GENETIC ANALYSIS OF PRODUCTIVE TILLER NUMBER AND GREEN LEAF DURATION UNDER LATE-SEASONED HEAT AND DROUGHT STRESS ENVIRONMENT IN SPRING WHEAT

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ABSTRACT

Climate change is affecting the growing environment for spring wheat (*Triticum aestivum L. em. Thell*) in the northern Great Plains, challenging breeders to identify traits and genes that will allow reliable grain yield under drought and heat stress conditions. The first objective of this study was to evaluate the genetic basis of productive tiller number (PTN) and its relationship to economic traits under a wide range of environments. Correlation of PTN with economic traits was determined using three recombinant inbred line populations. Quantitative trait loci (QTL) analysis was conducted with a mapping population generated from a cross between Reeder and Conan. Our results showed a consistent positive correlation between PTN and grain yield under drought and heat stress conditions as well as well-watered conditions across three spring wheat populations. The major stable QTL, *QTn.mst-6B,* was consistent across environments and populations, and the positive allele from Reeder increased grain yield. The second objective of this study was to evaluate the genetic basis of green leaf duration (GLDAH) which has been reported as a drought and heat stress resistant trait in several crops. Additionally, the relationship of GLDAH to agronomic traits and a root trait was assessed using the Reeder/Conan population. Correlation analysis showed a positive relationship between GLDAH and test weight, seed weight, seed diameter under heat and drought stress conditions but not cool, well-watered conditions. In contrast, GLDAH had a neutral relationship with grain yield under the stress conditions, but showed negative correlation under well-watered conditions. Major QTL *QGfd.mst-4A* had a consistent effect under hot, dry conditions for the populations. The Reeder allele of *QGfd.mst-4A* resulted in longer GLDAH and also increased the amount of xylem exudate, indicating higher root mass and/or activity. These results suggested that i) *QTn.mst-6B* may be useful for improvement of spring wheat production under a wide range of environment and ii) *QGfd.mst-4A* may contribute to heat and drought stress resistance potentially through root function, but may negatively affect grain yield under well-watered conditions in the northern Great Plains of North America and similar environments.

CHAPTER 1

INTRODUCTION

The food crisis of 2006 to 2008 suggests that climate change is affecting crop production and our food security. During this period, wheat production was lowered due to drought and heat in several wheat producing countries. In 2010, a devastating drought impacted grain crops in Russia, combined with anticipated lower outputs in Kazakhstan and Ukraine. Based on this production situation in the Russian Federation, the United Nations Food and Agriculture Organization has cut its global wheat production forecast (http://www.un.org/).

Several studies reported the effect of high temperature on crop production (Lobell et al. 2008; Ortitz et al. 2008; Schlenker et al. 2008). Lobell et al. (2008) showed that increasing temperatures and declining precipitation over semiarid regions are likely to reduce yields for corn, wheat, rice, and other primary crops in the next two decades. Ortitz et al. (2008) stated that global warming could reduce productivity in zones where optimal temperatures currently exist. For example, by 2050, as a result of possible climate shifts, current favorable cultivation areas with high potential due to irrigation might be reclassified as a heat-stressed, irrigated, short-season production mega-environments. This shift would also represent a significant reduction in wheat yields, unless appropriate cultivars and crop management practices are developed (Ortitz et al. 2008).

The area of wheat production is about 225.5 million hectares per year, which makes wheat the most widely grown crop in the world. Wheat constitutes approximately 21 % of the world's food demands (http://www.fao.org). The United States is ranked as the fourth major producer of wheat in the world. Northern states including Montana are the primary source of hard red spring wheat (Small Grains 2010 Summary, http://usda.mannlib.cornell.edu/usda/current/SmalGraiSu/SmalGraiSu-09-30-2010.pdf). Since approximately half of spring wheat produced in US is exported, spring wheat production in the northern Great Plains is responsible for not only domestic food but also contributes to the international food supply (http://www.ndwheat.com/).

According to climate change projections by Battisti et al. (2008), during 2040 to 2060, mean growing season temperatures in parts of the northern Great Plains will be hotter than the hottest summer yet recorded with a probability of greater than 0.50. Lanning et al. (2010) reported that temperatures have increased in wheat growing regions of Montana over the past 58 years. They found that high temperatures in July have a negative effect on both yield and grain volume weight.

Adaptation is a key factor that will shape the future severity of climate change impacts on food production (Lobell et al. 2008). Two principal ways to adapt to climate change include application of crop production techniques and development of new crop varieties. Crop production techniques, such as shifting planting dates and switching to a different crop may be immediately applicable for current stress conditions although its benefit might be limited where changes are moderate. Development of new crop varieties may have a bigger effect under any stress conditions. Breeding for a new wheat

cultivar usually takes at least 10–12 years if the target traits are known and the environment in which to test new lines is available (Semenov and Halford 2009). Therefore, it is an essential first step to develop a breeding strategy by assessing target climatic conditions and new ideotypes with short-, mid- and long-term breeding objectives. Trethowan et al. (2010) suggested successful breeding has four sequential steps, including (1) identifying the target traits, which are a function of the target growing environment; (2) identifying sources of genetic variability for these traits; (3) crossing these sources of variability with existing varieties that possess other traits of economic importance such as disease resistance and high yield or quality; and (4) testing these new varieties across a range of on-farm environments.

High grain yield potential and yield stability is usually one of the most important traits in any wheat breeding program. Grain yield is a quantitative trait which involves a several physiological process affected by the wheat genotype, the environment, and genotype by environment interactions (Sleper and Poehlman, 2006). Therefore, it might not be sufficient to identify key traits for yield improvement and abiotic stress resistance only through analyzing the genetics of grain yield. Genetic dissection of quantitative traits controlling the adaptive response of crops to abiotic stress is a prerequisite to allow cost-effective applications to breeding programs aimed at improving the sustainability (Collins et al. 2008).

Grain yield in cereals may be dissected into three primary yield components, including spike number per area, seed number per spike, and seed weight (Ma et al. 2007). Therefore, improvement of key yield components could potentially improve yield

stability and/or yield potential under a wide-range of environments including specific stress environments. For example, spike number per area is a function of productive tiller number per area and high productive tiller number has been reported as an important character in relation to phenotypic plasticity in response to drought (Baum et al. 2003; Reynolds et al. 1999).

Breeding progress might be accelerated if physiological, biochemical and morphological characteristics were used as selection criteria (Feil 1992). Late onset and/or a slower rate of leaf senescence has an advantage in several crops, particularly in water stress environments (Borrell et al. 2000; Banziger et al. 1999; Hafsi et al. 2000; Foulkes et al. 2007) and heat stress environments (Dias and Lidon 2009; Kumari et al. 2007). Genetic variants called 'stay-green' retain green color in plants and are categorized into functional- and non-functional types (Thomas and Howarth 2000). Functional stay-green types maintain photosynthetic ability longer and may be important for yield improvement. Studies have shown that plants with better-developed root systems exhibit higher physiological activity in their roots and delayed leaf senescence in several crops (Jiang et al. 1988; Hirasawa et al. 1998; Kondo et al. 2000; Nakamura et al. 2003; Nakagami et al. 2004). As an example of stress-adaptive traits, deeper roots enable plants to remain hydrated under drought and permit canopy cooling under heat stress (Reynolds et al. 2010)

Many of the traits determining abiotic stress tolerance and the quality and quantity of yield are controlled by a large number of genes, which have minor individual effects but which act together (quantitative trait loci, QTL) (Barnabas et al. 2008). QTL-based

approaches contribute to a better understanding of the genetic basis of crop performance under environmentally constrained conditions. This knowledge can help breeders accelerate the release of cultivars that are able to cope with abiotic constraints (Collins et al. 2008). In sorghum, four QTL controlling stay-green (*Stg1–Stg4*) have been identified, and near-isogenic lines (NIL) for these QTL have been generated for a further analysis of stay-green physiology and positional cloning of the underlying genes (Harris et al. 2007). Yadav et al. (1997) identified QTL controlling root morphology and architecture in rice. Following the confirmation of selected QTL with NIL derived from several original population and recurrent parents by Shen et al. (2001), Steele et al. (2006) used marker-assisted backcrossing to introgress these alleles for increased root length in upland rice varieties. Although there are several QTL studies for heat and drought stress resistance in wheat (Fleury et al. 2010), the physiological and genetic basis of heat and drought stress resistance are only partially understood.

This research project was conducted to evaluate the genetic basis of novel traits contributing to grain yield and heat and drought stress resistance in spring wheat, integrating physiological aspects under multiple environments in Montana. We used QTL analysis to dissect the genetic basis of productive tiller number and green leaf duration after heading and their relationship with agronomic and physiological traits, and tested the effects of the QTL in multiple genetic backgrounds. Results from different water regimes and years have implications for development of spring wheat cultivars in areas facing the possibility of late season heat and drought stress.

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CHAPTER 2

GENETICS OF PRODUCTIVE TILLER NUMBER AND ITS RELATIONSHIP TO ECONOMIC TRAITS IN SPRING WHEAT

Introduction

Climate change is affecting the spring wheat growing environment in the northern Great Plains (Lanning et al. 2010), challenging breeders to identify traits and genes that will allow reliable grain yield under increasing temperatures. Grain yield is a complex trait, controlled by multiple genes and environmental interactions across all plant developmental stages. Grain yield in cereals may be dissected into three primary yield components, including spike number per area, seed number per spike, and seed weight (Ma et al. 2007). Therefore, increasing any yield component could potentially improve yield. Spike number per area is a function of productive tiller number per area (PTN). PTN, the number of tillers that produce spikes and seeds, is determined by both tiller initiation and tiller survival to the point of spike production. High PTN is also an important character in relation to phenotypic plasticity in response to drought (Baum et al. 2003; Reynolds et al. 1999).

Tiller initiation and development has been described as having three stages: 1) initiation of an axillary meristem; (2) development of an axillary bud; and (3) outgrowth of the axillary bud (Schmitz and Theres 2005). In cereal crops, genes controlling axillary meristem development have been identified and characterized. For instance, *teosinte branched* (*tb1*) in maize causes a complete loss of apical dominance, allowing the

unrestrained outgrowth of axillary buds (Doebley et al. 1997). *Monoculm1* (*MOC1*) in rice results in a lack of axillary buds (Li et al. 2003). In rye, the monoculm (*mc*) gene on the proximal region of chromosome 6RL controls axillary bud formation (Malyshev et al. 2001). Several mutations have been reported in barley that effect axillary bud formation and thus tiller production. The recessive mutation *uniculm2* (*cul2*) on chromosome 6HL allows initiation of axillary meristems that fail to develop tillers (Franckowiak 1996; Babb and Muehlbauer 2003). The recessive mutation *absent lower laterals* (*als*) on chromosome 3H results in development of axillary buds for primary tillers, but not for secondary tillers (Dabbert et al. 2009).

Although single genes affecting tiller number have been identified in several cereal crops, tiller number per plant in most segregating populations is inherited as a quantitative trait with low to moderate heritability. For example heritability of tiller number per plant was 0.34 and 0.51 in rice (Miyamoto et al. 2004; Rahman et al. 2007), 0.51 in barley (Tapsell and Thomas 1983) and 0.62 in wheat (Li et al. 2002). Quantitative trait loci (QTL) for tiller number and spike number were identified at several chromosome locations in wheat (Richards 1988; Shah et al. 1999; Li et al. 2002; Huang et al. 2003; Spielmeyer and Richards 2004; An et al. 2006; Narashimhamoorthy et al. 2006; Kumar et al. 2007). In two studies, QTL for grain yield also were identified in the same region as the tiller number QTL on 4D and 6DL (Huang et al. 2003; Kumar et al. 2007).

Not all tillers produce spikes, and many tillers abort before anthesis (Gallagher and Biscoe, 1978). Loss and Siddique (1994) found that many older Mediterranean

wheat varieties have a large number of tillers that are unable to produce spikes, while newer varieties produce fewer total tillers, but more survive to spike production. Yan et al. (1998) found in rice that QTL affecting tiller number varied depending on the growth stage, with some QTL controlling tiller growth in early stages undetectable at later stages. Therefore, QTL controlling PTN may be more critical for yield improvement than QTL controlling tiller initiation.

In the past, abundant tillering has been predicted to be an undesirable trait for dry-land cereal production due to lack of productive spike formation on later tillers (Donald, 1968). Elhani et al. (2007) found that productive tiller number was an important contributor to grain yield under high moisture conditions, but had no detectable effect under rain-fed conditions. Importantly, in semiarid region of the northern Great Plains, high-yielding and widely-grown spring wheat cultivars vary in tiller production (Hansen et al. 2005). For this report, we determined the genetic basis for variation in productive tiller number and its impact on productivity for three spring wheat recombinant inbred line (RIL) populations. Results from different water regimes and years have implications for development of spring wheat cultivars in areas facing the possibility of late season heat and drought stress.

Materials and Methods

Plant Materials

RIL were developed by single-seed descent starting with the F_2 generation from three crosses, including Reeder (PI 613586)/Conan (PI 607549) (WestBred, LLC), McNeal (PI 574642) (Lanning et al., 1994) /Thatcher (CI 10003) and McNeal/Reeder

(Table 1). The parents, McNeal, Reeder and Conan are all widely grown hard red spring wheat cultivars in Montana (Montana Agricultural Statistics, 2010), while Thatcher is a historically important cultivar in the Great Plains. A total of 91 Reeder/Conan, 160 McNeal/Thatcher and 50 McNeal/Reeder RIL were derived from individual F_6, F_5 and F_4 plants, respectively. RIL in these three populations were segregating for height because of parental differences at the *Rht* loci. The McNeal/Thatcher population consisted of 80 semi-dwarf and 80 standard height RIL. These RIL were randomized within eight blocks in groups of 20 for either the tall or semi-dwarf genotypes to eliminate the effects of shading. The Reeder/Conan and McNeal/Reeder RIL initially consisted of dwarf, semi-dwarf, and standard height genotypes, but only semi-dwarf and standard height RIL were included in these studies.

Experimental Design

The three RIL populations were evaluated in replicated field trials with a randomized complete block design in different irrigation regimes in subsets of years from 2004 to 2009 at the Arthur H. Post Research farm near Bozeman, Montana (latitude 45.41˚N, longitude 111.00˚W, elevation 1455m) (Table 1). Each plot consisted of 3.3 m-single rows seeded 2.5 g m^{-1} for rain-fed trials and 3.3 g m^{-1} for irrigated trials. Growing season precipitation and temperature data were collected from NOAA climatological measurements for the Post Field Research farm near Bozeman, Montana (National Climatic Data Center, 2010).

Phenotypic Data Collection

Phenotypic data collected in all experiments included PTN, days to heading, plant height, grain yield, test weight, seed weight, grain protein concentration and seed number per spike. PTN was defined as number of tillers with fertile spikes per 30 cm of each plot. Heading dates were taken by assigning a day of the year when 50% of the heads were completely emerged. Plant height was the average of two measurements made from soil surface to the top of the spikes, excluding awns. Grain yield was determined from the raw grain weight of each plot. Test weight was measured from a sample of cleaned grain on a Seedburo (Chicago, IL) test weight scale. A subsample of seed was taken from each plot and was analyzed in the Single Kernel Characterization System 4100 (Perten, Huddings, Sweden) to determine seed weight. Grain protein concentration was obtained on whole grain samples using a Foss Infratec 1241 Grain Analyzer (Tecator, Höganäs, Sweden). For seed number per spike, the number of seed were counted and averaged from 10 spikes that were randomly sampled from each plot. This trait was measured only in Reeder/Conan and McNeal/Thatcher RIL.

Statistical Data Analysis

Data for each phenotypic trait in each population was analyzed via analysis of variance (ANOVA) using a model for a randomized complete block for each environment (irrigation regime and year). Also, ANOVA was combined over environments where the model included environment, replications within environment, entry and environment x entry with PROC GLM in SAS (SAS Institute, 2004). All factors except environment were considered random effects. Narrow-sense heritability

for PTN was computed on an entry mean basis as described in Knapp et al. (1985) combined over environments. Pearson correlations were computed using PROC CORR of SAS (SAS Institute, 2004) between PTN and agronomic traits using the entry mean value from each environment and combined over environments for each population.

QTL Mapping

Creation of a genetic map with 431 markers and a total size of 2608.75 cM for the Reeder/Conan RIL was described previously (Sherman et al. 2010). The map contained 232 SSR markers, 190 DArT markers (Akbari et al. 2006), six markers for major genes controlling development (*Ppd-A1*, *Ppd-B1*, *Ppd-D1*, *Rht-B1*, *Rht-D1*, *Vrn-B1*) and three markers for storage proteins. Markers were subjected to a chi-square test for fit to a 1:1 ratio using MapDisto (http://mapdisto.free.fr). Markers with significant distortion were indicated in the genetic map (Sherman et al., 2010). QTL analysis was conducted for PTN using the entry means for each environment by QTL Cartographer (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). QTL were identified with composite interval mapping (CIM) (Zeng 1993, 1994) using standard CIM model, Forward and Backward Regression method with a window size of 10.0 cM with probability in and out of 0.1 and a walking speed of 2cM as described by Sherman et al. (2010). LOD value was set by 1000 permutations at an experiment-wise $P < 0.01$. QTL were established by the map position of the peak LOD score in the interval between two flanking markers. A one-LOD fall-off (from the QTL peak) method was used to estimate the left-and right-flanking map positions of a confidence interval surrounding the mean QTL map position (Chaky, 2003).

Single Marker Analysis

In order to confirm QTL identified in the Reeder/Conan mapping population in different backgrounds, RIL populations derived from the McNeal/Thatcher and McNeal/Reeder crosses were used for a single marker analysis. A single-factor ANOVA was conducted using PROC GLM in SAS (SAS Institute, 2004) where the single factor was the segregating marker locus with two allelic classes. The segregating markers linked to PTN QTL in Reeder/Conan mapping population included PCR markers for *Ppd-B1* (Blake et al. 2009), microsatellite markers wmc453 and barc55 for the QTL on 2B, barc204, barc23 and wmc753 for the QTL on 6A, gwm88 and barc354 for the QTL on 6B (GrainGenes 2.0, http://wheat.pw.usda.gov/GG2/index.shtml). Entry means of PTN and other traits averaged over environments for each population were used for this analysis. Differences between allele class means were tested with an F ratio. This was done for each segregating marker locus for each population. The proportion of variation attributable to the segregating marker locus (R^2) was calculated as the sum of squares for segregating genes divided by the total sum of squares

Results

Meteorological Difference among Experiments

Data were gathered over five years for genetic analysis of PTN by evaluating the three RIL populations under rain-fed and irrigated conditions, spanning different time frames (Table 1). Table 2 shows that environmental conditions varied widely during these years. In 2006 and 2007, above average temperature and below average

precipitation, particularly during the grain-fill stage in July and August, resulted in heat and drought stress. In 2007, the conditions were the most severe with nine days above 35 °C maximum temperature and approximately 36 % precipitation relative to the 50-year average data in July and August. No other year had such extended period of high temperature and drought conditions. The years 2004, 2005 and 2009 showed similar temperature and precipitation to the 50-year average.

Mean Performance and Heritability of Productive Tiller Number in the Populations

There were significant differences for PTN between parents of each RIL population. Reeder had more PTN than Conan and McNeal, and Thatcher had more PTN than McNeal (Table 3). Both Reeder and McNeal were parents for two populations, and showed different mean PTN for each RIL population due to the different environments encountered by each experiment (Table 1; Table 3). There was significant genetic variation for PTN in all three populations (Table 3), with the RIL mean intermediate between the respective parents. Heritability of PTN ranged from 0.65-0.75 in the three RIL populations, based on combined means over environments and years.

Correlation between Productive Tiller Number and Agronomic Traits

The parents and RIL of the three populations varied for agronomic traits including PTN and seed weight in addition to heading date, plant height, test weight and grain yield as previously reported (Blake et al. 2009). Seed number per spike varied in the Reeder/Conan and the McNeal/Thatcher RIL. Correlations between PTN and other traits

for the Reeder/Conan, McNeal/Thatcher, and McNeal/Reeder RIL are shown in Table 4. PTN showed a positive correlation with grain yield in all RIL populations across all years and environments. Significant negative correlations of PTN with plant height were observed for the Reeder/Conan RIL in the 2006 and 2007 rain-fed environments and across all years and environments for the McNeal/Thatcher RIL. PTN was negatively correlated with seed number per spike in the 2006 McNeal/Thatcher rain-fed and irrigated environments and the 2006 Reeder/Conan rain-fed environment. Seed weight was also negatively correlated with PTN in the 2006 McNeal/Thatcher rain-fed environment, the 2006 and 2007 McNeal/Thatcher irrigated environments, the 2004 and 2005 McNeal/Reeder rain-fed environment and the 2005 McNeal/Reeder irrigated environment. In sum, correlation analysis showed consistent significant positive correlation between PTN and grain yield in all RIL populations in all experiments across years and environments (Table 4), though the correlation between PTN and seed number per spike, and PTN and seed weight, tended to be negative.

QTL Analysis for Productive Tiller Number

QTL analysis was conducted for each year and environment for the Reeder/Conan RIL population using the genetic map generated by Sherman et al. (2010). CIM showed seven significant QTL in at least one environment (Table 5). Four of these QTL occurred in only one environment. Two of the most significant QTL only occurred in 2007. QTL on chromosome 2B, 6A, and 6B were significant in multiple years and environments (Table 5). The Reeder allele for the 2B QTL flanked by Ppd-B1 and barc55 reduced PTN and accounted for 12 % and 13 % of the variation in the 2009 irrigated and rain-fed

environment, respectively (Table 5). Reeder carries *Ppd-B1a* that confers photoperiod insensitivity (Blake et al. 2009). The Reeder allele for the QTL on chromosome 6A was associated with greater PTN and accounted for 12% of variation in the 2009 irrigated environment and 11% of variation in the 2006 rain-fed environment (Table 5). The 6A QTL identified in 2006 rain-fed and 2009 irrigated environment were overlapping, flanked by barc204.1 and barc1055 and co-segregated with test weight (Table 5). A QTL was identified on chromosome 6B in three environments where the position of the QTL was localized within a 21.4 cM region flanked by gwm88 and wPt3581 (Table 5; Fig. 1). The Reeder allele caused greater PTN and accounted for variation as follows: 19% in 2006 rain-fed, 13% in 2009 irrigated and 9% in 2009 rain-fed environments (Table 5). The 6B QTL co-segregated with grain yield where the Reeder allele was associated with greater yield in the 2009 rain-fed environment (Table 5). Although there were also significant QTL for PTN identified on chromosome 1D, 3D, 6D and 7B, we did not pursue these QTL for further analysis because they were identified in only a single environment or year. As previously described (Sherman et al. 2010), segregation distortion was observed in this map. However, no markers associated with PTN were distorted except loci barc204.1 and wPt7599 linked to the 6A QTL.

QTL Confirmation for Productive Tiller Number

Based on the identification of the 2B QTL, the 6A QTL and the 6B QTL in the Reeder/Conan mapping population, single marker analysis was conducted in the McNeal/Thatcher and the McNeal/Reeder RIL using polymorphic markers for each QTL region to confirm the effect of these QTL in different populations. The 6B QTL (gwm88

and barc354) significantly increased PTN for the respective RIL populations (Table 6). The Reeder allele explained up to 28% of the variation for PTN in the McNeal/Reeder RIL, while the Thatcher allele explained 3% of the variation for PTN in the McNeal/Thatcher RIL. Due to its consistent effect on PTN and the apparent importance of this QTL in all three crosses, we have designated the 6B QTL as *QTn.mst-6B.* Polymorphic markers linked to the 2B QTL (wmc453 and PpdB) did not show a significant effect of allelic variation for PTN in the McNeal/Thatcher or the McNeal/Reeder RIL. The 6A QTL (barc204, wmc753 and barc23) did not show a significant effect of allelic variation for PTN in the McNeal/Reeder RIL. There were no polymorphic markers linked to the 6A QTL in the McNeal/Thatcher RIL.

The Impact of *Tn.mst*-6B on Agronomic Traits

To determine the impact of *QTn.mst-6B* on agronomic traits in three RIL populations, single marker analysis was also conducted. The Thatcher allele conferring high PTN was also associated with increased test weight, but decreased seed number per spike for the McNeal/Thatcher RIL (Table 7). The Reeder allele conferring high PTN at *QTn.mst-6B* was associated with increased test weight, grain yield, and grain protein in the McNeal/Reeder RIL (Table 7). Neither the Thatcher allele nor the Reeder allele at *QTn.mst-6B* affected heading date, plant height or seed weight (Table 7).

Discussion

Measurement of tiller number may be conducted at different stages of development. Assessment of tiller number in vegetative stages provides a measurement

of maximum tillering, while assessment at or after heading provides a measurement of productive tiller number. Several genetic studies with mutant populations have focused on pre-heading stages of tiller development (Li et al. 2002; Richards 1988; Spielmeyer and Richards 2004; Kuraparthy et al. 2007; Dabbert et al. 2009), while agronomic studies more often involve measurement of productive tiller number or spike number (Kato et al. 2000; Huang et al. 2003; Quarrie et al. 2006; Kuchel et al. 2007). A complication is that the tillering trait is measured two different ways with some studies using tiller number per plant, and others using tiller number per area. Here we report tiller number per area, although analyses using tiller number per planted seed gave similar results, including a significant positive correlation with yield in every environment for every cross, and an important QTL controlling tiller number on chromosome 6B for every cross (data not shown).

There was a consistently significant positive correlation between PTN and grain yield in each environment for all three RIL populations, even though the genetic background of these populations and environmental conditions of the experiments were different. This result is consistent with previous studies which reported positive correlations between tiller number and yield in wheat (Sidwell et al. 1976; Kato et al. 2000; Huang et al. 2003; Kumar et al. 2007). Despite the positive correlation with grain yield, there were negative correlations between PTN and seed weight in the McNeal/Thatcher and McNeal/Reeder RIL, and between PTN and seed number per spike in the Reeder/Conan and McNeal/Thatcher RIL. Other studies have reported similar negative correlations (Kato et al. 2000; Narasimhamoorthy et al. 2006), suggesting

difficulty in improving grain yield by only increasing PTN. There were negative correlations between plant height and PTN in the Reeder/Conan and McNeal/Thatcher RIL, during hotter and drier years, as shorter plants tended to have more productive tillers. However this relationship seems not to be a general trend in the few studies showing the effect of *Rht* genes on tiller number (Allan et al. 1986; McClung et al. 1986; Youssefian et al. 1992; Flintham et al. 1997). In this study, we did not identify any significant association between PTN and the *Rht-B1*or *Rht-D1* segregating in the Reeder/Conan RIL and the Reeder/McNeal RIL, although there was a significant association between PTN and the *Rht-D1* in the McNeal/Thatcher RIL, as semi-dwarf lines had increased PTN (data not shown).

QTL for PTN observed across environments were identified on three chromosomes in the Reeder/Conan mapping population (Table 5). The 2B QTL co-segregated with a marker for the *Ppd-B1* locus, whereby the allele for photoperiod insensitivity from Reeder resulted in fewer tillers. Other authors have reported a relationship between photoperiod sensitivity and tiller number (reviewed by Dyck et al. 2004). We identified significant QTL on all group 6 chromosomes, whereby the Reeder allele showed a significant positive effect on PTN. Others have reported QTL for tiller number on chromosome 6A and 6D (Li et al. 2002; Huang et al. 2003; An et al. 2006; Kumar et al. 2007). There is no previous report of a PTN QTL on 6B. However, in barley, Babb and Muehlbauer (2003) positioned *cul2* on chromosome 6HL. This gene co-segregates with molecular marker cdo524 that is closely linked to gwm88, which is linked to *QTn.mst-6B* reported here (GrainGenes 2.0, http://wheat.pw.usda.gov/GG2/index.shtml).

Moreover, Malyshev et al. (2001) identified monoculm growth habit (*mc)* on chromosome 6RL in rye (*Secale cereale L.)* that also appears to occupy a similar chromosomal position. These studies support the possibility that the chromosome 6 QTL for PTN identified in the present study and the barley and rye genes controlling tillers are homoeologous.

More QTL for PTN were identified in 2009, which was cooler and wetter during the grain-fill stage than 2006 and 2007. Cool temperatures along with adequate moisture may have allowed higher tiller survival and thus higher numbers of productive tillers. QTL identified in 2009 alone, located on chromosomes 1D and 2B might have a primary effect on tiller initiation. The only significant QTL for PTN observed in three environments was *QTn.mst-6B*. It was also the only PTN QTL confirmed by the McNeal/Thatcher and the McNeal/Reeder RIL, indicating stability between genetic background and environments. It is possible that this QTL has a more stable effect across environments. The only QTL identified in 2007 were unique to that year. The severe high temperature and drought conditions during the grain-fill stage in 2007 caused abrupt senescence and negatively impacted productive tiller number. The QTL on 7B, that increased PTN under the stress conditions, might be of particular interest. By combining with the more environmentally stable QTL on 6B, PTN might be improved in a broader range of environments.

Two of the populations in this study had Reeder as a parent, and the Reeder allele at *QTn.mst-6B* conferred increased PTN and yield potential in both populations. Although no PTN QTL on 6B has been previously reported, several QTL for yield on

chromosome 6B have been identified (Marza et al. 2006). Our results suggest that selection for the positive allele at *QTn.mst-6B* in crosses involving Reeder is likely to increase PTN and grain yield. Conversely, there was no significant effect on grain yield for alternative alleles at this QTL for the McNeal/Thatcher RIL. It is likely that the negative effect of the Thatcher allele for high PTN on seed number per spike at *QTn.mst-6B* (Table 7) offset the positive effect on increased PTN and resulted in no effect on yield in the McNeal/Thatcher RIL.

Segregation distortion was found for several markers linked to the 6A QTL. Distorted markers (barc204 and wPt7599) linked to the 6A QTL were either not polymorphic in the McNeal/Thatcher and McNeal/Reeder RIL or did not show significant effect of allelic variation in these populations. Xu (2008) showed that the effect of segregation distortion on QTL detection is likely to be negligible, though slight positive or negative effects are possible. An et al. (2006) identified a QTL for tiller number on chromosome 6A flanked by markers (wmc179 and wmc256), that also were linked to the 6A QTL reported here (GrainGenes 2.0, http://wheat.pw.usda.gov/GG2/index.shtml). Therefore, this report is probably a verification of the previously reported 6A QTL.

Crop modeling efforts have suggested that low tiller number may be beneficial for wheat productivity in water-stressed environments (Donald, 1968). However, high tiller survival may be related to increased tolerance to drought stress (Reynolds et al. 1999). Baum et al. (2003) reported that under stress conditions, the number of productive tillers contributes largely to grain yield in barley. High tillering is an important character in

relation to phenotypic plasticity in response to drought. An et al. (2006) showed significant correlation between root dry weight and tiller number and QTL for these traits in a wheat population derived from parents which differed in drought tolerance. As the wheat root system develops in a coordinated pattern along with tillers (Weaver 1926), plants with more tillers may develop more roots, therefore high tiller number may contribute tolerance to drought stress through increased root development.

Conclusion

Our results showed a consistent positive correlation between PTN and grain yield under drought and heat stress conditions as well as well-watered conditions across three spring wheat populations. *QTn.mst-6B,* for high productive tiller number on chromosome 6B from Reeder was consistent across environments and populations, and increased grain yield in crosses involving Reeder. *QTn.mst-6B* may be useful for improving spring wheat in the northern Great Plains of North America and similar environments.

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Table 1. Populations and environments used for genetic analysis of productive tiller number.

Parents	No. RIL[a]	Year of experiment	Irrigation regime
McNeal/Thatcher	160	2006	Rain-fed, irrigated
		2007	Rain-fed, irrigated
Reeder/Conan	91	2006	Rain-fed
		2007	Rain-fed
		2009	Rain-fed, irrigated
McNeal/Reeder	50	2004	Rain-fed, irrigated
		2005	Rain-fed, irrigated

[a]Number of recombinant inbred lines

Table 2. Growing season precipitation (cm) and average monthly temperature (°C) at the Post Research farm in Bozeman, MT.

Years	Precipitation (cm)					Average monthly temperature (°C)				≥35°C (d)
	May	June	July	August	Total	May	June	July	August	
2004	6.7	5.7	3.8	4.1	20.2	10.4	14.2	19.8	17.2	1
2005	3.0	7.7	2.7	4.4	17.8	9.4	12.1	19.8	19.1	2
2006	4.4	8.7	1.4	1.8	16.2	11.1	14.8	21.5	18.9	4
2007	12.7	6.7	0.8	1.6	21.7	12.2	16.1	23.4	19.2	9
2009	4.1	6.7	7.1	3.8	21.7	12.1	14.1	18.6	18.4	0
50-year average 1958-2009	6.7	6.9	3.5	3.2	20.3	10.7	14.8	18.7	18.1	

[a] Number of days recorded greater than or equal to 35°C maximum temperature in July and August

Table 3. Mean and range for productive tiller number for parents and RIL populations.

Populations	Reeder/Conan		McNeal/Thatcher		McNeal/Reeder	
	Reeder	Conan	McNeal	Thatcher	McNeal	Reeder
Parental	59.9	47.9*	56.4	65.6*	66.4	76*
RIL mean	54.8		60.7		71.6	
RIL range	42.5-67.8		46.9-78.8		59.9-88.7	
Pr>F	<.0001		<.0001		<.0001	
Heritability	0.70		0.75		0.65	

* Differences between parents significant (P<0.05) based on LSD

Table 4. Pearson's correlation between productive tiller number and agronomic traits[a] in three RIL populations.

Population	Year/ Environment	Heading date	Plant height	Seed weight	Seed number per spike	Test weight	Grain yield
Reeder/Conan	2006/Rain-fed	-0.28**	-0.43***	-0.19	-0.27**	0.08	0.39***
	2007/Rain-fed	-0.29**	-0.25*	-0.08	-0.05	0.05	0.36***
	2009/Rain-fed	0.22*	-0.07	-0.14	0.09	0.08	0.54***
	2009/Irrigated	-0.05	-0.07	-0.13	-0.06	0.33***	0.32**
	Combined	-0.14	-0.39***	-0.20	-0.18	0.12	0.39***
McNeal/Thatcher	2006/Rain-fed	-0.04	-0.32***	-0.18*	-0.25**	-0.03	0.47***
	2006/Irrigated	-0.04	-0.43***	-0.18*	-0.24**	0.02	0.56***
	2007/Rain-fed	0.16*	-0.47***	-0.18*	0.11	-0.13	0.54***
	2007/Irrigated	0.08	-0.41***	-0.37***	0.11	-0.23	0.49***
	Combined	-0.05	-0.46***	-0.25**	-0.28***	-0.04	0.55***
McNeal/Reeder	2004/Rain-fed	-0.02	-0.10	-0.29*	nd	-0.07	0.31*
	2004/Irrigated	-0.08	-0.07	-0.24	nd	0.12	0.30*
	2005/Rain-fed	-0.06	-0.02	-0.33*	nd	-0.15	0.42**
	2005/Irrigated	0.02	0.00	-0.35**	nd	-0.11	0.34*
	Combined	-0.05	0.14	-0.43**	nd	-0.03	0.38**

Significance levels: *P<0.05, **P<0.01, *** P<0.001

nd: no data

[a]Heading date, plant height, seed weight (mg), seed number per spike, test weight (kg m^{-3}) and grain yield (kg ha^{-1})

Table 5. Significant QTL for productive tiller number identified through composite interval mapping of the Reeder/Conan RIL in all environments and years.

Chromosome	Flanking markers	Year/Environment	Additive effect[a]	R^{2b}	LOD[c]	Coincident QTL
1D	gwm458-wPt0077	2009/Irrigated	2.31	0.08	2.7	Seed number per spike
2B	PpdB-barc55	2009/ Rain-fed	-2.80	0.13	3.2	None
		2009/ Irrigated	-2.92	0.12	4.1	
3D	barc316-gwm645	2007/Rain-fed	-3.24	0.19	5.2	None
6A	barc204.1-barc753	2009/Irrigated	2.92	0.12	2.7	Test weight
	wpt8833-barc1055	2006/Rain-fed	3.17	0.11	3.2	None
6B	gwm88-barc354	2006/Rain-fed	4.05	0.19	4.4	None
	gwm88-wPt3581	2009/Irrigated	2.99	0.13	3.7	None
	barc354-wPt3581	2009/Rain-fed	2.35	0.09	2.6	Grain yield
6D	barc196-cfd88	2006/Rain-fed	3.41	0.13	3.6	None
7B	wmc273-barc303	2007/Rain-fed	2.94	0.15	5.1	None

[a]Additive effect of the Reeder allele

[b]R^2 The phenotypic variation explained by the QTL

[c]LOD logarithm of odds. Only QTL with LOD scores above 2.5 are shown

Table 6. Single marker analysis of productive tiller number using markers for the 6B QTL (*QTn.mst-6B*) identified in the Reeder/Conan RIL.

Population	Marker	Allele	No. RIL[a]	PTN[b]	P value[c]	R^{2d}
McNeal/Thatcher	gwm88	McNeal	89	59.7	0.04	0.03
		Thatcher	64	62.0		
	barc354	McNeal	97	59.7	0.03	0.03
		Thatcher	62	62.1		
McNeal/Reeder	gwm88	McNeal	26	68.9	<0.0001	0.28
		Reeder	23	74.9		
	barc354	McNeal	25	69.3	0.003	0.18
		Reeder	21	74.2		

[a]Number of recombinant inbred lines with allele

[b]Mean of productive tiller number of lines with allele

[c]Compares the difference of the QTL allele from Reeder or Thatcher allele and the alternate parent

[d]The phenotypic variation explained by the marker

Table 7. Single marker analysis of agronomic traits[a] using markers linked to *QTn.mst-6B* identified in the Reeder/Conan RIL.

Population	marker	Allele	No. RIL[b]	Heading date (d)	Plant height (cm)	Seed weight (mg)	Seed number per spike	Test weight (kg m^{-3})	Grain yield (kg ha^{-1})	Grain Protein (%)
McNeal/Thatcher	gwm88	McNeal	89	179.2	91.5	30.0	36.1	735	3,652	15.4
		Thatcher	64	179.5	95.8	29.9	34.6	744	3,558	15.6
		P value[c]	-	0.292	0.067	0.871	0.009	0.006	0.317	0.098
	barc354	McNeal	97	179.3	91.9	30.1	36.0	735	3,645	15.4
		Thatcher	62	179.3	95.9	29.8	34.6	744	3,544	15.6
		P value	-	0.883	0.078	0.563	0.013	0.005	0.267	0.064
McNeal/Reeder	gwm88	McNeal	26	184.8	97.3	36.1	nd	771	5,689	14.5
		Reeder	23	184.4	98.7	35.4	nd	776	5,918	14.9
		P value	-	0.479	0.221	0.265	nd	0.052	0.050	0.023
	barc354	McNeal	25	184.3	97.5	36.1	nd	772	5,636	14.7
		Reeder	21	184.8	99.0	35.4	nd	776	5,979	14.8
		P value	-	0.415	0.178	0.290	nd	0.114	0.004	0.379

nd: No data

[a]Heading date, plant height, seed weight (mg), seed number per spike, test weight (kg m^{-3}), grain yield (kg ha^{-1}) and grain protein (%)

[b]Number of recombinant inbred lines

[c]Compares the difference between the QTL allele from Reeder or Thatcher and alternate parent

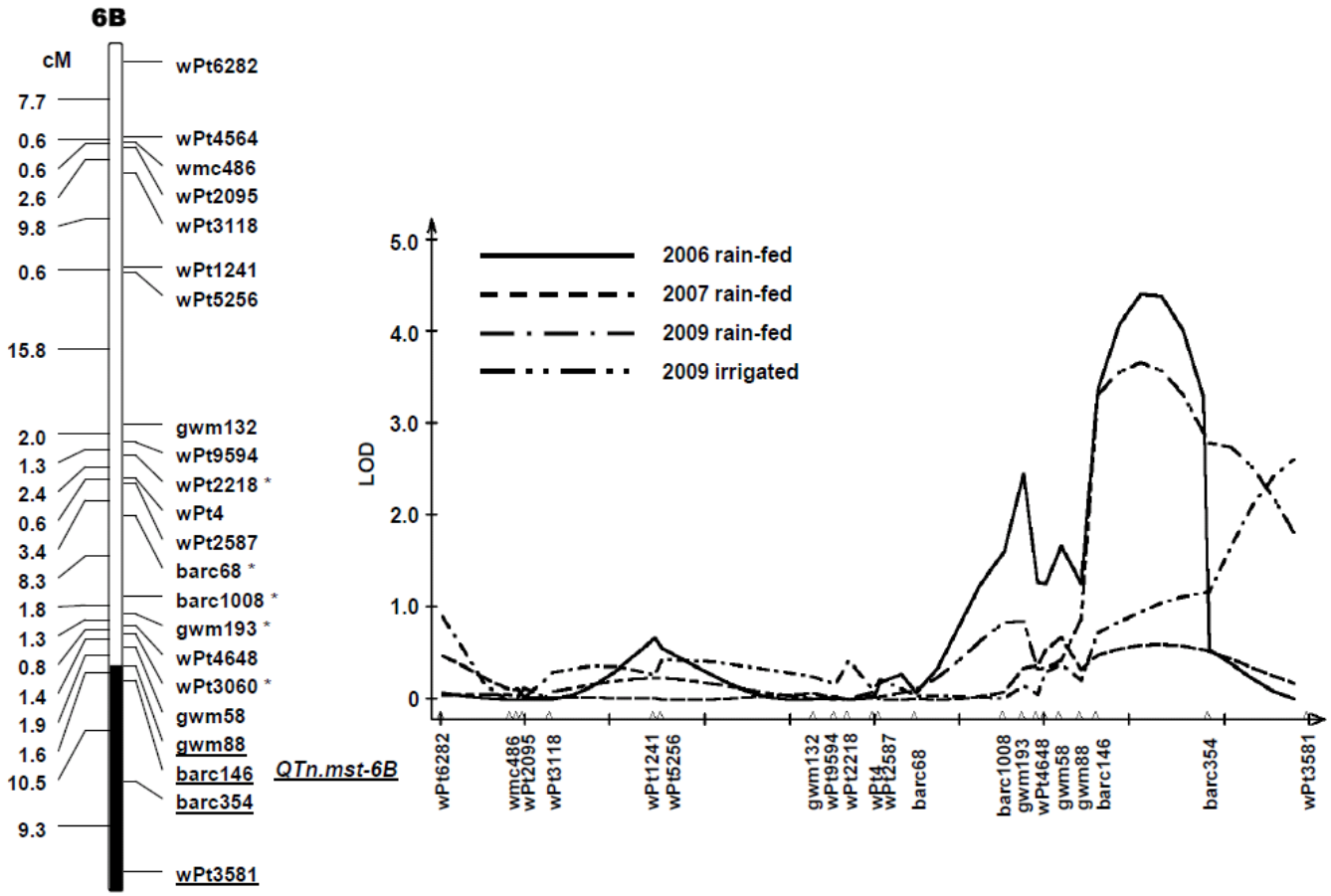

Figure. 1 Linkage map of chromosome 6B from Sherman et al. (2010) with the removal of co-segregating DArT markers. Chromosome region of *QTn.mst-6B* indicated in black. The markers defining *QTn.mst-6B* are underlined. Asterisks at the end of marker name denotes significantly distorted loci (*P<0.05). Position and LOD score of the QTL for PTN on chromosome 6B in 2006 rain-fed (solid line), 2007 rain-fed (dashed line) and 2009 rain-fed (single dotted line) and irrigated (double dotted line) environments are shown on the right.

CHAPTER 3

GENETIC ANALYSIS OF GREEN LEAF DURATION AND ITS RELATIONSHIP TO ECONOMIC TRAITS UNDER LATE SEASON STRESS ENVIRIONMENTS IN SPRING WHEAT

Introduction

Hard red spring wheat (*Triticum aestivum* L.) is primarily grown in the northern Great Plains of North America and its production generally is characterized by water deficit, where plants are largely dependent on stored soil moisture for growth and maturity (Talbert et al. 2001; http://www.nass.usda.gov/QuickStats/Create_County_All.jsp). In addition to the water limited environment for spring wheat production, global climate change is affecting spring wheat production with increasing temperatures (Lanning et al. 2010). Temperatures during the critical grain fill period for spring wheat in Montana, primarily encompassing the month of July, are historically above optimal and are projected to continue to increase (Lanning et al. 2010). Optimal temperature for anthesis and grain filling are approximately 18-22°C, with maximum temperatures approximately 31-35°C (Porter and Gawith 1999). High temperatures during grain filling result in faster senescence and decreased grain filling duration (Porter and Gawith 1999).

Under most circumstances, 90-95% of the carbohydrate in grain is derived from carbon dioxide fixation after anthesis (Evans 1975). Flag leaf photosynthesis of wheat contributes about 30-50% of assimilates for grain filling (Sylvester-Bradley et al. 1990; Lupton et al. 1966). Late onset and/or a slower rate of leaf senescence confers a yield

advantage in several crops, particularly under water stress environments (Borrell et al. 2000; Banziger et al. 1999; Hafsi et al. 2000; Foulkes et al. 2007) and heat stress environments (Dias and Lidon 2009; Kumari et al. 2007). Genetic variants called 'stay-green' retain green color in plants and are categorized into functional- and non-functional types (Thomas and Howarth 2000). Functional stay-green types maintain photosynthetic ability longer and may be important for yield improvement. Talbert et al. (2001) reported correlation between agronomic traits and grain fill duration measured as a period between heading and physiological maturity, approximated to coincide with the time when heads lose green color. They found that longer grain fill duration was often correlated with higher test weight in non-irrigated environments. However, there was no significant correlation between grain fill duration and grain yield in most instances (Talbert et al. 2001). Associations between grain fill duration and grain yield in wheat vary among studies. Nass and Reiser (1975) found no association between grain fill duration and grain yield, but Gebeyehou et al (1982) found that grain fill duration was strongly correlated with grain yield. Hansen et al. (2005) compared agronomic and physiological traits for twenty spring wheat cultivars, and suggested that flag leaf maturity may be related to yield potential. Blake et al. (2007) found a positive correlation between the green leaf duration of flag leaves (GLDAH) and grain volume and kernel weight in two different recombinant inbred line (RIL) populations segregating for GLDAH. However, a positive correlation between GLDAH and grain yield was found in only one of the populations.

Talbert et al. (2001) also found that earlier heading was associated with longer grain fill across twelve spring wheat crosses. Some studies indicate similar association between heading date and grain fill duration (Bruckner et al. 1987; Tewolde et al. 2006; Wang et al. 2009), while others do not find a clear association between heading date and percent green leaf area at a specific time after heading (Verma et al. 2004; Foulkes et al. 2007). Blake et al. (2009) reported that variation at photoperiod and vernalization loci which are major heading and flowering date determinants, impacted maturity characteristics including GLDAH.

The physiological mechanisms of stay-green related traits have been widely studied. Studies in rice, soybean, maize and wheat indicate that better-developed root systems exhibit higher physiological activity and delayed leaf senescence (Jiang et al. 1988; Hirasawa et al. 1998; Kondo et al. 2000; Nakamura et al. 2003; Nakagami et al. 2004). However, little is known about the genetics and genes affecting delayed leaf senescence and root traits and their relationship in wheat, partially due to complications in root phenotyping. Root activity in rice has been estimated through the measurement of 'bleeding' from the basal part of the stem (Hirasawa et al. 1983; Lee et al. 1994; Yamaguchi et al. 1995). Bleeding is the xylem sap exuded from detopped plant stumps, caused by root pressure (Schurr 1998). Sakaigaichi et al. (2007) found a significant positive correlation between the exudation rate and root length throughout the growing period in rice. Exudation rates in wheat plants grown in the dry plots were higher than those grown in excess-watered plots, corresponding to a better-developed root system in dry pots (Nakamura et al., 2003). Similar results also were observed in soybean plants

(Hirasawa et al. 1998). Therefore, the amount of xylem exudate might be a reasonable estimation of root mass and/or activity under field condition.

Heritability of grain fill duration has been reported to be 0.73 to 0.77 in wheat (Blake et al. 2007). Several authors have reported of QTL for stay-green related traits in bread wheat. Verma et al. (2004) identified QTL for percentage of green leaf area on chromosome 2B and 2D for optimal and drought-stressed environments. Wang et al. (2009) identified six QTL for grain fill duration. Kumar et al. (2010) identified three stay-green QTL on chromosome 1A, 3BS and 7DS. Although a large effect of epistatic interactions on stay-green has been reported in other crops (Subudhi et al. 2000; Jiang et al. 2004; Yoo et al. 2007), epistasis including the stay-green trait has not been investigated in wheat.

In this report, we determine the genetic basis of green leaf duration after heading (GLDAH), a stay-green trait, and its impact on agronomic traits for a spring wheat RIL population under various environments. Our goals were to (i) determine the impact of green leaf duration after heading on agronomic traits under different environments, (ii) identify genomic regions linked to green leaf duration after heading, particularly from hot, dry environments (iii) confirm identified GLDAH QTL in different genetic backgrounds from the mapping population, and (iv) explore the genetic association between green leaf duration and root traits.

Materials and Methods

Plant Materials

A total of ninety-one F_6-derived recombinant inbred lines (RIL) were developed by single-seed descent starting with the F_2 generation from a cross between Reeder (PI 613586) and Conan (PI 607549) spring wheat (Sherman et al. 2010). The parents, Reeder and Conan are widely grown hard red spring wheat cultivars in Montana (Montana Agricultural Statistics 2010). The Reeder/Conan RIL was segregating for height because of parental differences at the *Rht* loci. Only semi-dwarf and standard height RIL were included in these studies.

Twenty of the Reeder/Conan RIL and twenty old to modern spring wheat varieties used in Hansen et al (2005) were used to determine the relationship between root dry weight and amount of xylem exudate. RIL populations derived from a cross between Reeder and Choteau (PI 633974) (Lanning et al. 2004), and McNeal (PI 574642) (Lanning et al., 1994) and Reeder were used for single marker analysis of QTL identified in Reeder/Conan RIL. The Choteau/Reeder RIL and McNeal/Reeder RIL consisted of 46 and 50 RIL respectively.

Experimental Design

The Reeder/Conan RIL were evaluated in replicated field trials using a randomized complete block design in different irrigation regimes in 2006, 2007, and 2009 at the Arthur H. Post Research Farm in Bozeman in Montana. A 2008 experiment was destroyed by hail. Ten RIL that varied for GLDAH were evaluated for

photosynthetic rates of flag leaves in three replicated field trials using a randomized complete block design in 2008 at the Northwestern Agricultural Research Center at Creston in MT (Creston), and at the Northern Agriculture Research Center at Havre in MT (Havre). In the trials at Post Research Farm in Bozeman, each plot consisted of 3.3 m-single rows seeded 2.5 g m^{-1} for rain-fed trials and 3.3 g m^{-1} for irrigated trials. In the trial at Creston and Havre, each plot consisted of 10 seeds per hill, with spacing of 0.61 m between adjacent hills. Growing season precipitation and temperature data were collected from NOAA climatological measurements for the Post Field Research farm near Bozeman, Montana (National Climatic Data Center 2010).

Phenotypic Data Collection

Phenotypic data collected in all experiments included green leaf duration after heading (GLDAH), days to heading, days to flag leaf senescence, amount of xylem exudate, grain yield, test weight, seed weight, seed diameter, and seed number per spike. Heading date for plot was the day of the year when 50% of the heads were completely emerged. Days to flag leaf senescence for a plot was the day of the year when 75% of the plot exhibited flag leaves showing complete loss of green color (Hansen et al. 2005). GLDAH was determined by subtracting heading date from days to flag leaf senescence. Xylem exudate was collected as follows: ten tillers per plot were cut at approximately five inches above ground. The xylem exudate from the cut ends was collected by applying a pre-weighed cotton ball covered with a polyethylene bag to prevent evaporation for approximately 24 hours,. The amount of xylem exudate was calculated as the increase in the weight of the cotton balls. Amount of xylem exudate per area were

estimated as amount of xylem exudate per productive tiller times the number of productive tillers in a 30cm section. Test weight was measured from a sample of cleaned grain on a Seedburo (Chicago, IL) test weight scale. A subsample of seed was taken from each plot and was analyzed in the Single Kernel Characterization System 4100 (Perten, Huddings, Sweden) to determine seed weight and seed diameter. For seed number per spike, the number of seeds were counted and averaged from 10 spikes that were randomly sampled from each plot. In addition to these data, photosynthetic rate of flag leaves was measured for ten selected RIL at two different growth stages, early maturity, when 25% of plots were senesced, and late-maturity, when 50% were senesced, at Creston and Havre. Ten RIL consisted of 5 individuals each from the highest and lowest mean GLDAH in the 2006 and 2007 experiments. Two flag leaves per plant and two plants per plot were tagged and measured for photosynthetic rates. The same leaves were used for all measurements. The measurements were made by a portable infrared gas analyzer (LI-6400-40; Li-Cor Biosciences, Lincoln, NE) with a simultaneous gas exchange/chlorophyll a fluorescence chamber (2 cm^2) under a red/blue LED light at 1200 μ mol photons m^{-2} s^{-1}. A mixer set reference CO_2 to 400 μL L^{-1} to reach the chamber and air flow was set at 250 μmol s^{-1}.

Greenhouse Experiment

Experiments were also conducted with three replications using a randomized block design in a greenhouse at the Montana State University Plant Growth Center in 2008 and 2009. Three plants were grown in 17.8 cm diameter pots in a 1:1 mix of Sunshine Mix #1 (Sun Gro Horticulture Canada Ltd.) and MSU soil mix that consisted of

equal parts loam soil, sphagnum peat moss medium, and sand. Greenhouse temperature was initially set at 20-21°C day and 18-21°C night and increased up to 23.9°C at maturity. Photoperiod of 15: 9 (L:D) was maintained initially, and increased 16: 8 (L:D) near flowering. Plants were watered well until maturity. Heading date, and days to flag leaf senescence were measured and GLDAH calculated. In addition to these experiments, root dry weight and amount of xylem exudate were determined for 20 of the RIL that represented the extremes for GLDAH. Ten of these lines were the same selected RIL used for photosynthesis measurements. The same experiments were conducted for twenty old to modern spring wheat varieties (Hansen et al. 2005). In these experiments, three plants were grown in 17.8 cm diameter pots of sand. Xylem exudate was collected at late-maturity. Plant roots were washed out from a pot, dried at 49 °C in a ventilated oven and weighed.

Statistical Data Analysis

Data for each phenotypic trait in the Reeder/Conan RIL from each environment was analyzed via analysis of variance (ANOVA) using a model for a randomized complete block for each environment (irrigation regime and year). All factors except environment were considered random effects. Narrow-sense heritability for GLDAH was computed on an entry mean basis as described in Fehr et al. (1987) from each environment. Pearson correlations were computed using PROC CORR of SAS (SAS Institute, 2004) between GLDAH and agronomic traits and amount of xylem exudate using entry mean value from each environment. Student's t-test (two-tail) was conducted

to determine the difference of photosynthetic rate between each 5 individual with highest GLDAH and with lowest GLDAH.

QTL Mapping

Creation of a genetic map for the Reeder/Conan RIL was described previously (Sherman et al. 2010). The map originally contained 232 SSR markers, 190 DArT markers (Akbari et al. 2006), six markers for major genes controlling development (*Ppd-A1*, *Ppd-B1*, *Ppd-D1*, *Rht-B1*, *Rht-D1*, *Vrn-B1*) and three markers for storage proteins. In this study, 12 SSR markers were added and the total size of the map was 2619.16 cM. Markers were subjected to a chi-square test for fit to a 1:1 ratio using MapDisto (http://mapdisto.free.fr). Markers with significant distortion were indicated in the genetic map (Sherman et al., 2010). QTL analysis was conducted for GLDAH and amount of xylem exudate using the entry means for each environment by QTL Cartographer (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). QTL were identified with composite interval mapping (CIM) (Zeng 1993, 1994) using standard CIM model, Forward and Backwards Regression method with a window size of 10.0 cM with probability in and out of 0.1 and a walking speed of 2cM as described by Sherman et al. (2010). LOD value was set by 1000 permutations at an experiment-wise $P < 0.01$. QTL was established by the map position of the peak LOD score in the interval between two flanking markers. A one-LOD fall-off (from the QTL peak) method was used to estimate the left-and right-flanking map positions of a confidence interval surrounding the mean QTL map position. QTL detected in different environments were considered to be the same if the estimated map position of their peaks was within 20cM (Maccaferri et al. 2008). Proportion of

variation attributable to a segregating marker locus (R^2) was calculated as the sum of squares for segregating genes divided by the total sum of squares. To evaluate the presence of epistatic interactions across the QTL for GLDAH in each environment, multiple interval mapping (MIM) (Kao et al. 1999; Zeng et al. 1999) was conducted by QTL Cartgrapher. Initial QTL models were generated by scan through CIM results for all traits and environments. Main additive effects and their epistatic interactions were tested for significance using the Akaike's information criteria (AIC) (Alkaike 1974; Sakamoto 1987). Then, final epistatic QTL for GLDAH with the R^2 value >5% were reported in this study (Liu et al. 2007).

QTL Confirmation

In order to confirm QTL identified in the Reeder/Conan mapping population in other backgrounds, RIL populations derived from a cross Reeder and Choteau, and McNeal and Reeder were used for single marker analysis. The Choteau/Reeder RIL and McNeal/Reeder RIL consisted of 46 and 50 RIL respectively. Both populations were planted at the Post Research Farm near Bozeman in MT with the same experimental design as the Reeder/Conan RIL. Experiments for the Choteau/Reeder RIL were conducted only in the 2007 rain-fed environment. For the McNeal/Reeder RIL, there were four environments within rain-fed and irrigated trials in 2004 and 2005. The segregating markers linked to the GLDAH QTL in the Reeder/Conan RIL included microsatellite markers wmc161 and barc1047 for the QTL on 4A (GrainGenes 2.0, http://wheat.pw.usda.gov/GG2/index.shtml). Entry means of GLDAH averaged over environments for the McNeal/Reeder RIL and a single environment for the

Choteau/Reeder RIL were used for this analysis. Student's t-test (one-tail) was conducted to determine if the Reeder allele increase GLDAH at wmc161 and barc1047. This was done for each segregating marker locus for each population. The proportion of variation attributable to segregating marker locus (R^2) was calculated as the sum of squares for segregating genes divided by the total sum of squares.

Results

Heat and Drought Stress during Grain Filling

Experiments in this study were conducted in a wide range of environments, including field environments in 2006, 2007 and 2009 and well-watered greenhouse environments in 2008 and 2009. Environmental conditions varied widely during field trials (Table 2). Environments in 2006 and 2007 were characterized by heat and drought stress, since above average temperatures greater than 35 $^{\circ}$C for several days and approximately 36 to 48 % precipitation relative to the 50-year average in July and August occurred. 2009 showed similar temperature and precipitation to the 50-year average except it received approximately twice the average precipitation for July. Greenhouse conditions included optimal temperature and water regimes.

Mean Performance and Heritability of Green Leaf Duration

There was significant genetic variation for GLDAH in the Reeder/Conan RIL in every experiment (Table 8) with the RIL mean intermediate between parents. Reeder showed relatively longer GLDAH than Conan in the 2006 and 2009 rain-fed environment, and significantly longer GLDAH in 2007. Conan had longer GLDAH than

Reeder in the 2009 irrigated environment and 2008 greenhouse environment. Heritability of GLDAH ranged from 0.50-0.81 (Table 8).

Comparison of Photosynthetic Rates in the Selected RIL

Photosynthetic rates of flag leaves were measured at Creston and Havre to determine whether the green leaf color indicated continued photosynthesis during maturation (Fig. 2). There were no significant differences between the longer and shorter GLDAH RIL groups at the early maturity stage on July 24th at Creston, and July 28th at Havre. However, the RIL group with longer GLDAH showed significantly higher photosynthetic rate of flag leaves than the shorter GLADAH group at later maturity, on August 4th at Havre and August 19th in Creston.

Correlation between Green Leaf Duration and Agronomic Traits

Correlations between GLDAH and other traits are shown in Table 9. GLDAH showed negative correlations with heading date in all experiments including greenhouse trials. There were no significant correlations between GLDAH and grain yield in the 2006 and 2007 rain-fed environments, but a negative correlation in the 2009 rain-fed and irrigated experiments. There were significant positive correlations of GLDAH with test weight, seed weight and seed diameter in the 2006 and 2007 rain-fed experiments, but this correlation was not observed in the 2009 rain-fed and irrigated environments. GLDAH was negatively correlated with seed number per spike in the 2006 and 2007 rain-fed environments and the 2009 irrigated environment.

QTL Analysis for Green Leaf Duration

QTL analysis was conducted for each environment for GLDAH in the Reeder/Conan RIL using a genetic map generated by Sherman et al. (2010), with additional markers subsequently added. Epistatic interactions for GLDAH were found by CIM and MIM. CIM identified nine significant QTL for GLDAH (Table 10). Three QTL were identified on chromosome 2D, 4A and 5B from more than one environment, other QTL were identified from a single environment. The Conan allele for the 2D QTL flanked by Ppd-D1 and barc168 increased GLDAH and accounted for 13 % of the variation in the 2006 rain-fed, 2009 irrigated and 2009 greenhouse environment. Conan carries *Ppd-D1a* that confers photoperiod insensitivity and causes early heading (Blake et al. 2009). The Reeder allele for the QTL associated with greater GLDAH on chromosome 5B is in the area of Vrn-B1, and accounted for variation as follows: 12% in the 2007 rain-fed environment, 20% in the 2008 greenhouse and 30% in the 2006 rain-fed environment. Reeder possesses the spring *Vrn-B1a* for spring habit that confers earlier heading. A QTL was identified on chromosome 4A in the 2006 and 2007 rain-fed environments where the position of the QTL was flanked by barc170 and gpw3238 (Table 10; Fig. 3). The Reeder allele caused greater GLDAH and accounted for 11% and 20% of the variation in the 2006 and 2007 rain-fed environments, respectively (Table 10). The 2D QTL and 5B QTL co-segregated with heading date. All three QTL also co-segregated with seed number per spike. Although there were significant QTL for GLDAH identified on chromosomes 1B, 2D, 3A, 3B and 7B, we did not pursue these QTL for further analysis because they were identified in a single environment. Positive

epistatic effects were detected in two pairs of loci explaining 6 % and 7% of the phenotypic variation in the 2009 rain-fed and 2008 greenhouse environments respectively (Table 11). There were two new QTL identified on chromosome 4A flanked by wmc757 and wmc491, and chromosome 4D flanked by Rht-D1 and wPt0615 by MIM. The 4A QTL flanked by barc170 and gpw3228 was involved in one of the epistatic interactions affecting GLDAH in 2008 greenhouse environment.

QTL confirmation of Green Leaf Duration and Its Impact on Agronomic Traits

The effect of the 4A QTL identified in Reeder/Conan population was examined for GLDAH by single marker analysis using polymorphic markers for the 4A QTL in two additional populations in: Choteau/Reeder and McNeal/Reeder (Table 12). Student's t-test (one-tail) was used to test whether Reeder allele at these marker loci increases GLDAH in the Choteau/Reeder RIL and McNeal/Reeder RIL. The 2D QTL and 5B QTL were excluded from this analysis in this study, since *Ppd-D1* and *Vrn-B1* found to be unassociated with GLDAH based on single marker analysis for maturity traits including GLDAH and grain yield in Blake et al. (2009). Of the markers linked to the 4A QTL, barc170, wmc161 and gpw3238 were polymorphic in the Choteau/Reeder RIL and barc170, wmc161, barc1047 and gpw3238 were polymorphic in the McNeal/Reeder RIL. However, barc170 and gpw3238 did not show significant allelic differences for GLDAH in either population. In the Choteau/Reeder RIL, the Reeder allele at wmc161 did not significantly increase GLDAH. In the McNeal/ Reeder RIL, the Reeder allele at wmc161 and barc1047 significantly increased GLDAH (P=0.05 and P=0.04, respectively). Due to

its consistent effect on GLDAH and the apparent importance of this QTL in two different genetic backgrounds, we have designated the 4A QTL *QGfd.mst-4A* (Fig. 3). *QGfd.mst-4A* was not associated with test weight, but was significantly associated to grain yield in the McNeal/Reeder RIL (Table 12). The Reeder allele at wmc161 and barc1047 decreased grain yield approximately 5.2% and 3.9%, respectively, in the McNeal/Reeder population (Table 12). Other agronomic traits, such as seed weight, seed diameter and seed number per spike were not analyzed in these RIL populations.

Correlation between Amount of Xylem Exudate and Root Dry Weight

It has been suggested that delayed leaf senescence is associated with a larger and more active root system. A method to assess root activity is through the measurement of xylem exudate after the removal of most of the above-ground portion of the plant (Hirasawa et al. 1983; Lee et al. 1994; Yamaguchi et al. 1995). To determine if the amount of xylem exudate of plants could estimate root mass in Reeder/Conan RIL, it was collected in 20 of Reeder/Conan RIL and 20 spring wheat varieties at a late-maturity stage in the greenhouse in 2008 and 2009. Subsequently, roots from the same plants were washed, dried and weighed. Fig. 4 shows the relationship between amount of xylem exudate and dry root weight. Correlation coefficients were relatively high, 0.76 in selected 20 RIL and 0.59 for 20 spring wheat varieties. Therefore, amount of xylem exudate could be used as an estimate of root mass in field trials and was collected for all Reeder/Conan RIL in the 2007 rain-fed, 2009 rain-fed and irrigated environments.

Mean Performance and Genetic Variation for Xylem Exudate

There was significant genetic variation for amount of xylem exudate in only 2007 rain-fed environment (Table 13). Reeder tended to have more xylem exudate than Conan. Amount of xylem exudate was lower in 2007 rain-fed environment than 2009 rain-fed and irrigated environments reflecting very low precipitation during grain filling period in 2007.

Correlation between Amount of
Xylem Exudate and Green Leaf Duration and Agronomic Traits

Correlation analysis showed that there were significant positive associations between amount of xylem exudate and GLDAH, test weight, seed weight, seed diameter and seed number per spike, but a negative correlation with heading date in the 2007 rain-fed environment (Table 14). In contrast, there were no significant correlations between amount of xylem exudate and these traits in 2009 rain-fed and irrigated environments.

QTL Analysis for Amount of Xylem Exudate

Due to positive correlations between GLDAH and amount of xylem exudate identified in 2007 rain-fed environments, CIM was conducted for amount of xylem exudate to examine if there were any genetic associations between these two traits. Table 15 showed that a total of five QTL for amount of xylem exudate were identified on chromosome 1B, 2D, 4A and 7A from 2007 rain-fed, 2009 rain-fed and irrigated environments. Each QTL was identified in single environment. QTL identified on chromosome 4A from 2007 rain-fed environment co-segregated with *QGfd.mst-4A*

(Table 15; Fig.3). This QTL explained 10% of the phenotypic variation and the Reeder allele at this QTL had positive additive effect on amount of xylem exudate.

Discussion

We conducted genetic analysis, including QTL mapping, for GLDAH as a stay-green trait integrating physiological aspects under a wide range of environments, focusing on the difference between hot, dry and cool, wet environments. Heritability for GLDAH in each environment was moderate to high as previously reported (Silva et al. 2000; Blake et al. 2007). Photosynthetic rates were measured and compared between selected RIL with longer GLDAH and shorter GLDAH in the Reeder/Conan RIL. RIL with longer GLDAH showed significantly higher photosynthetic rates than RIL with shorter GLDAH at late-maturity. Therefore, GLDAH used in this study was classified as functional stay-green according to Thomas and Howarth (2000).

Across all environments, there were negative correlations between GLDAH and heading date. This result was consistent with many previous reports in wheat (Bruckner et al. 1987; Talbert et al. 2001; Tewolde et al. 2006; Hansen et al. 2005; Blake et al. 2009; Wang et al. 2009).

There were a distinctive difference between hot, dry environment and cool, wet environment in correlations between GLDAH and agronomic traits. There were positive correlations between GLDAH and seed weight only under hot, dry environments. Seed weight is the most sensitive yield component to high temperature stress after anthesis in

wheat (Gibson et al. 1999). Therefore, longer GLDAH appeared to be an important trait for retaining stable seed weight under drought and heat stress.

In contrast, there were neutral relationships between GLDAH and grain yield under hot, dry environments but, negative correlations under cool, wet environment. A negative correlation between grain yield and stay-green has been reported in rice and in maize (Bolanos and Edmeas 1996; Jiang et al. 2004). This result may be explained by analyze of grain yield components. Grain yield may be dissected into three primary yield components, spike number per area, seed number per spike, and seed weight (Ma et al. 2007). In this study, seed weight and seed number per spike had negative correlations in all environments, while spike number per area (productive tiller number in Chapter 2) did not correlate with any of traits including GLDAH in the Reeder/Conan RIL (data not shown). Since longer GLDAH increased seed weight and decreased seed number per spike in hotter, drier environments, GLDAH might have neutral effect on grain yield in such environments. Conversely, in cool, wet environment, GLDAH did no correlate with seed weight, but was negatively correlated with seed number per spike. Thus, GLDAH negatively affected grain yield in such environments. Therefore, longer GLDAH would only improve grain yield potential under certain environments.

We found two stable QTL for GLDAH across wide range of environments on chromosome 2D and 5B in Reeder/Conan mapping population. These QTL co-segregated with *Ppd-D1* and *Vrn-B1* whereby Conan allele confers photoperiod insensitivity and Reeder allele confers spring habit (Blake et al. 2009). Both alleles were associated with longer GLDAH due to early heading. Blake et al (2009) suggested that

the recent transition to higher temperatures at heading in northern Great Plains may favor wheat lines that head earlier to avoid heat stress. Tewolde et al. (2006) also suggested early heading is an important trait defining wheat cultivars adapted to production systems prone to high temperature stress during the post heading period. Since there were no significant correlations between either *Ppd-D1, Vrn-B1* (Blake et al. 2009) and *Ppd-D1* (Foulkes et al. 2004) and grain yield, they might indirectly contribute yield potential through increasing seed weight and seed size by exhibiting longer GLDAH only under heat stress and drought environments. However, the RIL population from McNeal and Reeder cross segregating with *Vrn-B1* did not show significant association with GLDAH (Blake et al. 2009). Verma et al (2004) showed no association between *Ppd-D1* and percent green flag leaf area at either 14 days or 35 days after flowering in a winter wheat doubled haploid population.

QGfd.mst-4A appeared to be involved in heat and drought resistance because it was stably identified under only the stress environments and did not co-segregate with heading date. It was also the only QTL for GLDAH that showed a significant effect of the Reeder allele in the different genetic mapping populations. The difference in the effect for GLDAH in the Reeder allele and the alternative allele in the McNeal/Reeder RIL was relatively small, maybe due to less severe heat stress during maturity in 2004 and 2005 than 2007 in Bozeman, MT (Table 2).

The 2D QTL, 5B QTL and *QGfd.mst-4A* co-segregated with seed number per spike. At these loci, the positive allele for GLDAH had a negative effect on seed number per spike in the Reeder/Conan mapping population. It is suggested that the selection of

only these QTL for longer GLDAH may not result in yield improvement under the stress environments, moreover may result in yield reduction in cool, wet environments as we found in correlation analysis in this study. In fact, the Reeder allele at wmc161 and barc1047 decreased grain yield though the effect of Reeder allele on seed number per spike was not known. Several QTL for seed number per spike not co-segregating with GLDAH were identified on chromosome 5A, 7A and 7D in the Reeder/Conan RIL (data not shown). Therefore, accumulation of positive alleles at these QTL for seed number per spike as well as the positive allele on the QTL for longer GLDAH may be important to keep stable grain yield under a wide-range of environments.

There were two epistatic interactions for GLDAH identified in the 2009 rain-fed environment and 2008 greenhouse environment. Since these epistatic interactions accounted for relatively small phenotypic variation compared to the three main QTL, we did not pursue them for further analysis. Epistatic interactions for stay-green traits also were reported in previous studies for sorghum and rice (Subudhi et al. 2000; Jiang et al. 2004; Yoo et al. 2007). Unlike our study, some epistatic interactions in these studies explained more phenotypic variation than main-effect QTL (Jiang et al. 2004; Yoo et al. 2007). It is suggested that wheat may have a different genetic system controlling system for this trait unique to other species.

To explore the genetic association between GLDAH and a root related trait, xylem exudate was collected from the field experiments in 2007 and 2009. Amount of xylem exudate in the 2007 rain-fed was smaller than the 2009 rain-fed and irrigated environments probably due to high heat and drought conditions during the grain filling

period. Significant genetic variation for amount of xylem exudate was found only in the 2007 rain-fed environment. Since there was approximately twice the July 50 year, average precipitation in 2009 (Table 2), genetic variation might be masked by abundant moisture in the soil in the 2009 field experiments.

Correlation analysis revealed that amount of xylem exudate had a significant positive correlation with GLDAH only in the 2007 rain-fed environment. This result suggested that greater root mass and/or root activity might be related to GLDAH under hot, dry conditions. Amount of xylem exudate was associated with other agronomic traits including test weight, seed weight and seed diameter, though there was no significant correlation with grain yield. In rice, Babu et al. (2003) reported that thousand grain weight and deep root dry weight were positively correlated under drought stress, but not under a non-stress environment. Genotypes with greater amounts of xylem exudate in the Reeder/Conan RIL might develop greater root system and/or higher activity so that they could take up deep soil moisture under heat and drought stress conditions. Larger amount of xylem exudate also was associated with higher seed weight and diameter in addition to positive association with GLDAH under such stress environments. There was a negative correlation between amount of xylem exudate and heading date in the 2007 rain-fed environment. Negative correlations between heading date and root traits also have been found in other studies under drought conditions in wheat (Derera et al. 1969; Babu et al. 2002). This relationship could be due to indirect and direct effects of heading date. Since longer GLDAH was associated with earlier heading date (Table 9), earlier heading might indirectly lead to a greater amount of xylem

exudate through longer GLDAH. Another possibility may be direct effects of heading date through pleiotropic or linkage of genes for heading, although CIM for amount of xylem exudates showed that any QTL for amount of xylem exudate did not co-segregate with heading date (Table 15).

The same QTL for different traits should be result in phenotypic correlation of the traits, if there is pleiotropy or genetic linkage among genes controlling to the traits (Paterson et al. 1991). We conducted CIM for amount of xylem exudate to assess if there were any associations with GLDAH. There was only one significant QTL for xylem exudate identified from the 2007 rain-fed environment. This QTL was coincident to *QGfd.mst-4A* where the Reeder allele increased both the amount of xylem exudate and GLDAH in the Reeder/Conan RIL in the hot, dry environment of 2007. We could not confirm the effect of *QGfd.mst-4A* for amount of xylem exudate because no measurements for xylem exudate were available in other RIL populations. Sanguineti et al (2007) reported that SSR marker gwm637 located on the long arm of chromosome 4A was associated with seminal root angle in durum wheat. Interestingly, SSR markers within *QGfd.mst-4A* (barc1047, wmc161 and gwm494) are also located on the long arm of chromosome 4A. wmc161 appeared to be linked to gwm637 within10cM region and both loci are located in a same chromosome bin (5AL13 0.59-0.66) (GrainGenes 2.0, http://wheat.pw.usda.gov/GG2/index.shtml; Gadaleta et al. 2009). Manschadi et al (2003) reported that drought tolerant cultivar SeriM82 had a uniform rooting pattern and greater deep root length relative to standard wheat. A more vertically oriented root system may be more beneficial for accessing residual moisture in deeper soil layers when

the water in surface soil is insufficient to enable the crop to complete its cycle (Sanguineti et al. 2007). We did not collect any further root measurements including root architecture in this study. In the future, we might be able to compare these traits using near isogenic lines for *QGfd.mst-4A* with relatively simple methods (Nakamoto et al. 1992; Kato et al. 2006).

In rice and maize, several QTL for root traits have been identified (Courtois et al. 2009; Hochholdinger and Tuberosa 2009). Coudert et al. (2010) reported that the concentration area of QTL for root traits in rice linked to short arm of chromosome 3 appeared to be orthologous to the maize QTL in bin 1.06, a region of the maize chromosome 1 where major effects on root traits have been reported. This region also had large stretches of genetic marker collinearity with wheat chromosomes 4L (Buell et al. 2005). Therefore, it is possible that *QGfd.mst-4A* which is associated with the amount of xylem exudate and GLDAH in this study is also orthologous to these QTL in rice and maize.

Despite physiological and genetic studies for stay-green and root traits in several crops, their interaction especially under stress conditions is not well elucidated. Plant hormones, especially cytokinin and abscisic acid are generally thought to be internal factors controlling leaf senescence (Yang et al. 2005). Cytokinin is a predominantly root-sourced plant hormone, translocated from the roots through the xylem to the aerial plant parts and controls shoot development (Letham and Palni, 1983; Letham, 1994). Cytokinin also has been reported to be involved in nitrogen and carbon partitioning in plants and delay whole plant senescence (Yang et al. 2002; Ookawa et al. 2004). In the

future, we might be able to compare these plant hormones using near isogenic lines for *QGfd.mst-4A*.

Conclusion

Our results showed a positive correlation between GLDAH and test weight, seed weight and seed diameter under heat and drought stress conditions but not cool, well-watered conditions in a spring wheat population. In contrast, GLDAH had a neutral relationship with grain yield under the stress conditions, but showed negative correlation under well-watered conditions. The allele for longer GLDAH at QTL, *QGfd.mst-4A* on chromosome 4A had a consistent effect under hot, dry conditions for the populations. The Reeder allele for this QTL also increased amount of xylem exudate indicating more root mass and/or activity. *QGfd.mst-4A* may be useful for improving spring wheat for heat and drought stress resistance in the northern Great Plains of North America and similar environments.

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Table 8. Mean performance of GLDAH of Conan and Reeder and the Conan/Reeder RIL in 2006, 2007 and 2009 field experiments and 2008 and 2009 greenhouse experiments.

Factor	2006/Rain-fed	2007/Rain-fed	2009/Rain-fed	2009/Irrigated	2008/Greenhouse	2009/Greenhouse
Conan	34.2	30.3*	49.6	58.4*	43.7*	46.8
Reeder	35.7	31.6	51.1	56.7	40.5	47.2
RIL mean	34.7	30.5	50.4	57.1	40.0	44.8
RIL range	29.0-39.7	26.1-35.5	44.6-58.3	48.4-63.7	32.3-46.7	32.5-63.2
Heritabilit	0.81	0.73	0.50	0.78	0.66	0.55

* Differences between parents significant (P<0.05) based on LSD
GLDAH: Green leaf duration after heading

Table 9. Pearson's correlation between GLDAH and agronomic traits[a] from 2006, 2007 and 2009 field experiments and 2008 and 2009 greenhouse experiments.

Trait	2006/Rain-fed	2007/Rain-fed	2009/Rain-fed	2009/Irrigated	2008/Greenhouse	2009/Greenhouse
Heading date	-0.82****	-0.67****	-0.23****	-0.50****	-0.49****	-0.51****
Grain Yield	0.01	0.01	-0.27**	-0.46**	-	-
Test weight	0.47****	0.44****	-0.04	-0.01	-	-
Seed weight	0.44****	0.36***	0.01	-0.12	-	-
Seed	0.50****	0.40****	0.08	-0.05	-	-
Seed number per spike	-0.66****	-0.23*	-0.13	-0.36***	-	-

Significance levels: *P<0.05, ***P<0.001, **** P<0.0001

Table 10. Significant QTL for GLDAH identified through composite interval mapping in the Reeder/Conan RIL in 2006, 2007 and 2009 field experiments and 2008 and 2009 greenhouse experiments.

Chromosome	Flanking markers	Year/Environment	Additive effect[a]	R^{2b}	LOD[c]	Coincident QTL
1B	wPt3477-barc119	2008/Greenhouse	-0.94	0.10	3.30	
2D	PpdD1-barc168	2006/Rain-fed	-0.76	0.13	3.90	Heading date
		2009/Irrigated	-1.05	0.13	2.70	Seed number per spike
		2009/Greenhouse	-2.74	0.13	3.86	
	gwm484-cfd11	2009/Irrigated	1.13	0.16	4.23	
3A	wmc264-barc324	2009/Grenhouse	2.35	0.17	3.24	
3B	barc295-barc147	2009/Rain-fed	-0.98	0.13	3.40	Test weight
	wPt5105-barc164	2009/Greenhouse	-1.89	0.11	3.56	
4A	barc170-gwm494	2006/Rain-fed	0.80	0.11	3.51	Seed number per spike
	Wmc161-gpw3238	2007Rain-fed	0.90	0.20	4.47	
5B	barc74-gwm604	2006/Rain-fed	1.34	0.30	7.58	Heading date
		2007/Rain-fed	0.72	0.12	2.92	Seed number per spike
		2008/Greenhouse	1.32	0.20	5.09	
7B	wmc273-barc303	2009/Rain-fed	-1.02	0.14	2.50	

[a]Additive effect of the Reeder allele

[b]R^2 The phenotypic variation explained by the QTL

[c]LOD logarithm of odds. Only QTL with LOD scores above 2.5 are shown

Table 11. Epistatic interactions for GLDAH analyzed by multiple interval mapping in the Reeder/Conan RIL in 2006, 2007 and 2009 field experiments and 2008 and 2009 greenhouse experiments.

Chr	Interval	Chr.	Interval	Year/Environment	A[a]	R^{2b}	LOD[c]
3B	barc295-barc147	4A	wmc757-wmc491	2009/Rain-fed	0.49	0.06	1.03
4A	barc170-gpw3228	4D	RhtD1-wPt0615	2008/Greenhouse	0.72	0.07	1.10

Only QTL with the R^2 value >5% were considered

[a]Additive effect of Reeder Allele

[b]R^2 The phenotypic variation explained by the QTL

[c]LOD logarithm of odds. Only QTL with LOD scores above 2.5 are shown

Table 12. GLDAH and agronomic traits in the McNeal/Reeder RIL in the presence of contrasting allele for the 4A QTL (*QGfd.mst-4A*).

Marker	Allele	No. RIL[a]	GLDAH(d)[b]	Grain yield (kg ha^{-1})	Test weight (kg m^{-3})
wmc161	McNeal	31	34.08	5901.2	774.4
	Reeder	17	34.72	5591.8	773.6
	P value[c]	-	0.05	0.01	0.48
barc1047	McNeal	35	34.12	5850.1	773.9
	Reeder	15	34.79	5623.5	773.0
	P value[c]	-	0.04	0.04	0.38

[a]Number of recombinant inbred lines

[b]Mean of GLDAH

[c]Compares the difference of the QTL allele from Reeder allele and the alternate parent

Table 13. Mean performance of amount of xylem exudate[a] of Conan and Reeder and the Reeder/Conan RIL grown in 2007 rain-fed and 2009 rain-fed and irrigated fields in Bozeman, MT.

Factor	2007/Rain-fed	2009/Rain-fed	2009/Irrigated
Conan	0.25	1.68	2.04
Reeder	0.40	1.70	2.58
RIL mean	0.34*	1.81	2.05
RIL range	0.08-0.61	0.66-3.67	1.20-5.30
LSD	0.31	1.38	1.54

[a] xylem exudate collected at 50% of plants showed flag leaf senescence

Table 14. Pearson's correlation between amount of xylem exudate and GLDAH and agronomic traits in 2007 rain-fed and 2009 rain-fed and irrigated field in Bozeman MT.

Trait	2007Rain-fed	2009Rain-fed	2009Irrigated
GLDAH	0.48****	0.2	0.02
Heading date	-0.41****	0.11	0.05
Grain yield	0.14	0.11	0.16
Test weight	0.28**	-0.15	0.07
Seed weight	0.25*	0.02	0.16
Seed diameter	0.29**	0.07	0.17
Seed number per spike	-0.16	0.09	-0.07

Significance levels: *P<0.05, **P<0.01, **** P<0.0001

Table 15. Significant QTL for amount of xylem exudate identified through composite interval mapping in the Reeder/Conan RIL in 2007 rain-fed and 2009 rain-fed and irrigated field experiments in Bozeman, MT.

Chromosome	Flanking markers	Year/Environment	Additive effect[a]	R^{2b}	LOD[c]
1B	cfd20-gm153	2009/Rain-fed	-0.21	0.15	4.0
2D	wPt4413-cfd44	2009/Irrigated	0.22	0.13	3.0
4A	wmc262-barc70	2009/Irrigated	0.21	0.10	2.8
	barc1047-gwm494 (*QGfd.mst-4A*)	2007/Rain-fed	0.03	0.10	2.9
7A	wmc786-gwm332	2009/Rain-fed	0.22	0.17	3.3

[a]Additive effect of the Reeder allele

[b]R^2 The phenotypic variation explained by the QTL

[c]LOD logarithm of odds. Only QTL with LOD scores above 2.5 are shown

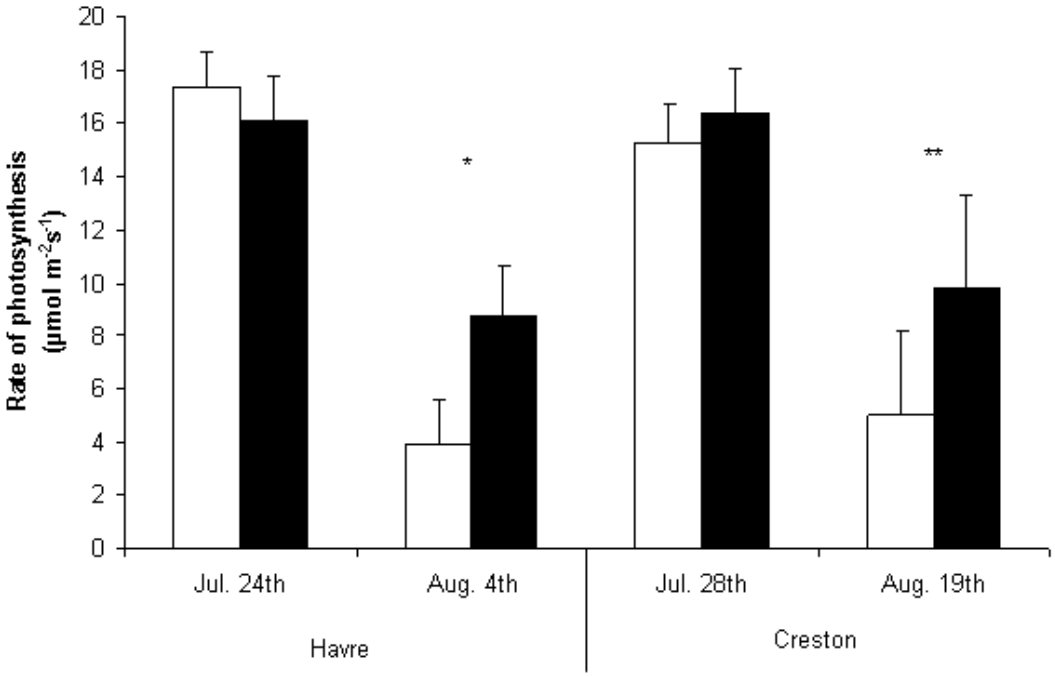

Figure.2 Rate of photosynthesis of flag leaves for five shortest GLDAH (□) and longest GLDAH (■) in the Reeder/Conan RIL determined in Havre and Creston. Bars represent standard deviation. Two plants and two leaves for each plant were used for the measurement of each plot. Same leaves were used for the measurement in early maturity (July 28th and 24th) and late maturity (August 19th and 4th). Asterisks indicates significant mean difference (*P<0.05, **P<0.01).

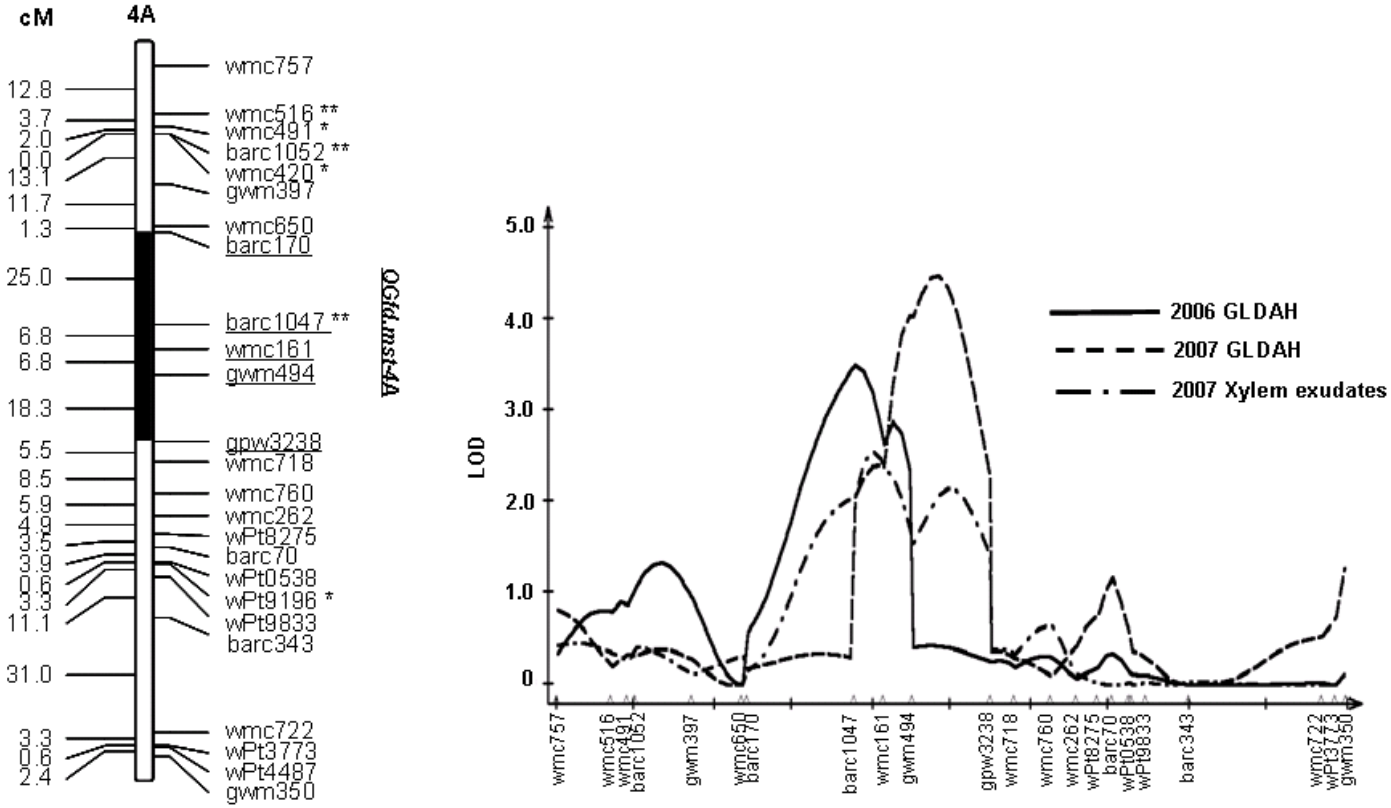

Figure.3 Linkage map of chromosome 4A from Sherman et al. (2010) with the additional markers and removal of co-segregating markers. Chromosome region of *QGfd.mst-4A* indicated in black. The markers defining *QGfd.mst-4A* are underlined. Asterisks at the end of marker name denotes significantly distorted loci (*P<0.05, **P<0.01). Position and LOD score of the QTL for GLDAH on chromosome 4A in 2006 rain-fed environment (solid line), 2007 rain-fed environment (dashed line) and QTL for amount of xylem exudate in 2007 rain-fed (single dotted line) environments are shown on the right.

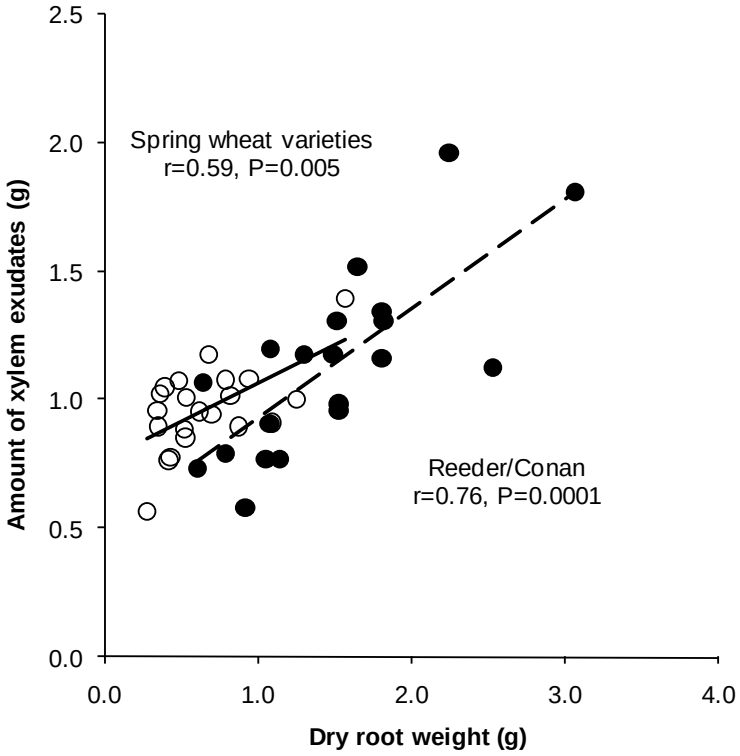

Figure.4 Relationship between dry root weight and amount of xylem exudate of selected RIL in Reeder/Conan (●, dashed line) and twenty spring wheat varieties (○, solid line) in 2008 and 2009 greenhouse experiment.