPO-A1* and *TaGW2-A1* were relatively unaffected by resource availability. The *QTn.mst-6B.1* allele at *QTn.mst-6B* conferred PTN plasticity resulting in significant GY increases in high resource environments. The *gw2-A1* allele at *TaGW2-A1* significant increased KWT, SNS and GPC offering a source of GY improvement without negatively impacting end-use quality. *QGw.mst-3B* allelic variation did not significantly impact KWT but did significantly impact SPS. Treatment effects in both experiments often resulted in significant positive impacts on GY and yield component traits when resource availability was increased. Results provide guidance for leveraging yield component QTL to improve GY performance in high and low-yield environments.

**Key Message**

Investigation of resource availability on allele effects for four yield component quantitative trait loci provides guidance for the improvement of grain yield in high and low yielding environments

**Keywords**

Grain yield, resource availability, yield components, productive tiller number, seeding densit

**Abbreviations**

DAP, Days after planting
FLS, Flag leaf senescence
GLDAH, Green-leaf duration after heading
GPC, Grain protein content
GY, Grain yield
HD, Heading date
HIF, Heterogeneous inbred family
KWT, Kernel weight
NIL, Near isogenic line
PTN, Productive tiller number
QTL, Quantitative trait loci
RIL, Recombinant inbred line
SNS, Spikelet number per spike
SPS, Seeds per spike

## Introduction

Hexaploid wheat (*Triticum aestivum* L., 2n = 6x = 42) accounts for 30% of global grain production, ranking in the top three major food crop species along with maize (*Zea mays* L.) and rice (*Oryza sativa* L.) (FAOSTAT, 2018). Grain yield (GY) is a determining factor of agronomic success and an important consideration in breeding programs. Grain yield must be increased by 2.0% annually to support projected population growth (Tshikunde et al., 2019). However, increases have been between 0.5% to 1.0% over the past decade (Dixon et al., 2009; Tshikunde et al., 2019). Therefore, further investigation of novel yield component alleles and their interactions with resource availability, agronomic practices, and genotype may provide insight into new avenues for improved GY potential.

Efforts to bridge the current yield gap between potential and actual GY have been unsuccessful due to constraints not limited to changing climate, nutrient management and water availability (Mueller et al., 2012; Sinclair and Rufty, 2012; Ray et al., 2015; Hatfield and Beres, 2019). Hatfield and Dold (2018) suggest that low precipitation during the grain filling stage constrains GY of wheat grown in the Great Plains. Reduced precipitation during grain filling can result in the early onset of plant senescence thereby reducing the period in which the plant produces biomass as well as reducing the translocation of nitrogen and assimilate from plant tissues to developing kernels (Talbert et al., 2001; Woo et al., 2013; Torrion and Stougaard, 2017). Suggested avenues to improve wheat GY potential can be achieved through the development of genotypes with tolerance to abiotic stress and enhanced nutrient, radiation, and water use efficiency (Tshikunde et al., 2019). However, these efforts may be negatively impacted by the reduction in genetic variation of breeding populations which limits the potential to find

novel allelic combinations to improve GY potential across changing climates (Charmet, 2011; Govindaraj et al., 2015). Improvements to GY are further complicated due to the fact that yield component traits are often influenced by genotype x environment interactions, resulting in variable allelic performance across environments (Allard and Bradshaw, 1964; Reynolds et al., 2002; Kuchel et al., 2007b; El-Soda et al., 2014; Slafer et al., 2014; Mohammadi et al., 2015).

Grain yield increases are dependent upon improved agricultural practices as well as improved germplasm (Sinclair and Rufty, 2012; George, 2014). For example, water is a major resource for agricultural crops and water deficits can reduce GY (Lollato et al., 2019). Torrion and Stougaard (2017) investigated eight hard red spring wheat (HRSW) cultivars under six Montana water regimes and reported that GY was increased in all irrigated water regimes compared with the rainfed check. Notably, Torrion and Stougaard (2017) also saw a significant GY reduction when no irrigation was applied during grain filling stage. Seeding density has also been associated with significant impacts on GY but variably reported in the literature. High seeding density (above 215 seeds $m^{-2}$) has been correlated with increases in GY for non-competitive wheat ideotypes (Reynolds et al., 1994; Chen et al., 2008). Chen et al. (2008) reported seeding density above 215 seeds $m^{-2}$ for wheat grown in central Montana was not associated with increased GY. Otteson et al. (2007) showed no significant impact of seeding density on GY. Plant competition is also an important consideration in regard to GY response in variable planting designs. Nasseer et al. (2016) investigated high and low tillering NIL in three competition level treatments and reported that GY was most significantly affected when competition was low and resource availability was high. Based on these reports wheat GY demonstrates a level of plasticity in response to seeding density and resource availability.

Grain yield in wheat results from the combined interactions and hierarchical plasticity of multiple yield component traits (Sreenivasulu and Schnurbusch, 2012; Slafer et al., 2014). Several quantitative trait loci (QTL) analyses in wheat have co-located loci for both GY and yield component traits illustrating genetic control overlap (Kuchel et al., 2007a; Cuthbert et al., 2008; Sukumaran et al., 2015; Schulthess et al., 2017). Grain yield component traits include productive tiller number (PTN, also referred to as number of spikes per area), seeds per spike (SPS), spikelet number per spike (SNS), and individual kernel weight (KWT). Interactions between yield component traits are often negative, such that important traits including seed number and KWT are negatively correlated (Slafer, 2003; Gupta et al., 2006; Sadras, 2007). This negative interaction of yield component traits has the potential to result in the improvement of a specific yield component without an overall improvement in GY (Jones et al., 2019). Several studies have suggested that GY is predominately sink-limited (Reynolds et al., 2005; Fischer, 2007; Miralles and Slafer, 2007; Zhang et al., 2010). As such, increases in GY have mainly been conferred by increasing the number of seeds per area rather than increasing individual KWT (Bustos et al., 2013; Philipp et al., 2018; Ballesteros-Rodríguez et al., 2019).

This study investigated four yield component QTL to better understand the impact of resource availability as a function of plant competition and seeding density on HRSW GY potential as influenced by genetic variation for specific yield components. The QTL included *QTn.mst-6B* for PTN, *WAPO-A1* for SNS, and *QGw.mst-3B* and *TaGW2-A1* for KWT. *QTn.mst-6B* was identified by Naruoka et al. (2011) in two recombinant inbred line (RIL) populations derived from the bi-parental crosses ‘Reeder’ (PI 613586) (Underdahl et al., 2008) / ‘Conan’ (PI 607549) (WestBred, LLC) and Reeder / ‘McNeal’ (PI 574642) (Lanning et al., 1994). Allelic

variation at *QTn.mst-6B* explained 9%, 15%, and 17% of the variation observed for PTN in three Montana environments. The high tillering allele from Reeder was associated with an additive effect of 2.35 to 3.70 tillers $m^{-1}$ (Naruoka et al., 2011). Nasseer et al. (2016) investigated *QTn.mst-6B* NIL derived from crosses Reeder / 'Choteau' (PI 633974) (Lanning et al., 2004) and 'Vida' (PI 642366) (Lanning et al., 2006) / McNeal in three levels of plant competition. Vida was derived from the cross 'Scholar' (PI 607557) (Lanning et al., 2000) / Reeder and inherited the high tillering allele at *QTn.mst-6B* (Nasseer et al., 2016). Nasseer et al. (2016) showed significant increases in PTN associated with the Reeder and Vida allele specifically when plant competition was reduced and resource availability increased.

Quantitative trait loci *QGw.mst-7A* and *QGw.mst-3B* were identified in a QTL analysis of a RIL population derived from a cross between Choteau spring wheat and 'Mountrail' (PI 607540) (Elias and Miller, 2000) durum wheat (*Triticum durum* Desf.). Both QTL were associated with KWT (Kalous et al., 2015). *QGw.mst-3B* explained 6.1% of KWT variation across multiple environments. Jones et al. (2019) investigated NIL for *QGw.mst-7A* derived from a Choteau/Mountrail//'Berkut' (International Maize and Wheat Improvement Center (CIMMYT); released in 2002) cross and found that the spring wheat allele was significantly associated with SNS with no significant impact on KWT. Zhang et al. (2018) also associated SNS with a QTL on 7A, which was determined to be the same locus as *QGw.mst-7A*, in two RIL populations (Kuzay et al., 2019). *WAPO-A1* was identified to be the causal gene underlying *QGw.mst-7A* (Kuzay et al., 2019). The *WAPO-A1* ortholog *APO1* in rice has been functionally characterized as impacting spike branch morphology (Kuzay et al., 2019). Hereafter *QGw.mst-7A* is referred to as *WAPO-A1*.

*TaGW2-A1*, located on chromosome 6A, is an ortholog to rice gene *OsGW2* (Song et al., 2007). The *OsGW2* locus impacts kernel weight and length (Song et al., 2007; Zhang et al., 2013; Simmonds et al., 2016). Transcriptional expression studies in wheat have shown *TaGW2-A1* is a negative regulator of kernel weight (Zhang et al., 2013). Simmonds et al. (2016) screened a tetraploid TILLING (Targeted Induced Local Lesion IN Genomes) population for *TaGW2-A1* mutations and identified an exon 5' splicing mutation. The resultant *gw2-A1* mutant allele was evaluated in $BC_2$ and $BC_4$ derived NIL from the cross tetraploid Kronos TILLING mutant line (T4-2235) by 'Paragon' (PI 675014) and was associated with a 7.8 % KWT increase in hexaploid wheat across four testing environments (Simmonds et al., 2016).

The four QTL in the present study were investigated in two experiments including a competition experiment and a seeding density experiment. The competition experiment was designed to address the biological response of plants to variable resource availability by using different levels of plant competition. The seeding density experiment examined the impact of normal and low seeding density on GY and yield component response. One common finding with the four selected QTL for this study is variability of allele effects related to competition and density treatments. This variability is reflected in the differential impact of alleles on multiple yield component traits (Naruoka et al., 2011; Kalous et al., 2015; Nasseer et al., 2016; Simmonds et al., 2016; Jones et al., 2019). The objective of the current research was to subject NIL of four QTL associated with GY and yield components to varying levels of resource availability. Results provide guidance for the potential selection of yield component QTL to improve GY potential in high and low-yielding environments. Further, results highlight the importance of investigating single QTL to determine allele response across a range of

environmental conditions.

## Materials and Methods

### Plant Materials

This study used HRSW NIL for four yield component QTL. Yield component traits and the number of NIL for each QTL are described in Table 1. Near-isogenic lines with contrasting alleles at *QTn.mst-6B* were developed from crosses between Reeder / Choteau and Vida / McNeal (Nasseer et al., 2016). Derivation of NIL was described by Nasseer et al. (2016) using the heterogenous inbred family (HIF) strategy (Haley et al., 1994; Pumphrey et al., 2007). In short, single seed descent (SSD) was used to generate inbred $F_4$ progeny from bi-parental crosses. Heterozygous $F_4$ individuals were identified using a QTL specific marker and self-pollinated to produce NIL with contrasting alleles at *QTn.mst-6B* (Table 1). Reeder and Vida were parents for the high PTN allele (designated *QTn.mst-6B.1*). Choteau and McNeal represented parents for the low PTN allele (designated *QTn.mst-6B.2*).

Near-isogenic lines for *WAPO-A1* and *QGw.mst-3*B were derived from the cross Choteau/Mountrail//Berkut using the HIF strategy as described by Jones et al. (2019). Heterozygous individuals were selected at the $F_5$ generation and self-pollinated to produce NIL with contrasting spring or durum alleles at the respective QTL. In the case of *WAPO-A1* the elite HRSW cultivars Choteau and Berkut contributed the high SNS allele (designated *WAPO-A1b*). Durum wheat Mountrail conferred the low SNS allele (designated *WAPO-A1a*). Allele designation for *WAPO-A1* NIL are based on Kuzay et al. (2019), where the allele contributed by spring wheat provides a similar phenotype as the low SNS allele from 'RAC875' (Zhang et al., 2018). For *QGw.mst-3B*, durum wheat Mountrail contributed the high KWT allele (designated *QGw.mst-3B.1*). Choteau and Berkut contributed the low KWT allele (designated *QGw.mst-*

*3B.2*).

Backcross-derived NIL for *TaGW2-A1* were described by Simmonds et al. (2016). Near-isogenic lines were derived at the $BC_4F_3$ generation by crossing the recurrent parent hexaploid HRSW variety Paragon with the donor parent tetraploid Kronos TILLING mutant line (T4-2235) containing the *gw2-A1* mutation. After the initial bi-parental cross four backcrosses were performed selecting progeny carrying the *gw2-A1* allele. In this study, a single backcross NIL from the original bi-parental cross with the *gw2-A1* allele and the parental variety Paragon were used to evaluate *TaGW2-A1*. The backcross mutant line containing the *gw2-A1* allele showed high KWT (was designated *gw2-A1*), and the wildtype *GW2-A1* allele in the Paragon background showed low KWT (designated *GW2-A1*) (Simmonds et al., 2016). Allele calls for the single *TaGW2-A1* NIL pair used in the present study were determined by markers provided by Simmonds et al. (2016) (Table 1).

**Competition Experiment**

The NIL pairs were grown with three competition levels, where plant competition was inversely related to resource availability. Competition levels included border (high competition), no-border (intermediate competition), and space-planted (low competition) (Figure 1). The border treatment plots consisted of three rows, spaced 30-cm apart x 3.0-m long, where the center row was a single NIL entry and border rows were HRSW cultivar ‘Lanning’ (PI 676978) (Heo et al., 2016). Only the center row was evaluated. No-border treatment consisted of a single row plot 3.0 m in length of a NIL entry planted without adjacent borders. Space-planted treatment included an individual NIL entry planted in a single row and thinned to a spacing of one plant per 0.3 m, with a total of 3.0 m of plot length. A seeding density of 217 seeds $m^{-2}$ was

used for the border and no-border treatments. The experiment was arranged in a randomized block split-plot design with three blocks. Competition levels were main plots with NIL entries as subplots. The experiment was grown in rainfed and irrigated environments at the Arthur H. Post Field Research Farm near Bozeman, MT (latitude 45.68°N, longitude 111.04°W, elevation 1469 m) in 2018 and 2019. The 2018 irrigated environment received an additional 16.5 cm of moisture through two applied irrigation events, and the 2019 irrigated environment received an additional 17.3 cm of moisture through two applied irrigation events. Irrigation events in both environments were applied prior to heading date. Climatological data for the 2018 and 2019 growing season and respective planting and harvest dates of all experiments are provided in Table 2.

**Seeding Density Experiment**

The same NIL pairs were grown in three environments at two seeding densities in a randomized block split plot design with three blocks. Seeding density treatment was the main plot and NIL entries were subplots. Seeding densities were normal (217 seeds $m^{-2}$) and low (76 seeds $m^{-2}$). The low seeding density had the same number of seed per unit area as the low competition and intermediate resource treatment of the no-border treatment in the competition experiment. The experiment was planted at the Arthur H. Post Research Farm near Bozeman, MT in both rainfed and irrigated conditions in 2019 and at a single rainfed environment at the Northwestern Agricultural Research Center in Kalispell, MT (latitude 48.19°N, longitude 114.32°W, elevation 900 m). The Bozeman irrigated and rainfed plots were three rows spaced 30-cm apart x 3.0-m long. Plots in the Kalispell environment were seven 4.6-m long rows spaced 15.2-cm apart. The Bozeman irrigated environment received an additional 17.2 cm moisture applied across two irrigation events prior to heading. Climatological data and planting and

harvest dates for the Bozeman and Kalispell environments are provided in Table 2.

**Phenotypic Data Collection**

Phenotypic data collected for the competition and seeding density experiments included physiological and agronomic traits. Physiological traits included heading date (HD), flag leaf senescence (FLS), and green leaf duration after heading (GLDAH). Agronomic traits included productive tiller number (PTN), spikelet number per spike (SNS), seeds per spike (SPS), kernel weight (KWT), grain yield (GY) and grain protein content (GPC).

Heading date was determined as days after planting (DAP) when 50% of spikes had emerged from the boot. Flag leaf senescence was determined as DAP when 50% of flag leaves had become 100% chlorotic. Green leaf duration after heading was determined as the difference in days between FLS and HD. Productive tiller number was determined as the number of tillers bearing a spike at plant maturity. In the competition experiment PTN for the border and no-border competition treatments was determined by the number of tillers in 1-m of row. The seeding density experiment determined PTN in the same manner. Productive tiller number for the space-planted treatment in the competition experiment was measured by counting all tillers from three representative plants. Spikelet number per spike was determined as the average number of fertile spikelets from five randomly sampled spikes per plot. Seeds per spike was determined as the average number of seeds from five randomly sampled spikes per plot. Thousand-kernel weight was determined by weighing a grain sample with pre-determined number of seeds. Kernel weight was characterized by taking 1000-seed weights per plot and converting to single kernel weight. Grain yield was determined from the raw grain weight of each plot in the competition experiment (border and no-border treatment) and seeding density experiments. Grain yield in the

competition experiment for space-planted treatment was determined by grain weight from all spikes of three representative plants per plot. Grain protein content was measured using near-infrared transmittance (Infratech 1241 Grain Analyzer).

**Statistical Analysis**

Data from the competition and seeding density experiments were analyzed separately. Analyses for the competition and seeding density experiments were done using PROC MIXED in SAS version 9.4 (SAS Institute Inc. 2012). Each QTL was analyzed separately. A model for split-plot design combined over environments was used for the competition experiment. The model included environment, competition level, NIL pair and allele, and their interactions as fixed effects and replication within environment and replication by competition level within environment as random effects. For *WAPO-A1* and *TaGW2*, the model did not include the NIL pair factor as a single NIL pair represented the QTL. Productive tiller number and GY for the space-planted treatment were analyzed separately as measurement units differed from the border and no-border competition level treatments. This analysis was similar to the one described above except that the treatment factor was dropped from the model. For the seeding density experiment a split-plot design combined over environments was used. The model for the seeding density experiment was the same as described for the competition experiment. An ANOVA was done on data for the competition and seeding density experiments using PROC GLM to determine overall treatment effect on all measured traits. The model included environment, replication within location, treatment, and treatment x location. PROC GLM was used to calculate least significant difference (LSD) among treatment means for the competition and seeding density experiments. Number of observations varied between the competition and seeding density experiments. For

loci *QTn.mst-6B* and *QGw.mst-3B* two NIL pairs were included in this study with a total of n = 72 and n = 36 allele observations in the competition and seeding density experiments, respectively. Only a single NIL pair was included for the loci *WAPOA-A1* and *TaGW2-A1* resulting in a total of n = 36 and n = 18 allele observations for each QTL in the competition and seeding density experiments, respectively.

## Results

### Reduced competition significantly impacted yield component traits

Treatment effect for both the competition and seeding density experiments was determined by using overall means combined across environments and genotypes (Table 3). Treatment effect was significant for most traits. Yield component traits PTN, SNS, SPS, and KWT were all impacted by competition level ($P < .05$) in the competition experiment. For all yield component traits, the difference in overall mean trait response increased between competition levels such that the greatest means were in the lowest competition and thus highest resource available treatment (space-planted) or in the case of PTN and GY the greatest means were observed in the no-border treatment. This same trend was also observed for GPC, such that GPC in the space-planted treatment was the highest. The most notable difference between treatments in the competition experiment was the treatment effect on GY and PTN. Between the border and no-border treatments GY for the harvested row was increased by 152.3 g $m^{-1}$ and PTN was increased by 56.5 tillers $m^{-1}$ in the no-border treatment. Heading date was delayed as competition level decreased resulting in a longer plant vegetative stage. Significant treatment differences for FLS and GLDAH were only detected between the space-planted treatment compared to the other two competition level treatments. Treatment effects were smaller for the density experiment. Yield component traits SNS and SPS were increased by 1.1 spikelets and 6.4 seeds respectively, in the low seeding density treatment relative to the normal seeding density treatment. However, for PTN and GY there was a decrease of 18.4 tillers $m^{-1}$ and 595.4 kg $ha^{-1}$, respectively in the low seeding density compared with the normal seeding density. Treatment effect was not detected ($P > .05$) for KWT, FLS, GLDAH or GPC.

### *QTn.mst-6B.1* allele increased PTN and GY when plant competition was reduced

Allele means over the competition and seeding density experiments for *QTn.mst-6B* NIL pairs are shown in Table 4. *QTn.mst-6B.1* increased PTN compared to *QTn.mst-6B.2* ($P < .05$) in all three competition level treatments and when combined across the border and no-border treatments. In the border and no-border treatment PTN was increased relative to *QTn.mst-6B.2* by 28.9 and 34.1 tillers $m^{-1}$, respectively. In the space-planted treatment *QTn.mst-6B.1* was associated with a 2.1 tiller increase per plant. *QTn.mst-6B.1* reduced KWT by 1.7 mg ($P < .0001$) averaged over competition treatments resulting in a 5.0% decrease. *QTn.mst-6B.1* reduced KWT for each competition level but was only significant in the no-border ($P < .05$) and space-planted ($P < .001$) treatments. Allele type did not significantly impact SPS or SNS. *QTn.mst-6B.1* increased GY 4.4% when averaged over the border and no-border competition treatments ($P < .05$). Additionally, *QTn.mst-6B.1* increased GY in the 2018 Bozeman rainfed border ($P < .01$) and space-planted ($P < .05$) treatments as well as in the 2019 Bozeman irrigated space-planted ($P < .05$) treatment (Supplemental File 1). Heading date and FLS were delayed for *QTn.mst-6B.1* compared with *QTn.mst-6B.2*. *QTn.mst-6B.1* delayed HD by 1.3 d and FLS by 1.1 d, averaged over treatments. There was no allele impact on GLDAH. *QTn.mst-6B.1* decreased GPC in the space-planted treatment ($P < .05$). Similar though non-significant ($P > .05$) differences were observed in the border and no-border competition levels. There was no significant treatment x allele interaction for any of the traits measured (Supplemental File 2).

Results from the seeding density experiment showed that *QTn.mst-6B.1* increased PTN. Differences between alleles for PTN was only significant when averaged over all seeding density

treatments ($P < .05$). Environment effect was significant for *QTn.mst-6B* (Supplemental File 4). Allelic variation at *QTn.mst-6B* did not impact SNS, SPS and GY (Table 4). However, *QTn.mst-6B.1* significantly reduced KWT ($P < .01$) at both seeding densities relative to *QTn.mst-6B.2*. *QTn.mst-6B.1* delayed HD by > 1.1 d ($P < .0001$) compared to *QTn.mst-6B.2* in both seeding densities. Flag leaf senescence was 1.4 d later and GLDAH was 3 d shorter for *QTn.mst-6B.1* compared to *QTn.mst-6B.2* in the low-density treatment. These differences were not observed at normal seeding density. Unlike the competition experiment, *QTn.mst-6B.1* reduced GLDAH in the seeding density experiment by 2.1 d averaged over treatments. The allelic difference on GLDAH was observed only in the 2019 Kalispell rainfed environment (Supplemental File 3) which gave rise to multiple significant interactions. Both HD and GLDAH were significantly impacted by the two-way interaction treatment x allele likely resulting from magnitude changes between allele effects across seeding density treatments (Supplemental File 4). *QTn.mst-6B.1* significantly reduced GPC in both seeding density treatments ($P < .05$) (Table 4). The 2019 Kalispell rainfed environment accounted for the majority of allelic differences for GPC (Supplemental File 3).

### *WAPO-A1b* allele significantly increased SNS with minimal yield component trait pleiotropic interaction

Means of NIL differing for alleles at *WAPOA-A1* in the competition and seeding density experiments are shown in Table 5. *WAPO-A1b* significantly increased SNS by 1.5 spikelets in the border ($P < .0001$) and 1.3 spikelets in the no-border ($P < .001$) competition level treatments (Table 5). Treatment x allele interaction was significant for SNS (Supplemental File 2), likely because the difference between alleles was small for the space-planted competition level

compared to border and no-border competition levels (Supplemental File 2). Seeds per spike and PTN were not significantly impacted by allelic variation for any of the competition treatments or when combined across competition level treatments. However, *WAPO-A1b* increased SPS in the 2018 Bozeman irrigated environment for the border treatment (Supplemental File 1). Kernel weight was significantly reduced with *WAPO-A1b* associated with a 1.3 mg and 2.8% decrease averaged across competition level treatments. This reduction in KWT likely resulted from the significant KWT reduction observed only in the 2018 Bozeman irrigated space-planted treatment (Supplemental File 1). *WAPO-A1b* resulted in a delayed HD ($P < .01$) for the space-planted treatment but showed no significant change for the border and no-border competition level treatments. This resulted in a significant ($P < .05$) treatment x allele interaction (Supplemental File 1). Allelic differences at *WAPOA-A1* did not impact GY, FLS, GLDAH or GPC.

Data summarized in Table 5 for the seeding density experiment showed similar allelic impacts as in the competition experiment. Yield component traits SPS and KWT were not significantly impacted by allele type ($P > .05$). *WAPO-A1b* was associated with a significant reduction in PTN in the low seeding density treatment ($P < .05$). *WAPO-A1b* caused a significant increase in SNS in the low seeding density ($P < .01$) treatment and when averaged over the two seeding treatments ($P < .01$). Grain yield and GPC were not impacted by allele type. For the normal seeding density, *WAPO-A1b* was associated with an earlier HD by 0.4 d ($P < .01$) and a 0.9 longer GLDAH ($P < .01$) relative to *WAPO-A1a*. The allele type means were nearly the same for the low seeding density for HD and GLDAH which resulted in significant treatment x allele interactions (Supplemental File 4).

**_QGw.mst-3B.1_ allele significantly increased GPC and KWT in the low seeding density treatment**

Means over the competition and seeding density experiment for NIL differing for *QGw.mst-3B* alleles are shown in Table 6. *QGw.mst-3B.1* significantly reduced SPS ($P < .05$) by 3.6 seeds for the no-border treatment, and when averaged over competition treatments. The difference for the no-border treatment was most pronounced in the two 2018 Bozeman environments (Supplemental File 1). *QGw.mst-3B.1* was also associated with increased GPC ($P < .0001$) for the no-border and space-planted competition level treatments. *QGw.mst-3B.1* was previously confirmed in multi-environment studies to confer an increase in KWT (Jones et al., 2019). Data from the competition experiment shows no significant difference between *QGw.mst-3B.1* and *QGw.mst-3B.2* on KWT. Allele type at *QGw.mst-3B* did not impact any additional traits in the competition experiment. Treatments were significantly different ($P < .01$) for all traits excluding PTN and GY, indicating trait variation between border, no-border, and space-planted treatments (Supplemental File 2). Treatment x allele interaction was not significant for any of the agronomic and phenological traits (Supplemental File 2).

Data for the seeding density experiment are shown in Table 6. Allelic variation at *QGw.mst-3B* significantly impacted PTN, SNS and KWT. *QGw.mst-3B.1* significantly increased PTN when averaged over both seeding density treatments ($P < .05$). Allelic differences were not detected at the normal seeding density for any of the measured traits for *QGw.mst-3B*. However, at the low seeding density *QGw.mst-3B.1* caused increased KWT (1.6 mg) ($P < .05$), FLS (2.9 d) ($P < .01$), GLDAH (1.5 d) *(*$P < .05$), and GPC (4.7 g $kg^{-1}$) ($P < .001$). *QGw.mst-3B.1* exceeded *QGw.mst-3B.2* for FLS ($P < .05$), GLDAH ($P < .05$), and GPC ($P < .001$) averaged over seeding

densities. In addition, *QGw.mst-3B.1* increased PTN and reduced SNS only when averaged over seeding densities. There were no significant treatment x allele or location x allele interactions for traits measured with the notable exception of GPC (Supplemental File 4). The two-way interactions treatment x allele and location x allele were significant for GPC resulting from larger differences observed between *QGw.mst-3B.1* and *QGw.mst-3B.2* across the two seeding density treatments and the three environments (Supplemental File 3).

**Mutant *gw2-A1* allele simultaneously increased SNS and KWT when competition level was reduced**

Means over the competition and seeding density experiment for NIL differing for alleles at *TaGW2-A1* are shown in Table 7. Allelic variation at *TaGW2-A1* impacted several yield component traits including KWT and SNS (Table 7). The *gw2-A1* allele at *TaGW2-A1* resulted in a significant 7.8 % KWT increase of 3.6 mg ($P < .0001$) relative to *GW2-A1* combined over competition level treatments. Allele type had no significant impact on SPS in the border and space-planted treatments or when combined across competition level treatments. However, in the no-border treatment *gw2-A1* significantly increased SPS by 6.7 seeds relative to *GW2-A1* ($P < .01$). Allele *gw2-A1* increased SNS in the no-border (0.8 spikelets) ($P < .05$) and space-planted (1.2 spikelets) ($P< .01$) and when averaged over treatments (0.7 spikelets) ($P < .001$). GY was not impacted by allelic variation and treatment at *TaGW2-A1* when averaged over environments. However, *gw2-A1* significantly increased ($P < .05$) GY and KWT in the 2018 Bozeman rainfed environment (Supplemental File 1). Allele *gw2-A1* decreased PTN in all competition level treatments and when combined over the border and no-border treatments. Though allele differences were not significant ($P > .05$) *gw2-A1* tended to delay HD in each treatment, but the

difference reached statistical significance only for the space-planted treatment ($P < .05$) and when averaged over treatments ($P < .05$). Flag leaf senescence and GLDAH were not impacted by allele type. Allele *gw2-A1* significantly increased GPC in all competition level treatments ($P < .05$) with an increase of 3.0 g $kg^{-1}$ relative to *GW2-A1* when combined over all competition level treatments. The treatment x allele interaction was non-significant for traits excluding SNS and GLDAH (Supplemental File 2). The significant interaction between treatment x allele for SNS resulted from a magnitude difference between treatment levels (Supplemental File 1).

Data for *TaGW2-A1* NIL planted in the seeding density experiment are summarized in Table 7. Allele type at *TaGW2-A1* did not result in significant impacts on yield component or phenological characteristics in the seeding density experiment averaged over combined seeding density treatments. However, *gw2-A1* significantly reduced GLDAH by 3.2 d ($P < .05$) relative to *GW2-A1* in the normal seeding density treatment. Reduction in GLDAH likely resulted from the 2019 Kalispell rainfed normal seeding density treatment (Supplemental File 3). Unlike in the competition experiment there was no impact on GPC from allelic variation in the seeding density experiment. Allele *gw2-A1* was associated with a significant reduction in GY of 476.9 kg $ha^{-1}$ in the normal seeding density treatment compared to *GW2-A1*. There was a significant treatment x allele interaction for GY (Supplemental File 4). This may be because GY trends for *gw2-A1* and *GW2-A1* for the low seeding density were opposite those for normal seeding density (Supplemental File 3).

## Discussion

The primary objective of the current study was to determine the impact of resource availability on QTL controlling yield component traits (Table 1). Grain yield is influenced by environment, genotype x environment interactions and pleiotropic interactions between underlying yield component traits, resulting in changes in relative genotype performance and stability across environments (Allard and Bradshaw, 1964; Kang et al., 2004; El-Soda et al., 2014). Resource availability in the form of water and soil nutrients significantly impact GY and yield component traits. The present study assessed NIL for four yield component QTL across different competition and seeding density treatments.

Treatment effect in the competition and seeding density experiments influenced GY and yield component trait responses. GY was increased by 74% (152.3 g $m^{-1}$)in the no-border relative to the border treatments averaged across all environments and genotypes of the competition experiment (Table 3). This increase is due to the fact that the harvested row in the no-border treatment had excess resource relative to the harvested row in the border treatment because adjacent border rows were not planted. Notably, the conferred increase of GY in the no-border treatment is not reflective of expected GY increases in large scale fields because two thirds more rows would be planted in a no-border versus border scenario. For example, GY was significantly decreased in the low seeding density treatment which was designed to be the same number of seed per unit area as the no-border competition treatment. However, this observed GY increase does suggest that on a biological scale plants respond positively to reduced competition and increased resource availability as observed in the no-border treatment. Additional yield component traits including PTN, SNS, SPS and KWT were also influenced by resource

availability such that as competition level decreased yield component trait response increased. In the seeding density experiment, treatment effect significantly impacted PTN, SNS, SPS and GY. Grain yield was 10% higher in the normal seeding density treatment compared to the low seeding density. Chen et al. (2008) similarly reported significant reduction in GY when seeding density was decreased from 215 seeds $m^{-2}$ to 108 seeds $m^{-2}$ for spring wheat grown in central Montana. Spikelet number per spike and SPS were both greatest in the low seeding density treatment. In general, wheat crops planted at higher seeding rates tend to produce fewer tillers and consequently, fewer seeds per plant compared with low density wheat (Tompkins et al., 1991; Chen et al., 2008; Walsh and Walsh, 2020). By reducing seeding density, it is plausible that each individual plant had access to more soil resources which allowed for the production of more SPS.

Allelic variation at three of the four QTL had a significant effect on yield components confirming previous reports (Naruoka et al., 2011; Kalous et al., 2015; Nasseer et al., 2016; Simmonds et al., 2016; Jones et al., 2019). *QTn.mst-6B* (Table 4), *WAPO-A1* (Table 5), and *TaGW2-A1* (Table 7) impacted PTN, SNS and KWT, respectively. Other yield component traits were also impacted by these QTL. *QGw.mst-3B* did not significantly impact KWT as seen in a previous experiment (Jones et al., 2019), but did impact SPS.

Resource availability affected allelic variation at *QTn.mst-6B* such that *QTn.mst-6B.1* increased PTN for all treatments of the competition experiment and combined over treatments in the seeding density experiment (Table 4). *QTn.mst-6B.1* increased PTN in the no-border treatment relative to the border treatment. The lack of a border row provided more available resources to the harvested row than when border rows were present. Findings for *QTn.mst-6B*

from the present study are consistent with those observed by Nasseer et al. (2016). Furthermore, much like in the present study Nasseer et al. (2016) detected GY increases only in the highest resource treatment and environments.

The *WAPO-A1b* allele at *WAPO-A1* was associated with significant SNS increases when averaged over treatments for both the competition and seeding density experiments (Table 5). Allelic variation had no significant effect on SNS in the space-planted treatment of the competition experiment when resources were high. A possible explanation for this result may be that SNS is predetermined early in plant development (Waddington et al., 1983; Slafer, 2003; Gonzalez et al., 2011; Gonzalez-Navarro et al., 2015). Therefore, allelic variation for SNS at *WAPO-A1* may not be influenced by resource availability, such that *WAPO-A1b* confers a genetic potential regardless of environment.

Little interaction between allelic variation and resource availability was observed for *QGw.mst-3B* with GY and yield component traits including KWT (Table 6). Both Kalous et al. (2015) and Jones et al. (2019) showed a significant increase in KWT associated with the durum wheat allele (*QGw.mst-3B.1* in the present study) at *QGw.mst-3B*. Results from the present study did not detect an impact of *QGw.mst-3B* on KWT, though SPS was reduced. The lack of significant allelic variation on KWT may reflect a difference in environments for this study and those evaluated by Kalous et al. (2015) and Jones et al. (2019). Allelic variation at *QGw.mst-3B* significantly increased GPC in both the competition and seeding density experiments. Therefore, it is possible that *QGw.mst-3B*, rather than effecting KWT, may explain variation for GPC.

Allelic variation at *TaGW2-A1* resulted in significant impacts on KWT. Specifically, *gw2-A1* resulted in significant KWT increases in all treatment levels of the competition experiment and

non-significant KWT increases in the seeding density experiment (Table 7). There was no significant treatment x allele interaction for KWT at *TaGW2-A1* suggesting differences in KWT were stable over environment (Supplemental File 2). Findings for *TaGW2-A1* in the present study are consistent with those observed by Simmonds et al. (2016) in which the mutant *gw2-A1* allele was associated with a stable increase in KWT resulting from increased grain width and length across the spike and within spikelets.

All four QTL in the present study showed pleiotropic effects on multiple yield component traits. Only two of the four QTL showed yield component trait interactions as influenced by resource availability. *QTn.mst-6B.1* at *QTn.mst-6B* for high PTN was associated with significant reduction in KWT in the no-border and space-planted treatments in the competition experiment as well as in normal and low treatments in the seeding density experiment. Increasing PTN and thereby producing more seed per plant results in a reduction in the weight of individual kernels (Slafer, 2003; Sadras, 2007; Gaju et al., 2009; Mitchell et al., 2012; Mitchell et al., 2013; Gaju et al., 2014; Brinton et al., 2018). The negative trade-off between KWT and PTN is likely influenced by resource availability. For example, the KWT in the competition experiment was only significantly decreased only in the no-border and space-planted treatments when PTN differences were the greatest due to increased resource availability.

A notable pleiotropic interaction for the *TaGW2-A1* NIL pair was that *gw2-A1* was associated with an increase of GPC, SNS and KWT in the competition experiment (Table 7). The increase in both KWT and SNS may result from lower PTN associated with *gw2-A1*. Differences between allele type for PTN approach significance at $P < .06$ and $P < .07$ for the border and space-planted treatments in the competition experiment, respectively (Supplemental File 1). Further, increase

of SNS in the high resource availability environments may relate to increases in KWT by raising the grain sink size through the development of additional spikelets (Gaju et al., 2009; Sreenivasulu and Schnurbusch, 2012). Increases in KWT are typically pleiotropically associated with decreases in GPC related to the dilution of grain protein and increase in grain carbohydrate (Groos et al., 2003; Oury et al., 2003; Oury and Godin, 2007; Blanco et al., 2012; Goel et al., 2019). Few studies have reported similar results showing an increase in both KWT and GPC. Oury and Godin (2007) suggested that the negative interaction between KWT and GPC is heavily influenced by environment such that in some environments the negative interaction is weak or undetected. In the present study allelic variation only affected GPC in the competition experiment and not the seeding density suggesting that environment and agronomic practice variation may influence allele response. The effect of the mutant *gw2-A1* allele on GPC in the present study is consistent with results from Zhang et al. (2018) which demonstrated that homoeologous gene edited mutants of *TaGW2-B1* and *TaGW2-D1* had higher GPC. There was a significant treatment x allele interaction for SNS suggesting that the difference in SNS between allele types was marginally increased as resource availability increased (Supplemental File 2).

Plant physiological traits including HD and FLS influence GY and yield component response (Trethowan et al., 2001; Blake et al., 2009; González et al., 2011; Jobson et al., 2019). All four QTL showed variability in HD, FLS and GLDAH in response to resource availability and allelic variation. However, only variation in HD, FLS and GLDAH at *QTn.mst-6B* was associated with yield component response. *QTn.mst-6B.1* increased PTN at *QTn.mst-6B* and was also associated with a significant overall postponement of HD and FLS in the competition experiment (Table 4). Changes in HD and FLS did not significantly influence GLDAH in the competition experiment

whereas, only HD was postponed in the seeding density experiment. In the seeding density experiment GLDAH was decreased by 2.1 d. Notably, in conjunction with increased PTN and decreased GLDAH in the seeding density experiment, *QTn.mst-6B.1* also significantly decreased KWT. Longer periods of GLDAH have been associated with increases in KWT in spring wheat (Talbert et al, 2001; Blake et al., 2007; Naruoka et al., 2012). It is therefore likely that reduced GLDAH further enhanced the negative pleiotropic interaction between PTN and KWT associated with *QTn.mst-6B.1*.

Two of the four QTL, *QTn.mst-6B* and *TaGW2-A1*, showed impacts of allelic variation on GY as influenced by resource availability. The allele impacts for *QTn.mst-6B* and *TaGW2-A1* on GY were isolated to treatments and environments where resource availability was high (Supplemental File 1). *QTn.mst-6B.1* at *QTn.mst-6B* significantly increased GY in the 2018 Bozeman irrigated border and space-planted treatments and the 2019 Bozeman irrigated space-planted treatment in the competition experiment (Supplemental File 1). Productive tiller number has been associated with increased GY in high resource availability environments when comparing free-tillering to tiller inhibition (*tin*) genotypes (Mitchell et al., 2013; Sadras and Rebetzke, 2013). Reynolds et al. (1994) suggested that breeding material capable of demonstrating phenotypic plasticity to changes in microenvironmental competition can confer a yield advantage. Arguably, the increases in GY associated with *QTn.mst-6B* lends support for the selection of traits demonstrating a level of plasticity. Plants with the beneficial *QTn.mst-6B.1* allele are capable of capitalizing on changes in resource availability in the environment and increasing PTN thereby promoting increased GY if resources are sufficient. Allelic variation at *TaGW2-A1* did not significantly impact GY across individual treatments in the competition

experiment. However, *gw2-A1* increased GY in the 2018 Bozeman rainfed border treatment. Kernel weight was also increased in this same environment. It is possible that when available precipitation was increased, *gw2-A1* at *TaGW2-A1*conferred a GY increase by increasing KWT. However, in the normal treatment of the seeding density experiment GY was significantly reduced in association with *gw2-A1*. The lack of consistent allele effect on GY with *TaGW2-A1* highlights the difficulty of improving overall GY potential when selecting for only a single yield component trait.

The present study highlights an important difficulty in selecting for improved GY in variable high and low resource environments and agronomic practices. All four of the QTL studied showed differences in GY and yield component trait response between the competition and seeding density experiments. Differences in GY, yield component and phenological traits by treatment were detected for both experiments (Table 3). However, the two experiments did not provide consistent statistical differences between alleles for the individual QTL. For example, allele responses of the four QTL studied were most evident in the competition experiment for *QTn.mst-6B* (Table 4) and *WAPO-A1* (Table 5). However, differences in phenotype due to allele types for KWT at *QGw.mst-3B* was only detected in the seeding density experiment (Table 6). Phenotypic differences due to allele types at *TaGW2-A1* were detected in both the competition and seeding density experiments (Table 7). Differences in GY and yield component trait response highlight the importance of investigating potential allele sources for GY improvement in both commercial production conditions and in conditions where plant competition is minimized as seen in the competition experiment.

The objective of the current study was to determine the impact of alleles at four yield

component QTL in HRSW across a range of plant competition conditions evaluated over several environments. The QTL tended to impact multiple yield components in addition to the specific yield component identified in previous work (Naruoka et al., 2011; Kalous et al., 2015; Simmonds et al., 2016). Physiological traits were also impacted by allelic differences at the QTL, which may also have contributed to yield component differences. Resource availability had a significant effect on GY and yield component traits and most often resulted in improved yield response when resource availability was high and intraspecific plant competition was low. Allelic variation had no significant effect on GY for *WAPO-A1*, *QGw.mst-3B* and *TaGW2-A1*. This lack of significant effect on GY likely stems from the pleiotropic interactions between yield component traits for all QTL in the present study. Notably, *gw2-A1* for *TaGW2-A1* led to increases in SNS, KWT, and GPC. Future investigation of the effect of combining yield related QTL such as *QTn.mst-6B* and *TaGW2-A1* in a single background may provide more guidance for breaking negative interactions between yield component traits. The response of QTL to differences in resources may help guide deployment of specific alleles in different environments and management systems to increase GY potential.

## Supplemental Material

*Supplemental materials can be found online at the Montana State University open access repository ScholarWorks*

Supplemental File 1. Grain yield, yield component and phenological trait means for four yield component quantitative trait loci grown in four environments for the competition level experiment.

Supplemental File 2. Allele, treatment, environment and all interaction combination significance values for grain yield, yield component and phenological traits measured in the competition level experiment.

Supplemental File 3. Grain yield, yield component and phenological trait means for four yield component quantitative trait loci grown in four environments for the seeding density experiment.

Supplemental File 4. Allele, treatment, environment and all interaction combination significance values for grain yield, yield component and phenological traits measured in the seeding density experiment.

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FIGURES


Figure 1. Competition experiment treatments including border (3.0 m row), no-border (3.0 m row) and space-planted (3.0 m row with 0.3 m spacing between individual plants).

TABLES

**Table 1.** Near-isogenic line (NIL) pedigree, allele designation and reference for yield component trait quantitative trait loci (QTL) evaluated across competition and seeding density experiments

| Trait[a] | QTL | Chr | Marker | NIL Pedigree | No. NIL pairs | No. Lines | Reference |
|---|---|---|---|---|---|---|---|
| PTN | *QTn.mst-6B* | 6B | Xgwm-88 | Reeder/Choteau Vida/ McNeal | 2 | 4 | Naruoka et al., 2011; Nasseer et al., 2016 |
| SNS | *WAPO-A1* | 7A | IWA5912 | Mountrail/Choteau//Berkut | 1 | 2 | Kalous et al., 2015 |
| KWT | *QGw.mst-3B* | 3B | IWA6375 | Mountrail/Choteau//Berkut | 2 | 4 | Kalous et al., 2015 |
| | *TaGW2-A1* | 6A | K1-K2/K3 KASP | Paragon*5/*gw2-A1* mutant | 1 | 2 | Simmonds et al., 2016 |

[a]PTN, Productive Tiller Number; SNS, spikelet number per spike; KWT, kernel weight

**Table 2.** Planting date, precipitation, temperature and harvest date for five environments used to compare near-isogenic lines for yield component QTL.

| | | **Experiment Planting Date** | | **Total Water Received[a]** | | **Mean Temperature** | **Experiment Harvest Date** | |
|---|---|---|---|---|---|---|---|---|
| Environments | Year | Competition Level | Seeding Density | Annual | Growing Season | Apr-Jul | Competition Level | Seeding Density |
| | | | | ----------*cm*---------- | | *°C* | | |
| Bozeman, MT - rainfed | 2018 | 4 May | . | 43.8 | 37.8 | 13.3 | 10 Sept | NA |
| Bozeman, MT - irrigated | 2018 | 4 May | . | 60.2 | 54.4 | 13.3 | 19 Sept | NA |
| Bozeman, MT - rainfed | 2019 | 9 May | 9 May | 41.8 | 24.9 | 12.2 | 18 Sept | 15 Sept |
| Bozeman, MT - irrigated | 2019 | 9 May | 9 May | 59.2 | 42.2 | 12.2 | 25 Sept | 25 Sept |
| Kalispell, MT - rainfed | 2019 | . | 22 Apr | 36.6 | 15.0 | 12.8 | . | 30 Aug |

[a]Total water received included precipitation and irrigation. Annual precipitation (Sept through Aug of the following year). Growing season precipitation (Apr through Jul).

**Table 3.** Treatment effect on overall means for agronomic and phenological characteristics measured combined across environments and genotypes[a]

**Competition Experiment**

| Competition Level | PTN[b] (*no m$^{-1}$*) | SNS (*no*) | SPS (*no*) | KWT (*mg*) | GY[c] (*g m$^{-1}$*) | HD (*d*) | FLS (*d*) | GLDAH (*d*) | GPC (*g kg$^{-1}$*) |
|---|---|---|---|---|---|---|---|---|---|
| Border (*high*) | 158.4$^{A}$ | 15.1$^{A}$ | 52.4$^{A}$ | 41.5$^{A}$ | 205.3$^{A}$ | 64.5$^{A}$ | 103.1$^{A}$ | 38.5$^{A}$ | 148.1$^{A}$ |
| No-Border (*intermediate*) | 214.9$^{B}$ | 15.9$^{B}$ | 59.1$^{B}$ | 42.8$^{B}$ | 357.6$^{B}$ | 65.2$^{B}$ | 104.7$^{A}$ | 39.5$^{A}$ | 152.0$^{B}$ |
| Space-planted (*low*) | 9.9 | 16.5$^{C}$ | 71.5$^{C}$ | 44.1$^{C}$ | 63.3 | 66.6$^{C}$ | 111.7$^{B}$ | 45.1$^{B}$ | 157.0$^{C}$ |
| LSD[d] | 10.4 | 0.4 | 1.7 | 1.0 | 109.8 | 0.6 | 4.0 | 3.9 | 0.3 |
| environment | 0.0576 | 0.0521 | 0.0036 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0015 | <.0001 |
| treatment | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0001 | <.0001 |
| environment x treatment | <.0001 | 0.1595 | 0.0033 | 0.8042 | <.0001 | 0.8737 | 0.4513 | 0.3015 | <.0001 |

**Seeding Density Experiment**

| Seeding Density | PTN (*no m$^{-1}$*) | SNS (*no*) | SPS (*no*) | KWT (*mg*) | GY (*kg ha$^{-1}$*) | HD (*d*) | FLS (*d*) | GLDAH (*d*) | GPC (*g kg$^{-1}$*) |
|---|---|---|---|---|---|---|---|---|---|
| Normal (*216 seeds m$^{-2}$*) | 125.7$^{A}$ | 13.8$^{A}$ | 46.4$^{A}$ | 42.0$^{A}$ | 5167.4$^{A}$ | 65.4$^{A}$ | 101.8$^{A}$ | 36.5$^{A}$ | 145.0$^{A}$ |
| Low (*76 seeds m$^{-2}$*) | 107.34$^{B}$ | 14.9$^{B}$ | 52.8$^{B}$ | 42.0$^{A}$ | 4572.0$^{B}$ | 66.4$^{B}$ | 102.8$^{A}$ | 36.4$^{A}$ | 145.7$^{A}$ |
| LSD | 8.1 | 0.3 | 1.8 | 1.1 | 235.3 | 0.6 | 1.3 | 1.1 | 0.2 |
| environment | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.8097 | <.0001 | <.0001 | <.0001 |
| treatment | <.0001 | <.0001 | <.0001 | 0.5090 | <.0001 | 0.0005 | 0.1795 | 0.7920 | 0.5199 |
| environment x treatment | 0.2685 | 0.0211 | 0.0288 | 0.0197 | 0.6766 | 0.0441 | 0.9234 | 0.5760 | 0.6813 |

[a]PTN, productive tiller number; SNS, spikelet number per spike; SPS, seeds per spike; KWT, single kernel weight; GY, grain yield; HD, heading date; FLS, flag leaf senescence; GLDAH, green leaf duration after heading; GPC, grain protein content

[b]Productive tiller number is counted per plant in the space-planted treatment and m$^{-1}$ for the border and no-border treatments, mean comparisons made only between border and no-border treatments, pair-wise p-values for main effects based on mean comparison of border and no-border

[c]GY is presented as grams per plant for the space treatment and g m$^{-1}$ for the border and no-border treatments, mean comparisons made only between border and no-border treatments, pair-wise p-values for main effects based on mean comparison of border and no-border

[d]Means within a column that share a common letter are not significantly different ($P > .05$)

**Table 4.** *QTn.mst-6B* near-isogenic lines (NIL) allele means for the competition and seeding density experiments. NIL allele means for yield, yield component and phenological traits representative of lines derived from crosses Reeder/Choteau and Vida/McNeal[a]

**Competition Experiment**

| Competition Level | Allele[b] | PTN[c] (*no m$^{-1}$*) | SNS (*no*) | SPS (*no*) | KWT (*mg*) | GY[d] (*g m$^{-1}$*) | HD (*d*) | FLS (*d*) | GLDAH (*d*) | GPC (*g kg$^{-1}$*) |
|---|---|---|---|---|---|---|---|---|---|---|
| Border (*high*) | *QTn.mst-6B.1* | 199.1* | 13.6 | 48.1 | 36.6 | 193.5 | 62.8**** | 103.2 | 40.4 | 147.6 |
| | *QTn.mst-6B.2* | 170.2 | 13.7 | 48.8 | 37.7 | 181.9 | 61.4 | 102.6 | 41.2 | 147.5 |
| No-Border (*intermediate*) | *QTn.mst-6B.1* | 273.2** | 14.3 | 56.5 | 37.6* | 345.7 | 63.5**** | 104.4* | 41.0 | 151.6 |
| | *QTn.mst-6B.2* | 239.1 | 14.4 | 58.0 | 39.0 | 333.7 | 62.0 | 102.6 | 40.6 | 152.8 |
| Space-planted (*low*) | *QTn.mst-6B.1* | 12.2*** | 15.0 | 70.4 | 39.3*** | 58.2 | 65.3**** | 111.3 | 46.1 | 158.9* |
| | *QTn.mst-6B.2* | 10.1 | 15.2 | 69.0 | 41.6 | 52.8 | 64.0 | 110.3 | 46.2 | 161.4 |
| Combined | *QTn.mst-6B.1* | 236.2*** | 14.3 | 58.3 | 37.8**** | 269.6* | 63.8**** | 106.3** | 42.5 | 152.7 |
| | *QTn.mst-6B.2* | 204.7 | 14.4 | 58.6 | 39.5 | 257.8 | 62.5 | 105.2 | 42.7 | 153.9 |

**Seeding Density Experiment**

| Seeding Density | Allele[b] | PTN (*no m$^{-1}$*) | SNS (*no*) | SPS (*no*) | KWT (*mg*) | GY (*kg ha$^{-1}$*) | HD (*d*) | FLS (*d*) | GLDAH (*d*) | GPC (*g kg$^{-1}$*) |
|---|---|---|---|---|---|---|---|---|---|---|
| Normal (*216 seeds m$^{-2}$*) | *QTn.mst-6B.1* | 158.8 | 13.1 | 45.3 | 37.4** | 4927.3 | 64.2*** | 101.6 | 37.4 | 144.4** |
| | *QTn.mst-6B.2* | 144.5 | 12.8 | 44.7 | 38.9 | 5122.8 | 63.3 | 101.8 | 38.5 | 147.1 |
| Low (*76 seeds m$^{-2}$*) | *QTn.mst-6B.1* | 139.9 | 13.5 | 48.8 | 36.8*** | 4586.5 | 65.6**** | 102.9* | 37.2**** | 144.5* |
| | *QTn.mst-6B.2* | 125.6 | 13.5 | 49.8 | 38.9 | 4730.2 | 64.1 | 104.3 | 40.2 | 146.8 |
| Combined | *QTn.mst-6B.1* | 149.3* | 13.3 | 47.0 | 37.1**** | 4756.9 | 64.9** | 102.3 | 37.3**** | 144.4*** |
| | *QTn.mst-6B.2* | 135.0 | 13.1 | 47.3 | 38.9 | 4926.5 | 63.7 | 103.1 | 39.4 | 146.9 |

[a] PTN, productive tiller number; SNS, spikelet number per spike; SPS, seeds per spike; KWT, single kernel weight; GY, grain yield; HD, heading date; FLS, flag leaf senescence; GLDAH, green leaf duration after heading; GPC, grain protein content

[b] *QTn.mst-6B.1* means are representative of NIL with the Reeder or Vida alleles, *QTn.mst-6B.2* means are representative of NIL with Choteau or McNeal alleles.

[c] Productive tiller number is counted per plant in the space-planted treatment and m$^{-1}$ for the border and no-border treatments, mean comparisons made only between border and no-border treatments

[d] GY is presented as grams per plant for the space treatment and g m$^{-1}$ for the border and no-border treatments, mean comparisons made only between border and no-border treatments

* , **, ***, **** Significant at the .05, .01, .001, and .0001 probability levels, respectively

**Table 5.** *WAPO-A1* near-isogenic lines (NIL) allele means for the competition and seeding density experiments. NIL allele means for yield, yield component and phenological traits representative of lines derived from cross Mountrail/Choteau//Berkut[a]

**Competition Experiment**

| Competition Level | Allele[b] | PTN[c] (*no m$^{-1}$*) | SNS (*no*) | SPS (*no*) | KWT (*mg*) | GY[d] (*g m$^{-1}$*) | HD *(d)* | FLS *(d)* | GLDAH *(d)* | GPC (*g kg$^{-1}$*) |
|---|---|---|---|---|---|---|---|---|---|---|
| Border (*high*) | *WAPO-A1b* | 141.6 | 16.8**** | 52.2 | 44.5 | 204.0 | 66.1 | 100.5 | 34.4 | 158.3 |
| | *WAPO-A1a* | 142.2 | 15.3 | 50.2 | 46.0 | 205.8 | 66.2 | 100.7 | 34.5 | 159.6 |
| No-Border (*intermediate*) | *WAPO-A1b* | 193.6 | 17.2*** | 55.1 | 45.7 | 352.6 | 66.4 | 101.8 | 35.4 | 161.9 |
| | *WAPO-A1a* | 192.2 | 15.9 | 55.5 | 47.1 | 360.1 | 66.9 | 102.1 | 35.2 | 159.7 |
| Space-planted (*low*) | *WAPO-A1b* | 8.6 | 16.8 | 66.9 | 46.5 | 62.4 | 67.3* | 104.3 | 37.1 | 163.4 |
| | *WAPO-A1a* | 8.9 | 16.7 | 68.1 | 47.3 | 61.5 | 66.6 | 103.7 | 37.1 | 166.1 |
| Combined | *WAPO-A1b* | 167.6 | 16.9**** | 58.1 | 45.5* | 278.3 | 66.6 | 102.2 | 35.6 | 161.2 |
| | *WAPO-A1a* | 167.2 | 16.0 | 57.9 | 46.8 | 283.0 | 66.6 | 102.1 | 35.6 | 161.8 |

**Seeding Density Experiment**

| Seeding Density | Allele[b] | PTN (*no m$^{-1}$*) | SNS (*no*) | SPS (*no*) | KWT (*mg*) | GY (*kg ha$^{-1}$*) | HD *(d)* | FLS *(d)* | GLDAH *(d)* | GPC (*g kg$^{-1}$*) |
|---|---|---|---|---|---|---|---|---|---|---|
| Normal (*216 seeds m$^{-2}$*) | *WAPO-A1b* | 112.2 | 14.4 | 41.5 | 43.4 | 4904.1 | 66.1** | 99.3 | 33.2** | 153.3 |
| | *WAPO-A1a* | 121.4 | 13.8 | 45.3 | 42.7 | 4711.6 | 66.9 | 98.2 | 31.3 | 151.0 |
| Low (*76 seeds m$^{-2}$*) | *WAPO-A1b* | 101.9* | 15.8** | 54.0 | 43.5 | 3884.7 | 66.9 | 98.9 | 32.0 | 152.8 |
| | *WAPO-A1a* | 77.7 | 14.6 | 53.0 | 45.3 | 4025.9 | 67.0 | 99.1 | 32.1 | 153.4 |
| Combined | *WAPO-A1b* | 107.1 | 15.1** | 47.7 | 43.5 | 4394.4 | 66.5** | 99.1 | 32.6* | 153.1 |
| | *WAPO-A1a* | 99.6 | 14.2 | 49.2 | 44.0 | 4368.7 | 66.9 | 98.7 | 31.7 | 152.2 |

[a] PTN, productive tiller number; SNS, spikelet number per spike; SPS, seeds per spike; KWT, single kernel weight; GY, grain yield; HD, heading date; FLS, flag leaf senescence; GLDAH, green leaf duration after heading; GPC, grain protein content

[b] *WAPO-A1b* means are representative of NIL with Choteau or Berkut spring allele, *WAPO-A1a* means are representative of NIL with Mountrail durum allele.

[c] Productive tiller number is counted per plant in the space-planted treatment and m$^{-1}$ for the border and no-border treatments, mean comparisons made only between border and no-border treatments

[d] GY is presented as grams per plant for the space treatment and g m$^{-1}$ for the border and no-border treatments, mean comparisons made only between border and no-border treatments

* , **, ***, **** Significant at the .05, .01, .001, and .0001 probability levels, respectively

**Table 6.** *QGw.mst-3B* near-isogenic lines (NIL) allele means for the competition and seeding density experiments. NIL allele means for yield, yield component and phenological traits representative of lines derived from the cross Mountrail/Choteau//Berkut[a]

**Competition Experiment**

| Competition Level | Allele[b] | PTN[c] (*no m$^{-1}$*) | SNS (*no*) | SPS (*no*) | KWT (*mg*) | GY[d] (*g m$^{-1}$*) | HD (*d*) | FLS (*d*) | GLDAH (*d*) | GPC (*g kg$^{-1}$*) |
|---|---|---|---|---|---|---|---|---|---|---|
| Border (*high*) | *QGw.mst-3B.1* | 144.8 | 16.0 | 54.9 | 43.2 | 204.6 | 63.9 | 100.8 | 36.8 | 155.2** |
| | *QGw.mst-3B.2* | 144.8 | 15.7 | 55.6 | 44.0 | 202.6 | 64.0 | 100.9 | 36.9 | 151.9 |
| No-Border (*intermediate*) | *QGw.mst-3B.1* | 193.9 | 16.5 | 58.2** | 44.6 | 347.2 | 64.6 | 102.6 | 38.0 | 158.4 |
| | *QGw.mst-3B.2* | 194.2 | 16.4 | 61.8 | 45.2 | 342.0 | 64.9 | 102.8 | 38.0 | 156.9 |
| Space-planted (*low*) | *QGw.mst-3B.1* | 8.6 | 16.8 | 70.4 | 45.6 | 62.9 | 66.5 | 105.8 | 39.4 | 161.7** |
| | *QGw.mst-3B.2* | 8.7 | 16.8 | 71.0 | 46.2 | 61.5 | 66.1 | 105.8 | 39.8 | 158.5 |
| Combined | *QGw.mst-3B.1* | 169.3 | 16.4 | 61.1* | 44.5 | 275.9 | 65.0 | 103.1 | 38.1 | 158.4**** |
| | *QGw.mst-3B.2* | 169.5 | 16.3 | 62.8 | 45.1 | 272.3 | 65.0 | 103.2 | 38.2 | 155.8 |

**Seeding Density Experiment**

| Seeding Density | Allele[b] | PTN (*no m$^{-1}$*) | SNS (*no*) | SPS (*no*) | KWT (*mg*) | GY (*kg ha$^{-1}$*) | HD (*d*) | FLS (*d*) | GLDAH (*d*) | GPC (*g kg$^{-1}$*) |
|---|---|---|---|---|---|---|---|---|---|---|
| Normal (*216 seeds m$^{-2}$*) | *QGw.mst-3B.1* | 119.9 | 14.0 | 46.3 | 43.6 | 4890.9 | 64.7 | 99.6 | 34.9 | 147.8 |
| | *QGw.mst-3B.2* | 110.0 | 14.4 | 47.7 | 43.3 | 4788.6 | 64.8 | 99.4 | 34.6 | 147.5 |
| Low (*76 seeds m$^{-2}$*) | *QGw.mst-3B.1* | 101.0 | 15.1 | 51.9 | 44.1* | 4157.3 | 65.5 | 100.2** | 34.7** | 150.1**** |
| | *QGw.mst-3B.2* | 91.9 | 15.5 | 53.0 | 42.5 | 4172.6 | 65.9 | 99.1 | 33.2 | 145.4 |
| Combined | *QGw.mst-3B.1* | 110.5* | 14.6* | 49.1 | 43.9 | 4524.1 | 65.1 | 99.9* | 34.8* | 148.9*** |
| | *QGw.mst-3B.2* | 100.9 | 15.0 | 50.4 | 42.9 | 4480.6 | 65.3 | 99.2 | 33.9 | 146.5 |

[a] PTN, productive tiller number; SNS, spikelet number per spike; SPS, seeds per spike; KWT, single kernel weight; GY, grain yield; HD, heading date; FLS, flag leaf senescence; GLDAH, green leaf duration after heading; GPC, grain protein content

[b] *QGw.mst-3B.1* means are representative of NIL with the Mountrail durum allele, *QGw.mst-3B.2* means are representative of NIL with Choteau or Berkut alleles.

[c] Productive tiller number is counted per plant in the space-planted treatment and m$^{-1}$ for the border and no-border treatments, mean comparisons made only between border and no-border treatments

[d] GY is presented as grams per plant for the space treatment and g m$^{-1}$ for the border and no-border treatments, mean comparisons made only between border and no-border treatments

* , **, ***, **** Significant at the .05, .01, .001, and .0001 probability levels, respectively

**Table 7.** *TaGW2-A1* near-isogenic line (NIL) allele means for the competition and seeding density experiments. NIL allele means for agronomic traits representative of lines derived from the cross *gw2-A1* mutant / Paragon[a]

| Competition Level | Allele[b] | PTN[c] (*no m$^{-1}$*) | SNS (*no*) | SPS (*no*) | KWT (*mg*) | GY[d] (*g m$^{-1}$*) | HD (*d*) | FLS (*d*) | GLDAH (*d*) | GPC (*g kg$^{-1}$*) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Competition Experiment** | | | | | | | | | | |
| Border (*high*) | *gw2-A1* | 138.3 | 15.7 | 55.6 | 44.0**** | 251.9 | 68.9 | 110.9 | 42.0 | 128.9* |
| | *GW2-A1* | 160.6 | 15.9 | 57.0 | 39.9 | 237.3 | 68.7 | 109.7 | 41.0 | 126.3 |
| No-Border (*intermediate*) | *gw2-A1* | 192.5 | 17.6* | 67.9** | 45.8**** | 424.6 | 69.9 | 114.6 | 44.7 | 133.0** |
| | *GW2-A1* | 199.9 | 16.8 | 61.2 | 42.1 | 416.9 | 69.8 | 112.8 | 43.1 | 130.3 |
| Space-planted (*low*) | *gw2-A1* | 10.7 | 19.4*** | 81.8 | 45.5** | 81.2 | 71.3* | 121.1 | 49.8 | 138.7** |
| | *GW2-A1* | 11.5 | 18.1 | 81.2 | 42.5 | 83.5 | 70.6 | 122.3 | 51.7 | 135.0 |
| Combined | *gw2-A1* | 165.4 | 17.6** | 68.4 | 45.1**** | 338.3 | 70.0* | 115.5 | 45.5 | 133.5**** |
| | *GW2-A1* | 180.3 | 16.9 | 66.4 | 41.5 | 327.1 | 69.7 | 114.9 | 45.3 | 130.5 |
| **Seeding Density Experiment** | | | | | | | | | | |
| **Seeding Density** | **Allele[b]** | **PTN** (*no m$^{-1}$*) | **SNS** (*no*) | **SPS** (*no*) | **KWT** (*mg*) | **GY** (*kg ha$^{-1}$*) | **HD** (*d*) | **FLS** (*d*) | **GLDAH** (*d*) | **GPC** (*g kg$^{-1}$*) |
| Normal (*216 seeds m$^{-2}$*) | *gw2-A1* | 98.3 | 14.6 | 51.7 | 44.9 | 6290.8* | 69.1* | 108.8 | 39.7* | 130.9 |
| | *GW2-A1* | 113.3 | 14.2 | 50.7 | 42.1 | 6767.7 | 68.2 | 111.1 | 42.9 | 130.9 |
| Low (*76 seeds m$^{-2}$*) | *gw2-A1* | 97.5 | 16.6 | 61.2 | 45.4 | 6036.2 | 70.3 | 111.6 | 41.2 | 134.8 |
| | *GW2-A1* | 94.2 | 16.0 | 57.9 | 44.7 | 5624.1 | 70.4 | 110.8 | 40.3 | 133.6 |
| Combined | *gw2-A1* | 97.9 | 15.6 | 56.4 | 45.2 | 6163.5 | 69.7 | 110.2 | 40.4 | 132.8 |
| | *GW2-A1* | 103.8 | 15.1 | 54.3 | 43.4 | 6195.9 | 69.3 | 110.9 | 41.6 | 132.2 |

[a] PTN, productive tiller number; SNS, spikelet number per spike; SPS, seeds per spike; KWT, single kernel weight; GY, grain yield; HD, heading date; FLS, flag leaf senescence; GLDAH, green leaf duration after heading; GPC, grain protein content

[b] *gw2-A1* means are representative of NIL with *gw2-A1* mutant allele, *GW2-A1* means are representative of Paragon with wildtype allele.

[c] Productive tiller number is counted per plant in the space-planted treatment and $m^{-1}$ for the border and no-border treatments, mean comparisons made only between border and no-border treatments

[d] GY is presented as grams per plant for the space treatment and g $m^{-1}$ for the border and no-border treatments, mean comparisons made only between border and no-border treatments

* , **, ***, **** Significant at the .05, .01, .001, and .0001 probability levels, respectively

# CHAPTER FIVE

# HIGH-RESOLUTION MAPPING AND CHARACTERIZATION OF *QTN.MST-6B,* A QTL FOR INCREASED TILLER NUMBER

## Introduction

Hexaploid wheat (*Triticum aestivum* L., 2n = 6x = 42) accounts for 30% of global grain production (FAOSTAT, 2018). Grain yield (GY) is a pivotal consideration in wheat breeding programs and an important determinant in agronomic success; however, annual increases of 0.5% to 1.0% in GY have been minimal in the last decade (Dixon et al., 2009; Tshikunde et al., 2019). Grain yield is a quantitative trait controlled by many genes, and as such GY potential is determined by genotype and genotype x environment interactions (Allard and Bradshaw, 1964; Kang et al., 2004; El-Soda et al., 2014). Furthermore, GY results from pleiotropic interactions and hierarchical plasticity of underlying yield component traits (Sreenivasulu and Schnurbusch, 2012; Slafer et al., 2014). Yield component traits include number of spikes or productive tiller number (PTN), seeds per spike (SPS), spikelet number per spike (SNS) and kernel weight (KWT) (Yoshida, 1972; Slafer, 2003; Nadolska-Orczyk et al., 2017). Additionally, yield component traits can be more heritable and less environmentally sensitive than GY and therefore the target in breeding programs to translate gains in agronomic performance (Kato et al., 2000; Slafer et al., 2014; Zhang et al., 2018). Due to the quantitative nature of yield component traits little is known of the underlying genetic architecture of these traits. Therefore, a possible avenue for increased GY is to further elucidate the genes controlling yield component traits.

Shoot branching or tillering in cereal crops determines plant morphology and ultimately GY (Kato et al., 2000; Schmitz and Theres, 2005; Kumar et al., 2007; Sherman et al., 2014; Tian and Jiao, 2015). Increases in PTN have been associated with reduced KWT and SPS and an increase in total seed number (Slafer, 2003; Sadras, 2007; Gaju et al., 2009; Mitchell et al., 2012; Mitchell et al., 2013; Gaju et al., 2014; Brinton et al., 2018). Several studies have also shown an increase in GY correlated with increased PTN in high-resource available environments (Mitchell et al., 2013; Sadras and Rebetzke, 2013; Nasseer et al., 2016; Jones et al., 2020). Reynolds et al. (1994) suggested that genotypes capable of demonstrating phenotypic plasticity to changes in environments may confer a GY advantage. These pleiotropic interactions between PTN and additional yield component traits suggest a potential source for overall GY improvement. However, the endogenous and environmental interactions responsible for controlling of tillering are not yet well understood.

Plant tillering involves two primary stages: formation of axillary meristems, and the outgrowth of the axillary bud from the axils of leaves (Schmitz and Theres, 2005; Domagalska and Leyser, 2011; Kebrom et al., 2013; Hussien et al., 2014; Tian and Jiao, 2015). In rice (*Oryza sativa* L.) the gene *Monoculm 1* (*MOC1*) a GRAS family transcription factor was characterized as being involved in the formation of axillary meristems and bud development (Li et al., 2003). Mutants with loss-of-function mutations in *MOC1* developed a single main stem and failed to develop axillary meristems (Li et al., 2003). *MOC1* is the homolog to Lateral suppressor (*Ls*) in tomato and *LATERAL SURPRESSOR* (*LAS*) in *Arabidopsis* (Schumacher et al., 1999; Greb et al., 2003; Li et al., 2003). Additional genes identified in rice including *LAX PANICLE1* (*LAX1*) *and LAX PANICLE2* (*LAX2*) have been identified to play a role in the formation and maintenance of

axillary meristems (Komatsu et al., 2003; Hussein et al., 2013). It is unclear if *MOC1*, *LAX1* and *LAX2* act independently or jointly in the formation and maintenance of axillary meristems (Hussein et al., 2014). Once an axillary bud has been formed the next step in the tillering process is the outgrowth of axillary buds into tillers.

The outgrowth of axillary buds into tillers in cereals is predominately controlled by hormonal, developmental (Hayward et al., 2009; Kebrom et al, 2013; Hussein et al., 2014). Primary of these plant hormones are auxin, strigolactones (SL), and cytokinins (CK) (Domagalska and Leyser, 2011; Kebrom et al., 2013; Hussien et al., 2014). Both auxin and SL act as inhibitors to bud outgrowth and are produced in the stem axis and in roots, respectively. Auxin in the stem and buds represses plant branching and upregulates the expression of *MORE AXILLARY GROWTH* (*MAX*) genes required for biosynthesis of SL in *Arabidopsis* (Brewer et al., 2009; Hayward et al., 2009; Kebrom et al., 2013). Strigolactones have been linked with the regulation of the *teosinte branched1* (*TB1*) gene originally identified in maize which is downstream of the *MAX* pathway (McSteen, 2009; Kebrom et al., 2013; Hussien et al., 2014). Belonging to the II TPC transcription factor family *TB1* encodes a protein which prevents cell growth and proliferation. *TB1* is highly conserved between species and has been well characterized in maize, sorghum (*SbTB1*), rice (*osTBI1/FC1*) and *Arabidopsis* (*BRC1*) as acting as a negative regulator of plant branching (McSteen, 2009; Choi et al., 2012; Kebrom et al., 2013). Mutations in the *TB1* gene result in a loss of apical dominance allowing for the outgrowth of buds. Cytokinins are synthesized in the roots and act as bud outgrowth activators by downregulating the expression of *TB1* (McSteen, 2009; Kebrom et al., 2013; Hussein et al., 2014). Auxin transcriptionally regulates CK through the downregulation of the *ISOPENTENYL*

*TRANSFERASE* (*IPT*) genes involved in the biosynthesis pathway of CK (Ferguson and Bevridge, 2009; Hussien et al., 2014). Levels of CK increase in axillary buds when the source of apical auxin is reduced (Ferguson and Beveridge, 2009; Domagalska and Leyser, 2011). Mutations in the *CYTOKININ OXIDASE* (*CKX*) gene which encodes an enzyme which degrades CK in rice resulted in plants with branching and conferred increase in GY (McSteen, 2009).

Genes controlling the outgrowth of axillary buds in wheat have also been explored. For instance, the recessive tiller inhibition (*tin*) gene located on the distal portion of chromosome 1AS (Spielmeyer and Richards, 2004). Wheat plants with the recessive *tin* allele demonstrate a reduction in number of tillers, increased leaf area index and increased kernel weight (Duggan et al., 2005; Kebrom et al., 2012; Moeller et al., 2014; Moeller and Rebetzke, 2017). Moeller and Rebetzke (2017) investigated near-isogenic lines (NIL) contrasting for reduced tillering *tin* and free tillering phenotype and associated a decrease in overall GY in lines with the recessive *tin* allele, likely due to the decrease in number of spikes per plant. Investigation of the orthologous *TB1* gene in wheat has also suggested that expression of *TB1* in wheat reduces tillering (Lewis et al., 2008; Dixon et al., 2018). Dixon et al., 2018 investigated wheat NIL differing for the maize *TB1* ortholog in maize on chromosome 4D. Comparison of NIL showed that increased expression of the *TB-D1* gene in wheat resulted in the formation of tiller buds, but outgrowth of buds was suppressed.

A large portion of the early tillers produced by the plant before stem elongation (Zadoks growth stage GS21- GS22) (Zadoks et al., 1974) are often aborted and only a few are taken to maturity and bear a fertile spike (Roy and Gallagher, 1985; Berry et al., 2003; Chen et al., 2019). Measurement of tiller number may be done at different stages of plant development. Assessment

of early tiller number (ETN) during the vegetative growth phase of plant development provides a measurement of maximum tillering potential whereas, measurement of tillers post-heading provides an estimate of PTN and thereby GY potential (Roy and Gallagher, 1985; Naruoka, 2010). Nasseer et al. (2016) showed correlation between increased ETN and PTN in NIL varying for high and low tillering alleles such that, an increase in ETN resulted in increased PTN when resource availability was high. However, Nasseer et al. (2016) also noted that increased ETN did not necessarily confer increased PTN in resource limited environments. Therefore, the measurement of ETN provides a better understanding of genetic potential and initial development is less impacted by environmental effects such as resource availability.

To better understand the genetic control mechanisms of tillering in wheat, the target for genetic dissection in the present study focused on a quantitative trait locus (QTL) associated with tiller production, *QTn.mst-6B,* identified by Naruoka et al. (2011). *QTn.mst-6B* was identified in a recombinant inbred line (RIL) population derived from the bi-parental crosses 'Reeder' (PI 613586) (Underdahl et al., 2008) / 'Conan' (PI 607549) and Reeder / 'McNeal' (PI 574642) (Lanning et al., 1994). The selected parents Reeder, McNeal and Conan were widely grown hard red spring wheat (HRSW) varieties in Montana. Quantitative trait loci mapping was described in Naruoka et al. (2011). The QTL *QTn.mst-6B* was significantly associated with PTN in multiple years and environments and accounted for a 21.7 cM region flanked by wPt4716 and wPt3581 on chromosome 6B. Productive tiller number combined across four Montana environments in the Reeder/Conan and Reeder/McNeal RIL populations was positively correlated with GY (Naruoka et al., 2011). The Reeder allele explained 9%, 15%, and 17% of the variation observed for PTN in three Montana environments. Additionally, the Reeder allele conferred an additive effect of

2.35 to 3.70 tillers $m^{-1}$ (Naruoka et al., 2011). Along with increased PTN the Reeder allele was associated with a 4.2 % increase in plot weight (kg $ha^{-1}$) relative to the McNeal allele (Naruoka et al., 2011).

Additional QTL analysis of additional bi-parental populations derived from crosses with Reeder and 'Vida' (PI 642366) (Lanning et al., 2006), a progeny line of Reeder carrying the high tillering allele at *QTn.mst-6B*, including Reeder / 'Choteau' (PI 633974) (Lanning et al., 2004) and Vida / McNeal associated *QTn.mst-6B* with PTN further suggesting QTL stability across genetic background and environment (Naruoka et al., 2011; Nasseer et al., 2016). Eight NIL derived from the cross Reeder/Choteau were evaluated in a RCBD with three replications in Bozeman, Montana in 2010 (Naruoka et al., 2011). The Reeder allele at *QTn.mst-6B* conferred a significant 13% PTN increase relative to the Choteau allele (Naruoka et al., 2011). The Reeder/Choteau and Vida/McNeal NIL pairs were further investigated by Nasseer et al. (2016) in a replicated field experiment grown over nine environments. The high tiller allele conferred by Reeder or Vida caused significantly higher PTN combined over nine environments and significantly higher ETN combined over five environments. Additional study by Jones et al. (2020) using a single NIL from the Reeder/Choteau and Vida/McNeal crosses was done to investigate the effect of resource availability in the form of plant competition and seeding density on PTN and GY. Results further supported findings from Naruoka et al. (2011) and Nasseer et al. (2016) in that the high tillering allele *QTn.mst-6B.1* was associated with significant increase in PTN across environments and increases in GY when resource level was increased by decreasing plant competition relative to the low tillering allele *QTn.mst-6B.2* (Jones et al., 2020). Jones et al. (2020) suggests that *QTn.mst-6B* provides an important level of plant plasticity to adapt to

various environments and potential to improve GY by stacking with additional yield component QTL.

Sufficient evidence has suggested that *QTn.mst-6B* is involved with the regulation of tillering in wheat and provides plant plasticity enabling improved GY performance in high resource environments. Therefore, further understanding of the genetic control of this QTL would provide information to better understand tillering in wheat and leverage new sources of GY improvement. The objective of the current research was to identify candidate genes within the *QTn.mst-6B* candidate gene region through the development of high-resolution mapping. Efforts in the current study delimited *QTn.mst-6B* to a 20 Mb region on chromosome 6B containing 98 putative candidate genes. Orthologous investigation of these putative genes identified *TraesCS6B02G197500* as the most likely candidate gene for *QTn.mst-6B.*

## Materials and Methods

### Linkage map construction and QTL analysis in Vida/MTHW0202 RIL Population

QTL mapping to confirm the presence of *QTn.mst-6B* in an additional bi-parental cross was done using a RIL population derived from the cross Vida / MTHW0202 (ID377S / MTHW9701) henceforth referred to as V/0202. MTHW0202 is a Montana State University spring wheat breeding line. The RIL population consisted of 155 $F_{6:7}$ individuals, generated using single seed descent (SSD) starting at the $F_2$ generation (Cook et al., 2020). The V/0202 RIL population was genotyped with the 90-K SNP array (Wang et al., 2014). A total of 711 polymorphic 90-K SNP markers were identified in the RIL population and used to construct a linkage map (Cook et al., 2020). In the present study, the linkage map generated by Cook et al. (2020) was supplemented with 10 additional KASP makers located on chromosome 6B, including 8 previously excluded 90-K SNP markers and two microsatellite markers *Xgwm88-6B* and *Xgwm58-6B* previously associated with the peak of *QTn.mst-6B* identified by Naruoka et al. (2011). The linkage map for 6B was regenerated to include these additional markers using R package ASMap (Taylor and Butler 2017). In short, only markers showing expected Mendelian allelic segregation ratios of 1:1 and that had less than 10% missing genotype data were included in the linkage map. The 'mstmap' function in R was used to cluster and group markers with a significance threshold of $P < 1x10^{-5}$ and genetic distances were calculated using the 'kosambi' function from the ASMap package (Taylor and Butler, 2017).

Phenotyping data for ETN was collected in the greenhouse at six weeks post-sowing. Planting in the greenhouse was done in a randomized complete block design (RCBD) with three blocks. Within each block a single RIL individual was represented once by sowing three plants

into a 17.8 cm pot. Early tiller number was determined as the average of tillers per plant. This was calculated by taking the sum of all tillers over three plants in a pot, excluding main stems and dividing by three.

Quantitative trait loci mapping for ETN in the V/0202 RIL population was performed using the R/qtl package (Broman et al., 2003). Initially, QTL were detected using the single interval mapping function 'scanone' in addition to the Haley-Knott regression method. A LOD significance threshold of 3.0 was determined by running 1,000 permutations at an experimental wise $P < 0.05$ to detect significant QTL. Additional QTL analysis was done using multiple interval mapping in R/qtl to determine significant QTL, associated QTL peaks and determine the percent of variation explained for ETN.

R/C and V/M near-isogenic lines

Near-isogenic lines for contrasting *QTn.mst-6B* alleles, *QTn.mst-6B.1* the high tillering allele and *QTn.mst-6B.2* the low tillering allele, were developed from bi-parental crosses made between Reeder / Choteau and Vida / McNeal henceforth referred to as R/C and V/M, respectively. Vida is a progeny line of Reeder and inherited the high tillering *QTn.mst-6B.1* allele. Development of NIL were described by Naruoka et al. (2011) and Nasseer et al. (2016) using the heterogeneous inbred family (HIF) strategy (Haley et al., 1994; Pumphrey et al., 2007) (Figure 1). In short, inbred $F_4$ progeny were generated using SSD from initial $F_1$ cross to the $F_4$ generation. Heterozygous $F_4$ progeny were identified using *Xgwm88-6B* and self-pollinated to produce NIL with the contrasting high and low tillering alleles at *QTn.mst-6B*. The high tillering allele was associated with increased PTN and ETN in replicated greenhouse and field

experiments in both the R/C and V/M backgrounds (Naruoka et al., 2011; Nasser et al., 2016; Jones et al., 2020).

Determination of optimum time to count ETN

To increase the efficiency and maximize resources it became necessary to determine the earliest possible date for ETN characterization in the greenhouse. The earliest date to count ETN for lines contrasting for the high and low tillering alleles at *QTn.mst-6B* was determined using R/C and V/M NIL pairs contrasting for the high and low tillering allele. Early tiller number was measured at four, five, six, and seven weeks post-planting in the greenhouse. It is during this physiological growth period in wheat development in which the plant transitions from the tillering stage (GS20 – GS29) to stem elongation (GS30 – GS39) (Zadoks et al., 1974). Seed from a single individual for the two NIL pairs were planted in 17.8 cm pots in triplicate with a soil mix containing equal parts loam soil, washed concrete sand and Sphagnum peat moss. The experimental unit was pot where four plants were sown, and ETN was determined by calculating the mean tillers (excluding the main stem) across four plants growing in a single pot. The mean ETN value was determined for each of the three pots per sisterline within a NIL pair. Significant differences between the high and low tillering alleles were determined with a two-tailed t-test, assuming equal variance with a significance level of $P < 0.05$ at each of the time-points separately. Greenhouse temperature averaged 21.1 °C and 20.0 °C during the day and night, respectively. Plants were grown under 16-hour day lengths and fertilized once a week using Peter's Professional 20-20-20 fertilizer mixture at approximately 100 ppm.

R/C and V/M HIF Development

Two HIF's were developed by crossing *QTn.mst-6B* R/C and V/M sisterlines within a single NIL pair, respectively (Figure 1). Both NIL pairs were previously confirmed to differ for the high and low tillering alleles and validated in field experiments for allele associated differences in ETN and PTN (Naruoka et al., 2011; Nasseer et al., 2016; Jones et al., 2020). Crosses between sisterlines of a NIL pair were made in order to further increase the number of meiosis events needed to reduce the *QTn.mst-6B* candidate gene region. Derived $F_{1\text{-HIF}}$ progeny lines were segregating at the *QTn.mst-6B* QTL region and highly homogeneous in genetic background. The largely homogeneous genetic background between the HIF lines allowed for early generation testing of subsequent self-pollinated generations denoted as $F_{2\text{-HIF}}$, $F_{3\text{-HIF}}$, and $F_{4\text{-HIF}}$ in the development of high-resolution maps.

Determination of *QTn.mst-6B* allele inheritance in HIF's

Due to limited seed for recombinant progeny tests further investigation of ETN was done using nonrecombinant R/C and V/M $F_{2\text{-HIF}}$ progeny, individuals without recombination within the *QTn.mst-6B* candidate region, to determine the number of replicates needed to detect significant ETN differences between the high and low tillering alleles with a statistical power of 0.80 to 0.90. The $F_{2\text{-HIF}}$ progeny were derived by self-pollinating $F_{1\text{-HIF}}$ progeny from the cross between individual sisterlines within the R/C and V/M NIL pairs, respectively. Non-recombinant homozygous high and low tillering allele lines within the *QTn.mst-6B* region as well as non-recombinant heterozygous progeny were identified by genotyping with markers specific to chromosome 6B between IWA7807 (126,136,002 bp) and 475.6_5800 (475,658,707 bp) (Supplemental File 1). Selected markers for genotyping included those polymorphic between

parental lines of the R/C and V/M HIF's from the 90-K SNP array (Wang et al., 2014) and KASP markers developed from the exome-capture assay as described by Krasileva et al., 2017 of elite hexaploid, durum and emmer WheatCAP project parental lines (parental SNP data deposited in T3/Wheat https://triticeaetoolbox.org/wheat/). Additionally, physical genomic coordinates of markers on the 6B chromosome were determined from the Chinese spring genome released by the International Wheat Genome Sequencing Consortium (IWGSC), henceforth referred to as RefSeq V1.0 (IWGSC 2018). A total of 44 homozygous high tillering, 41 homozygous low tillering and 68 heterozygous non-recombinant lines were identified in the V/M $F_{2\text{-HIF}}$ progeny. In the case of the R/C HIF $F_{2\text{-HIF}}$ progeny 27 homozygous high tillering, 47 homozygous low tillering and 65 heterozygous non-recombinant lines between the before-mentioned markers were identified. Dominance of the high tillering allele was determined by comparing the genotypic value of the heterozygote to the mid-parent value for both the R/C and V/M $F_{2\text{-HIF}}$ progeny (Bernardo, 2010). Sample size required to detect significant differences between high and low tillering alleles with a power of 0.80 was calculated using the means of the homozygous high and homozygous low genotype classes and pooled standard deviation with an alpha level equal to 0.05.

Construction of R/C high-resolution map

A high-resolution map was developed for the R/C HIF to resolve candidate genes within the *QTn.mst-6B* chromosomal region. Original QTL identification of *QTn.mst-6B* identified Xgwm88-6B (430,082,160 bp - 430,082,179 bp) to be most closely linked to the QTL (Naruoka et al., 2011). Markers were created for the HIF population approximately every 1 Mb upstream and downstream of Xgwm88-6B using parental SNPs identified from exome-capture assay

(Krasileva et al., 2017). The *QTn.mst-6B* candidate gene region was further represented using markers from the 90-K SNP array (Wang et al., 2014). The initial candidate gene region on chromosome 6B spanned 126,136,002 bp - 475,658,707 bp (Supplemental File 1). The exome-capture assay identified 15,914 SNP between parental lines of the R/C and V/M HIF's (https://triticeaetoolbox.org/). Early progeny testing of the V/M HIF suggested the potential of multiple segregating genes in the candidate region rather than a single gene thereby complicating high-resolution efforts and as such the V/M HIF was not used to identify candidate genes.

The high-resolution map for the R/C HIF was developed by genotyping R/C $F_{2\text{-HIF}}$ and $F_{3\text{-HIF}}$ progeny using markers IWA7807 (126,136,002 bp) to 475.6_5800 (475,658,707 bp) (Supplemental File 1). Plants showing recombination within the target region were self-pollinated and $F_{4\text{-HIF}}$ homozygous recombinant and non-recombinant sisterlines were grouped into haplotype sub-groupings based upon identified recombination events. Due to the genetic identity between sister-lines within the R/C HIF population it was possible to group by recombination events. Recombinant and non-recombinant sisterlines were then evaluated for ETN in replicated greenhouse experiments and pair-wise comparisons using a Bonferroni multiple comparison adjustment was used to detect significant differences in ETN.

All greenhouse experiments done for the high-resolution mapping were designed in a completely randomized design where a plant represented a single replication. Two seeds were sown in 7-inch pots with PGC soil mix. Early tiller number measurements were made at six and seven weeks post-sowing. Greenhouse temperature averaged between 21.1 °C and 20.0 °C during the day and night, respectively. Plants were grown under 16-hour day lengths and

fertilized once a week using Peter's Professional 20-20-20 fertilizer mixture at approximately 100 ppm.

Putative candidate genes

In combination with high-resolution mapping a list of putative candidate genes within the delimited region for *QTn.mst-6B* were identified using the updated RefSeq V1.1 annotation by the IWGSC (IWGSC, 2018). Further, expression of genes within the *QTn.mst-6B* candidate gene region was accessed from the expVIP wheat expression database (Borrill et al., 2016; Ramìrez-González et al., 2018). Gene expression was specific to hexaploid wheat Chinese Spring genetic background. Expression levels in vegetative tissues including first leaf blade, first leaf sheath, fifth leaf blade, fifth leaf sheath, shoot axis, shoot apical meristem (SAM) as well as, shoot and leaf tissues for genes were compiled (Supplemental File 2). Homologous expression of genes that showed moderate to high expression in vegetative tissues, greater than 10 transcripts per million (TPM), was determined by querying the same vegetative tissues in the expVIP wheat expression database (Supplemental File 3). Putative candidate gene function was determined by comparison of protein amino acid sequence to functionally characterized *Arabidopsis thaliana* proteins using the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) and Universal Protein Resource (UniProt) (https://www.uniprot.org/) databases (Supplemental File 4).

Frequency of *QTn.mst-6B.1* allele in WheatCAP Parental Panel

In order to determine the frequency of the high tillering allele (*QTn.mst-6B.1*) exome-capture data from 6 durum wheats, 2 *T. turgidum subsp dicoccoides* and 55 hexaploid wheats comprised of the WheatCAP project parental lines (https://www.triticeaecap.org/wheatcap-

germplasm-list/) was obtained. A subset of the diagnostic *QTn.mst-6B* markers derived from exome-capture including 226.8_755340 (226,868,798 bp) through 247.2_756072 (247,209,591 bp) were used to evaluate the *QTn.mst-6B* region in the afore mentioned sample (Supplemental File 5).

## Results

### *QTn.mst-6B* associated with ETN identified in QTL mapping of V/0202 RIL population

Original QTL identification of *QTn.mst-6B* was done using a RIL population derived from a cross between Reeder / Choteau characterized for PTN. To further confirm *QTn.mst-6B* in additional genetic backgrounds and determine significant association with ETN the V/0202 RIL was analyzed for significant ETN association with chromosome 6B. A total of 711 markers were assigned to 28 linkage groups, of which 82 markers were grouped onto linkage group 6B, representing chromosome 6B (Supplemental File 6). The total genetic size of the 6B linkage group was 260.7 cM. Using this map, multiple interval mapping in R/qtl identified two significantly associated QTL with ETN on linkage group 4A and 6B, with significant LOD thresholds of 3.70 and 4.41 (Figure 2a), respectively. The QTL on 6B accounted for 7.37 % of the total variation for ETN and the QTL on 4A accounted for 6.38% of total variation for ETN. The Vida allele at the 6B QTL increased ETN by 0.32 early tillers (Figure 2b) and 0.29 early tillers at the 4A QTL (Figure 2c) relative to MTHW02020. Results from QTL mapping of the V/0202 RIL population confirm the presence of the *QTn.mst-6B* QTL on chromosome 6B as previously identified in the R/C and V/M population and the increase in ETN due to the Vida high tillering allele.

### Allele differences in ETN detected at five, six, and seven weeks

Significant differences in ETN were detected between the high and low tiller alleles at *QTn.mst-6B* for both the R/C and V/M NIL pairs (Figure 3). Significant differences were detected between the high and low tillering allele for NIL pair R/C at six ($P < 0.03$) and seven (*P*

< 0.02) weeks post-sowing (Figure 3a). Additionally, significant differences in ETN were detected at five ($P < 0.01$), six ($P < 0.001$), and seven ($P < 0.01$) weeks post-sowing for the V/M NIL (Figure 3b). For both NIL pairs the production of ETN appeared to plateau after six weeks. Physiologically the time point of between five and six weeks corresponds to the Zadoks GS31 stage in which the first internode is detectable (Zadoks et al., 1974). Additionally, the number of ETN produced by V/M plants was higher than those produced by the R/C NIL pair. Given the differences detected between the high and low tillering alleles at five- and six-weeks post-sowing, additional evaluation of ETN in the construction of a high-resolution map was performed at five- and six-weeks post-sowing.

High tillering allele at *QTn.mst-6B* demonstrated partial dominance

Early tiller number differences between homozygous high, homozygous low and heterozygous genotypes at *QTn.mst-6B* were detected for $F_{2\text{-HIF}}$ R/C (Figure 4a) and V/M progeny (Figure 4b). Significant differences were detected between the homozygous non-recombinant high and low tillering genotypes. The high tillering allele conferred a 0.6 and 0.5 ETN increase in the R/C and V/M $F_{2\text{-HIF}}$ progeny, respectively. In both cases no difference was detected between the homozygous high genotype from the heterozygous genotype ($P > .05$). However, significant differences were detected between the homozygous low tillering genotype and the heterozygous genotype progeny ($P < .05$) for both the R/C and V/M $F_{2\text{-HIF}}$ progeny. This significant difference suggests the high tillering *QTn.mst-6B.1* allele is dominantly inherited. A calculation of dominance based upon genotype breeding value determined that the high tillering allele expresses partial dominance with d = 0.23 and d = 0.13 for the V/M and R/C $F_{2\text{-HIF}}$ progeny, respectively. Additionally, calculation of sample size required to detect significant ETN

in this population based on differences between high and low tillering alleles and their variances with a statistical power of 0.80 and 0.90 suggested a sample size of 50 to 70 are required, respectively. The large number of replications required to detect significant differences in ETN was applied for all additional phenotyping of ETN done in the development of high-resolution maps in the HIF populations.

High-resolution mapping efforts delimit *QTn.mst-6B* to 20 Mb region

Near-isogenic lines developed from the original bi-parental cross between R/C (Naruoka et al., 2011) were used to generate a HIF population for the high-resolution mapping of *QTn.mst-6B* associated with ETN. A total of 178 recombinants were identified from the 517 $F_{2\text{-HIF}}$ and $F_{3\text{-HIF}}$ progeny screened with markers between IWA7807 (126,136,002 bp) and 475.6_5800 (475,658,707 bp) with a total genetic map distance between markers of 34.4 cM (Figure 5). Recombinants were self-pollinated to increase seed and $F_{4\text{-HIF}}$ progeny lines were evaluated in the greenhouse for ETN. Recombinant sister-lines with the same recombination break points were grouped together into haplotype groups H1, H2, H3, and H4, and 45 – 100 plants were evaluated per haplotype (Table 1). Recombinant haplotype groups were compared to both high and low tillering allele non-recombinant sister-line checks. Haplotype group H1, H3, and H4 were significantly ($P < .05$) different than the high non-recombinant check (Table 1). Significant differences between recombinant haplotype groups and non-recombinant sister-line checks suggests that the candidate gene region is where allele calls differ between the lines. Haplotype group H2 was not significantly ($P > .05$) different than the high non-recombinant check (Table 1). Using the combined genotype data from markers described above and pair-wise comparisons for the detection of significant differences in ETN between recombinant and non-recombinant

haplotype groups the candidate gene region was reduced to 226.8_755340 (226,868,798 bp) and 247.2_756072 (247,209,591 bp).

98 high confidence genes were identified in the 20 Mb candidate region

Candidate gene region expression analysis was used to supplement high-resolution mapping efforts. High-resolution mapping delimited the candidate region to 20 Mb (Figure 5). Using the updated IWGSC RefSeq V1.1 a total of 98 high-confidence protein encoding genes were identified within the 226.8_755340 (226,868,798 bp) and 247.2_756072 (247,209,591 bp) region (Supplemental File 2). A heat map was created showing differences in expression levels in vegetative tissues for each of the 98 genes using the expVIP wheat expression database (Supplemental File 2). Of the 98 genes only 13 genes appeared to be expressed in levels greater than 10 TPM in vegetative tissues including: *TraesCS6B02G192900, TraesCS6B02G193400, TraesCS6B02G194600, TraesCS6B02G194700, TraesCS6B02G197100, TraesCS6B02G197200, TraesCS6B02G198500, TraesCS6B02G198900, TraesCS6B02G200200, TraesCS6B02G200300, TraesCS6B02G200800, TraesCS6B02G201600* and *TraesCS6B02G201800* (Supplemental File 2). For each of these 13 genes homologous gene expression was also determined for the same vegetative tissues using expVIP wheat expression database. All 13 genes showed similar expression levels on the A and D genomes (Supplemental File 3). For all 98 high-confidence genes orthologous genes in *Arabidopsis thaliana* were determined based off protein similarity (Supplemental 4). Of the 98 genes 39 encoded uncharacterized proteins therefore, function has yet to be determined. A subset of 10 genes were further investigated based upon expression levels in vegetative tissues and gene function (Table 3). Of these 10 genes investigated only *TraesCS6B02G197500* located at 234,870,167 – 234,845,758 was associated with plant tillering

in *Arabidopsis thaliana* (Table 3). Notably, *TraesCS6B02G197500* expression levels were not detectably high in vegetative tissues (Supplemental Table 2). The encoded *TraesCS6B02G197500* protein sequence in wheat shares 38%, 39%, 68%, identity to proteins in *Arabidopsis thaliana*, soybean, and rice respectively. However, only in *Arabidopsis thaliana* has this protein been functionally characterized.

Low frequency of the high tillering haplotype at *QTn.mst-6B* in elite germplasm

The frequency of the high tillering haplotype in the candidate region was determined using diagnostic markers of the *QTn.mst-6B* candidate region as developed from the exome-capture assay on a panel of WheatCAP parental lines (Supplemental Table 5). From the total panel of 67 lines a subset of 16 lines including the high tillering checks Reeder and Vida were fixed for the high tillering haplotype in the current candidate region suggesting 23.9 % of elite WheatCAP parental lines are fixed for the high tillering haplotype. Notably of the 6 durum wheats included in this panel 4 were fixed for the high tillering haplotype (Supplemental File 5).

## Discussion

Tiller number ultimately determines GY and as such is an important consideration for the improvement of plant potential. Investigation in the present study focused on the genetic dissection of *QTn.mst-6B* a QTL explaining variation in both PTN and ETN in spring wheat. *QTn.mst-6B* has been detected in multiple genetic backgrounds and environments indicating a level of stability associated with the high tillering allele (Naruoka et al., 2011). Further, extensive investigation has been done to better understand the response of the high tillering allele (*QTn.mst-6B.1*) to variation in competition and resource availability (Nasseer et al., 2016; Jones et al., 2020). Results from said investigations suggest that the high tillering allele is associated with increased PTN as well as, increases in GY when resource availability is high and competition is low (Nasseer et al., 2016; Jones et al., 2020). As such the *QTn.mst-6B* QTL was a strong candidate for high-resolution mapping in order to identify underlying putative candidate genes and better explore the genetic control mechanisms associated with tillering in hexaploid wheat and positive pleiotropic interactions with GY.

Quantitative trait loci mapping in the current study identified *QTn.mst-6B* in a V/0202 RIL population as explaining 7.37 % variation for ETN (Figure 2b). An additional QTL was also detected on 4A as explaining 6.38 % variation for ETN (Figure 2c). Cook et al. (2020) evaluated the V/0202 RIL population in three rainfed and two irrigated environments across 2013 and 2014. The parental line Vida showed significant increases in PTN and a significantly decreased thousand kernel weight (TKW) relative to the MTHW02020 parent (Cook et al., 2020). Further, Cook et al. (2020) showed significant correlation between GY and PTN in irrigated environments. In the QTL analysis done by Cook et al. (2020) a significant QTL was detected on

6B as explaining variation for TKW at the peak marker IWB61228 (RAC875_c98074_60) (Supplemental File 1). This is the same peak marker as *QTn.mst-6B* as detected in this and previous studies (Naruoka et al., 2011), suggesting this is the same QTL. However, rather than explaining variation for PTN the 6B QTL explained 11.2 % variation in TKW such that the Vida allele significantly reduced TKW relative to MTHW0202 (Cook et al., 2020). A possible explanation for this may be the negative pleiotropic interaction observed between PTN and TKW (Slafer, 2003; Sadras, 2007; Gaju et al., 2009; Mitchell et al., 2012; Mitchell et al., 2013; Gaju et al., 2014; Brinton et al., 2018). It is therefore possible that the Vida allele increased PTN but not significantly so resulting in a decrease in TKW in the RIL progeny. Several studies have noted a lack of significant difference in PTN associated with the high tillering allele when resources are not optimal (Naruoka et al., 2011; Nasseer et al., 2016; Jones et al., 2020). This highlights the advantage of measuring ETN in order to estimate early plant potential.

High-resolution mapping was facilitated to identify putative candidate genes within the *QTn.mst-6B* region (Figure 5). This included the genotyping of R/C HIF progeny combined with progeny tests conducted in the greenhouse where ETN was evaluated for all identified recombinant individuals. Four unique recombination haplotypes were detected with a total genetic distance of 34.4 cM between markers IWA7807 (126,136,002 bp) and 475.6_5800 (475,658,707 bp) (Table 1). The current *QTn.mst-6B* candidate gene region was delimited to a 20 Mb region between the markers 226.8_755340 (226,868,798 bp) and 247.2_756072 (247,209,591 bp) (Figure 5). This candidate region contained 98 high-confidence protein encoding genes from the IWGSC RefSeq V1.1 (Supplemental File 2). The limited amount of recombination and number of high confidence genes in the current candidate region may be

explained by the centromeric proximity of *QTn.mst-6B*, where the chromosome 6B centromere is approximated to be located at 325 - 350 Mb (RefSeq V1.0).

Of the 98 genes between markers 226.8_755340 (226,868,798 bp) and 247.2_756072 (247,209,591 bp), 10 potential candidate genes were selected based upon high expression levels in vegetative tissues or associated impacts on plant development and growth (Table 3). Several of these genes are discussed here. *TraesCS6B02G192900* showed high expression in all vegetative tissues (TPM > 20) (Supplemental Table 2) and is orthologous to *SAP2*, a gene associated with plant response to abiotic stressors including drought and heat stress (Vij and Tyagi, 2007; Priya et al., 2019). Transgenic rice plants expressing *OsiSAP1* showed improved germination and seedling establishment under water-deficit conditions and reduced yield losses compared to the non-transgenic rice plants (Dansana et al., 2014). *TraesCS6B02G193400*, a gene which encodes a lipoxygenase protein has been shown to be involved in plant physiological growth and development, pest resistance and senescence (Bell et al., 1995; Howe and Schilmiller, 2002; Weber, 2002). Interestingly gene expression of *TraesCS6B02G193400* in SAM and shoot axis tissues was low, suggesting perhaps that this gene is more likely to be expressed in leaf tissue rather than determining tiller development (Supplemental File 2). *TraesCS6B02G194700* was moderately expressed in all vegetative tissues (TPM > 12) and the protein has been functionally characterized to be involved in plant metabolism specifically the mitochondrial tricarboxylic acid cycle (Schmidtmann et al., 2014). Gene expression of *TraesCS6B02G197200* was primarily isolated to the SAM and shoot axis tissues (Supplemental File 2) and has been characterized as a component in the production of noncellulosic polysaccharides found in plant stem tissues (Liepman et al., 2005; Goubet et al., 2009).

*TraesCS6B02G200800* was detected to have moderate to high expression in most vegetative tissues and is orthologous to *GHS1* which encodes a histone protein involved broadly in plant germination, development and photosynthesis (Morita-Yamamuro et al., 2004).

Most notable of the putative candidate genes investigated was *TraesCS6B02G197500* orthologous to the *Arabidopsis thaliana AtMBD9* gene encoding a methyl-CpG binding domain 9 protein transcription factor (Table 3). In *Arabidopsis* the AtMBD9 transcription factor has been associated with histone acetylation and increased gene expression (Yaish et al., 2009). Specifically, in Arabidopsis the AtMBD9 protein has been shown to directly bind to chromatin at the *flowering locus C* (*FLC*) known to repress flowering and acetylates histone H3 and H4 (Yaish et al., 2009). Further Peng et al. (2006) identified *Arabidopsis* loss-of-function insertion mutants *atmbd9-1* through *atmbd9-3* showing an earlier flowering time and increased axillary branching. Peng et al. (2006) suggested that *atmbd9-1* mutant lines showed increased branching independent of auxin. Mutant *atmbd9-1* lines showed equivalent auxin production as wild type lines and in fact lateral shoot branching was increased in double *atMBD9-1* and *axr1-12* (auxin resistant mutant) knockout mutants suggesting additive effect of the *atMBD9* and auxin on shoot branching (Peng et al., 2006). Yaish et al. (2009) further investigated *atMBD9* mutant lines and determined that the mutation in *AtMBD9* functionally decreased histone acetylation and increased DNA methylation, specifically at the FLC locus thereby decreasing the expression of the FLC repressor explaining the earlier flowering time. It is still yet unknown the downstream branching gene target of the transcription factor atMBD9 protein however transcriptional expression studies indicated branching hormone-related gene expression was increased in *atMBD9-1* mutant lines relative to *ATMBD9* wild type lines (Yaish et al., 2009). In the current

study gene expression using data from the expVIP wheat expression database did not suggest that *TraesCS6B02G197500* was highly expressed in vegetative tissues (Supplemental File 2). Therefore, further investigation should be taken to determine differential expression of *TraesCS6B02G197500* and additional genes within the candidate region between the parental lines Reeder and Choteau.

Of further interest in the current study was to determine the frequency of the high tillering haplotype using exome-capture data for a panel of WheatCAP parental lines (Supplemental File 5). A total of 16 parental lines out of 67 from the WheatCAP for a frequency of 23.9% were fixed for the high tillering haplotype. Further, of the 6 durum wheats included in the panel 4 were fixed for the high tillering haplotype as well as a single emmer wheat (*Triticum turgidum* L. ssp. *dicoccoides*). It is possible that in current day hexaploid wheat the high tillering haplotype was inherited from historical hybridization between Emmer wheats (A and B donor) and the D genome donor *Aegilops tauschii* Coss. (Faris, 2014; Feuillet, Langridge, &Waugh, 2007; Kabbaj et al., 2017). Of additional note is that due to the fact that high tillering *QTn.mst-6B* haplotype is only present in 23.9% of elite varieties grown across the Great Plains suggest that this may be a source of novel increased tillering and potential for GY improvement in current breeding programs.

In summary the purpose of the current investigation was to delimit *QTn.mst-6B* a QTL associated with increased ETN and PTN to a 20 Mb region spanning 226,868,798 bp to 247,209,591 bp on chromosome 6B. A total of 98 candidate genes were identified in this region however only a single gene, *TraesCS6B02G197500*, has been functionally characterized to impact plant tillering and as such presents as the most plausible candidate gene. Further, the

dominantly inherited high tillering haplotype associated with *QTn.mst-6B* presents a novel source of germplasm improvement for current breeding programs and a potential avenue to increase GY potential and improve plant plasticity. Further investigation is needed to definitively determine the causal candidate gene underlying the *QTn.mst-6B* region however, results from the current study further support the effect of the high tillering allele on increasing ETN and potential as a stable source of plant improvement in multiple genetic backgrounds.

## Supplemental Materials

*Supplemental materials can be found online at the Montana State University open access repository ScholarWorks*

Supplemental File 1: Genotyping markers used for the high-resolution fine mapping of *QTn.mst-6B*

Supplemental File 2: Gene expression heatmap of potential candidate genes in vegetative tissues on chromosome 6B between 226,868,798 bp and 247,209,591 bp.

Supplemental File 3: Homologous gene expression for a subset of the candidate genes on chromosome 6B between 226,868,798 bp and 247,209,591 bp.

Supplemental File 4: Putative candidate gene function of genes on chromosome 6B between 226,868,798 bp and 247,209,591 bp

Supplemental File 5: Allele haplotypes for the *QTn.mst-6B* candidate region between chromosome 6B between 226,868,798 bp and 247,209,591 bp for a panel of WheatCAP parental lines

Supplemental File 6: Chromosome 6B Linkage Map derived from the Vida/MTHW0202 RIL population

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FIGURES

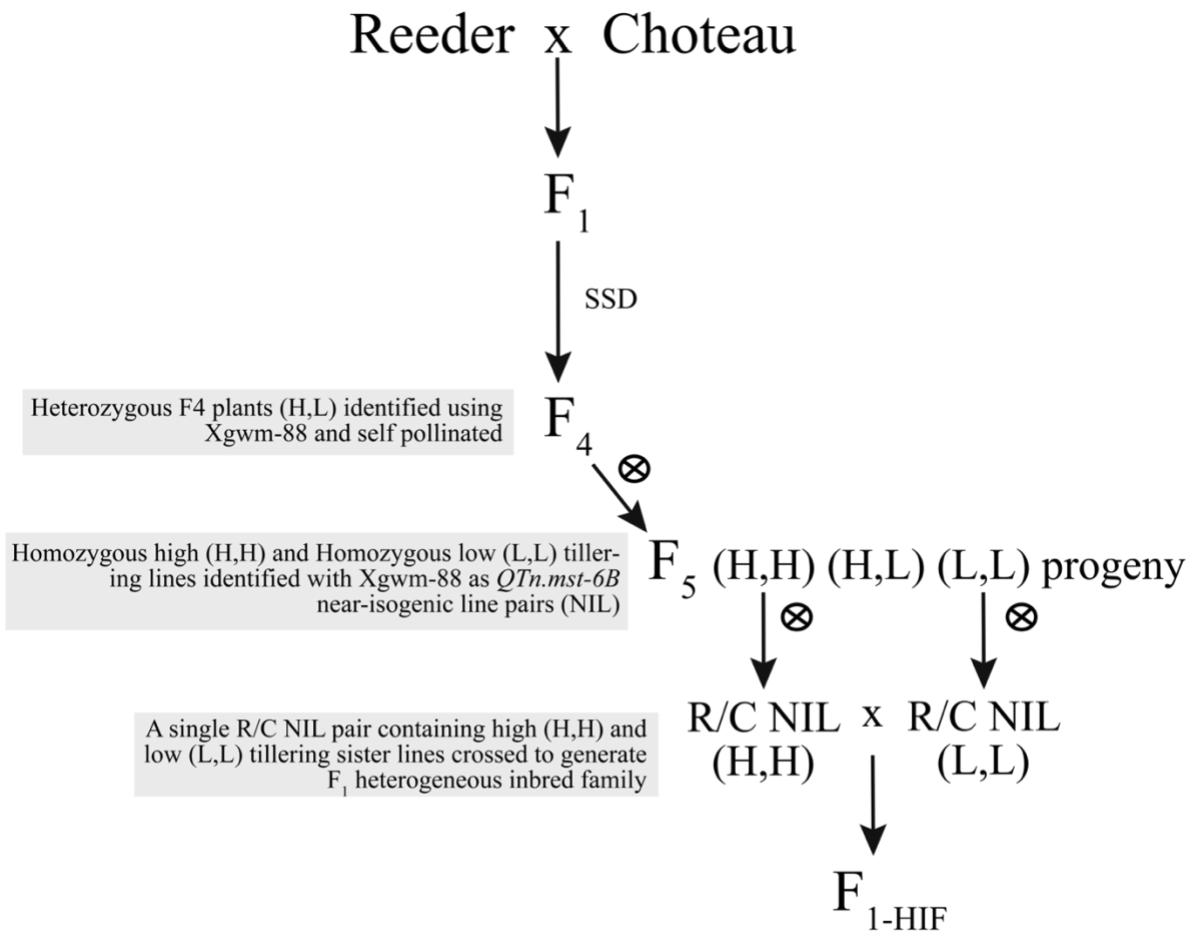


Figure 1. Single seed descent (SSD) crossing scheme used to generate near-isogenic line pairs (NIL) for the bi-parental cross Reeder/Choteau. The same crossing scheme was used to generate NIL from the cross between Vida/McNeal. Small crossed circles indicate a self-pollination. The *QTn.mst-6B.1* high tillering allele denoted as 'H' and the *QTn.mst-6B.2* low tillering allele denoted as 'L'.

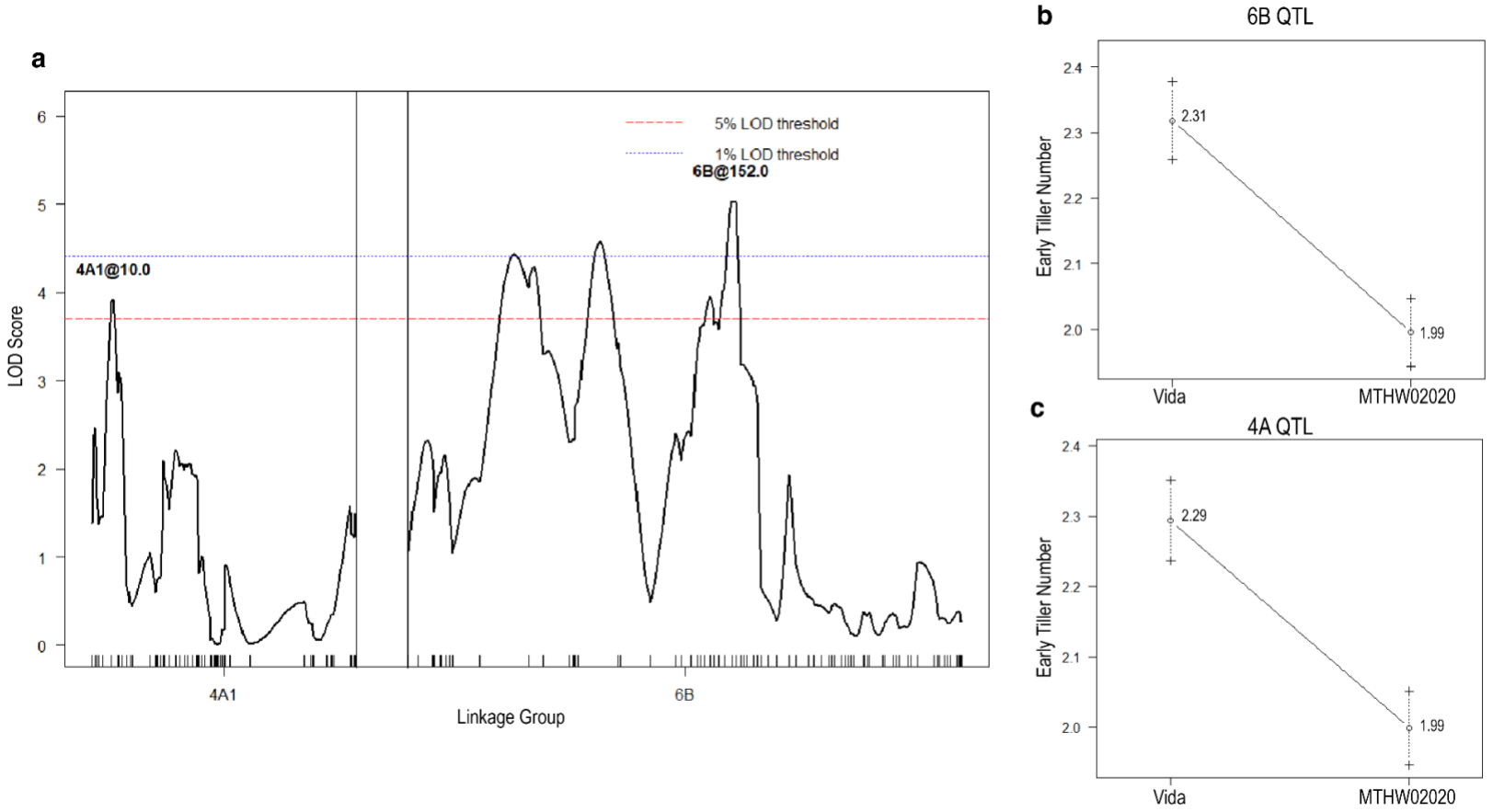


Figure 2. Identification of early tiller number (ETN) quantitative trail loci (QTL) on (a) linkage groups 4A and 6B in the Vida/MTHW0202 RIL population and Vida allele effect plots for the (b) 6B QTL and (c) 4A QTL relative to the MTHW0202 allele.

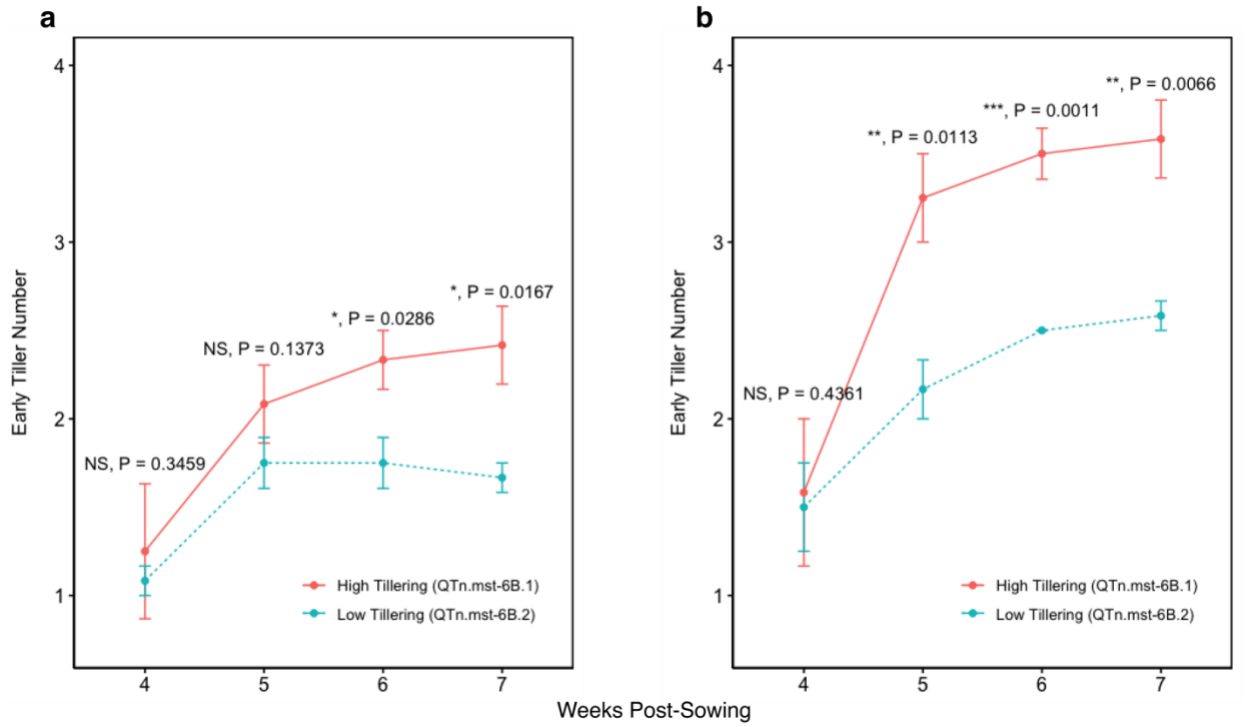


Figure 3. Schematic of mean early tiller number (ETN) differences between the high (*QTn.mst-6B.1*) and low (*QTn.mst-6B.2*) tillering alleles at *QTn.mst-6B* for the (a) R/C and (b) V/M NIL pair at four, five, six, and seven weeks post-sowing. Error bars represent the standard error of the mean of three replicates. Asterisks indicate significant difference between alleles using t-test comparisons at each weekly time point post-sowing: NS $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, ***, $P < 0.001$.

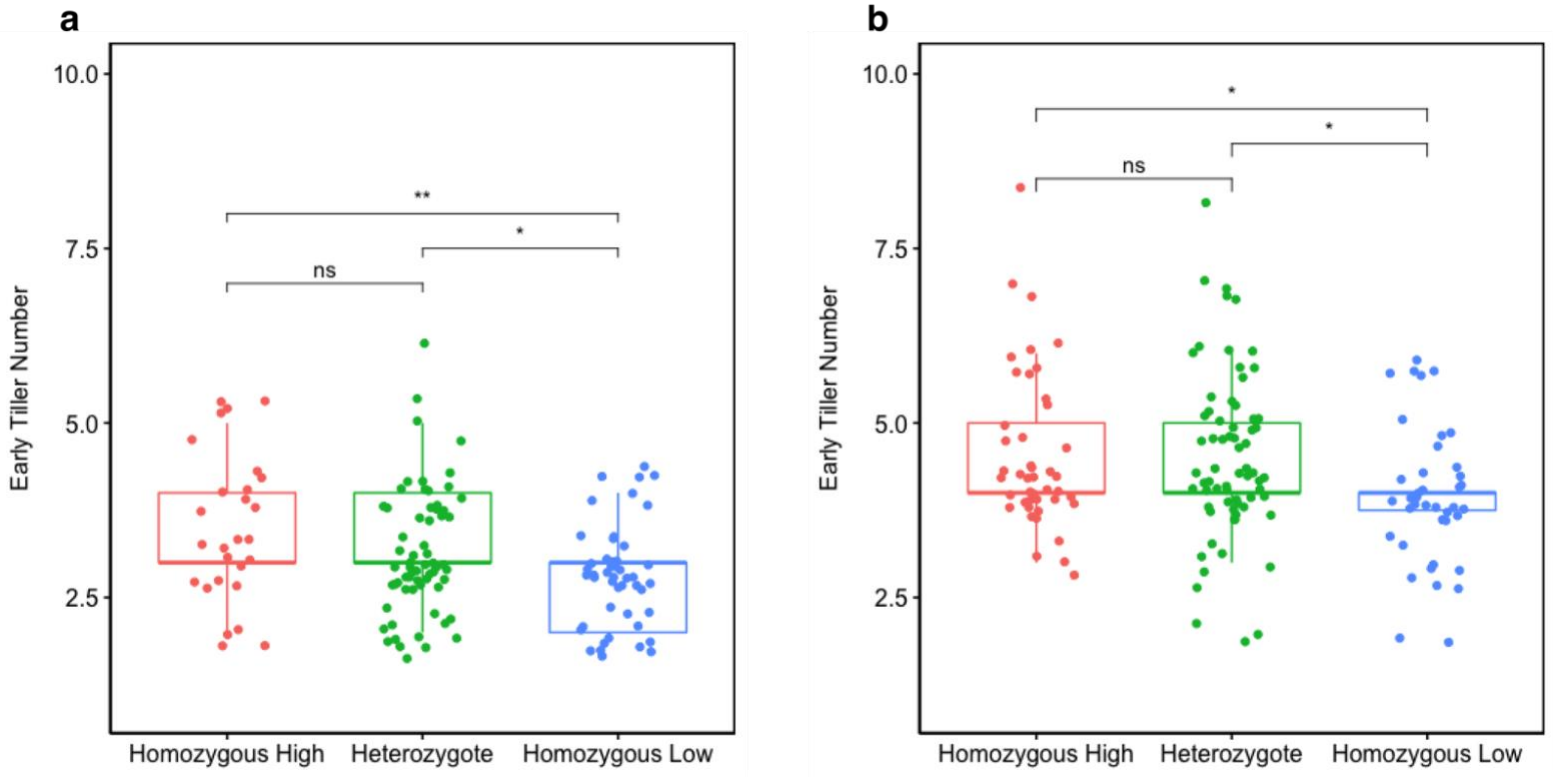


Figure 4. Early tiller number (ETN) mean of (a) R/C and (b) V/M homozygous high, homozygous low, and heterozygous *QTn.mst-6B* non-recombinant $F_{2\text{-HIF}}$ progeny measured at five weeks post-sowing. Asterisks indicate significant difference between alleles using t-test comparisons at each weekly time point post-sowing: NS $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, ***, $P < 0.001$.

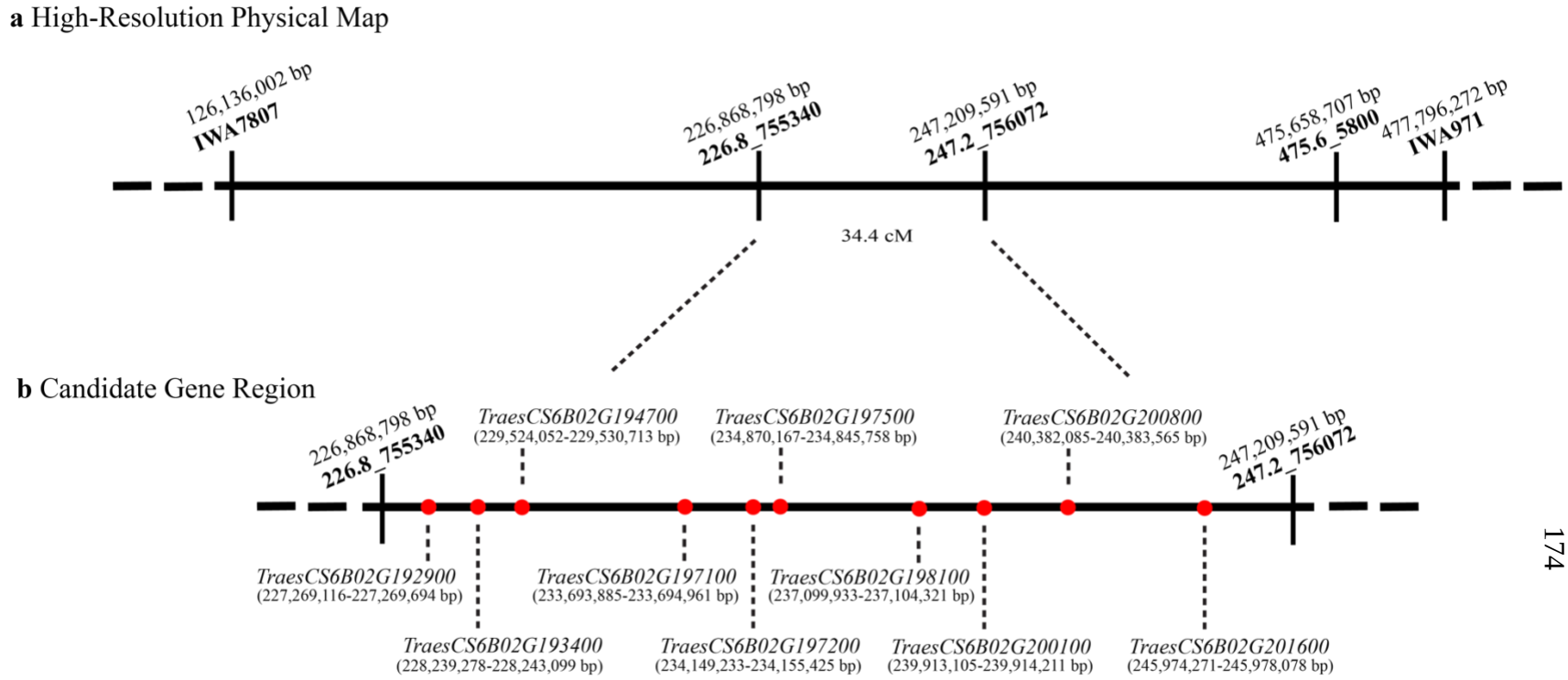


Figure 5. *QTn.mst-6B* high-resolution physical map and delimited candidate gene region (a) Physical map generated by genotyping $F_{2\text{-HIF}}$ and $F_{3\text{-HIF}}$ with markers listed in Supplemental File 1. (b) Subset of putative candidate genes within the region flanked by markers 226.8_755340 (226,868,798 bp) and 247.2_756072 (247,209,591 bp) associated with plant growth and development (Table 2).

# TABLES

Table 1. Phase 1 high-resolution map of the Reeder (R) x Choteau (C) HIF population based off early tiller number (ETN) progeny tests from $F_{4\text{-HIF}}$ sisterlines combined into similar haplotype groups based upon same recombination event.

| **Marker** | **RefSeq v1.1 Position** | **Homo R** | **Homo C** | **H1** | **H2** | **H3** | **H4** |
|---|---|---|---|---|---|---|---|
| IWA7807 | 126,136,002 | R[a] | C | R | C | C | R |
| 140.8_7491 | 140,859,812 | R | C | R | C | C | R |
| 175.7_7527 | 175,718,883 | R | C | C | R | C | R |
| 207.4_7545 | 207,452,956 | R | C | C | R | C | R |
| 210.2_7547 | 210,282,326 | R | C | C | R | C | R |
| 226.8_7553 | 226,868,798 | R | C | C | R | C | R |
| 247.2_7560 | 247,209,591 | R | C | C | R | R | C |
| 280.0_7565 | 280,099,809 | R | C | C | R | R | C |
| IWB57747 | 309,755,056 | R | C | C | R | R | C |
| 465.1_4150 | 465,146,641 | R | C | C | R | R | C |
| 475.6_5800 | 475,658,707 | R | C | C | R | R | C |
| Average ETN | | 1.0 | 0.5 | 0.7 | 0.9 | 0.7 | 0.5 |
| vs Homozygous R *P*-value[a] | | 1.0000 | 0.0010 | 0.0217 | 0.2723 | 0.0233 | 0.0020 |
| vs Homozygous C *P*-value | | 0.0010 | 1.0000 | 0.2053 | 0.0192 | 0.3963 | 0.9803 |
| N | | 99 | 59 | 100 | 97 | 50 | 48 |
| Inferred ETN Allele[b] | | R | C | C | R | C | C |

Progeny tests were done by combining $F_{4\text{-HIF}}$ sisterlines genotyped as having the same recombination break-points into four haplotype groups (H1, H2, H3, and H4) and compared to non-recombinant sisterlines (Homo R and Homo C) for the QTn.mst-6B region spanning 126,136,002 bp – 475,658,707 bp. Cells with R indicate loci homozygous for the Reeder high tillering allele, whereas cells with a C indicate loci homozygous for the Choteau low tillering allele

[a] *P*-value for average ETN difference of haplotype group versus the homozygous R or C non-recombinant sisterlines was determined through pair-wise comparisons of all haplotype groups and non-recombinant homozygous R and C sisterline checks.

[b] Inferred candidate region and allele assignment was determined to be the same as the non-recombinant sisterline check without significant difference in average ETN

Table 2. Putative candidate genes within the QTn.mst-6B 20 Mb region spanning 226,868,798 bp to 247,209,591 bp

| **Gene ID** *IWGSC RefSeq V1.1* | **Orthologous Gene** *Arabidopsis thaliana* | **Function** |
|---|---|---|
| TraesCS6B02G192900 | SAP2 (NP_001077694.1) | Plant stress response to abiotic factors including heat and drought (Vig and Tyagi, 2008; Priya et al., 2019) |
| TraesCS6B02G193400 | LOX2 (NP_566875.1) | Plant physiological growth and development, pest resistance, and senescense or response to wounding (Bell et al., 1995; Howe and Schilmiller, 2002; Weber, 2002) |
| TraesCS6B02G194700 | CSY4 (NP_001324514.1) | Catalyzes the first step of the mitochondrial tricarboxylic acid cycle (TCA cycle) for plant metabolism (Schmidtmann et al., 2014) |
| TraesCS6B02G197100 | PRXIIE (NP_190864.1) | Protection of photosynthetic apparatus by detoxifying reactive oxygen species (Rouhier and Jacquot, 2005; Dietz et al., 2006) |
| TraesCS6B02G197200 | CSLA2 (NP_197666.1) | Production of noncellulosic polysaccharides found in plant cell walls in stem tissue (Liepman et al., 2005; Goubet et al., 2009) |
| TraesCS6B02G197500 | AtMBD9 (NP_186795.1) | Proposed involvement in regulation of shoot branching and flowering time through histone acetylation and DNA methylation (Peng et al., 2006; Yaish et al., 2009 ) |
| TraesCS6B02G198100 | FLXL2 (NP_001320766.1) | Involved in flowering time through the transcriptional activation of the FLC locus (Choi et al., 2011; Lee and Amasino, 2013) |
| TraesCS6B02G200100 | HT1 (NP_176430.2) | Negative regulator of $CO_2$ induced stomatal closing (Hashimoto et al., 2006; Tian et al., 2015) |
| TraesCS6B02G200800 | GHS1 (NP_001327188.1) | Regulation control in sugar response in plant germination, development and photosynthesis (Morita-Yamamuro et al., 2004) |
| TraesCS6B02G201600 | CAD4 (NP_188576.1) | Lignin biosynthesis in the floral stem (Kim et al., 2004; Sibout et al., 2005) |

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