

GENETIC DISSECTION OF STAY-GREEN AND OTHER TRAITS RELATED  
TO DRY LAND SPRING WHEAT PERFORMANCE

by

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DEDICATION

To my parents Khemunath and Laxmi, my sister Rita and brother in law Bhes and my brother Milan and sister in law Shrijana who are always encouraging and supportive for my study since I left home after high school.

To my super funny nephews Aryaman and Achyutam who never get tired to spend their whole day in video chat asking too many questions and demanding different play items.

To my small and very cute niece AaroHi who is not ready to talk yet but talent enough to make me happy by her childish activities.

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## ABSTRACT

Spring wheat production in the upper Great Plains is at risk due to increased occurrence of low moisture and high temperatures during grain-fill. Wheat yield is impacted by several often counteracting traits, including seed number per head, seed weight and number of productive tillers. A variety of morphological and phenological plant characteristics impact performance in conditions of low moisture and high temperatures during grain-fill. In this study, we dissect traits associated with grain yield using a recombinant inbred line (RIL) mapping population derived from parents with contrasting phenotypes for several yield and drought related traits. Importantly, differences in quantitative trait loci (QTL) for yield and yield components were observed in the RIL between rain-fed and irrigated environments. We report important QTLs associated with yield and drought stress in spring wheat, with the long term goal of identifying traits useful to molecular breeding programs for the creation of lines better adapted to our changing environments. We also confirmed six grain-fill QTLs in association mapping (AM) panel which were identified by using this RIL population.

## CHAPTER ONE

GENETIC DISSECTION OF STAY GREEN AND OTHER TRAITS RELATED TO  
DRY LAND SPRING WHEAT PERFORMANCEIntroduction

Wheat, particularly adapted to dry land farming, is a primary food source for the world. In Montana, of the 1.21 million hectares of spring wheat planted each year, more than 80 percent is under rain-fed. Challenges with spring wheat production include low moisture and high heat during grain filling stages in Northern Great Plains. Researchers have found that yield can vary by as much as 5.4 ton/ha depending if grown in dry or irrigated winter wheat fields in U.S. southern plains (Musick et al., 1994). On average, one irrigation increased grain yield by 21 to 43% and four irrigations by 60 to 100% in Northern China (Zhang et al., 1999). The impact of lack of water is global, with developing countries at even greater risk where the dry land production is 50-90% lower than when under irrigation (Reynolds et al., 2000).

Wheat yield is a complicated trait impacted by a variety of components. A line can be high yielding for a variety of reasons, including high productive tiller numbers (Ma et al., 2007; Naruoka et al., 2011), greater number of seeds per head (del Morel et al., 2003; Ma et al., 2007) and bigger seed (Gupta et al., 2006; Ma et al., 2007). Various researchers have identified different chromosome segments controlling yield and yield components, with some variation in quantitative trait loci (QTLs) identified depending on environment.

Plants have developed a variety of strategies for abiotic stress tolerance. The strategies vary depending on the timing of the abiotic stress in relationship to plant phenology. In Montana, a critical time when abiotic stress greatly impacts wheat yields is during grain-fill. In recent years, increased temperature has become more common during grain-fill (Lanning et al., 2010). Likewise, dryness is also going to be a huge problem in next two decades in North America (Dai, 2011). Therefore, breeders must keep or enhance their drought breeding program to understand different plant strategy for drought tolerance because the future growing condition has been predicted to be more intense in terms of heat and drought throughout the world (Battisti & Naylor, 2009). Potential strategies for abiotic stress resistance during grain fill include:

#### Increased Grain-fill Duration Even in Abiotic Stress

Grain-fill is the developmental stage when photosynthates are stored in the seed as starch. Starch is the largest compound contributing to yield. The majority of carbohydrates accumulated in grain comes from CO<sub>2</sub> fixation during grain fill period (Evans, 1975). Extending grain-fill duration increases yield by allowing more time for starch to be deposited. Delayed senescence has been positively correlated with yield (Verma et al., 2004). Drought stress is usually associated with early maturity in a majority of wheat cultivars. Early maturity as a response to drought tends to reduce grain-fill duration and thereby reduce starch deposition and yield. In cotton, a yield penalty has been observed for early maturing cultivars in dry land whereas a single day delay in maturity resulted in significant increase in cotton lint (Stiller et al., 2004). Delayed maturity, which often results in extended grain-fill duration and is often referred to as

stay-green, has been developed for a number of crops including soybean, cotton, wheat and cowpea (Lopes & Reynolds, 2012; Luquez & Guiamét, 2002; Muchero et al., 2013; Yan et al., 2004). Thomas & Howarth (2000) note that although there are a variety of ways to stay-green, only extended green that includes extended photosynthetic capacity, also known as functional stay green, can increase yield and resist abiotic stress. In Montana environments, positive correlations have been found between green leaf duration after heading (GLDAH) and test weight, seed weight, and seed diameter in late season heat and drought stress conditions but not in cool, well-watered conditions (Naruoka et al., 2012).

#### Maintenance of Photosynthetic Capacity

In order to have functional stay-green, photosynthesis must be maintained under abiotic stress. Photosynthesis is a complex photochemical reaction where carbon dioxide and water are utilized in the presence of light to generate chemical energy in the form of carbohydrates. This energy is utilized to fuel overall plant physiological activities and storage in the sink or seed. Chlorophyll is the only molecule which performs photosynthesis. A proposed model for drought induced leaf senescence indicates that the drought stress perception of plants acts in signal transduction to decrease cytokinin, increase abscisic acid (ABA) and reactive oxygen species (ROS) to promote degradation of chlorophyll (Munn et al., 2004). Therefore, preventing early degradation of chlorophyll as a response to drought could be important in breeding success for drought tolerance.

### Maintenance of Plant Water Status

An important factor influencing photosynthesis during drought is water status. Plants require water not only for photosynthesis to build carbohydrates, but also to allow gas exchange. Opening and closing of stomata for gas exchange and heat loss (via transpiration) depends upon turgidity of guard cell (Raber, 1937), which is lost in drought-stressed conditions. Drought stress results in closed stomata and an inadequate supply of carbon dioxide for photosynthesis (Kramer & Boyer, 1995). Canopy water status is a prime indicator of water stress. Genotypes vary in their ability to hold water in their tissue. Drought resistant genotypes maintain better canopy water content throughout all the developmental stages than susceptible genotypes (Gutierrez et al., 2010). Water status could be maintained in drought due to more extensive root systems (Comas et al., 2013; Nezhadahmadi et al., 2013). A study shows positive correlation between tiller numbers and root surface area in spring wheat (Narayanan et al., 2014) indicating plants with high tillering capacity produced bigger root system (especially nodal roots) to maintain higher plant water status. This could be a very useful strategy of plants to extract more water during stress anticipating the return of favorable condition. Naruoka et al. (2012) found that an extended grain-fill QTL was associated with increase root functionality, which translated into higher performance in low moisture condition. In a dry land wheat stay green line, stay green correlated with a larger root system as indicated by root length density (i.e. length of roots per volume of soil) in a deep soil layer (80-100 cm), providing up to a 28 % yield increase over the standard cultivar (Waisel et al., 2002).

Another mechanism of maintaining water status can be water storage in plant organs. Solid stems in wheat have been reported to provide water and photosynthate storage that could be utilized during grain fill when photosynthesis is inhibited by heat or drought (Blum, 1998; Monasterio, 2001; Rawson & Evans, 1971). Solid stem wheat has pith filled in with undifferentiated parenchyma cells which act as a store room for soluble carbohydrates and water. Plants with more solid stems could be a component of an ideal plant ideotype to improve yield and adaptation to drought conditions (Pierre et al., 2010). The solid stem trait is highly heritable and stems tend to be more solid if plants are exposed to drought and high temperature stress during stem elongation (Pask et al. 2012). Likewise, stem reserve was also found highly correlated with grain yield and plant height under dry environments using a set of hollow-stemmed wheat lines. When the mean seed weight was measured under desiccated and in controlled condition, the contribution of stem reserve in seed weight was found to be 15 to 36% in some genotypes (Mohammadi et al., 2009).

#### Increased Productive Tillers

Wheat plants can vary from a mono-culm to multiple tillers. The productive tiller number (PTN) is a yield component. Wheat breeders have tended to select for lines with few culms to increase uniformity of grain development (Atsmon & Jacobs, 1977; Kuraparthi et al., 2007). Mono-culm wheat might get access to more resources to increase seed number, higher seed weight and bigger seed than free tillering wheat. However, in a favorable environment, the ability to tiller can provide a yield boost. The number of tillers are generally higher in irrigated environments (Bose, 2008) but a

considerable degree of tiller mortality is equally possible if crop receives temperature stress at a later stage (Chaturvedi et al., 1981). As a response to terminal drought stress, the number of abortive tillers can increase causing poor crop stand and lower yield. However, in case of drought in earlier stages, plants may produce late tillers on its recovery, which can contribute to yield (Blum et al., 1990). Productive Tiller Number (PTN) can provide phenotypic plasticity in response to drought (Naruoka et al., 2011). The high PTN allele on chromosome 6B identified by (Naruoka et al., 2011; Nasseer et al., 2016) has different pleiotropic effects depending on the environment. Increased tillers can be associated with higher yields; however, in many environments no association with yield was observed due to negative effects on seeds per head and or seed diameter (Nasseer et al., 2016).

#### Phenotypic Proxies for Abiotic Stress Resistance

An important limiting factor for genetic dissection of abiotic stress resistance is the precise and reproducible measurement of phenotypes. Field-based phenotyping using traditional yield trials is laborious and time consuming, and the unpredictable occurrence of drought adds to the complication. Therefore, proxies to yield trial traits are appealing as selection tools. Unfortunately, it is harder to select for below ground phenotypes, and so several proxies have been suggested (Comas et al., 2013, Richard et al., 2015, Wasson et al., 2012). One such proxy is Canopy Temperature Depression (CTD). Plants lose water and heat through stomata, which can have a cooling effect on the canopy. CTD is the degree to which the canopy temperature is decreased. Difference in CTD could be due to difference in soil moisture, ability of plant to access that moisture, stomata index

(number of stomata per unit leaf area), and leaf size and leaf number. Canopy Temperature (CT) can be measured by using infrared thermometer. Various reports have shown that plants with stay-green trait generally have higher CTD under terminal heat stress in wheat (Kumari et al., 2013; Kumari et al., 2007) and CTD can be used as a potential selection criteria for yield and drought tolerance in wheat (Bahar et al., 2008; Balota et al., 2007, Balota et al., 2008). Higher CTD indicates a higher rate of transpiration, which indicates access to more water due to a larger root system (Atta et al., 2013; Tuberosa, 2012). Naruoka et al. (2012) found that stay green lines produced greater xylem exudate and increased xylem exudate was correlated with higher root mass and stay-green. The amount of exudate was also associated with major yield traits in dry environments.

Another proxy is Canopy Spectral Reflectance (CSR) given its versatility and potential for high throughput (Chapman, 2008; Montes et al., 2007). Plants vary in their ability to absorb and reflect different wavelengths of light for reasons that include amount of biomass, water content, plant morphology, growth stage, chlorophyll concentration, and disease. Depending upon the degree of absorption and reflection of particular wavelengths of light, various proxies have been created. Measurement of reflected wavelengths from the red zone (R800 & R680) to calculate Normalized Difference Vegetation Index (NDVI) and far red zone (R900 & R970) to calculate Water Index (WI) have been used to estimate chlorophyll content and canopy water status respectively (Ollinger, 2011; Penuelas et al., 1993; Penuelas et al., 1997). Studies have reported NDVI associates with yield and yield components at heading and grain filling stages of

wheat (Babar et al., 2006), especially in drought and drought combined with heat stressed environments (Lopes & Reynolds, 2012). WI (measured as a ratio of R900/R970) and four other normalized water indices have been used to explain yield variation in wheat and maybe a useful selection tool (Babar et al., 2006; Prasad et al., 2007).

Recent years have seen an influx of spring wheat cultivars with longer periods of green leaf duration after heading and an increased number of tillers into arid regions of the Northern Great Plains (Naruoka et al., 2011; Nasseer et al., 2016). Acreage in Montana exemplifies this trend, as leading cultivars Reeder (PI 613586) and its progeny line Vida (Lanning et al., 2006) share these characteristics. For the present study, a recombinant inbred line (RIL) population was developed between Vida and an experimental line with a contrasting phenotype, MTHW0202. Vida and MTHW0202 appear to have different strategies for high performance. Vida has significantly more tillers, while MTHW0202 has more seeds per head, higher seed weight, test weight and greater seed diameter. The objectives of the study were to 1) determine the relationship of extended green leaf duration after heading (i.e. stay green) on yield and its components 2) identify QTL associated with these traits and other traits in this population that impact agronomic performance, and 3) to gain information on the combination of traits for improved performance under Montana dry land conditions. The results from this study could have implications for development of spring wheat cultivars for the dry land growing condition in the Northern Great Plains.

## CHAPTER TWO

## MATERIALS AND METHODS

Plant MaterialRecombinant Inbreed Line

One hundred and eighty RILs (F6:7) were developed via single seed descent method by crossing two spring wheat lines: Vida (Lanning et al., 2006) and MTHW0202. Vida, with later heading and maturity and longer grain-fill in most environments, is widely grown and well adapted to Montana; whereas MTHW0202, with earlier heading and maturity and shorter grain-fill, is a hard white experimental line. Vida is semi-solid and MTHW0202 is solid stem. Vida is semi-dwarf due to *Rht-B1b* while MTHW0202 is semi-dwarf due to *Rht-D1b*. During the creation of the population semi-dwarf lines were selected, while height extremes were eliminated. The pedigree records indicate that these two parental lines (i.e. Vida and MTHW0202) share a common parent called 'Gallo' four generation back.

Association Mapping Panel

Association Mapping (AM) panel was comprised of 247 elite hard spring wheat cultivars from 10 wheat-breeding programs in North America (Varella et al., 2015).

## Field Trials

### Recombinant Inbreed Line

In 2013, nurseries were planted in single row plots at Arthur H. Post Research Farm, Bozeman (45° 40' 16.06" N & 111° 8' 15.17" W), with rain-fed and irrigated treatments. Seed rate was 8 g per row. Row length was 2.3 to 2.7 m long with 30 cm row space between the single rows planting. In 2014, the nurseries were planted at same location in Bozeman rain-fed, [Southern Ag Research Center](#), Huntley (45° 55' 27.95" N & 108° 14' 43.87" W) rain-fed and [Eastern Ag Research Center](#), Sidney (47° 43' 34.77" N & 104° 9' 0.19") irrigated land. Seeding rate at Sidney was 38 g per plot and each plot had 6 rows 2.5 m whereas in Huntley and Bozeman, seed rate was 36 g per plot and had 4 2.7 m rows in an average. The plot size was variable across research stations. The distance between rows was 30 cm. All the experiments were planted with an augmented design. RILs were randomly planted in 4 blocks in 2013 and in 6 blocks in 2014 nurseries. 4 check varieties (Vida, MTHW0202, Reeder and Fortuna) were randomly planted in each block in all experimental trials.

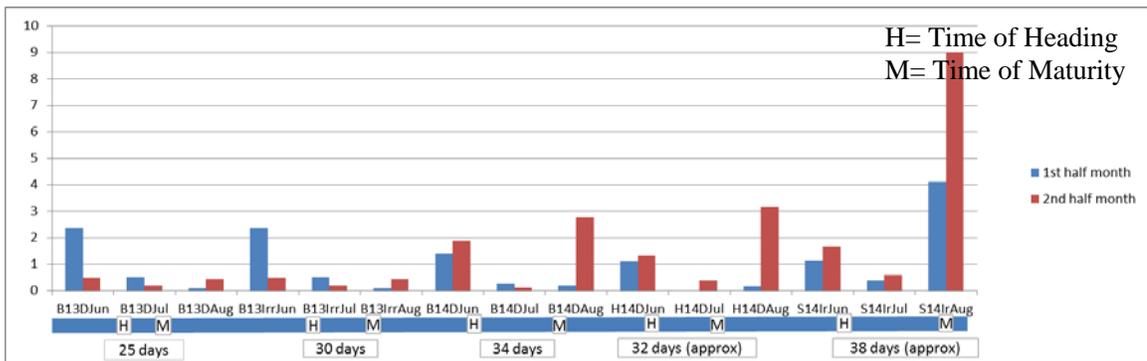
### Association Mapping Panel

The AM panel was also planted in an augmented field design in 2012 and 2013 Bozeman (Montana) and Saskatoon (Canada). The trial was composed of 5 blocks with 6 check varieties repeated in each block. This paper includes only grain-fill and related data from this population.

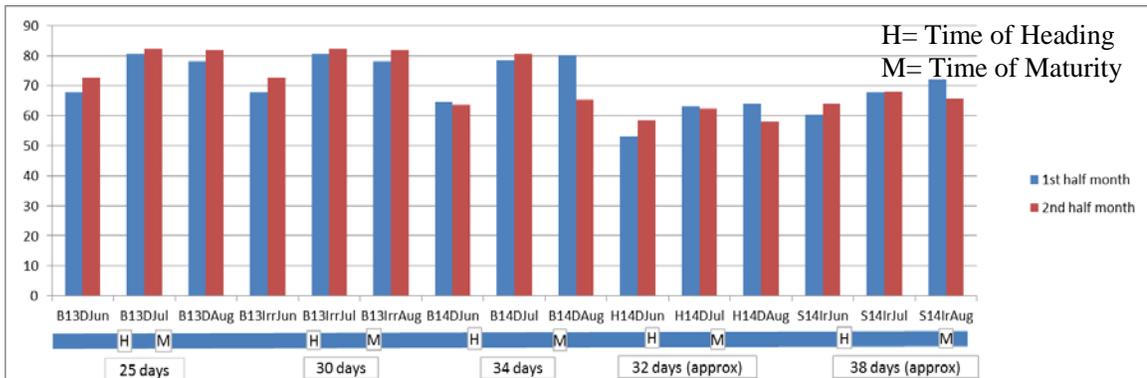
Description of Treatments

Recombinant Inbreed Line

Different nurseries were categorized into rain-fed and irrigated treatments. Based on figure 1, the rain-fed treatment is the average of 2013 Bozeman rain-fed, 2014 Bozeman rain-fed and 2014 Huntley rain-fed nurseries whereas the irrigated treatment consist of average of 2013 Bozeman irrigated and 2014 Sidney irrigated nurseries.



(a.)



(b.)

Figure 1: Bi-weekly precipitation in inch (a.) and temperature in °F (b.) from June to August in 2013 Bozeman rain-fed (B13D), 2013 Bozeman Irrigated (B13Irr), 2014 Bozeman rain-fed (B14D), 2014 Huntley rain-fed (H14D) and 2014 Sidney Irrigated (S14Irr) nurseries.

### Association Mapping Panel

The Triticeae Coordinated Agricultural Project (TCAP) AM panel is a collection of 237 elite spring wheat lines from 10 public wheat programs in the US, Canada and CIMMYT. The panel originally composed of 250 entries but was reduced to 237 after filtering for >5% missing data and allele similarity. This panel has been widely used among TCAP member institutions in different association mapping studies like drought tolerance, stem solidness and resistance to stripe rust, leaf rust and stem sawfly. All the nurseries were planted in rain-fed condition.

### Phenotypic Measurements

#### Recombinant Inbreed Line

In total, 13 phenotypic traits were measured. Days to heading was noted when 50% of the heads emerged from the boot. Days to maturity was noted when 75% main spike glumes were senesced. Grain-fill was calculated as a difference between days to heading and maturity. CTD was calculated as a difference between ambient temperature and canopy temperature. The canopy temperature was measured by Infrared Thermometer (2011 Raytek Corporation). NDVI and WI were calculated by using light wavelengths from red and far red zone as described by (Ollinger, 2011) and measured with the help of CROPSCAN (CROPSCAN, Inc.). Stem solidness was measured as an average of 3 stems by scoring the degree of solidness on a scale of 1-5 by cutting 5 internodes from the base where 1 is hollow and 5 is solid. Plant height was measured as an average of two measurements from the soil surface to the top of the spike excluding

awns. Tiller number was measured by counting the fertile spike per 30 cm of the row. Seeds per head were measured as an average number of seeds per five spikes. Test weight was calculated using Seedburo (Chicago, IL) test weight scale. Grain yield was measured from raw grain yield of each plot. Seed diameter and seed weight were measured using Single Kernel Characterization System 4100 (Perten, Huddings, Sweden).

#### Association Mapping Panel

Grain-fill duration was measured as days from heading to physiological maturity (i.e. when the glumes turn yellow). Other traits measurement not included here.

#### Statistical Analysis

##### Recombinant Inbreed Line

Phenotypic values from the augmented design were adjusted by computing Best Linear Unbiased predictors (EBLUPs) for each environment using PROC MIXED in SAS (Wolfinger, Federer, & Cordero-Brana, 1997), treating RIL as random effects. The mean for RILs and parental lines were calculated for all treatments and combined environment. Also, correlations between traits were computed for each treatment using PROC CORR in SAS (SAS Institute, Inc.2010).

##### Association Mapping Panel

For each location, spatial adjustments were conducted using PROC MIXED considering check varieties as fixed and genotypes and blocks as random effects. Then, combined data analysis across locations was implemented, considering all factors in the

model as random, to obtain the best linear unbiased predictors (BLUPs) and variance components for each trait. Statistical summary of grain-fill duration (GFD) was computed across all the four environments where it was measured.

### Genetic Analysis

#### Recombinant Inbred Lines

A total of 155 of the 180 hexaploid wheat recombinant inbred lines (RILs) obtained from a cross between Vida and MTHW0202 were genotyped by the USDA-ARS Small Grain Genotyping Center, North Dakota (<http://wheat.pw.usda.gov/GenotypingLabs/>) using the Illumina iSelect 90K SNP Assay. A total 81,587 SNP markers were assayed.

Genotyping and SNP Calling: The procedure for validating SNPs was similar to that previously described (Sukumaran et al., 2015). Polyploid clustering and genotype calling was conducted using the software Genome Studio Polyploid Clustering v1.0. Assays that produced distinct clusters corresponding to AA and BB genotypes as expected in inbred bi-parental populations were identified. The default clustering parameters implemented in the software was used. The algorithm used to assign the clusters in genotyping data was OPTICS (Ordering Points To Identify Cluster Structure). Manual curation was done for assays that produced compressed SNP allele clusters and could not be discriminated using the default algorithm. Severely distorted SNPs were excluded by selecting only those assays for which the population segregated nearly in 1:1

ratio. A total of 5,635 polymorphic SNPs markers were selected for use in linkage MAP construction.

Linkage Map Construction and QTL Analysis: SNP's chromosome position was determined using the 90k Wheat Consensus Map (<http://129.130.90.211/snp/>) and chromosome names were concatenated at the end of most of the markers. The map was created without reference to the consensus map, but after map creation the consensus map was used to predict chromosomes for linkage groups. At first framework markers and co-segregating markers were identified using Multipoint software version 3.3 ([www.multoqtl.com](http://www.multoqtl.com)), setting the maximum Chi2 segregation distortion and percentage of missing marker to 15. LOD score and recombination fraction were set to 5 and 0.2 respectively. Kosambi mapping function was used. The map was constructed in Mapdisto 1.7.7.0.1.1 by using only framework markers from Multipoint program. In Mapdisto, maximum missing marker was set to 13%. Kosambi mapping function was used. Minimum LOD and recombination threshold used were 3 and 0.3, respectively. Estimation of recombination fraction used the Bailey function. The file was exported in INP file format to be used in Windows QTL Cartographer V2.5\_011 (M. Wang et al., 2012). The linkage map including co-segregating markers is reported in supplemental table 1.

QTL analysis was accomplished with QTL cartographer. The dSUM function was useful to check the population distribution for each trait. Then, an initial model was created using composite interval mapping (CIM) function (Zeng, 1994). The model consisted of standard model 6 (default), window size 10 cM and forward and backward

regression method. The walking speed was selected 1 cM and minimum permutation was set to 500. Minimum significance level was 0.05. After creating initial model, more QTLs were identified and tested by scanning through QTL mapping result file (i.e. CIM result file) through multiple interval mapping (MIM) (Kao et al., 1999). QTL search standard in MIM included threshold LOD score of 2, minimum distance between QTLs was 5 cM, minimum walking speed of 1 cM and walking speed of 10cM. Significant QTLs and QTLs interactions were searched and tested for significance. All significant QTLs were reported. Likewise, flanking markers were reported at 2 LOD drop scale on either side of peak (Chaky, 2003). The reported LOD was the maximum among the three treatments.

#### Association Mapping Panel

Fresh leaf tissues were harvested at 3-leaf stage and pooled for genome DNA extraction following CTAB procedure (Saghai-Maroo et al., 1984). DNA samples were sent for genotyping to the USDA-ARS genotyping laboratory in Fargo, North Dakota.

Genotyping and SNP Calling: SNP genotyping was implemented using the Illumina iSelect 90K SNP Assay as described in (S. Wang et al., 2014). Genotype data was processed using Genome Studio Software v2011.1. SNP markers that were monomorphic, low-quality and as minor allele frequency (MAF) <0.05 were discarded. Biallelic SNPs which showed distinct clusters that correspond to AA, AB and BB genotypes were identified using the default clustering algorithm. Manual curation was done for compressed SNP allele clusters that could not be discriminated using the clustering algorithm as described in (Cavanagh et al., 2013). A total number of 19,192

SNP markers with map positions identified in the wheat 90K consensus map were selected and using the association analysis.

Marker Trait Association (MTA): Marker-traits associations were tested using a mixed linear model (MLM) approach that included the BLUPs for each trait, marker data, kinship (K matrix) and first 3 principal components (PC3). Including the K and PC3 in an MLM approach greatly improves the reliability of the MTAs by reducing false positive and increasing power for MTA.

Association analysis was conducted using the software Genomic Association and Prediction Integrated Tool (GAPIT) implemented in R software (Lipka et al., 2012). MTAs were tested for individual and combined data across locations. Significant MTAs were described as P values and magnitude of QTL effects  $R^2$  were evaluated by comparing to “null allele” (missing plus allele) model for each locus (Brescaglio & Sorrells, 2006). Consistency across environments was given additional attention to further select stable MTAs. Strong, significant MTAs from the combined analysis which were stable across environments were reported. However, in this paper, only those QTLs which had close position to the QTLs identified in Vida X MTHW0202 populations were reported.

## CHAPTER THREE

## RESULTS

Data from 10 different environments across the state of Montana gathered by the spring wheat breeding program indicate that on average Vida significantly out yields MTHW0202, with largest differences in water-stressed environments (data not included). Characteristics that distinguish Vida from MTHW0202 and other traditional spring wheat cultivars include an extended grain fill period (S.P. Lanning et al., 2006) and a relatively high number of productive tillers (Naruoka et al., 2011). MTHW0202 on average headed 5 to 9 days earlier than Vida and tended to mature earlier based on breeder observations in the yield trials (data not shown). Therefore, an RIL population was created from a cross between Vida and MTHW0202 and were grown in a total of five location in 2013 (Bozeman Rain-fed and Irrigated) and 2014 (Bozeman Rain-fed, Huntley Rain-fed and Sidney Irrigated) to identify QTLs related to performance in moisture stressed spring wheat production.

Means for Parents and  
Recombinant Inbreed Population

Testing of the parents across the five environments confirmed that Vida and MTHW0202 differ for several phenotypic traits (Table 1). Vida has significantly more tillers, while MTHW0202 has more seeds per head, higher seed weight, test weight and greater seed diameter. Vida tends to be taller and with later heading and maturity, while MTHW0202 has more solid stems. There was significant genetic variation among RILs

for all traits. For most traits, the mean of the RIL was between the two parents. However, the mean of the RIL was higher than either parent for height and test weight. The mean of the RIL was lower for seeds per head and yield.

Table 1: Means for spring wheat parents and recombinant inbred lines (RIL) for grain yield, yield components, and agronomic traits in different environments.

Trait	Combined <sup>1</sup>			Rain-fed <sup>2</sup>			Irrigated <sup>3</sup>		
	Vida	MTHW0202	RIL	Vida	MTHW0202	RIL	Vida	MTHW0202	RIL
Heading (day)	182.4***	177.5***	180.3	181	176.3	179.1	184.5	179.2	182.1
Maturity(day)	215.3***	210.4***	212.6	215*	209.2*	211.4	218	212.2	214.9
Grainfill(day)	30.2	29.30	29.4	30.2	29.5	29.45	30.3	28	29.5
Height (cm)	84.9	82.1	93.1	84.1	81.7	94.1	86.1	82.7	91.5
Tillers/m <sup>2</sup>	628***	461***	557	653	470	574	591*	447*	531
Seed/head	37.6***	45.8***	37.5	38**	46.4**	39.5	37.3	45.2	42.7
Test wt. (Kg/m <sup>3</sup> )	737	742	755	722	724	745	758	768	775
Yield (kg/ha)	4496	4289	4170	3683	3465	3419	5715	5525	5284
Seed wt. (g)	33.6**	36.6**	34.6	33.1	34.8	33.3	34.1	38.4	35.8
Seed diameter (mm)	2.71***	2.91***	2.84	2.71	2.85	2.81	2.71*	3.06*	2.90

Note: <sup>1</sup>Population means of agronomic traits (all trials combined); <sup>2</sup>Population means of rain-fed trials combined (2013 Bozeman rain-fed + 2014 Bozeman rain-fed + 2014 Huntley rain-fed); <sup>3</sup>Population means of irrigated trials combined (2013 Bozeman Irrigated + 2014 Sidney Irrigated). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001; Sample size= 180

### Correlation Analysis

Correlations between yield and yield components varied depending on the environment (Table 2). In the rain-fed environments, yield was most positively correlated with grain-fill, seed diameter and weight but negatively correlated to heading date. In irrigated environments, yield was most positively correlated with heading date and increased number of seed through more seeds per head or more tillers. In the rain-fed

environments, yield had higher correlation with traits that have been reported to be related to drought tolerance, including length of grain-fill and CTD. WI and NDVI, which were only measured in rain-fed environments, also showed correlation with yield (Table 4).

Most of the agronomic traits correlated with grain-fill (Table 3). Heading and maturity correlated with grain-fill though with opposite signs, as expected since both traits are components of grain-fill. Grain-fill also correlated with a number of traits in both environments showing a positive correlation with seed diameter and weight and a negative correlation with number of seeds per head in both environments. Interestingly, grain-fill only significantly correlated with yield and CTD in the rain-fed environments and correlated with test weight negatively in the rain-fed and positively in the irrigated environments.

Table 2:- Correlation between yield and other traits in rain-fed and irrigated environments for recombinant inbred lines from a cross of Vida x MTHW202 spring wheat parents.

	Test Wt.	Grain-fill	Height	Tiller	Seed/head	Heading	Maturity	Seed diameter	Seed Wt.	Stem solid	CTD
Rain-fed	-0.21**	0.49***	0.14*	0.13ns	0.02ns	-0.31***	0.09ns	0.23**	0.26***	0.06ns	0.14*
Irrigated	-0.07ns	0.03ns	-0.04ns	0.26***	0.39***	0.23**	0.01ns	-0.19**	-0.08ns	0.04ns	-0.02ns

**Note: Level of significance: \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, Sample Size= 180**

Table 3:- Correlation between grain-fill and other traits in rain-fed and irrigated environments for recombinant inbred lines from a cross of Vida x MTHW202 spring wheat parents.

	Test Wt.	Height	Tillers	Seed/head	Heading	Maturity	Seed diameter	Seed Wt.	Stem solid	CTD
Rain-fed	-0.18*	0.26***	0.03ns	-0.17*	-0.40***	0.41***	0.41***	0.38***	0.03ns	0.14*
Irrigated	0.33***	0.14*	0.10ns	-0.26***	-0.43***	0.29***	0.26***	0.27***	-0.01ns	0.12ns

**Note: Level of significance: \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, Sample Size= 180**

Table 4: Correlation of water index (WI) and normalized difference vegetation index (NDVI) with yield, grain-fill and maturity in rain-fed environment for recombinant inbred lines from a cross between Vida and MTHW0202 spring wheat parents.

	Yield	Grain-fill	Heading	Maturity	Plant Height
WI	0.32***	0.30***	0.05ns	0.35***	0.41***
NDVI	0.16*	0.11ns	0.32***	0.46***	0.29***

**Note: Level of significance: \*P<0.05, \*\*P<0.01, \*\*\*P<0.001; Sample Size=180**

### Linkage Map and QTLs Analysis

The total number of polymorphic markers used in linkage map construction was 5635. A total of 714 formed the skeleton map, while the remaining markers all co-segregated with different skeletal markers. The total genome coverage was 2057.8 cM with 29 linkage groups. The marker order in the linkage map is similar to the 90k Wheat Consensus Map (<http://129.130.90.211/snp/>).

A QTL analysis was conducted using the map constructed with 714 markers (supplemental Table 1). We identified QTLs specific to rain-fed and irrigated environments as well as QTLs observed across environments. A total of 133 individual QTLs were identified. Here we focus on QTL that for the most part were associated with a number of traits and appear to play an important role in moisture stressed spring wheat production (Table 5).

### Yield and Yield Component QTLs

Nine yield QTLs were observed and in all cases they co-segregated with other traits, including the yield components such as tiller number, seed diameter and seeds per head, as well as agronomic traits and/or drought related traits (Table 5). In some cases, the yield QTL was only significant in a given environment; in others it was significant across environments. Positive alleles came from either parent, depending on the QTL. The yield QTL on chromosome 6B had the largest effect on yield, but was only significant in the rain-fed environment with the positive allele from MTHW0202. This favorable allele at this QTL was negatively associated with tiller but positively associated

with seed diameter, seed weight and test weight. Other yield QTLs were observed on 2D and 3D where yield and tillers were in opposition to seed diameter, with positive alleles for yield and tillers contributed by Vida and positive alleles for seed diameter contributed by MTHW0202. Importantly, for both 2D and 3D, the yield QTL was only significant in the irrigated environment, but both QTLs for seed diameter were significant in all environments. Chromosome 3A also has a significant yield QTL. In irrigated environment, higher yield is associated with more tillers, with MTHW0202 contributing the positive allele. However, in the rain-fed environment this QTL has a negative impact on yield. On 3B, yield co-segregated with seed diameter significantly in the rain-fed environment and test weight in irrigated environment with MTHW0202 contributing the positive allele that is not in a significant negative association with any other yield component, including tillers. Another strategy for increased yield is increased seeds per head. A significant QTL for yield and seeds per head was observed on 1D for all environments with the positive allele contributed by MTHW0202. No negative yield components were significantly associated with this QTL. Several yield QTLs on 6D, 6B and 5B also segregated with traits that have been previously associated with drought tolerance, including grain-fill, WI, and NDVI (all detailed below). In some cases, yield components were mapped that did not significantly associate with yield and had two or more yield components in negative association, including 1) 3A QTL with tiller number, seed weight and seed diameter in opposition to seeds per head 2) 3B QTL with seeds per head in opposition to test weight 3) 4A QTL with seeds per head in opposition to seed

weight, seed diameter and test weight 4) 7B QTL with tiller and seed diameter in opposition to seeds per head.

### QTLs Impacted by Environments

As mentioned above, several QTLs had significant effect across environments (Table 5). However, a number of QTL have a greater effect or were only significant in either the rain-fed or irrigated environment (Table 5). Of particular importance may be the yield and yield component QTLs with positive effects in the rain-fed, but no effect or even a negative effect in the irrigated environment. Examples include tiller on 2D and 7B; seed weight on 3A, 4A, 5B and 7B; yield on 3B, 5B, 6B and 6D; seed diameter on 3B, 4A and 7B; Test weight on 3D, 6D, 7B and 7D; and seeds per head on 6A and 7B. Interestingly, positive alleles in rain-fed environments were contributed by both Vida and MTHW0202.

### Grain-fill QTLs

Nine grain-fill QTLs are reported in Table 5 on chromosomes 2D, 3A, 3B, 5B, 6B, 6D, 7D, and two separate QTLs on 4A separated by 36 cM. Several of the grain-fill QTLs co-segregated with maturity, including 3A, 3B, 4A, 6B and 6D. However, the second 4A long grain-fill QTL (located at 111 cM) allele from Vida is associated with earlier heading and earlier maturity. The 6B long-grain fill allele is also associated with earlier heading. Interestingly, the longer grain-fill allele is the Vida allele for the two 4As, 3A and 6D QTLs, but the MTHW0202 allele for 3B and 6B QTLs. The first 4A

(74.0 cM) grain-fill QTL is significant in all environments due to Vida allele and did not co-segregate with any yield component but with late maturity and late solid stem in irrigated environment. Many of the grain-fill QTLs co-segregated with a yield or yield component QTL, including 2D favorable allele with seed diameter but its opposite allele with yield and tiller number; 3A favorable allele with tiller number, seed weight, and seed diameter, but its opposite allele with seeds per head; 3B favorable allele with seeds per head, but its opposite allele with test weight; the second 4A favorable allele with seed weight, seed diameter, and test weight, but its opposite allele with seeds per head; 5B with test weight; 6B favorable allele with seed weight, seed diameter, test weight and yield, but its opposite allele with tiller number; 6D with yield and test weight. Most of the grain-fill QTLs were either expressed in both environments or when the environments were averaged (2D, 3A, 3B, both 4As, 6B). However, the 5B grain-fill QTL was only expressed in the irrigated environment and the 6D and 7D grain-fill QTLs were only expressed in rain-fed environment. In 2D, a very strong early heading QTL was identified in all environments very close to the position of *Ppd-D1*, a photoperiod insensitive QTL (Naruoka et al., 2012; Blake et al., 2009) where Vida contributed the positive allele.

#### Height and Solid Stem QTLs

Height QTLs were observed on 4B and 4D at the *Rht-B1b* and *Rht-D1b* loci (not reported), but did not co-segregate with any other trait. Minor height QTLs were observed on chromosomes 2A, 3A, 3D, 6B and 7A (Table 5). The 6B height QTL co-segregates with yield and WI. The 3A and 3D taller alleles co-segregate with lower tiller

number and yields, while the 3D taller allele also co-segregates with increased test weight, seed diameter and seed weight. Two minor height QTLs, 2A and 7A, co-segregate with solid stems. However, the 2A allele for increased height co-segregates with less solid stems; while the 7A QTL for increased height co-segregates with more solid stems. Other solid stem QTLs are reported (Table 5), including 1D, 5B, 6A and 6B. The 1D increased solid stem allele co-segregates with increased yield and seeds/head; 5B increased solid stem allele co-segregates with shorter grain-fill and lower test weight; 6A increased solid stem allele co-segregates with lower seeds per head; and 6B increased solid stem allele co-segregates with lower tiller, but greater seed diameter, seed weight, test weight and yield.

#### QTLs Associated with Proxies for Abiotic Stress Resistance

QTL for NDVI and WI were observed on 2A, 5B and 6D (Table 5) with an additional QTL for WI on 6B. For 2A and 6D QTLs, the positive alleles come from Vida for both WI and NDVI; while for 5B, the positive allele comes from MTHW0202 for both NDVI and WI. All three QTLs co-segregate with other traits: increased height on 2A, increased yield on 5B, and increased grain-fill, yield and test weight on 6D. The positive allele for WI on 6B from Vida was also associated with increased yield and height. Two significant QTLs for CTD were observed on chromosomes 3B and 7B. The 3B QTL for increased CTD co-segregates with increased test weight, but in opposition to increased grain-fill and seeds per head; while the 7B QTL co-segregates with increased tiller and seed diameter but in opposition to seeds per head.

QTLs from Association Mapping Panel

The statistical summary (Table 6) indicates variation for grain-fill duration in all the four spring wheat nurseries in Bozeman and Saskatoon during 2012 and 2013. In total, 34 significant grain-fill QTLs were identified in individual and combined environments. Among them, only 6 QTLs, which are in close agreements to the QTLs identified using Vida/MTHW0202 population, are reported. They are located on chromosomes 3B, 4A, 5B, 6B and 6D in association mapping population (Table 7).

Table 6:-Statistical summary for GFD across different locations in 2012 and 2013.

Location	Plant date – Harvest date	Mean	Min	Max	St. Dev.	Variance
2012_Bozeman, MT	5/4/12 – 8/17/12	30.48	28	34	1.05	1.10
2012_Saskatoon, CA	5/3/12 – 8/22/12	44.06	40	47	1.63	2.66
2013_Bozeman, MT	5/15/12 – 9/5/12	27.85	23	33	1.62	2.62
2013_Saskatoon, CA	-	43.95	39	49	2.02	4.10

**Note: Population Size (N= 247)**

Table 7:-MTA identified for GFD for individual locations in AM panel in close agreements to QTLs identified using RIL.

SNP ID	Chromosome	Position (cM)	<i>P-value</i>	R <sup>2</sup>	Stability*
IWB31458	3B	95.10	0.0005	1.8-3.5	3(4)
IWB9549	4A	83.42	0.0001	1.3-3.1	3(4)
IWB70641	4A	127.12	0.008	1.5-3.0	3(4)
IWB56834	5B	57.18	0.004	1.6-3.6	3(4)
IWA7571	6B	64.82	0.0006	1.6-3.7	2(4)
IWB35645	6D	83.44	0.0001	1.9-2.0	2(4)

**Note: \* Indicates number of environments where the QTL is identified**

## CHAPTER FOUR

## DISCUSSION

Vida and its parent Reeder are two of the most widely grown varieties in Montana due to their high yield in rain-fed condition. Two of the traits that set Vida and Reeder apart are extended grain-fill and high tillering. MTHW0202 has a different strategy for yield that appears to be due to increased seeds per head, test weight and seed size. During this study, Vida out yielded MTHW0202 in all environments; while, the greatest yield difference between the two was observed in the rain-fed environment (218 vs. 190 kg). The biggest difference in tillers between the two parents was also observed in the rain-fed environment (183 vs. 144). On the other hand, the yield components contributing to the yield of MTHW0202 had the greatest difference in irrigated environments (e.g. seed weight and seed diameter) or seemed to be independent of environment (e.g. seeds per head). Correlation analysis of the RIL did not confirm the relationship between tiller, grain-fill and rain-fed production. However, difference in correlations between environments for yield components and abiotic resistance traits necessitated analyzing the traits by environment. An RIL population from Vida X MTHW0202 provides us with the ability to determine what combination of yield and abiotic resistant QTLs are highest performing for dry land or rain-fed production.

Linkage map of the Vida X MTHW0202 population was constructed and QTL analysis for yield and abiotic stress traits was conducted. The map and its size is comparable to other genetic maps for wheat (Bajgain et al., 2015; Buerstmayr et al.,

2002) with no identity issue for any linkage group. Markers are less concentrated in D genome with numbers similar to other studies. The map consists of 29 linkage groups instead of the expected 21 and certain regions had lower marker density. Vida and MTHW0202 share a common parent 'Gallo' back four generations. This relatedness would maintain approximately 6% genetic similarity in the parental lines creating some non-recombined genomic blocks that explain regions low in markers and increased numbers of linkage groups.

### Yield

QTL analysis of the Vida X MTHW0202 population allowed for the genetic dissection of yield and yield components. Yield was significantly associated with nine QTLs. The fact that yield components, agronomic traits and abiotic stress traits were also phenotyped allowed the prediction of how each QTL impacts yield. Increased tillers is an obvious strategy to increase yield and three yield QTLs co-segregate with increased tillers (2D, 3A, 3D). Although Vida has more tillers, some of the positive yield/tiller alleles (e.g. 3A) come from MTHW0202. Two of the increased tiller QTLs also co-segregated with shorter height (3A, 3D). Generally, restricted tillering is a strategy to conserve water uptake during drought (Richards et al., 2010). For example, the positive impact of tiller QTLs 2D and 3D was only observed in irrigated environment. The increased tiller QTL on 6B reported here was previously identified in a population including a parent of Vida and called *QTn.mst-6B* (Naruoka et al., 2011). Observing NILs for the *QTn.mst-6B* indicated that the QTL enhances early tiller formation regardless of

the environment, but that the environment determines if each tiller becomes productive. Also, high tiller did not provide a yield boost under low moisture conditions (Nasseer et al., 2016). In the current study, the Vida 6B allele for increased tillers actually decreased yield in rain-fed environment. Interestingly, the allele for increased tillers on 3A QTL had a positive impact on yield in rain-fed environment but negative in irrigated environment with almost equal effect, cancelling net impact when environments are combined. A possible explanation of improved productivity with more tillers in low moisture environments lies in the relationship between tillers and roots. Several studies have found a positive correlation between tiller numbers and root surface area in spring wheat (Narayanan et al., 2014; Waisel et al., 2002).

Increasing the number of seeds per head is another strategy for increased yield. One yield QTL co-segregates with increased seeds per head (1D). MTHW0202 has more seeds per head than Vida and the increased seeds per head allele originated from the former. The 1D yield and seeds per head QTL do not negatively co-segregate with any other yield component and have similar effects in both rain-fed and irrigated environment. Similar effects were observed at 3B and 6A seeds per head QTLs, although yield did not co-segregate with these traits. The number of seeds per head is determined before grain-fill stage depending upon accumulation of nitrogen, stem reserve and carbon assimilation (Sinclair & Jamieson, 2006). However, the fate of those seeds could be determined by drought induced abscisic acid dependent plant response, causing ovule abortion and pollen sterility (Barnabas et al., 2008).

Seed diameter is an important yield component trait selected during crop domestication and further breeding (Shomura et al., 2008). Since growers sell crops by weight, denser seed is an economic advantage. It has also been reported that recurrent selection for increased seed size in spring wheat has increased flour extraction (Wiersma et al., 2001). Although there is very little evidence of direct impact of seed size on yield, bigger seed could also be a route to high yield (Sayre et al., 1997). In this research, one yield QTL co-segregates with seed diameter (3B) with the positive allele originating from MTHW0202.

Canopy Spectral reflectance (CSR) has been investigated for its potential in high-throughput phenotyping. There have been several reports on the relationship between CSR indices and yield especially in drought and drought combined with heat stressed environments (Babar et al., 2006; Lopes & Reynolds, 2012; Prasad et al., 2007). In this study, three yield QTL co-segregate with CSR indices (i.e. NDVI and WI) with the positive allele on 5B originating from MTHW0202 and positive alleles on 6B and 6D originating from Vida. The 6B QTL also co-segregated with increased height. Selection of semi dwarf genotypes during the creation of this population removed the impact of Rht loci, allowing us to identify minor height genes. Tall semi dwarf plants are found to have higher yield and/or better water status (e.g. 2A and 6B QTLs), indicating that tallness increases access to soil moisture and/or tissue storage. A previous paper indicated the importance of solid stems in improving water status where there was high variation for the trait (Pierre et al., 2010). However, in the current population height is more important for increasing water status than solid stems. Vida is semi-solid and MTHW0202 is solid

and the population ranged from semi-solid to solid. Perhaps to have an observable effect on water status the population must vary from hollow to solid.

In this study, a number of yield components were mapped that did not co-segregate with yield. For many of these QTLs, multiple yield components co-segregated in opposition, resulting in a neutral impact on yield. For example, the Vida allele increases tillers and seed diameter while the MTHW0202 allele increases seeds per head in QTLs on 3A and 7B. A number of the QTLs for high tillers also associated with smaller seed, although in most case the increase in tillers was still enough to positively impact yield, except with the 5B QTL (due to opposition to seed weight) and 6B QTL (due to opposition to seed diameter, seed weight and yield). Seeds per head QTLs were observed that co-segregated negatively with seed diameter e.g. 3A, 4A and 7B. Similar results of yield components co-segregating in opposition have been reported previously (Sherman et al. 2014; Azadi et al., 2014; Paliwal et al., 2012). In some cases, the QTLs co-segregating for yield components responded to the two environments differently. There was a tendency for the tiller trait to have the largest effect in the irrigated environment, while the trait in opposition had its largest effect in the rain-fed environment. However, the 4A and 7B QTLs greatest effect was observed in rain-fed environment. Competition between sinks is more common in dry environment where rate of carbon assimilation, soil moisture and nutrients are more limited (Paul & Lawlor, 2014).

### Grain-fill

Starch, the largest component of yield in cereal seed, is stored in the seed during grain fill. Extending the time in which starch can be deposited in the seed can increase yields. Premature senescence due to drought reduces yields. Therefore, breeders have an interest in extending grain-fill especially in low moisture conditions and have done so successfully with varieties like Reeder and Vida.

In the Vida /MTHW0202 population, we observed nine significant grain-fill QTLs. In most cases, grain-fill co-segregated with other traits the exception being the 7D QTL. There is a potential for grain-fill to be effectively shortened by the increased demand of the plant on limiting resources. For example, the increased demand on water and nutrients by more tillers could cause those resources to be used more quickly hastening maturity. Several of the grain-fill QTL co-segregated in opposition with a sink. For example, a 2D QTL increased grain-fill and was in opposition to tiller and yield, a 6B QTL increased grain-fill and was in opposition to tiller, while a 3A and a 4A QTLs increased grain-fill QTLs and are in opposition to seeds per head. We assume that grain-fill is pleiotropic to the yield sink in these QTL and that the sink is shortening grain-fill. However, several grain-fill QTLs positively co-segregated with yield components, especially 6D. Several grain-fill QTL also co-segregated with improve water status (6D and 5B), indicating potential for a positive impact for rain-fed farming. Likewise, increased grain-fill QTL often co-segregated with increased seed diameter and seed weight. Finally, one of the 4A grain-fill QTLs was previously identified in a population with Reeder as a parent (Naruoka et al., 2012).

Six grain-fill QTLs at 3B, 4A, 4A, 5B, 6B and 6D have also been identified in AM panel population (Table 7). Although different markers are listed associated with these QTL in Table 7, the marker positions are very close when compared in the 90k wheat consensus map, confirming grain-fill QTLs effect in a more diverse population. The variation explained by these QTLs in AM panel population is slightly lower than in this study population.

An important goal of this study was to identify trait combinations that would benefit rain-fed farming. Breeders often select for genotypes that perform the best across environments to ensure trait stability. Some studies have shown little difference between QTLs depending on the environment (Sherman et al. 2014). However, other studies have reported QTLs specific to a given environment. In this study, only one of the yield QTLs (i.e. 1D) was effective in both rain-fed and irrigated condition. It is presumed that this QTL would be a good candidate for yield improvement across environments. On the other hand, the 3A QTL for yield/tillers was positive in rain-fed environment while negative in irrigated environment. Two yield/tiller QTLs were only effective in irrigated environment (2D and 3D), while yield QTLs on 3B, 5B, 6B and 6D were all only effective in rain-fed environment. It is important to note that these five QTL did not negatively impact yield in irrigated so might be neutral in more favorable environments. The 6D QTL is of particular interest as it did not negatively associate with any yield component and might improve yield due to improved water status.

Yield potential is frequently not met when environmental conditions are not favorable. Here, we have identified a number of QTL that could increase yield potential,

but in unfavorable environments still might not be fully effective. It was our hope that by pairing increased yield potential with extended grain-fill that yield potential could be more fully realized. We identified nine QTLs that were associated with grain-fill. Many of these were believed to be the result of pleiotropic interactions with co-segregating yield components. However, 4A, 5B and 6D seemed to be independent of other traits.

Finally, yield is the ultimate criterion for selection for most crop breeding projects and it is a function of multiple yield components. However, in the case of moisture stressed environments, yield is a function of yield components as well as drought associated traits. Therefore, understanding of the genetic mechanisms controlling not only yield and abiotic stress resistance individually, but also the genetic control of the interaction between these phenotypes is critical for dryland crop improvement. A major finding of this work is that all the grain-fill QTLs may not contribute to stay-green phenotype. The extended grain-fill QTL on 4A and 6D seem to increase grain-fill in a way that is resistant to early senescence due to low moisture. Creation of NILs with combinations of grain-fill QTL and yield component QTLs would allow for further study of interactions between these QTL and environments.

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Table 5:- QTLs identified for yield, yield components and drought associated traits in rain-fed, irrigated and average over environments using Vida/MTHW0202 recombinant inbred line (RIL) population.

Chr	Marker Name	Flanking Marker Name	Arm	Position (cM)	LOD	Effect	R <sup>2</sup> in percentage			Traits
							Rain-fed	Irrigated	Average environment	
1D	CAP12_c46_3331D	BobWhite_V7474_65/Kukri_V2870_91	S	41.75	2.7	0.72	X	6.1	X	Stem solid
					2.1	41.73	3.4	4.2	8.1	Yield
					3.3	1.11	6.4	3.3	6.7	Seed per head
2A	BobWhite_c41527_2012A	Kukri_rep_V07630_127/Kukri_M3597_324		104.1	2.5	-2.00	4.1	X	X	Height
					9.1	-0.02	18	#	#	NDVI
					5.1	-0.004	9.9	#	#	WI
2A	TA002087-10002A	RAC875_rep_M07961_348/RFL_Conting4324_13482A		119.9	2.0	0.53	5.3	X	X	Stem solid
2D	TA012840-03692Dx	BobWhite_M1626_243/BS00011109_512Dx	S	49.59	2.8	-10.9	4.8	X	7.7	Tiller
2D	BS00011109_512Dx	BobWhite_M1626_243/Excalibur_c7347_1067	L	54.21	3.1	0.20	5.9	2	6.2	Grain fill
				54.21	2.9	0.02	0.6	5.1	11	Seed diameter
2D	Excalibur_c65531_147	Excalibur_c65531_147/Tdurum_contig92425_16732Dx		52	3.7	-88.5	X	18	X	Yield
3A	BS00067499_513A	Kukri_rep_V02131_826/BobWhite_s67516_1593A	S	68.65	2.9	-14.9	2	4.5	2.8	Tiller
				68.65	2.5	0.89	6.5	3.5	X	Seed per head

3A	Kukri_rep_V02131_826	Kukri_rep_V02131_826/BS00067499_513A		Near 75	1.5	-0.36	X	X	2.5	Maturity
3A	Excalibur_c6040_7623A	RAC875_V8582_204/Jagger_c791_623A	L	85.3	1.5	-0.13	X	X	2.4	Grain fill
				85.3	5.8	-0.95	9.2	X	X	Seed Wt.
				85.3	3.4	-0.01	X	X	6.8	Seed Diameter
3A	BS00041742_513A	Tdurum_contig34075_983A/RAC875_rep_V09228_400	L	151.7471	1.4	10	X	3	2.8	Tiller
3A	RAC875_rep_V09228_400	w SNP_BE604885A_Ta_2_23A/BobWhite_c46361_3313A		NEAR 170	1.6	-1.1	X	2.5	X	Height
3A	BobWhite_M3661_275	Kukri_M2212_182/Kukri_V3354_369	L	NEAR 175	4.7	-0.41	X	4.5	X	Heading
3A	w SNP_Ex_c361_7089273A	BobWhite_c46361_3313A/RAC875_M5873_273	L	177.2	4.3	-0.3	6.3	X	X	Maturity
3A	Kukri_rep_c70441_1323A	RAC875_M5873_273/IACX59803A		188.3	2.3	39.5	4.1		X	Yield
						-41.8		2.9		****
3B	BS00070455_513B	BobWhite_s65848_176/Tdurum_contig49753_1913B		34.1	3.3	48.7	6.2	X	X	Yield
3B	BS00062690_513B	BS00070455_513B/BS00062690_513B		35.76	3	2.1	X	4.5	X	Test Wt.
				35.7	2.2	0.03	4.8	X	X	Seed diameter
3B	Ra_c965_27023B	RAC875_c33825_11093B/BobWhite_s65848_176		61.6	4.1	1.32	X	8.6	9.9	Maturity
				61.6	3.4	1.5	3.8	6.5	3.6	Seed per head
3B	RAC875_rep_V09105_57	BS00035878_513B/IACX112873B		NEAR 70	3	-0.18	X	X	5.3	CTD
				NEAR 70	4	-2.4	X	3.2	X	Test Wt.
3B	BS00099633_513B	BS00099633_513B/IACX112873B		82.1	4.2	0.62	X	X	8.9	Grain fill
3D	BS00023210_51	IAAV56353D/ BobWhite_V7541_67	L	112.5	3.8	-22.4	X	7.1	15	Tiller
3D	BobWhite_V7541_67	IAAV56353D/ tpb0029j24_2118		112	12	9.33	17	X	13	Test Wt.

3D	tplb0029j24_2118	tplb0029j24_2118/BS00054496_513D		115	7.5	2.55	11	4.6	8.7	Height
					2.9	-66.9	X	12	X	Yield
3D	RAC875_V01793_136	BS00054496_513D/RAC875_V01793_136 /CAP12_V615_128		NEAR 121	2.6	0.04	6.7	7.5	11	Seed diameter
3D	wsnp_BE497740B_Ta_2_13B	BS00054496_513D/RAC875_V01793_136/CAP12_V615_128/RFL_Contig532_2_2193D		NEAR 121	3.9	0.48	X	4.2	10	Seed Wt.
4A	RAC875_c34231_8124A	Kukri_c6224_266/Tdurum_contig47148_6514A/BS00021727	L	74.9	2.9	-1.13	x	2.7	1.9	Maturity
			L	74.9	3.3	0.81	X	7.7	X	Stem solid
4A	Excalibur_V4511_1196	Kukri_c6224_266/Tdurum_contig47148_6514A/BS00021727	L	NEAR 74.9	2.4	-0.06	3.3	4.2	4.7	Grain-fill
4A	BobWhite_V0610_1096	Tdurum_contig65718_2094A/BobWhite_M3141_92		102	2.2	0.54	X	4.9	7.2	Maturity
4A	tplb0062M4_1758	Tdurum_contig65718_2094A/RAC875_c59673_5004A		106	9.9	2.16	23	19	28	Seed per head
				106	9.7	-0.81	10	X	X	Seed Wt.
				106	5.5	-0.02	6.5	X	6.1	Seed diameter
4A	RAC875_c35979_2634A	Tdurum_contig65718_2094A/BobWhite_M3141_92	L	107.6	13	-5.50	X	13	5.7	Test Wt.
				107.6	11	0.56	15	8.5	12	Heading
4A	tplb0046a02_8044A	Tdurum_contig65718_2094A/RAC875_c59673_5004A		111.1	4.2	-0.36	9.3	7.2	11	Grain fill
5B	BS00064297_515B	BS00064297_515B/Excalibur_c58520_785B		29.11	7.7	84.12	3.2	X	5.5	Yield

5B	BS00050707_515B	Excalibur_V4594_182/tplb0021f14_984 5B	S	38.50	4.9	0.04	20	#	#	NDVI
5B	BS00058876_515B	RAC875_c33387_11025B/Excalibur_re p_c67473_1975B	L	60.31	6.1	0.10	X	11	X	Grain fill
			L	60.31	2.7	-0.75	X	2.8	X	Stem solid
			L	60.31	4.7	0.01	5.8	X	#	WI
5B	Excalibur_rep_c67473_19 75B	BobWhite_V4323_288/Excalibur_rep_c 67473_1975B/BobWhite_c6306_424	L	68.36	2.1	1.89	X	3	X	Test Wt.
5B	Excalibur_c3165_7305B	Tdurum_contig13773_3215B/ Excalibur_c3165_7305B	L	114.9	1.6	-10.7	X	2.6	X	Tiller
5B	wsnp_Ra_c48052_53424 4905B	wsnp_Ku_c8953_15094606/Tdurum_co ntig13773_3215B	L	130.3	7.2	0.68	7.4	X	X	Seed Wt.
6A	BS00059860_516A	BS00010811_516A/Tdurum_contig5536 3_2976A	L	80.10	2.7	0.67	12	5.3	11	Stem solid
6A	Tdurum_contig55363_29 76A	Kukri_c8148_27196A/Tdurum_contig6 1448_4126A	L	90.53	1.9	-0.77	3.1	X	2.8	Seed per head
6B	RAC875_c41316_278	RAC875_c41316_278/Tdurum_contig8 740_1096B		57	2.2	-10.7	5.8	X	X	Tiller
6B	Tdurum_contig8740_109 6B	Tdurum_contig8740_1096B/Tdurum_co ntig54967_5816B	S	57.07	14	-0.60	17	9.6	13	Heading
6B	Tdurum_contig54967_58 16B	BS00074041_516B/RAC875_c98074_6 06B		59.16	5.9	3.01	X	X	5.9	Test Wt.
6B	Tdurum_contig77500_36 9	BobWhite_c36228_536B/RAC875_c68 525_2846B		NEAR 70	6.3	0.02	4.5	6.1	13	Seed diameter
				70	5.3	0.57	7.9	4.7	7.7	Seed Wt.

				70	1.3	0.42	x	2.8	X	Maturity
6B	RAC875_M2539_1978	Tdurum_contig47140_2036B/BS000377 84_516B		74	3.2	0.07	6	6.3	6	Grain fill
6B	Excalibur_c82444_220	Tdurum_contig47140_2036B/BS000377 84_516B		Near 75	9.7	615	45	X	X	Yield
6B	RAC875_c68525_2846B	Tdurum_contig47140_2036B/BS000377 84_516B	L	76.79	4.9	1.05	X	11	5.8	Stem solid
6B	BS00065357_516B	Ra_M557_2531/tp1b0046e21_6616B	L	110.4	1.4	-31	2.5	X	2.7	Yield
6B	D_contig31987_446	BS00065357_516B/Kukri_c66579_2066 A		113	4.3	-0.004	8.3	#	#	WI
				113	2.3	-1.46	X	X	4.5	Height
6D	wsnp_BF428701D_Ta_1_16D	wsnp_BF428701D_Ta_1_16D/RAC875 _c25839_225			1.7	S	83.4	X	X	Yield
			S	83.4	5.1	-5.57	4.2	X	X	Test Wt.
6D	Excalibur_rep_c70026_1136D	wsnp_BF428701D_Ta_1_16D/RAC875 _c25839_225		83.4	4.1	-0.32	4.8	X	X	Maturity
6D	RAC875_c25839_225	wsnp_BF428701D_Ta_1_16D/RAC875 _c25839_225		83.4	2	-0.16	6.7	X	7.2	Grain fill
				83.4	3.2	-0.003	6.3	#	#	WI
				83.4	3.5	-0.01	2.1	#	#	NDVI
7A	Kukri_V4194_2659	Kukri_V4194_2659/BobWhite_c761_29 37A		NEAR 45.9	4.8	-0.35	7.3	X	3.4	Maturity
				NEAR 45.11	3.5	0.91	8.1	X	15.3	Stem solid

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7A	wsnp_CAP11_rep_c4066_19218947A	BobWhite_c761_2937A/Excalibur_rep_c68458_15367A	S	62.7	4.2	1.89	2.4	4.7	3.1	Height
7B	BobWhite_c41356_627B	BobWhite_c41356_627B/Tdurum_contig16244_3357B		36.07	2.22	0.38	X	5	X	Heading
7B	BobWhite_c41356_627B	BobWhite_c41356_627B/Tdurum_contig16244_3357B		36.07	5.82	-7.08	11.5	X	X	Test Wt
7B	BobWhite_c41356_627B	BobWhite_c41356_627B/Tdurum_contig16244_3357B		36.07	6.11	-0.66	8.5	X	7.7	Seed Wt.
7B	RAC875_c48671_1727B	RAC875_c48671_1727B/CAP12_c3699_807B	S	167.5	4.7	-20.8	22	X	18	Tiller
				167.5	1.7	1.2	3.3	X	X	Seed per head
				167.5	2.6	-0.20	X	X	6.3	CTD
				167.5	2.7	-0.01	4.9	X	X	Seed diameter
7D	BS00111202_517D	Ra_M573_3696/BS00111202_517D	S	3.46	2.4	0.17	4.1	X	4.6	Grain fill
7D	Kukri_c38283_263	Kukri_c38283_263/Kukri_rep_M03248_582		NEAR 91.2	4.1	-0.38	13	X	X	Maturity
				NEAR 91.2	7	9.4	8.3	X	X	Test Wt.
7D	Kukri_rep_M03248_582	Kukri_c38283_263/tplb0024a09_21067D		NEAR 91.2	6.6	-0.44	9.5	11	22	Heading

**Table 5 contains chromosome name, marker name, flanking markers name, chromosomal arm (S= small arm, L= long arm), position of marker in consensus map, LOD score, marker effect, R<sup>2</sup> in percentage (rain-fed, irrigated and combined environments) and co-segregating traits. MTHW0202 allele is tracked in QTL analysis. Note: ‘X’ indicates QTL not detected; ‘#’ indicate trait not measured in that environment; Chr = Chromosome; Position = Position in 90 k wheat consensus map (<http://129.130.90.211/snp/>). Note: \*\*\*\* Yield QTL at 3A has positive effect on rain-fed but opposite in irrigated condition.**

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