DISSERTATION

GENOME-WIDE ASSOCIATION STUDY AND DROUGHT TOLERANCE EVALUATION OF A WINTER WHEAT ASSOCIATION MAPPING PANEL

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2015

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ABSTRACT

GENOME-WIDE ASSOCIATION STUDY AND DROUGHT TOLERANCE EVALUATION OF A WINTER WHEAT ASSOCIATION MAPPING PANEL

Drought is one of the most important environmental challenges farmers face around the globe, with water stress the main cause for yield loss. Therefore, the objectives of the first part of this study were to 1) evaluate a hard winter wheat association-mapping panel (HWWAMP) in multiple environments differing for soil moisture for agronomic and drought tolerance related traits; 2) determine the relationship between yield and other agronomic and physiological traits; and 3) identify QTL involved in drought tolerance through association analysis. The HWWAMP consists of 299 entries (cultivars and breeding lines) adapted to the U.S. Great Plains region. The panel was characterized using a high-density 90 000 single nucleotide polymorphism (SNP) genotyping platform. Field evaluations for the HWWAMP were conducted in two sites in a side-by-side experiment under two soil moisture regimes in two years (2011-2012 at Greeley, CO and 2012-2013 at Fort Collins, CO). In addition, a replicated confirmation study was conducted in two sites (Greeley and Fort Collins in 2013-2014) to evaluate performance in field trials of a subset of 50 entries.

At Greeley 2011-2012, genotypes differed significantly for grain yield (GY) in both well-watered (WW) and water-stressed (WS) trials ($P<0.001$). Genotypes also differed significantly ($P<0.001$) for total biomass (TBM), biomass grain weight (BGW), harvest index (HI), plant height (Ht), relative water content (RWC), and carbon isotope discrimination (CID) in the WW trial and

differed significantly (*P*<0.001) for BGW, HI, Ht, canopy temperature at late heading stage ($T_c$lh), and RWC in the WS trial. At Fort Collins 2012-2013, genotypes differed significantly (*P*<0.001) for GY in both WW and WS trials. Moreover, genotypes differed significantly (*P*<0.001) for BGW, HI, Ht, RWC, and canopy temperature at booting stage ($T_c$bs) in the WW trial and for TBM, BGW, HI, Ht, RWC and $T_c$bs in the WS trial.

Markers with MAF <5 % and SNPs with ≥10 % of calls missing were removed to produce a set of 16,052 filtered SNPs. These 16,052 SNPs were used by the software package GAPIT in R to perform the genome-wide association study (GWAS). A kinship matrix was estimated in the rrBLUP package for R and was incorporated in the analysis. Several models were considered in the analysis with principal components and kinship (P+K) to control for kinship and structure. A total of 331 significant (*P*<0.001) marker-trait associations (MTA) was detected in one or more environment for 10 measured or calculated traits (GY, TBM, BGM, HI, Ht, $T_c$bs, $T_c$lh, canopy temperature at grain filling stage ($T_c$gf), CID, and drought susceptibility index (DSI)). For the five main traits that were measured in all four environments, the highest number of MTA was recorded for HI (58) followed closely by GY (57), while the lowest number of MTA was recorded for BGW (18). The MTA for HI and GY were spread along 14 and 12 chromosomal regions, respectively in four environments. Amongst the three different T_c measurements analyzed for genome-wide association study, canopy temperature at late heading stage ($T_c$lh) had the highest number of detected MTA (50). Carbon isotope discrimination was measured in the Greeley 2011-2012 WW trial, where the number of detected MTA was 29. Multi-trait chromosome regions were detected on chromosomes 4A and 4D associated with GY and CID, which may be useful in marker-assisted selection, following proper validation. In the

confirmation study at Greeley 2013-2014, genotypes differed significantly for GY under WW conditions only (P<0.001) and for GY under both WW and WS conditions at Fort Collins 2013-2014 (P<0.001).

The development of a deep and extensive root system is a drought adaptation mechanism to allow water and nutrient extraction from the soil profile. We conducted two studies to investigate the variation in root architecture and related physiological and morphological traits in winter wheat under drought stress. The first study evaluated 30 entries primarily from Colorado, and the second study included 30 entries from seven Great Plains states. Entries were evaluated in a greenhouse in 2012 and 2013 in 1 m x 10 cm plastic tubes filled with a fritted clay medium. Drought stress was imposed by withholding water after the emergence of the fourth leaf. After three weeks without watering, above ground biomass was harvested and roots were separated from the growing medium, washed, scanned, and digitally analyzed. Colorado entries differed significantly (P<0.05) for estimated transpiration, above ground biomass, average root diameter, total root length for bottom, middle, and top sections, and root length in most diameter classes. Great Plains entries differed significantly (P<0.05) for above ground biomass, stomatal conductance, water use efficiency, total root length, and root length for several diameter classes. Total root length adjusted for above ground plant size of Colorado entries ranged from 5212 to 7279 cm and average diameter ranged from 0.33 to 0.40 mm. Total root length correlated positively (P<0.05) with leaf elongation rate and RWC for Colorado entries and total root length correlated negatively with average root diameter for entries of both studies. No significant differences were observed for any root trait between entries with and without *Rht* semi-dwarf alleles. The variation in root traits among Colorado

and Great Plains winter wheat entries can be exploited in breeding programs to help develop plants with the best adapted root systems to withstand drought stress.

Because it is very important to test root performance of entries in the actual environment where they grow, a soil coring study was conducted at multiple environments to directly quantify variation in root traits. The objectives of this study were 1) to determine the variation in root architecture traits among US Great Plains winter wheat germplasm in the field, under water-stressed field conditions, 2) to examine correlations among the evaluated root traits and yield, canopy temperature, CID, plant height and harvest index, and 3) to examine correlations between evaluated root traits in the field and greenhouse. The study was conducted in three location-year environments, each with a different set of entries, all part of the HWWAMP. In all three environments, soil cores were collected from the WS treatment using a 1 m high, 5 cm diameter truck- or tractor-mounted hydraulic soil probe at three depths. Entries at Greeley 2011-2012 WS trial differed significantly for TL, TLTS, TLMS, AD, average diameter in the middle root section (ADMS), and average diameter in the bottom root section (ADBS) (P<0.001). Total root length correlated negatively with $T_c$lh (r=-0.51, P<0.05). The 25 entries at Fort Collins 2012-2013 WS trial differed significantly for TL, TLTS, TLMS, TLBS, AD, and average diameter in the top root section (P<0.01). There was significant (P<0.05) negative correlation between $T_c$bs and three root traits, TLTS, TLMS, and TL (r=-0.42, -0.24, and -0.36, respectively). The 12 entries at Fort Collins 2013-2014 WS trial differed significantly for TL and TLMS (P<0.01). There was a significant negative correlation between TL and $T_c$vg (r=-0.23, P<0.05) and a significant negative correlation between TLMS and canopy temperature at grain filling stage (r=-0.40, P<0.05). Root

traits collected from this study should be regarded as a useful resource to gain insights about wheat adaptation to water stress in the field.

Our results demonstrate the relative importance of several physiological and morphological traits for drought tolerance evaluation in wheat. The most important traits, which showed significant association with grain yield, include root length, carbon isotope discrimination, and canopy temperature. The important chromosomes that comprised QTL for yield and drought tolerance traits in this study are chromosomes 4A, 4D, 2D, 3B, 3A, 2A, 2B, and 1D. Insights gained from this research will help aid our understanding of drought tolerance mechanism of winter wheat and help us define the morphological and physiological traits that define productivity under drought stress.

ACKNOWLEDGEMENTS

It gives me great pleasure to express my gratitude to all those who have supported me and who contributed in making this dissertation possible. First, my supervisor Dr. Patrick Byrne for his support, guidance, motivation and help during my PhD course at CSU. Dr. Byrne's dedication to his students and myself is beyond any expectations, I never wished for another advisor.

I express my deepest gratitude to my committee members, Dr. Scott Haley, Dr. Louise Comas, and Dr. Yaling Qian for their guidance and supervision of this dissertation, as well as their valuable advice throughout the process.

I would like to show my thanks to everyone in the Byrne lab, especially Mr. Scott Reid who was of great help to me during this PhD, as well as the wheat breeding program crew for their help in harvesting and providing the necessary machinery.

Finally, I would like to thank every individual of my family, especially my wife Asmaa, my mother, my father, my sisters, and my brothers for their continuous support and prayers during the course of four years.

DEDICATION

I dedicate this dissertation to three persons. First, my son, Adam who was the major motive for me to finish writing this dissertation to be able to see him again as soon as possible. Second, my brother Mohammed who was the best brother, father, and friend and if it was not for him, I would have never made it that far with education. Third, I dedicate this dissertation to Aude, who was always there for me in the hardest times of this journey.

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CHAPTER 1: LITERATURE REVIEW

Wheat production and uses

Wheat (*Triticum aestivum* L.) is among the world's most important food crops and is grown on a larger land area than any other crop worldwide (Reynolds et al., 2011). Wheat is the third most important cereal crop next to maize (*Zea mays)* and rice (*Oryza sativa* L.) in annual production (Graybosch and Peterson, 2012). The Food and Agriculture Organization of the United Nations (FAO, 2013) estimates that one-fifth of the total calories of the world's population comes from wheat, making it an important component of global food security (FAO, 2013). In human history, wheat cultivation enabled sufficient amounts of food to be produced for the first time, which in turn supported the growth of cities and enabled the rise of human civilizations, starting with the great Middle Eastern empires of Babylon and Egypt (Curtis and Halford, 2014).

Wheat origin and domestication

The domestication of wheat approximately 10,000 years ago in the Fertile Crescent helped humankind's transition from hunter-gathering and nomadic herding to sedentary farming. The earliest cultivated forms of wheat were diploid (genome AA) (einkorn, *Triticum monococcum*) and tetraploid (genome AABB) (emmer, *Triticum dicoccon*) species that originated from the south-eastern part of Turkey (Shewry, 2009). A natural cross between two wild grass species, *T. urartu* (genome AA, 2n=2x=14) with an unknown species of which the closest relative is *Aegilops speltoides* (genome BB, 2n=2x=14), resulted in the appearance of wild emmer, *T. dicoccoides* (tetraploid genome AABB, 2n=4x=28) (Daud and Gustafson, 1996).

Wild emmer selection for larger heads resulted in its subsequent domestication (*T. diccocum*) and its evolution into modern durum wheat (*T. turgidum ssp. durum*). A second natural cross occurred between cultivated emmer (tetraploid genome AABB, 2n=4x=28) and the wild grass *Ae. tauschii* (genome DD, 2n=2x=14). This rare combination resulted in the creation of the hexaploid wheat *T. aestivum* (hexaploid genome AABBDD, 2n=6x=42) (Kihara, 1944; McFadden and Sears, 1945, 1946). Hexaploid wheat has a large genome size of about 17,300 Mb which is approximately 35 times and 110 times larger than that of rice and *Arabidopsis thaliana*, respectively (Hussain et al., 2007). Repetitive DNA elements account for approximately 90% of the wheat genome, and transposable elements make up 80% (Wanjugi et al., 2009).

Plant abiotic stresses and water scarcity

Environmental stresses, such as drought, salinity, extreme temperatures, and radiation represent the most limiting factors for the growth of plants and agricultural production. The set of mentioned stresses, termed as abiotic stress, is the main cause of crop loss worldwide (Rodríguez et al., 2005). Every year a very large amount of annual crop production is lost due to abiotic stress. Furthermore, the amount of productive arable land is continuously decreasing, meaning that crop production will be forced to move to lands where abiotic stresses, specially drought, is even harsher (Passioura, 2007). Drought stress is a major limiting factor for agriculture in arid and semiarid areas and is considered the most important cause of yield reduction in crop plants. The worldwide population is growing and the demand for water is increasing at an alarming rate, therefore the availability of water is becoming extremely limited and there is an increasing demand for water efficient crops (Condon et al., 2004).

Drought effects on plants

The effects of drought occur at the morphological, biochemical, and physiological levels and are evident at all phenological stages of plant growth, at whatever stage the water shortage takes place (Kadam et al., 2012). Photosynthesis is one of the major metabolic processes that are directly affected by drought. Responses to drought include a reduction in photosynthesis, decrease in leaf expansion, stomatal closure, impaired photosynthetic machinery, enhanced formation of reactive oxygen species (ROS), premature leaf senescence, decrease in assimilate translocation, and associated reduction in crop production (Farooq et al., 2009a). In addition, the stress imposed by drought conditions affects water relations, such as water use efficiency, relative water content, leaf water potential, stomatal resistance, rate of transpiration and canopy temperature (Farooq et al., 2009b).

Drought stress mechanisms in plants

Plants respond to drought stress by the induction of several morphological, physiological and molecular mechanisms that enable the plant to withstand the stress. Drought resistance mechanisms can be grouped into three categories, i.e., drought escape, drought avoidance and drought stress tolerance (Mitra, 2001). First, drought escape is the ability of a plant to complete its lifecycle before serious soil and/or plant water stress develops. Plants can escape from drought by early flowering and maturity before the stress becomes severe, in the case of terminal drought (Zaman-Allah et al., 2011b). The drawback of the escape mechanism is that it is associated with a yield penalty under favorable growing conditions. Additionally, breeders for well-developed agricultural regions have already optimized crop flowering time to match the growing environments (Passioura, 2007). Secondly, drought avoidance is the ability of plant to

endure periods without significant rainfall, while maintaining a higher water status. Plants can withstand the drought stress by either reducing the water loss by one or more of these mechanisms (I) increased stomatal and cuticular resistance (Meinzer, 1993); (II) reduced radiation absorbed through leaf rolling (Ehleringer and Cooper, 1992); (III) reduced leaf area and/or increased water uptake. Increasing water uptake by roots can be achieved using two mechanisms (I) increased root density and length; (II) increased liquid phase conductance (Jackson et al., 2000). Thirdly, drought tolerance at low water potential is the ability to endure periods without significant rainfall and to endure low tissue water potentials. In this mechanism the plant can resist the drought stress by reserving high turgor with osmotic adjustment, increasing elasticity, or decreasing cell size and/or desiccation tolerance by protoplasmic tolerance (Morgan, 1984). There is also reducing xylem cavitation or increasing embolism repair (Comas et al 2013).

Challenges of breeding for drought tolerance

Drought tolerance is a complex quantitative trait with low heritability. It has a high level of genotype by environment (G×E) interaction and traits such as phenology and plant height can confound plant responses to it (Fleury et al., 2010). Another complexity level of drought is that it is also confounded and usually accompanied with other stresses like heat and salinity (Fleury et al., 2010). Plant breeding has been successful in improving crop performance under drought conditions in the past (Cattivelli et al., 2008), but with the increasing world population and climate change, plant breeders are asked to do more. Combining knowledge about physiological traits, genetic control of drought, and improved understanding of target environments can speed up this process. However, the success of physiology-based breeding

for drought tolerance depends on the genetic correlation of the physiological trait of interest with final grain yield, extent of genetic variability, level of heritability and extent of G×E interactions (Mir et al., 2012) . High throughput and precise phenotyping in target environments is a key to accurately associate the massive amount of genotypic data available today with phenotypic expression of a trait (Tuberosa, 2012).

Breeding for drought tolerance in wheat

Wheat is best adapted to temperate regions but it is also the major cereal grown in arid and semi-arid regions of the world, where nearly 50 % of the area sown to wheat is affected by drought on an annual basis (Trethowan and Reynolds, 2007). The end goal for breeders is increasing productivity under both favorable and limited growing conditions. Trethowan et al. (2002) and others suggest that selecting for maximum yield potential in an ideal environment will correspondingly increase the productivity of elite lines under drought conditions. Nevertheless, the cross-over effect that is often seen between yield potential and stress tolerance presents a challenge in selecting a single, ideal variety that will maximize yield in multiple environments (Blum, 2011). The negative relationship between yield and stress tolerance may only hold true for extreme drought conditions, while selection for improved drought tolerance in water stressed environments shows a greater increase in yield gain when the varieties are targeted for drought stressed environments (Trethowan et al., 2002). Trethowan et al. (2001) suggested that a selection technique that may be beneficial for improving both maximum yield potential and drought tolerance is alternating the selection environment between stressed and ideal conditions. Consequently, selection for elite lines should involve the improvement of certain constitutive traits, such as basic root architecture,

plant size and vigor, and early maturity that are known to be beneficial in the majority of environments (Blum, 2011). The constitutive traits (depending on the population of target environments) can be utilized as secondary selection criteria to allow for maintenance of high yield potential while increasing the rate of gain for yield in water-stressed environments.

Traits for improving productivity under drought

In the past few decades, there have been many studies attempting to identify physiological traits that can contribute to drought stress tolerance in wheat and other plants (Foulkes et al., 2007; Jongdee et al., 2002; Mir et al., 2012; Rampino, et al., 2006; Reynolds et al., 2007; Richards et al, 2007; Richard, 2006; Waines and Ehdaie, 2007). However, in order for a drought tolerance trait to be introgressed into a breeding program, any potential negative impact of the trait in a favorable environment must be avoided or at least minimized, especially when it comes to yield. Associating physiological traits with their contribution to yield for the target environment has been shown to increase success of parental selection through trait-based strategic crosses (Mir et al., 2012). However, the specific traits to be selected depend on the population of target environments in which the crop will be grown, therefore making it difficult to generalize the benefit of a single trait in multiple regions of the world (Chenu et al., 2011).

Passioura (1977) suggested that the conceptual framework for yield drought adaptation has three important drivers: (1) water uptake (WU), (2) water-use efficiency (WUE) and (3) harvest index (HI). This framework stimulates thinking about trait-based breeding and genetic dissection of drought-adaptive mechanisms. Several traits have been found to be associated with these yield component drivers. For WU, direct selection for variation in root characteristics

may be unfeasible; therefore, measurements associated with stomatal conductance such as canopy temperature (T_c) provide indirect indicators of water uptake by roots (Reynolds and Tuberosa, 2008). For water use efficiency (WUE), carbon isotope discrimination seems to be the best estimate and is based on higher affinity of the carbon-fixing enzyme (Rubisco) for the more common ^{12}C isotope over the less common ^{13}C. A lower discrimination value indicates higher WUE (Mir et al., 2012). The extreme sensitivity of reproductive processes to drought may result in reproductive failure, which is associated with low HI, and may eliminate benefits associated with favorable WU or WUE. Considering the overall contributions of these three yield drivers, WU is the most important for improving the yield potential in drought environments, while stable HI is associated with higher yield potential in general (Blum, 2009). These traits are often not all present in a single genotype, reflecting the complexity of drought tolerance and the need to pyramid several beneficial traits through plant breeding.

Stomatal conductance

Stomatal conductance measures the rate and passage of carbon dioxide (CO_2) entering or water vapor exiting the stomata via transpiration and plays a significant role in water stress avoidance. For example, stomatal conductance decreases under stress, i.e., plants close stomata to reduce water loss. Reduction in stomatal conductance results in reduction in photosynthetic rate, which in turn has a direct impact on yield and yield components under water–stressed conditions (Lawlor and Cornic, 2002). The opening and closing of stomata in order to control the influx of CO_2 is regulated by environmental conditions such as light, CO_2 concentration, and atmospheric humidity (Blum, 2011). Condon et al. (2007) reported substantial genotypic variation for stomatal attributes among bread wheat varieties. Because of

the difficulty of accurately quantifying leaf stomatal conductance in field conditions, it is usually measured in controlled conditions. Under field conditions, traits such as carbon isotope discrimination and relative water content that are often associated with stomatal conductance are often studied.

Relative water content (RWC)

Relative water content is a measure of the amount of water held in the leaves relative to full turgor and is considered a measure of plant water status. Relative water content reflects the metabolic activity in tissues and is used as a meaningful index for dehydration tolerance (Asif and Kamran, 2011). Maintaining high water content allows normal growth to occur as water becomes scarce. One way a plant can stay closer to full turgor is to lose water at a slower rate by closing stomata or accumulating insulating wax layers (Suprunova et al., 2004). The goal of breeding for drought tolerance is to develop cultivars that have high yield potential under drought conditions instead of being merely able to survive. Plants that are able to maintain high levels of RWC under drought stress are less affected by the stress and able to maintain their growth rate.

Relative water content of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. A decrease in RWC in response to drought stress has been noted in a wide variety of plants as reported by Nayyar and Gupta (2006). Exposure of plants to drought stress substantially decreases leaf water potential, relative water content and transpiration rate, with a concomitant increase in leaf temperature.

Root system architecture

The development of a deep and extensive root system is a drought adaptation strategy that allows the plant to efficiently acquire water and nutrients from deep in the soil profile and helps the plant to meet evapotranspiration demands. Root growth and distribution play an important role in plant response to water availability, are central in determining the growth and yield of crops in water limited environments, and are determined by both plant genotype and the soil environment (Hund et al., 2008). Moreover, a plant root provides a dynamic interface between plants and soil by providing the chlorenchyma cells of stems and leaves with a steady supply of water and dissolved minerals.

There is evidence that under moderate stress, when crop production is economically feasible, mechanisms providing drought avoidance (such as water acquisition from deeper in the soil profile) and increased WUE are effective in maintaining yield under stress conditions without detrimental effects on yield under favorable conditions (Collins et al., 2008). Improved water acquisition as a result of size, depth and architecture of the root has been related to increased drought tolerance in wheat (Christopher et al., 2008) and small cereals (Richards, 1991).

It is crucial to target specific physiological mechanisms and to identify those traits most relevant to the patterns of drought stress found in the target environment. For instance, in crops grown with residual soil moisture that experience terminal drought, genotypes with deeper, more profuse roots have an advantage through better water extraction deeper in the soil profile (Kashiwagi et al., 2005). In other crops, also a deeper/profuse root system was found to increase plant access to water from deeper soil layers and support greater crop growth

under drought conditions (Sinclair, 2011). Therefore, deeper, profuse roots are targeted to improve grain yield under rainfed conditions in wheat (Reynolds et al., 2007).

Most of the previous studies on root morphology have indicated that they are intrinsically difficult to conduct, due to the plastic nature of root development, which shows a high interaction with the experimental environment (Gregory et al., 2009; Kato et al., 2006; McKenzie et al., 2009; Passioura, 1983; Richards and Passioura, 1989; Smith and De Smet, 2012). In addition, because of their underground location, it is difficult to observe roots, especially in a non-destructive manner during the growing season. It is also difficult to extricate roots from the soil, because roots tend to be fragile (Lynch, 2007; Passioura, 1983). The majority of root studies have therefore relied on above ground estimates of root characteristics (such as root pulling force, root lodging, and number of brace roots) or on the phenotype of root systems from early stages of plant development, grown under artificial conditions that are more amenable to measurement, such as hydroponics, artificial media or root tubes in the greenhouse. Using such methods, QTL for root traits have been identified in Arabidopsis (Rauh et al., 2002), rice (Courtois et al., 2000), and maize (Tuberosa et al., 2002). The challenge with these systems is to demonstrate that the root characteristics of young plants grown under these conditions are correlated with the root characteristics of the mature field-grown plants, their response to nutrient- and water stress, and final yield. Evidence in support of the use of these 'artificial' systems includes the identification of overlapping QTL for root traits measured in hydroponic systems, root traits measured in the field under two different water regimens, and grain yield in maize (Tuberosa et al., 2002). Major QTLs for root length were detected in rice using a soil-column-based system (Courtois et al., 2000). These results support the

exploration and implementation of artificial systems as an alternative to laborious and expensive field-based phenotyping and can be applied on wheat.

Depending on the specific method used, root trait screening in controlled environments may offer the advantage of examining a relatively large number of genotypes with reduced environmental variation. However, it is unclear if these controlled environment screens, generally conducted on seedling root systems, translate to larger root systems in the field at the time of grain development (Wasson et al., 2014). Therefore, screening for deep roots in the field may have an advantage over controlled environments for breeding programs as an appropriate field site better represents the target environment. There have been few attempts from researchers to develop field-based root phenotyping. “Shovelomics”, for example, has been used in maize for field excavation of mature root crowns and followed by root separation and digital imaging which enables a relatively high throughput analysis needed for breeding and quantitative genetics (Colombi et al., 2015a). Soil coring at multiple field environments can directly quantify variation in root traits (Wasson et al., 2014).

Carbon isotope discrimination (CID) and Water use efficiency

Drought tolerance is a complex trait that can be estimated as the ratio of yield under water stress vs. non-stressed conditions (Reynolds et al., 1999). However, measuring yield is expensive and time consuming. Several parameters with high correlations with drought tolerance have been proposed as indirect indicators including carbon isotope discrimination (CID), canopy temperature depression (T_c), and canopy spectral reflectance (CSR) (Prasad et al., 2008). Carbon isotope discrimination integrates the response of the plant over the growing season and is negatively correlated with transpiration efficiency. Carbon isotope discrimination

has been used in wheat as a selection tool for improved yield in rainfed environments of Australia (Condon et al., 2004).

Selection efficiency for CID in breeding programs will be enhanced through a better understanding of its underlying genetic control and this can be achieved through identification of QTL and linked markers for CID (Chen, et al., 2012). Quantitative trait loci for CID have been reported across a range of plant species including *Arabidopsis thaliana* (Juenger et al., 2005), barley (*Hordeum vulgare*) (Forster et al., 2004), and rice (Takai et al., 2009). However, few QTL have yet been described for CID in wheat. Rebetzke et al. (2008) reported that QTL for CID are repeatable across environments and wheat mapping populations. The same researchers also showed that some of the CID genomic regions were associated with variation in heading date and/or plant height to confound genotypic associations between CID and grain yield. However, after removing the effect of height and heading date, strong correlations were observed for CID and both yield and biomass across populations (Rebetzke et al., 2008).

Canopy temperature (T_c)

Canopy temperature is a non-destructive high-throughput measurement and is related to the plant's ability to cool leaves through transpiration. It reflects the ability of the roots to access water (Reynolds and Tuberosa, 2008). Canopy temperate is highly correlated with several canopy spectral reflectance (CSR) indices, biomass, and leaf water potential (Babar et al., 2006). Canopy temperature can be related to many aspects of plant water status, as leaf cooling is associated with the ability of the plant to transpire. Moreover, increases in water stress through lower water status will slow transpiration and increase T_c. Olivares-villegas et al. (2007) conducted trials on wheat over three years in various environmental conditions in

Mexico and reported that T_c was the single-most drought-adaptive trait contributing to higher performance. The trait was highly heritable and consistently associated with grain yield. Canopy temperature has been found to be strongly associated with several water stress related traits such as stomatal resistance (Jones et al., 2009), leaf water potential (Blum et al., 1982), soil moisture extraction (Olivares-villegas et al., 2007), CID (Zhu et al., 2008), and soil water content (Lopes and Reynolds, 2010). These studies indicate the importance of maintaining a low T_c through more efficient soil moisture extraction, which is translated into higher yields under drought.

Quantitative trait loci (QTL) mapping

Many important traits for drought tolerance like yield, leaf area and length, and flowering time, are controlled by many genes and are known as quantitative traits. To facilitate detecting and estimating the effect of the QTL controlling those traits, and then to utilize them for crop improvement, high density genetic maps (linkage maps) constructed with molecular markers are helpful. Identifying QTL influencing the response of yield and its components to water deficits aids in our understanding of the genetics of drought tolerance and helps in development of more drought tolerant cultivars.

To effectively utilize new alleles for yield, drought tolerance, and WUE, genetic characterization is required. The standard approach is to construct bi-parental crosses between tolerant and susceptible parents for water stress response and then phenotype and genotype (with molecular markers) progeny populations to determine the number and chromosomal location of loci controlling the trait. Traditional (biparental) QTL mapping involves (1) development of mapping populations segregating for drought tolerance related traits, (2)

identification of polymorphic markers, (3) genotyping of the mapping populations with polymorphic markers, (4) construction of genetic maps, (5) precise phenotyping for drought tolerance-related traits, as mentioned above, and (6) QTL mapping using both genotypic and phenotypic data (Mir et al., 2012).

As reviewed in Cattivelli et al. (2008) and Fleury et al. (2010), a large number of linkage mapping studies have been conducted in several crops to identify QTLs linked to drought tolerance. However, linkage mapping does not provide precise information on QTL. This is because of some essential limitations associated with each mapping population such as (1) insufficient time for recombination to occur and shuffle the genome into small fragments, and as a result the QTLs identified are generally localized to large genomic regions/chromosomal segments, (2) insufficient phenotypic variation for the trait present in the mapping population and (3) segregation of different QTL for the same trait in different mapping populations (Myles et al., 2009).

Association mapping (AM)

An alternative approach that does not require development of biparental crosses and several generations of progeny is association mapping. It was initially used in human genetics, and has been suggested as an alternative approach for linkage mapping in crop species (Rafalski, 2010). Association mapping (AM) analysis, also known as Genome-wide Association Study (GWAS) and linkage disequilibrium (LD) mapping is a population-based survey used to identify marker-trait relationships based on LD (Flint-Garcia et al., 2003). With association mapping, statistical assessments are made for associations between genotypes based on molecular markers and phenotypes of various traits in reference germplasm sets (Buntjer et al.,

2005). Theoretically, this technique can be applied to any set of germplasm to detect QTL for as many traits as show variation.

According to Mir et al. (2012), AM has many advantages compared to traditional QTL mapping. These include: "(1) exploitation of all the recombination events that took place during the evolutionary history of a crop species, resulting in much higher mapping resolution, (2) less time required in mapping QTL as there is no need to develop a specialized mapping population, rather a natural germplasm collection of a crop species is sufficient, (3) cost-effectiveness because the same AM panel and genotyping data can be used for mapping of different traits, (4) populations can be structured to avoid randomly generated lines (recombinant inbred lines; RILs), many of which express substandard agronomic type and (5) a higher number of alleles can be sampled compared to linkage mapping where only two alleles are usually surveyed". The most important advantages of AM include increased resolution for mapping QTL, greater capacity for detecting more alleles, and faster completion time (Zhu et al., 2008). Since its first use with plants in 2001 (Thornsberry et al., 2001), AM has gained wide application in many important crop plants because of advances in high throughput genotyping technologies, increased interest in identifying novel alleles, and improvements in statistical methods (Zhu et al., 2008).

Population structure

To minimize statistical error in AM, correction for population structure is critical in a collection of genotypes, especially when genetic relationships among breeding lines are highly variable. In essence, this is due to the fact that genotypes in the collection for AM analysis are rarely independent samples given the geographical origins, local adaptation, and breeding

history of these genotypes (Zhu et al., 2008). Population structure refers to populations that deviate from Hardy-Weinberg proportions. False positive association between markers and traits can result from the absence of physical proximity due to population structure caused by admixture, mating system, and genetic drift or by artificial or natural selection during evolution, domestication, or plant improvement (Hirschhorn and Daly, 2005). False associations can also result from alleles occurring at very low frequencies in the original population (Breseghello and Sorrells, 2006). Population structure creates LD between loci that are not physically linked and cause a high rate of false positives between markers and phenotypic traits and should be corrected for (Crossa et al., 2007). Thus, separating LD due to physical linkage from LD due to population structure is a critical prerequisite in association analyses. In modern breeding populations, population structure can be caused by complex pedigrees derived from crosses of parents with different levels of relatedness (Crossa et al., 2007).

To decrease type I error rates (false positives) Yu et al. (2006) incorporated the outcome of population structure (Q matrix), with the estimation of relatedness between individuals obtained through the marker-based kinship matrix (K) into a unified linear mixed-model approach. This approach was effective in increasing the power of the marker–trait association tests through removing the confounding effects of population structure.

While the overall approach of association mapping in plants varies based on the methodology chosen, assuming structured population samples, the performance of association mapping includes the following steps as described by Abdurakhmonov and Abdukarimov (2008):

1) Selection of a group of individual lines or cultivars with wide genetic and phenotypic diversity to form the mapping population or panel;

2) Recording the phenotypic characteristics;
3) Genotyping the mapping population with available molecular markers;
4) Quantification of the extent of LD for a chromosome and/or a genome using molecular marker data of the mapping panel;
5) Assessment of the population structure and kinship (coefficient of relatedness between each pair of individuals);
6) Determination of association of phenotypic and genotypic data based on the information gained from LD and population structure using appropriate statistical methods.

QTL discovery for yield and drought tolerance-related traits

Genetic studies conducted for wheat under water-stressed environments have detected QTL underlying yield and yield component traits (El-Feki, 2010; Pinto et al., 2010; Pinto et al., 2007) using linkage mapping. Quantitative trait loci linked to drought tolerance traits and identified using AM, have been reported in wheat (Maccaferri et al., 2011), barley (Varshney et al., 2012) and maize (Lu et al., 2010). These QTL were identified for a variety of important traits including yield and yield contributing traits under water-deficit conditions, as well as physiological traits like CID and T_c[1].

Many QTL with minor effects have been detected for yield, but repeatable QTL across environments and genetic backgrounds are rare. This situation has undermined the

[1] An updated compilation of mapped QTL and major genes associated with abiotic stress tolerance including drought tolerance in crop plants is available at PLANTSTRESS site (http://www.plantstress.com/biotech/index.asp?Flag=1).

transferability of QTL information into practice in plant breeding programs to increase yield genetic gain for under water-stressed environments. Therefore, focusing on the identification and utilization of QTL for traits related to drought tolerance alongside yield per se may be an appropriate approach. After validation, QTL detected can be utilized in breeding through marker-assisted selection (MAS).

CHAPTER 2: GENOME-WIDE ASSOCIATION STUDY OF A WINTER WHEAT ASSOCIATION MAPPING PANEL UNDER TWO WATER REGIMES

Summary

Drought is one of the most important environmental challenges farmers face around the globe, with water stress the main cause for yield loss. Therefore, the objectives of this study were to 1) evaluate the hard winter wheat association mapping panel (HWWAMP) for agronomic and drought tolerance related traits in multiple environments differing for soil moisture; 2) determine the relationship between yield and other agronomic and physiological traits, and 3) identify chromosomal regions/quantitative trait loci (QTL) involved in drought tolerance through association analysis. The HWWAMP consists of 299 entries adapted to the U.S. Great Plains region. The panel was characterized using a high-density 90 000 single nucleotide polymorphism (SNP). Field evaluations for the HWWAMP were conducted in two sites in a side-by-side experiment under two soil moisture regimes in two years (2011-12 at Greeley, CO and 2012-13 at Fort Collins, CO). In addition, a replicated confirmation study was conducted in two sites (Greeley and Fort Collins in 2014) to evaluate field performance of a subset of 50 entries.

At Greeley 2011-2012, genotypes differed significantly for grain yield (GY) in both well-watered (WW) and water-stressed (WS) trials ($P<0.001$). Moreover, genotypes differed significantly ($P<0.001$) for total biomass (TBM), biomass grain weight (BGW), harvest index (HI), plant height (Ht), relative water content (RWC), and carbon isotope discrimination (CID) in the

well-watered (WW) trial and differed significantly (*P*<0.001) for BGW, HI, Ht, canopy temperature at late heading stage ($T_c$lh), and RWC in the water-stressed (WS) trial. Under WW conditions, GY correlated positively (*P*<0.0001) with BGW and HI (*r*=0.71 and 0.76, respectively) and negatively with Ht (*r*=-0.60). Under WS conditions, GY correlated significantly (*P*<0.001) with TBM, BGW, and HI (*r*=0.18, 0.50 and 0.61, respectively), and negatively with Ht (*r*=-0.17, *P*<0.05). At Fort Collins 2012-13, genotypes differed significantly (*P*<0.001) for GY in both WW and WS trials. Moreover, genotypes differed significantly (*P*<0.001) for BGW, HI, Ht, RWC, and canopy temperature at booting stage ($T_c$bs) in the WW trial and differed significantly (*P*<0.001) for TBM, BGW, HI, Ht, RWC and $T_c$bs in the WS trial. Under WW conditions, GY correlated significantly (*P*<0.0001) with TBM, BGW, and HI (*r*=0.39, 0.52, and 0.42, respectively) and with Ht (*r*=0.13, *P*<0.05). GY also correlated negatively with $T_c$bs (*r*=-0.35, *P*<0.0001). Under WS conditions, GY correlated significantly (*P*<0.0001) with TBM, BGW, HI, Ht, and $T_c$bs (*r*=0.37, 0.53, 0.51, 0.27, and -0.38, respectively).

A total of 331 significant (*P*<0.001) marker-trait associations (MTA) was detected in one or more environment for 10 measured or calculated traits (GY, TBM, BGM, HI, Ht, $T_c$bs, $T_c$lh, canopy temperature at grain filling stage ($T_c$gf), CID, and drought susceptibility index (DSI)). Multi-trait chromosome regions were detected and particularly the region on chromosomes 4A and 4D associated with GY and CID may be useful in MAS, following proper validation. This study confirmed some of the major QTL for GY and drought related traits previously described in biparental mapping populations and highlighted a novel set of MTA for yield and drought-related traits that should be further validated and utilized in MAS. In the confirmation study at Greeley 2013-2014, genotypes differed significantly for GY under WW conditions only

(P<0.001). At Fort Collins 2013-2014, genotypes differed significantly for GY under WW and WS conditions (P<0.0001).

The insights gained from this research will aid our understanding of drought tolerance mechanisms of winter wheat and help us identify the morphological and physiological traits that define productivity under drought stress. The MTA detected for yield and other important agronomic and physiological traits under WW and WS conditions can be utilized in breeding programs through marker-assisted selection after being validated in other environments and genetic backgrounds.

Introduction

Drought is one of the most important environmental challenges farmers face around the globe. Water stress is the main cause for yield loss every year in dry regions of both developed and developing countries. Because of climate change, water stress will likely increase, further jeopardizing food security if mitigation efforts are not undertaken. Identifying QTL influencing the response of yield and its components to water deficits will aid our understanding of the genetics of drought tolerance and will help in development of more drought tolerant cultivars. To effectively utilize new alleles for yield, drought tolerance, and water use efficiency (WUE), genetic characterization is required. The standard approach is to construct bi-parental crosses between water stress tolerant and susceptible parents and then phenotype and genotype (with molecular markers) progeny populations to determine the number, size, and chromosomal location of loci affecting relevant traits. An alternative approach that does not require development of bi-parental populations is association mapping (AM) or linkage disequilibrium (LD) mapping. With AM, statistical assessments are made for associations between genotypes

based on molecular markers and phenotypes of various traits in reference germplasm sets (Buntjer et al., 2005). Theoretically, this technique can be applied to any set of germplasm and detect QTL for as many traits as show variation.

Compared to traditional QTL mapping, AM has three main advantages: increased resolution for mapping QTL, greater capacity for detecting more alleles, and faster completion time (Zhu et al., 2008). Since its first use with plants in 2001 (Thornsberry et al., 2001), AM has gained wide application in many important crop plants because of advances in high throughput genotyping technologies, increased interest in identifying novel alleles, and improvements in statistical methods (Zhu et al. 2008). However, AM has some limitations including the risk of Type I error (false positives), which can be higher in AM compared to biparental QTL mapping because of population structure. Estimates of population structure or kinship are used in a linear mixed effects model to reduce the frequency of false positive associations. In addition, principal components analysis (PCA) corrects for stratification in genome-wide association studies (Price et al., 2006). Another drawback of AM is the high sampling variance of rare alleles. In AM, rare alleles are excluded from the analysis. On the other hand, the relative value of rare alleles can be assessed in biparental cross populations.

Carbon isotope discrimination has been used in wheat as a selection tool for improved yield in rainfed environments of Australia (Condon et al., 2004). Several studies suggest that CID, through its negative relationship with water-use efficiency (WUE), is a good index for selecting stable yielding crops in rain-fed environments (Chen et al., 2012). Identification of QTL for CID will enhance its use efficiency in breeding programs. Chen et al. (2012) phenotyped two populations of barley containing 200 and 127 recombinant inbred lines (RILs) for leaf CID and

agronomic traits under rain-fed environments in Alberta, Canada and detected a total of 12 QTL for leaf CID in one of the populations and 5 QTL in the other. For one of the populations, a major QTL located on chromosome 3H was identified and overlapped with several agronomic traits, such as plant height, leaf area index, grain yield, harvest index and days to maturity. Quantitative trait loci for CID were also identified in rice using backcross inbred lines (Xu et al., 2009). Quantitative trait loci for CID were repeatable across Australian environments and wheat mapping populations (Rebetzke et al., 2008). However, there are not enough studies of QTL discovery for CID in either winter wheat or using genome-wide association mapping; this study will try to fill this knowledge gap.

Canopy temperature (T_c) is a non-destructive high-throughput measurement and is related to the plant's ability to cool leaves through transpiration and reflects the ability of the roots to access water (Reynolds and Tuberosa, 2008). Canopy temperate is highly correlated with several canopy spectral reflectance (CSR) indices, biomass, and leaf water potential (Babar et al., 2006). Olivares-Villegas et al (2007) concluded that "canopy temperature was the single-most drought-adaptive trait contributing to a higher performance, highly heritable and consistently associated with yield" after conducting wheat trials over three years and several environmental conditions in Mexico. Canopy temperature has been found to be strongly associated with several important traits related to drought tolerance, including soil moisture extraction (Olivares-Villegas et al., 2007) and CID (Zhu et al., 2008). Lopes and Reynolds (2010) reported phenotypic correlations of yield with vegetative T_c and soil water content at depth (r=-0.91 and -0.96, respectively). Canopy temperature was measured by Mason and Singh (2014) in both heat and drought stressed field experiments in northwest Mexico on 18 breeding trials

totaling 504 spring wheat lines from the International Maize and Wheat Improvement Center (CIMMYT) Irrigated Bread Wheat program and low T_c was associated with high yield (r=−0.26) across all trials. These findings points out the importance of plant's ability to maintain a low T_c under drought stress conditions through efficient soil moisture extraction, which is related to higher productivity.

There have been several studies attempting to discover QTL for T_c and yield under water stress, most of them using biparental QTL mapping. Using a double haploid wheat population in multiple heat and water stress environments, Bennett et al (2012) detected two QTL, located on chromosome 3B. They had a large effect on canopy temperature and grain yield, accounting for up to 22 % of the variance for these traits. One locus on chromosome arm 3BL was detected under all three treatments tested but had its largest effect under the heat stress conditions.

Using a GWAS for grain yield and related traits spring wheat population of 287 elite spring wheat lines grown under temperate irrigated high-yield potential conditions in Obregón, Mexico, during four crop cycles, and using high-density Illumina iSelect 90K single nucleotide polymorphisms (SNPs) assay for genotyping, Sukumaran et al. (2014) were able to identify SNPs in chromosome 5A and 6A that were significantly associated with yield and yield components. Four loci were detected for yield on chromosomes 3B, 5A, 5B, and 6A and the locus on 5A explained 5 % of the variation for grain number per m^2. Using GWAS in in a spring wheat association mapping panel (n = 285–294) in two contrasting water regimes, Edae et al. (2014) were able to identify a stable QTL for grain yield on chromosome 2DS both under irrigated and rain-fed conditions and a multi-trait region significant for yield and yield components on chromosome 5B. Grain yield QTL on chromosome 1BS co-localized with a harvest index QTL.

A limited number of studies have investigated drought susceptibility index (DSI) via QTL analysis in wheat and was mostly using the biparental QTL mapping approach. Kirigwi et al. (2007) identified QTL for DSI on chromosome 4A in a recombinant inbred line wheat population (n=127) with a range of 13 to 48% of phenotypic variation. After validation in relevant genetic backgrounds and environments, QTL detected for yield, yield components and drought tolerance-related traits in such studies may be used in marker-assisted selection in wheat breeding programs.

To my knowledge, there are few studies especially in the Great Plains region that have used association mapping as a methodology to identify QTL for yield and drought related traits such as T_c and CID in winter wheat.

The overall goal of this project was to use phenotypic and genotypic data from Great Plains winter wheat germplasm to detect QTL that improve yield under drought stress conditions in winter wheat. The specific objectives of this study were

I. To evaluate the Hard Winter Wheat Association Mapping Panel (HWWAMP) for yield and drought tolerance related traits in multiple fully irrigated and moisture-stressed environments;
II. To examine correlations among the evaluated traits; and
III. To use phenotypic data in combination with genomic data to perform a GWAS, to identify QTL for agronomic and physiological traits (grain yield, biomass weight, harvest index, plant height, canopy temperature (T_c), and carbon isotope discrimination (CID)).

Materials and Methods

Plant materials

Hard Winter Wheat Association Mapping Panel (HWWAMP)

The entire HWWAMP developed by the Triticeae Coordinated Agricultural Project (TCAP) (http://WWW.triticeaecap.org), consists of 299 cultivars and advanced lines (Table A.1). Genotypes or "entries" in the panel included 193 cultivars and 106 breeding lines, of which 258 were hard red winter wheat and 41 were hard white winter wheat. Nine public breeding programs contributed 270 entries and four private breeding programs contributed 27 entries. The public breeding programs that contributed to the HWWAMP were Colorado, Kansas, Michigan, Montana, Nebraska, North Dakota, Oklahoma, South Dakota, and Texas. The private breeding programs were AgriPro-Syngenta (APS), WestBred-Monsanto (WES), Goertzen Seed Research, and Hardeman Grain and Seed. The historic entries included in this panel were the landrace Turkey; the two ancestral cultivars Cheyenne and Kharkof; and five cultivars released before 1960 (Comanche, Wichita, Kiowa, Bison, and Tascosa). More information about the HWWAMP can be found in the T3 database (https://t3sandbox.org/t3/sandbox/wheat/).

Confirmation subset of HWWAMP

A subset of 50 cultivars and lines of the HWWAMP was used in a confirmation study of grain yield and canopy temperature and their relationship in two sites in Colorado (Greeley and Fort Collins) in 2013-14. The entries were chosen based on several criteria from the previous two years. Thirty-three of the 299 entries with very extreme phenological data were excluded based on BLUPs of heading date for 2011-12 and 2012-13 trials (both well-watered and water-stressed). The entries that were included in the confirmation subset are described below.

1. The two repeated check cultivars in the modified augmented field design (Hatcher and Settler CL).
2. The five wheat varieties with greatest planted acreage in Colorado in 2012- 2013 (Hatcher, Ripper, TAM 111, Snowmass, and Bill Brown).
3. Four entries (Prairie Red, TAM 112, TX06A001, and CO07W24) that were in the top 90% quantile for yield in both years' water-stressed trials.
4. The highest-yielding entries in the dry treatment in both years (listed in descending order of yield).
    A. Greeley 2011-12: TX06V7266, TX99U8618, Endurance, and Ronl.
    B. Fort Collins 2012-13: TAM 112, NE05548, TX06A00113, and OK06336
5. Four entries (TAM 112, Ogallala, TX06A00113, and NI08708) that were ranked high for yield in both years trials (WW and WS).
6. Three entries (OK05134, OK06318, and CO03W043) that yielded high in the dry treatment, and low in the wet treatment in both years.
7. Three entries (Nell, Bronze, and Comanche) that yield low in wet and dry treatments in both years.
8. Four entries (HV9W03-1379R, Spartan, Platte, and Yumar) that yielded high in wet treatment and low in the dry treatment in both years.
9. Thirteen entries (OK06319, Sturdy 2k, Larned, TX06V7266, NE05548, Prairie Red, Ronl, TX06a001386, Triumph64, OK05134, OK06318, CO03w043, OK10119*)* with below-average reduction under stress compared to the well-watered treatment in both 2012 and 2013, that also had above-average yield under stress in both 2012 and 2013.

10. Three entries with high yields in the dry treatment that also had high yields (among top 10%) in the wet treatment, for both 2012 and 2013.
    a. Greeley 2011-12 (TX06V7266, Ronl, and Prairie Red).
    b. Fort Collins 2012-13 (NE05548, Prairie Red, and OK05134).
11. Eight entries were select based on grain-filling (GF) duration ("long" >97.5% quantile, "short" <2.5%) and yield ("high" >50%, "low" <50%) in the dry treatment of each year: Cheyenne (2012, long GF, low yield), SD05118 (2012, long GF, high yield), HV9W06-504 (2013, long GF, high yield), Shocker (2013, short GF, low yield), Caprock (2013, long GF, low yield), TX99U8618 (2012, short GF, high yield), Karl 92 (2013, short GF, high yield), Dumas (2012, short GF, low yield).
12. Top five ranked entries across all environments (TAM 112, Endurance, TX06A001132, Byrd, and CO07W245).
13. Bottom four ranked entries across all environments (Wichita, Comanche, Bronze, and Kharkof).
14. Three entries chosen based on breeders recommendations (Akron, CK050, and CO940610).
15. Three entries based on root traits in greenhouse and field studies (Jules (high bottom root length), Hail (low bottom root length), and CO04393 (high total root length).

Experimental design and growing conditions

Greeley, 2011-12

Evaluation of the HWWAMP in 2011-12 was conducted at the USDA-Agricultural Research Service Limited Irrigation Research Farm in Greeley, CO (latitude 40.4484 N; longitude 104.636

W; elevation 1427 m). The soil at the Greeley site is mesic Usti Haplargids and mesic Aridic Arguistolls. It is well drained with fine sandy loam to clay loam texture and a pH range of 7.4-8.4. Side-by-side trials were planted on October 30, 2011, one designed to experience moisture stress through limited irrigation, and the other well-watered to avoid significant moisture stress throughout the growing season. The experimental design was an unreplicated modified augmented design with 15 repeated check plots each of two elite cultivars: "Hatcher" and "Settler CL" to control for spatial variation. Water was applied using a surface drip tube irrigation system. Irrigation was supplied to both treatments beginning approximately at the booting stage (Zadoks stage 40, Zadoks et al., 1974) targeted at reducing yield of the limited irrigation trial to 50% of the full irrigation trial. Water-stressed (WS) and well-watered (WW) trials received a total of 181 and 417 mm water between January 1 and physiological maturity, respectively (including 82 mm of precipitation). Climatic data were collected by an on-site weather station and indicated that the Greeley site experienced 28 days with highs above 30 °C (86 °F) between January 1 and July 1, 2012. The water stressed (WS) and well-watered (WW) treatments were harvested on July 3d and 13th 2012, respectively. Average plot length was 305 cm, average plot width was 158 cm, and average harvested plot area was 3.6 m^2. Seeding density was approximately 700,000 seeds ha^{-1} in both treatments.

Fort Collins, 2012-13

In 2012-13 the HWWAMP was evaluated at CSU's Agricultural Research, Development, and Education Center (40.652 N, 104.996 W, and elevation 1558 m) in Fort Collins. The trials were designed as modified augmented designs with one replicate and incorporating the two repeated checks (15 each) as described for Greeley 2011-12. Side-by-side trials were grown

with two moisture levels: fully irrigated (well-watered) and partially irrigated (water stressed). The trials were planted on October 2, 2012. The soil at this site is mesic Aridic Haplustalf. More moisture was received during the spring of 2013 than 2012, including late snow around jointing stage but the temperatures were still hot during grain filling. Water was applied using a linear overhead sprinkler irrigation system. Supplemental irrigation of 38 mm was applied prior to planting for both treatments. Irrigation was delayed at approximately the booting stage (Zadoks scale 40) until 20 May 2013, due to early spring rainfall as well as cool temperatures at this site. Once initiated, irrigation was applied on a weekly basis to the well-watered trial only, with the first three applications at 31 mm, the fourth, fifth and sixth applications at 38 mm, and the seventh application at 12 mm; the total amount of water applied was 260 mm for the well-watered treatment only. Trials were harvested on July 16-17, 2013 and plot size and seeding rate were as described for Greeley 2011-12.

Greeley, 2013-14

Three side-by-side trials were intended to provide severe drought stress, moderate drought stress, and well-watered conditions for evaluations of a subset of 50 HWWAMP entries. Each trial was a Latinized row-column design with two replications. The trials were planted on October 9, 2013 and harvested on August 5, 2014. Irrigation was applied using a surface drip tube irrigation system. Due to plentiful rain pre-planting (107 mm) and in spring, three distinct moisture levels could not be arranged, so the severe and moderate stress trials were combined into a single rainfed trial with four replications and the well-watered trial received 108 mm of irrigation water, starting May 26 until June 21.

Fort Collins, 2013-14

The experimental design used to evaluate this subset of 50 HWWAMP entries was a Latinized row-column design with two moisture levels, each with three replications. The trial was planted on October 6, 2013 and harvested on July 24-25, 2014. Irrigation was provided by a linear overhead sprinkler system. The well-watered treatment received 95 mm of irrigation water starting May 2 until June 18. The total amount of pre plant precipitation received at this trial was 178 mm.

Agronomic and physiological measurements

Plant height (Ht) was measured when plots in the dry treatment were near or at physiological maturity. At least three measurements were taken from each plot. Plant height was measured to the nearest cm, from soil to tip of spike, excluding awns. The plants sampled were representative of main tillers from across the inner four rows of the plot, and excluded plants at any edge of the plot.

Above ground biomass (TBM) at maturity was measured as the dry biomass of a 1 m long section of a single row per plot cut at ground level. After drying and weighing, the biomass grain was threshed with a Vogel thresher and weighed (BGW) and harvest index (HI) was calculated as follows:

HI = Biomass grain weight / Total biomass weight

Grain yield (GY in kg ha^{-1}) from six-row field plots was collected using a Hege combine (Wintersteiger, Salt Lake City, Utah) after plants reached maturity. Grain samples were dried for at least 3 d at approximately 40°C prior to calculation of GY.

Canopy temperature (T_c)

A CS-1000 datalogger (Cambell Scientific, Logan, Utah) was used to record data from an SI-121 infrared radiometer sensor (Apogee Instruments, Logan, Utah). Measurements for T_c were made in a serpentine walking pattern from south to north of the field at repeated intervals through the late vegetative to early senescence period. Canopy temperature data were the means of approximately 20 measurements per plot, respectively, between 1100 and 1300 h. Canopy temperature was measured with the sensor 30 cm above the outside row of the six-row plots at a perpendicular angle to the rows of the plot. Canopy temperature data were collected from both treatments at Greeley 2011-12 on 6 June (late heading stage). At Fort Collins 2012-13 T_c data were collected for both moisture levels on 23 May (mostly booting stage and beginning of heading), for the dry treatment only on 11 June (grain filling stage), and for the wet treatment only on 25 June (grain filling stage). In the 2014 confirmation study, three T_c measurements from both water treatments in the two experimental locations (Greeley and Fort Collins) were collected at the same times and represented three growth stages (vegetative growth stage ($T_c$vg) which is heading stage, early grain filling stage ($T_c$egf), and late grain filling stage ($T_c$lgf).

Canopy temperature data were recalculated based on an error in the calibration program of the data logger, according to a notification from Campbell Scientific, because our program contains a constant with a number greater than 2,147,483,647, our data had to be corrected through post-processing using the correct calibration coefficients of the IR radiometer. When mC0 exceeds 2,147,483,647 CRBasic inputs this value for the mC0 coefficient, rather than the actual value for mC0, causing an error in the calculation of target temperature. Errors are

dependent on the difference between the actual value of the mC0 coefficient and 2,147,483,647, and on the difference between target temperature and detector temperature. Errors due to calculations with the incorrect coefficient were corrected and in cases where sufficient data had not been retained to correct the target temperature, data were excluded from analysis.

Carbon Isotope Discrimination (CID)

CID was evaluated for the Greeley 2011-12 WW trial. A total of 658 samples were collected from both Greeley 2011-12 treatments as follows: 5 to 10 cm segments near the distal end of flag leaves were collected from seven plants at the grain filling stage in the western row of each plot and dried at 80° C for 48 h. Four to six 4.5 mm steel ball BBs (www.daisy.com, Daisy Rogers, AR) were placed into the test tube with the dried leaf samples, and ground with a paint shaker (Fluid Management SK-650, Wheeling, IL) for 1 min. Approximately 2 mg of the ground leaf tissue was weighed and sealed into a tin cup (Tin Capules 5 x 9 mm cups, Costech Analytical Technologies Inc., Valencia, CA). The cups were then placed in 96-well tray boxes, in which four wells were allocated to control samples of ground maize leaves. The tray-boxes were sealed and sent to the Stable Isotope Facility at the University of California-Davis for CID analysis. Only samples from the WW treatment were sent for analysis for two reasons. This was for two reasons: first because from the literature it seems that it is better to measure CID when soil moisture is favorable and measurements of CID reflects transpiration efficiency and WUE and not confounded by the variation in soil moisture (Condon et al., 1992; Richards et al., 2010) and second, because of the high cost per sample.

Relative water content (RWC)

Relative water content was measured for a subset of 70 entries from Greeley 2011-12 on May 10, 2012 (average heading dates were May 17 and May 14, 2012 for the WW and WS trials, respectively). Relative water content was measured for the 30 Colorado entries and 30 additional entries that were randomly selected as well as 10 check plots of Hatcher and Settler CL from each treatment. Likewise, RWC was measured for a subset of 70 HWWAMP entries from Fort Collins 2012-13 on June 17, 2013 (average heading dates were May 30 and May 29, for the WW and WS trials, respectively). A section of the leaf just below the flag leaf (about 7-10 cm long) was collected from three separate leaves in the outer row of the plot and bulked together in tubes. The leaf sections were immediately weighed for fresh weight value (FW) followed by sealing them in a 50 ml tube containing 5 ml of de-ionized water. The leaf samples were allowed to saturate for 4 h and then re-weighed to obtain the turgid weight (TW). Leaf samples were then placed in a dryer for about 48 hours at 68°C. Following drying, samples were measured for dry weight (DW) and RWC was calculated using the following formula from Barr and Weatherley (1962):

RWC (%) = [(FW - DW) / (TW - DW)] * 100

Drought susceptibility index (DSI)

A drought susceptibility index (DSI) was calculated from mean GY following the method of Fischer and Maurer (1978): DSI = (1-Yd/Yw)/D, where, Yd = mean yield of an entry under drought, Yw = mean yield of an entry under well-watered conditions, and D = environmental stress intensity = 1- (mean yield of all entries in the water stressed treatment/mean yield of all genotypes in the well-watered treatment). A higher value for DSI indicates greater susceptibility to stress.

Phenotypic data analyses

All statistical analyses were conducted in SAS (version 9.3, SAS Institute Inc., Cary, NC), unless otherwise stated. Spatial variation in experiments with a modified augmented design was accounted for by using the best fit of six different SAS spatial models (row-column, spherical, exponential, power, anisotropic power, and Matérn) using the PROC MIXED procedure. The best model was chosen based on the Akaike Information Criterion (AIC) fit statistic (the lower the better), and best linear unbiased predictions (BLUPs) were calculated. Entries, rows, and columns were considered as random effects. The significance of entries was tested in every environment by using the covtest option in the mixed model statement. In the Fort Collins 2012-13 WS trial, Row 1 was located on the very western edge of the TCAP HWWAMP field, adjacent to a field of sorghum and separated by a single pass of 'Prairie Red' wheat. The entire row 1 pass had very poor growth, probably due to differences in irrigation (not having access to sprinkler heads on both sides). In the analysis, plots in row 1 were given a border effect value of one, while all other rows received values of zero. This border effect was used as a covariate in the analyses. In the 2014 confirmation study, replications were included in the class statement and were treated as random effects as well as entry, row and column. Pearson correlation coefficients were obtained for all pairs of traits using BLUPs in the CORR procedure. Graphs were constructed using Microsoft Office Excel (Microsoft, 2013) and JMP (version 11, SAS Institute Inc., Cary, NC).

Genome-wide association study (GWAS)

A genome-wide association study (GWAS) was performed on multiple traits including GY, TBM, BGW, HI, Ht, and T_c in four environments, Greeley 2011-12 (WW and WS) and Fort Collins

2012-13 (WW and WS), as well as for CID in one environment Greeley 2011-12 WW (Gr12 WW). The BLUPs calculated from the best-fit model were used as means for all traits in the analysis. DNA from the HWWAMP was characterized at the USDA-ARS Small Grains Genotyping Lab in Fargo, North Dakota using a high-density 90 000 single nucleotide polymorphism (SNP) Illumina iSelect genotyping platform as described in Wang et al. (2014). Genotype calling was performed using the GenomeStudio Polyploid Clustering Module v1.0 Software developed in collaboration with Illumina, Inc. Semi-automated curation removed monomorphic SNPs, multi-allelic SNPs, SNPs with low call rate, SNPs that produced diffuse clusters, and SNPs with smaller cluster distances to produce a set of 26,553 SNPs. Manual curation returned 21,555 SNPs calls on 299 genotypes, of which 21,546 SNP were missing <10 % of calls. The minor allele frequency (MAF) of 5,497 SNPs was <5 %. SNPs with MAF <5 % and SNPs with ≥10 % of calls missing were removed to produce a set of 16,052 filtered SNPs (M. Guttieri, University of Nebraska-Lincoln, personal communication). These 16,052 SNPs were used by the software package GAPIT (Genomic Association and Prediction Integrated Tool) in R (Lipka et al., 2012) to perform the GWAS. Map positions were obtained from http://wheatgenomics.plantpath.ksu.edu/ (download date: May 22, 2015). Of the 16,052 filtered SNPs, 14,829 were uniquely mapped and an additional 1,223 markers were unmapped and assigned chromosome number 99 and sequential positions. Distribution of SNPs varied among genomes, and included 5,971 in the A-genome, 7,244 in the B-genome, and 1,614 in the D-genome (Table 2.1).

The GWAS was conducted to identify genomic regions associated with traits in different treatments and years. A kinship matrix was estimated by M. Guttieri (University of Nebraska-Lincoln, personal communication) in the rrBLUP package for R (Endelman, 2011) and was

incorporated in the analysis. Several models were considered in the analysis including 1) the compressed mixed linear model (MLM)(Yu et al., 2006; Z. Zhang et al., 2010) with principal components (PC, Price et al., 2006) and kinship (P+K), where kinship was calculated externally by rrBLUP, 2) regular mixed linear model, where each individual is considered as a group, with P+K, with K calculated externally by rrBLUP, 3) the compressed mixed linear model with P+K, with K calculated internally by GAPIT, and 4) regular mixed linear mixed model with P+K, with K calculated internally by GAPIT.

Because the degree of correlation with population structure varies from trait to trait, the number of PCs selected to account for population structure in the GWAS may vary. Therefore, GAPIT was allowed to use the Bayesian information criterion (Schwarz, 1978) (BIC)-based model selection to find the optimal number of PCs . Between zero and three PCs were allowed in the P+K models and different models were allowed with different traits. The percentage of variation explained by the marker (R^2) was calculated as the difference between R^2 for the model with the SNP and R^2 for the model without the SNP. The false-discovery rate (FDR) adjusted *P*-value (Benjamini and Hochberg, 1995) was very stringent (Müller et al., 2011). For example, adjusted *P*-value ranged from 0.21 to 0.91 for GY significant MTA (based on unadjusted *P*-value) at Gr12 WS environment. Because the false-discovery rate (FDR) adjusted *P*-value failed to identify many significant markers, marker-trait associations (MTA) were concluded based on an unadjusted *P*-value at a less stringent significance level ($P<0.001$).

Results

The Greeley 2012 field season recorded earlier warm temperatures in the spring and many hotter days compared to the Fort Collins 2013 season. Recorded mean daily temperature

from January to June at Greeley 2012 was higher than the mean daily temperature at Fort Collins 2013 by 3.2°C. The highest difference in temperature was recorded in April, when the difference in the mean daily temperature was 7.2°C (11 % higher at Greeley). This resulted in accumulation of more growing degree days by heading in Greeley 2012 than Fort Collins 2013. Calendar days to heading ranged from 125 to 148 d with an overall mean of 137 d at Gr12 WW, and from 127 to 141 d with an overall mean of 134 d at Gr12 WS. At FC13 WW, calendar days to heading ranged from 146 to 156 d with an overall mean of 150 d and from 146 to 160 d with an overall mean of 149 d at FC13 WS. Total amount of rainfall differed considerably between Gr12 and FC13 with 82.1 mm at Gr12 and 124.4 mm at FC13.

Greeley, 2011-2012

Genotypes differed significantly for GY, BGW, HI, Ht, and RWC in both the WW and WS trials (P<0.001) (Table 2.2). Genotypes differed significantly (P<0.0001) for TBM in the WW trial and for $T_c$lh in the WS trial. Percent reduction from WW to WS ranged from 2 % (RWC) to 51 % (TBM). Details about trait means, ranges and percent reduction from WW to WS are contained in Table 2.2. Genotypes differed significantly for DSI (P<0.001). Drought susceptibility index ranged from 0.12 to 1.37 with an overall mean of 0.97 (Table 2.2). This range indicates that some entries (with DSI >1.00) had greater yield reductions than the overall mean, and others (with DSI <1.00) were less susceptible to drought stress. Moreover, genotypes differed significantly for CID in the WW trial (the only environment tested) (P<0.0001, Table 2.2). In the WW trial, BLUPs for CID ranged from -28.74 to -25.38 with an overall mean of -27.07 (Table 2.2). Moisture level (WW vs. WS) or treatment effect was significant (P<0.001) for five traits (TBM, BGW, HI, GY, Ht) but not significant for two traits (T_c and RWC) (P>0.05, Table 2.2).

Trait correlations

Total biomass correlated significantly (P<0.001) with BGW (r=0.46, P<0.0001) under WW conditions, and with BGW, GY, and Ht (r=0.54, 0.18, and 0.19, respectively) under WS conditions (Table 2.3). Biomass grain weight correlated positively (P<0.0001) with HI and GY (r=0.81, and 0.71, respectively), and negatively with Ht (r=-0.46) under WW conditions. Under WS conditions, BGW also correlated positively and significantly (P<0.0001) with HI and GY (r=0.57 and 0.50, respectively) (Table 2.3). Harvest index correlated positively with GY and negatively with Ht (P<0.0001) under both WW and WS conditions (r=0.76 and -0.61 under WW and r=0.61 and -0.29 under WS). Moreover, grain yield correlated negatively with Ht under WW and WS conditions (r=-0.60, P<0.0001, and r=-0.17, P<0.05, respectively). Carbon isotope discrimination (CID) in the WW treatment correlated significantly (P<0.05) with BG, HI, and Ht (r=0.13, 0.15, and -0.14, respectively) (Table 2.3). When CID was tested for correlation with traits measured under WS conditions, it only correlated significantly and negatively with TBM (r=-0.15, P<0.05) (data not shown). Under WW conditions, DSI correlated significantly (P<0.0001) and positively with $T_c$lh, BGW, HI, and GY (r=0.12, 0.57, 0.63, and 0.81, respectively) and negatively with Ht (r=-0.48 and P<0.0001) (Table 2.3). Under WS conditions, DSI correlated significantly (P<0.0001) and positively with HI (r=0.32) and negatively with TBM and Ht (r=-0.15 and -0.38, respectively, P<0.05) (Table 2.3).

Correlations of traits between moisture treatments

To investigate the extent of genotype by environment interaction, a correlation analysis was conducted between BLUPs for the same trait from both WW and WS environments. Only $T_c$lh had no significant correlation between wet (WW) and dry (WS) environments (Table 2.4),

and all other traits had a varied range of significant positive correlations between the two environments (*P*<0.0001 for all traits except TBM, *P*<0.05). Harvest index had the strongest correlation between the WW and WS trials, followed by GY, Ht, BGW and finally TBM (*r*=0.61, 0.48, 0.48, 0.24, and 0.14 respectively) (Table 2.4).

Fort Collins, 2012-2013

Genotypes differed significantly for GY, BGW, HI, Ht, RWC, and $T_c$bs in both WW and WS trials (*P*<0.001, Table 2.5). Genotypes differed significantly (*P*<0.05) for TBM in the WS trial. Canopy temperature at early grain fill stage ($T_c$egf) was collected from the WS trial only and genotypes differed significantly for it (*P*<0.0001), while canopy temperature at late grain fill stage ($T_c$lgf) was collected from the WW trial only and genotypes differed significantly for it (*P*<0.0001) (Table 2.5). Percent reduction from WW to WS ranged from 10 % (HI) to 46 % (GY). Details about trait means, ranges and percent reduction from WW to WS are contained in Table 2.5. Genotypes differed significantly for DSI (*P*<0.001). Drought susceptibility index ranged from 0.65 to 1.20 with an overall mean of 0.99 (Table 2.5). Moisture level (WW vs. WS) or trial effect was significant (*P*<0.001) for all traits (GY, TBM, BGW, HI, Ht, and $T_c$bs) collected from both trials except for RWC (*P*-value=0.066, Table 2.5).

Trait correlations

Canopy temperature at booting stage correlated negatively with TBM, BGW, Ht, and GY under WW (*r*=-0.32, -0.25, -0.14, and -0.35, respectively, *P*<0.05). Under WS conditions $T_c$bs correlated significantly (*P*<0.0001) with TBM, BGW, Ht and GY (*r*=-0.24, -0.24, -0.28 and -0.38, respectively). Total biomass correlated significantly (*P*<0.0001) with BGW, Ht, and GY (*r*=0.87, 0.35, and 0.39, respectively) under WW conditions, and with BGW, HI, GY, and Ht (*r*=0.88, 0.12,

0.22, and 0.37, respectively, $P<0.05$) under WS conditions (Table 2.6). Biomass grain weight correlated positively ($P<0.001$) with HI, GY, and Ht (r=0.48, 0.52, and -0.18, respectively) under WW conditions and with HI, GY, and Ht (r=0.53, 0.53, and 0.21, respectively) under WS conditions (Table 2.6). Harvest index correlated positively with GY and negatively with Ht ($P<0.0001$) under WW (r=0.42 and -0.32) and correlated positively with GY under WS (r=0.51, $P<0.0001$). Moreover, GY correlated significantly ($P<0.05$) with Ht under WW and WS conditions (r=0.13 and 0.27, respectively) (Table 2.6). Under WW conditions, DSI correlated significantly and positively with TBM, BGW, HI, Ht and GY (r=0.33, 0.40, 0.28, 0.14, and 0.78, respectively, $P<0.0001$) and negatively with $T_c$bs (r=-0.25 and $P<0.0001$) (Table 2.6). Under WS conditions, DSI correlated positively ($P<0.0001$) with $T_c$bs (r=0.24) and negatively with TBM, BGW, HI, Ht, and GY (r=-0.16, -0.24, -0.24, -0.22, and -0.36, $P<0.001$) (Table 2.6).

Correlations of traits between moisture treatments

Three traits ($T_c$bs, TBM, and BGW) had no significant correlation between WW and WS environments (Table 2.7). Plant height, HI, and GY had a significant positive ($P<0.0001$) correlation between WW and WS environments (r=0.59, 0.28, and 0.24, respectively) (Table 2.7).

Genome-Wide Association Study

A total of 331 significant ($P<0.001$) marker-trait associations (MTA) were detected in one or more environment for 10 measured or calculated traits (GY, TBM, BGW, HI, Ht, $T_c$bs, $T_c$lh, $T_c$gf, CID, and DSI) in four environments: Greeley 2012 WW, Greeley 2012 WS, Fort Collins 2013 WW, and Fort Collins 2013 WS. A summary of MTA in different environments for each phenotypic trait is presented in Table 2.8. For the five main traits that were measured in all four

environments, the highest number of MTA was recorded for HI (58) followed closely by GY (57), while the lowest number of MTA was recorded for BGW (18).

Five major genomic regions (2B, 4A, 5A, 5B, and 1D) contained most of the MTA for GY (Table 2.9). The total number of significant (P<0.001) MTA for GY in the four environments was 57, five of them unmapped. The SNPs explained from 3.4 to 5.7 % of the phenotypic variation (Table 2.8). The number of MTA detected for GY under both WW and WS treatments at Greeley 2012 were similar (15 in the WW and 16 in the WS), while at Fort Collins 2013 the number of MTA detected for GY under the WS treatment was much lower than that detected under WW treatment (21 in the WW and 5 in the WS) (Table 2.8).

The total number of significant MTA for TBM in the four environments was 34, all of them mapped. The SNPs explained from 3.7 to 4.9 % of the phenotypic variation (Table 2.8). Four major genomic regions (1A, 2A, 4D, and 7A) contained most of the MTA for TBM (Table 2.10).

The total number of significant MTA for BGW in the four environments was the second lowest for all traits (18), two of them unmapped. The SNPs explained from 3.6 to 5.0 % of the phenotypic variation (Table 2.8). Three major genomic regions (2B, 4D, and 5B) were detected for BGW (Table 2.11). The number of MTA detected for BGW under both WW and WS treatments at Greeley 2011-2012 were the same (three in each environment), while at Fort Collins 2012-2013 the number of MTA detected under WW was double the number at WS (four and eight MTA, respectively) (Table 2.8).

The number of significant MTA for HI in the four environments was the highest amongst all traits (58), six of them unmapped. MTA were distributed across several chromosomes and

genomic regions, however, the three genomic regions (within 10 cM) that contained most of the MTA were 2A, 3A, and 6B, respectively (Table 2.12). The significant SNPs explained from 3.4 to 6.8 % of the phenotypic variation (Table 2.8). At Greeley 2012, the number of MTA for HI was 3 times more in the WW compared to the WS treatment, while at Fort Collins 2013 the number of MTA detected for HI under both treatments was similar (25 in the WW and 21 in the WS (Table 2.8).

The total number of significant MTA for Ht in the four environments was 23 distributed among 10 chromosomes where two genomic regions (4A and 4D) showed relative importance (Table 2.13). The SNPs explained from 3.7 to 6.2 % of the phenotypic variation (Table 2.8). The number of MTA detected for Ht in the WW environment was similar to that detected in the WS (seven and five, respectively) at Greeley 2012, while at Fort Collins 2013, the number of MTA detected under WS was more than WW conditions (eight and three MTA, respectively) ((Table 2.8).

Amongst the three different T_c measurements analyzed for GWAS, canopy temperature at late heading stage ($T_c$lh) had the highest number of detected MTA (50), three of them unmapped. This trait was measured in two environments (Gr12 WW and Gr12 WS) where the number of detected MTA in the WW environment (40) was much higher than that recorded in the WS environment 10) (Table 2.8). The percentage of phenotypic variation explained by the SNPs for $T_c$lh ranged from 3.7 to 5.1 % (Table 2.8). Two genomic regions in chromosome 2D contained most of the MTA for T_cLH in both environments (Table 2.15). These two regions were only 20 cM apart. Moreover, 27 MTA were detected within 6 cM distance in chromosome 3B in the WW treatment. Canopy temperature at booting stage ($T_c$bs) was measured in FC13 under

both water treatments. Two major genomic regions (2D and 6A) had most of the MTA under WS , while under WW conditions, MTA were distributed across 3 chromosomes and one MTA was unmapped (Table 2.14). Canopy temperature at the grain filling stage ($T_c$gf) was measured only at FC13 WS environment and the number of MTA for this trait was 21, all of them with a map position. These MTA were concentrated on three major genomic regions in chromosomes 2A, 2B, and 5A (Table 2.16). The R^2 values ranged from 3.7 to 7.3 % (Table 2.8).

Carbon isotope discrimination (CID) was measured in one environment (Gr12 WW). The number of detected MTA at Gr12 WW was 29, with only one unmapped marker (Table 2.8). Most of the MTA were distributed in three genomic regions (2D, 4A, and 4D) and only one MTA was on chromosome 3D (Table 2.17). R^2 for these SNPs ranged from 3.8 to 5.0 %.

The drought susceptibility index was calculated based on GY in both the WW and WS treatments. The number of detected MTA at Greeley 2012 was more than double the number of MTA detected at Fort Collins 2013 (19 and eight, respectively). Two different major genomic regions were detected in each site: 2A and 5B at Greeley 2012 and 4D and 5D at Fort Collins 2013. Two markers detected at Fort Collins 2013 were unmapped (Table 2.18). R^2 for SNPs ranged from 3.7 to 5.2 %.

A detailed description (SNP name, chromosome number, position, *P*-value, minor allele frequency, and R^2 per SNP) of MTA for measured traits in all environments is presented in Tables 2.9 to 2.18 and Manhattan plots for these traits in different environments is presented in Appendix 2 (A 2.1 to A 2.25).

Confirmation study

Greeley, 2013-2014

Grain yield (GY)

Genotypes differed significantly for GY under WW conditions only (P<0.001). In the WW treatment, BLUPs for GY ranged from 3016 to 6467 kg ha^{-1} with an overall mean of 5130 kg ha^{-1} (Tables 2.19 and 2.20). In the WS treatment, GY ranged from to 2965 to 4203 kg ha^{-1} with an overall mean of 3630 kg ha^{-1} (Tables 2.19 and 2.20). There was significant variation due to moisture level (treatment) (P<0.001, Table 2.19). Yield reduction due to water stress was 29 %.

Canopy temperature (T_c)

Genotypes did not differ significantly for any of the T_c measurements ($T_c$vg; canopy temperature at vegetative stage, $T_c$egf; canopy temperature at early grain fill stage, $T_c$lgf; canopy temperature at late grain fill stage) under either moisture level (WW and WS) (Table 2.19). However, there were significant differences (P<0.0001) due to moisture level in two T_c measurements ($T_c$vg and $T_c$egf) (Table 2.19). The widest range was recorded for $T_c$lgf (from 25 to 32.5 in the WW, and from 26.3 to 32.3 in the WS) (Table 2.19). The highest difference between WW and WS treatments was recorded at the $T_c$egf (1.6°C) followed by $T_c$vg (1.5°C) (Table 2.19). At the Greeley, there were no significant correlations between GY and any of the T_c measurements recorded (P>0.05, Table 2.21).

Correlations of traits between moisture treatments

To investigate the extent of genotype by environment interaction, a correlation analysis was conducted between the same trait BLUPs from both WW and WS environments. Only one

trait, GY, had a significant (P<0.0001) correlation between WW and WS environments (r=0.55, Table 2.22).

Fort Collins, 2013-2014

Gain yield (GY)

Genotypes differed significantly for GY under WW and WS conditions (P<0.0001). In the WW treatment, BLUPs for GY ranged from 4168 to 8324 kg ha^{-1} with an overall mean of 6614 kg ha^{-1} (Table 2.20). In the WS treatment, GY ranged from 3378 to 6718 kg ha^{-1} with an overall mean of 5360 kg ha^{-1} (Table 2.20). There was significant variation in GY due to moisture level (treatment) (P<0.0001, Table 2.20). Yield reduction due to water stress was 15 %.

Canopy temperature (T_c)

Genotypes did not differ significantly for either of the two T_c measurements ($T_c$vg, canopy temperature at vegetative stage; $T_c$gf, canopy temperature at grain fill stage) under either moisture level (WW and WS) (Table 2.20). However, there were significant differences (P<0.0001) due to moisture level in the two T_c measurements ($T_c$vg, P<0.0001 and $T_c$gf, P<0.001) (Table 2.20). Canopy temperature at the vegetative stage ($T_c$vg) ranged from 19.3 to 23.0°C with an overall mean of 21.7°C in the WW treatment and from 18.7 to 21.7°C with an overall mean of 20.3 in the WS treatment (Table 2.20). Canopy temperature at grain fill stage ($T_c$gf) ranged from 17.7 to 23.0°C with an overall mean of 21.6°C in the WW treatment and from 18.7 to 23.0°C with an overall mean of 20.8°C in the WS treatment (Table 2.20). At the Fort Collins WS trial, GY had a significant (P<0.05) negative correlation (r=-0.23) with $T_c$vg in the WW treatment (Table 2.23).

Correlations of traits between moisture treatments

To investigate the extent of genotype by environment interaction, a correlation analysis was conducted between the same trait BLUPs from both WW and WS environments. Only GY had a significant (P<0.0001) correlation between WW and WS environments (r=0.86, Table 2.24).

Discussion

Greeley 2011-12 and Fort Collins 2012-13

Grain yield varied significantly (P<0.001 or P<0.0001) in all four environments studied with a high percentage of yield reduction due to water-stress (47 % at Greeley 2012 and 46 % at Fort Collins 2013). The highest recorded GY grand mean was in the Fort Collins WW trial (4973 kg h^{-1}), and the lowest recorded mean GY was in the Greeley WS trial (2539 kg h^{-1}). In general, GY was higher in the Fort Collins 2012-13 trials compared to the Greeley 2011-12 trials; this may be due to the very hot temperatures and dry conditions recorded in 2012, starting prior to anthesis stage throughout the grain-filling period. The entries in the Greeley 2012 trial were exposed to water stress (as low as 82 mm of rainfall, from January to June) and heat stress (maximum temperature > 30˚C). These conditions may have led to the early heading in the Greeley 2012 trials as an escape strategy. Mean calendar days to heading was lowest in the Greeley 2012 WS (134.3 d) followed by the Greeley 2012 WW (136.7 d), while at Fort Collins 2013, days to heading were almost the same for both trials (149.5 d in the WS and 149.9 in the WW). The optimum temperature for wheat anthesis and grain filling ranges from 12 to 22°C (Farooq et al., 2011) and temperatures above 30°C during floret formation in wheat may lead to complete sterility (Pradhan et al., 2012).

Total biomass weight (TBM) was the only agronomic trait that did not vary significantly in all environments. It varied significantly only in the WW treatment at Greeley 2012 and in the WS treatment at Fort Collins 2013. The range of TBM in the WW treatment at Greeley was very wide compared to the range in the WS treatment (187 to 604 in the WW and 232 to 236 in the WS) (Table 2.2). The range was the opposite at Fort Collins 2013, where it was wider in the WS compared to the WW treatment (Table 2.5). The percent reduction in TBM due to water stress was very high in Greeley 2012 (51 %) compared to Fort Collins 2013 (25 %), and this may be due to the high temperatures and low rainfall recorded in 2012 that led plants to stop investing in biomass (vegetative phase) and transition to the reproductive phase (anthesis) earlier.

Biomass grain weight (BGW) varied significantly in all four environments and a high reduction percentage due to water stress was observed (47 %) which is very close to GY and TBM. Due to the way these traits are measured, they are naturally always associated. The HI grand mean was surprisingly similar in the WS compared to the WW treatment at Gr12 (0.38 and 0.36, respectively, Table 2.2). However, the range of HI was higher in the WW trial (0.11 to 0.59) compared to WS (0.25 to 0.55). Harvest index is calculated as the ratio of BGW to TBM and due to the high water use efficiency under drought stress; plants invest less in above ground biomass, which may lead to high HI under drought stress conditions. At FC13, the grand mean for HI was higher in the WW than WS trial by 10 % (Table 2.5). Harvest index is a complex trait and indicates the efficiency of a crop in converting photosynthetic products or assimilates produced before and after anthesis into final grain yield. Even though HI was not used as a direct selection criterion in wheat yield improvement in the past, the realized yield progress was actually due to an increase in the number of kernels per unit area and a genetic shift

towards greater HI (Zhang et al., 2012). The response of HI to stress depends on the intensity of the stresses. In the absence of stresses or with mild stresses, HI is fairly constant for several crops. Cotton (*Gossypium hirsutum*) and sorghum are two crops for which HI increases under moderate stress (Fereres and Soriano, 2007). However, more severe stresses that are sufficient to reduce biomass production by 30-40 % can reduce HI, and the reduced biomass indicates the intensity of stress a crop has experienced (Fereres and Soriano, 2007). In wheat, HI is determined by the pattern of water use of the crop in the period before and after anthesis (Passioura, 1977).

Plant height (Ht) was severely reduced due to water stress, and the reduction rate was 36 and 22 % at Gr12 and FC13, respectively. The reduction in Ht was reflected in the TBM at both sites. More than 70% of wheat cultivars grown worldwide have a semi-dwarf phenotype controlled by the major genes *Rht-B1b*, *Rht-D1b*, and *Rht8c* (Guedira et al., 2010). The semi-dwarfing genes *Rht-B1b* and *Rht-D1b* were introduced into wheat germplasm as a key part of the Green Revolution and led to significant increases in grain yields after the 1960s (Reynolds and Borlaug, 2006). Most of the HWWAMP entries have a semi-dwarf gene (*Rht-B1b* or *Rht-D1b).* Optimum Ht is required for yield improvement in wheat, as tall plants are susceptible to lodging and excessively short plants are often associated with a yield penalty in stressed environments (Griffiths et al., 2012).

Relative water content (RWC) was measured for a subset of 70 entries only to serve as an indicator of the plant's water status. Relative water content is a measure of the amount of water held in the leaves relative to full turgor and reflects the metabolic activity in tissues. It is used as a meaningful index for dehydration tolerance (Asif and Kamran, 2011). Entries differed

significantly (P<0.05 and 0.001) for RWC in the four environments. The magnitude of reduction due to water stress was very high at FC13 compared to Gr12. Reduction percentage in RWC was 2 and 26 % at Gr12 and FC13, respectively. This may indicate that plants in the FC13 WS treatment were more stressed than plants in the Gr12 WS treatment. However, the water treatment effect was not significant for RWC at either of the two sites (Gr12 and FC13) for this subset of entries. The very small difference in RWC between the two trials in Greeley 2012 is due to unknown reasons.

Canopy temperature was measured at different growth stages in different environments, and we had to exclude many of the collected measurements due to a manufacturer error in the data logger. Canopy temperature at late heading stage was measured at the Greeley 2012 site and entries differed significantly (P<0.0001) in the WS treatment only (Table 2.2). Canopy temperature recorded at the WS treatment was very high compared to the WW; T_c was 16 % higher in the WS treatment compared to the WW one. However, analysis of variance shows this difference as statistically not significant. At FC13, T_c was measured at the booting stage ($T_c$bs) and the grain filling stage ($T_c$egf and $T_c$lgf) and entries differed significantly for both traits under both WW and WS treatments (P<0.0001). Water treatment effect was significant for $T_c$bs, which suggest that we may be able to observe the maximum difference in T_c due to water stress at this stage. Canopy temperature is an important trait and the reduction in T_c is often an indicator of increased soil moisture extraction capabilities due to increased soil exploration by roots (Lopes and Reynolds, 2010). Carbon isotope discrimination (CID) was measured in one environment only (Gr12 WW) and entries differed significantly (P<0.0001) for this trait (Table 2.2). Carbon isotope discrimination is reported to be the best estimate for WUE (Mir et al.,

2012) and is based on higher affinity of the carbon-fixing enzyme (Rubisco) for the more common ^{12}C isotope over the less common ^{13}C.

Grain yield (GY) correlated highly and positively with DSI, HI and BGW (*r*=0.81, 0.76, and 0.71) and negatively with Ht (*r*=-0.60) (table 2.3) in the WW treatment. In the WS treatment GY correlated positively again with HI, BGW, in addition to TBM (*r*=0.61, 0.0.50, and 0.18) and negatively again with Ht (*r*=-0.17) (Table 2.3). Most of these associations were repeated at FC13, where GY correlated positively with DSI, BGW, HI, TBM (*r*=0.78, 0.52, 0.0.42, and 0.39, respectively, Table 2.6) in the WW treatment. Grain yield correlated positively again with BGW, HI, TBM (*r*=0.53, 0.51, and 0.27, respectively) in the WS treatment. All these correlations were expected, but what is interesting is the significant negative correlation between GY and canopy temperature at booting stage ($T_c$bs) in both WW and WS environments (*r*=-0.35 and -0.38 in WW and WS, respectively, Table 2.6). Canopy temperature ($T_c$bs) also correlated negatively with TBM, BGW, and DSI (*r*=-0.32, -0.25, and -0.25, respectively, Table 2.6) in the WW treatment, and negatively with TBM, and BGW (*r*=-0.24 and -0.24 respectively) in the WS treatment. Canopy temperature was correlated negatively with DSI under the WW treatment and negatively with the same trait under the WS treatment (*r*=-0.25 and 0.24, respectively, Table 2.6). These results mean that a reduction in Tc at booting stage was highly correlated with increasing GY under both WW and WS conditions, which is similar to previous studies (Lopes and Reynolds, 2010; Olivares-Villegas et al., 2007). The plant's ability to maintain transpiration and thus a cooler canopy temperature under stress is associated with the crop's ability to maintain soil moisture extraction despite drying soils (Lopes and Reynolds, 2010).

In this study, $T_c$bs, which can be expressed as canopy temperature at vegetative stage was found to be more negatively associated with GY values than canopy temperature at heading and grain fill stages under both water treatments. This may be because variation in spike morphology after heading as well as the number of spikes per area can confound results for T_c at grain filling stage compared to Tc at the vegetative stage (Ayeneh et al., 2002).

Drought susceptibility index (DSI) was not significantly associated with grain yield in most of the environments. The drought susceptibility index (DSI) is derived from the yield difference between non-stress and stressed environments and has been used as a criterion for distinguishing drought tolerant genotypes from susceptible ones. It is best to benefit from this trait through comparisons among entries, which is not feasible in this study, giving the large number of entries being studied.

Genotype by environment interaction analysis

In this study, the treatment effect was significant for all traits except two (RWC and $T_c$lh) in both sites (Gr12 and FC13). The analysis of the magnitude of Genotype × Environment interaction showed that all traits had a significant correlation between WW and WS except $T_c$lh (P>0.05) at Gr12. The highest correlation at Gr12 was recorded for HI followed by GY and Ht, while the lowest correlation was recorded for TBM followed by BGW (Table 2.4) This analysis suggests that the latter traits (TBM and BGW) had a high magnitude of G × E compared to the earlier mentioned ones (HI, GY, and Ht). Meanwhile, $T_c$lh shows the highest magnitude of G × E amongst all measured traits at Greeley 2012 site. This was relatively confirmed at Fort Collins 2013 (FC13) site, where the same three traits (HI, GY, and Ht) recorded the highest associations

between WW and WS with different rank. Total biomass and BGW, in addition to canopy temperature at booting stage did not show a significant correlation at this site (Table 2.7).

Genome-wide association mapping

The purpose of this study was to characterize the HWWAMP with the intention of identifying QTL underlying yield and drought tolerance-related traits. While the panel was assembled with the intention to restrict the range of phenology which otherwise can mask detection of QTL (Pinto et al., 2010; Reynolds et al., 2009), entries in the panel had wide diversities in morphological characters and agronomic traits, heading dates ranged from 125 to 160 d in the four environments tested. We applied the genome-wide association mapping approach to study the underlying genetic basis of phenotypic variation for traits evaluated under different water regimes in two different sites and years. The panel was phenotyped under well-watered and water-stressed (primarily rainfed) conditions, and the amount of water applied through irrigation or received through precipitation ranged from as high as 417.3 mm in Gr12 WW to as low as 123 mm in FC13 WS. Moreover, entries at Gr12 experienced higher mean daily temperatures than FC13 for the majority of the days before anthesis and throughout the grain filling stage. Given these environmental differences and expected G × E interactions, different GWAS results were anticipated for the four environments.

Population structure can lead to false MTA if not taken into consideration during GWAS (Crossa et al., 2007). The newly developed GWAS statistical methods based on mixed linear model (MLM) hold great promise to overcome such challenges (Lipka et al., 2012). To decrease type I error rates (false positives), the unified linear mixed-model approach (Yu et al. 2006), which incorporates the outcome of population structure (Q matrix), with the estimation of

relatedness between individuals obtained through the marker-based kinship matrix (K) was used in GAPIT. The kinship matrix was estimated in rrBLUP and by GAPIT's Van Raden algorithm and was incorporated in the analysis, while the number of PCs varied among traits based on the best model fit. This approach is effective in increasing the power of the MTA tests through removing the confounding effects of population structure.

The total number of significant MTA for GY was 57, with the highest number detected in the FC13 WS (21) followed by Gr12 WS (16) (Table 2.8). MTA for GY were distributed across 11 chromosomes, including, 15 SNPs on the A-genome, 21 on the B-genome, 17 on the D-genome, and 4 unanchored SNPs. The range of phenotypic variation in GY explained by these MTA was from 3.4 to 5.6 %. The largest number of significant MTA was detected on chromosome 2B (13), followed by 1D (11), 5A (8), 4A (6), 5B (5), and 2D (4), respectively. One MTA was detected on chromosomes 6B, 3D, and 4D. Similarly, Edae et al. (2014) reported QTL for GY on chromosomes 4A, 1B, 5B, and 2B. Moreover, Maccaferri et al. (2008) reported QTL for GY on chromosomes 5A, 1B, 2B, and 4B, Sukumaran et al. (2014) detected QTL for GY on chromosome 5A, Lopes et al. (2014) detected QTL for GY on chromosome 2D, and Crossa et al. (2007) detected QTL for GY on chromosome 1B. Pinto et al. (2010) detected a yield QTL on 4A which explained 27 and 17% of variation under drought and heat stress, respectively. At the same location, a QTL explained 28% of the variation in T_c under heat.

The MTA for GY detected in this study were environment specific, which is probably due to the complex genetic basis of GY and its interaction with environmental conditions and because different genotypes can attain similar phenotypes via different morpho-physiological traits and corresponding gene networks (Maccaferri et al., 2011). Dodig et al. (2012) also

detected QTL on chromosome 2D that explained about 22 % of the phenotypic variation for GY. The highly significant grain yield MTA on 2D is probably due to a grain yield QTL in proximity to the *Ppd-D1* locus, or due to *Ppd-D1* itself, which is known for its influence on wheat yield through optimization of flowering time (Worland, 1996).

Furthermore, many markers consistently associated with traits such as TB, HI, BH, BGY, and DSI reside near the yield QTL position on 5B, indicating the importance of this region in influencing yield and yield components. In previous studies, yield QTL have been detected on both long and short arms of chromosome 5B (Crossa et al., 2007; Neumann et al., 2011) and some of those QTL may overlap with the QTL detected here on chromosome 5B.

Fifteen out of the 29 (more than half) MTA detected for CID in the GR12 WW environment (the only environment tested), were located on chromosome 4A, within 5 cM, while eight MTA were located on chromosome 4D that were spaced less than 5 cM apart, suggesting two important genomic regions or QTL for CID on these chromosomes. The QTL effects were fairly large for this trait, accounting for 3.8–5.0 % of the total phenotypic variance (Table 2.17). Many MTA for GY were detected on chromosome 4A, suggesting that this region may have pleiotropic genes for GY and CID. Marker *Tdurum_contig50698_601* (position 64.9) on chromosome 2D had significant associations with both GY and CID, and marker *BS00043664_51*, which is significantly associated with GY, colocalized with three significant markers for CID on chromosome 4A (position 71.7), suggesting a pleiotropic effect. Two more MTA for GY (Excalibur_c44713_137 and IACX3190) were within only 3 cM from three significant markers for CID on chromosome 4A (Tables 2.9 and 2.17). Similarly, Rebetzke et al. (2008) detected stable QTL for CID on chromosome 4A and concluded that QTL for CID are repeatable

across environments and wheat mapping populations. These researchers also showed that after removing the effect of plant height and heading date, strong genotypic correlations were observed for CID and both yield and biomass across populations (r= 0.29–0.57, P<0.05) as might be expected for the favorable experimental conditions. Therefore, selection for CID appears beneficial in increasing grain yield and biomass in favorable environments. However, care must be taken to avoid confounding genotypic differences in CID with plant height and flowering time when selecting for improved biomass and GY especially in environments experiencing terminal drought (Rebetzke et al., 2008).

Twenty-seven MTA for T_c at late heading stage were detected on chromosome 3B, these markers were within 7 cM, suggesting a QTL for T_c at this genomic region (positions from 60.6 to 66.7 cM). Pinto et al. (2010) detected a QTL at the same location, which explained 14 % of T_c variation under drought. On chromosome 4D, two significant markers for CID (*BobWhite_rep_c64527_134* and *Tdurum_contig53072_1935*) were located less than 1 cM apart from two significant markers for $T_c$gf (*Excalibur_c33675_410* and *wsnp_Ex_c33675_42124657*).

Environment-specific MTA were detected for TB, BGW, HI, and Ht, showing the presence of higher genotype by environment interaction for these traits. While there was a wide range in mean phenotypic values for Ht, we detected plant height MTA in the regions of previously reported QTL on chromosomes 3B (Maccaferri et al., 2011), 5B (McCartney et al., 2005) and 6A (Spielmeyer et al., 2007). Many chromosome regions were associated with drought tolerance-related traits such as DSI, T_c, and CID. Overall, the most important chromosomes that comprised QTL for drought tolerance in this study are chromosomes 4A, 4D, 2D, 3B, 3A, 2A, 2B,

and 1D. Stacking QTL that control traits of interest from different chromosome regions into one background is challenging and time-consuming in plant breeding. However, using multi-trait markers in marker-assisted selection (MAS) may increase QTL pyramiding efficiency. Traits sharing the same MTA could be under the same genetic control and markers in those multi-trait regions could be used in the future for improvement of these traits through MAS (Edae et al., 2014).

Multi-trait chromosome regions have been detected and particularly the region on chromosome 4A and 4D associated with GY and CID traits may be useful in MAS, following proper validation. QTL regions that control CID can imply the possibility of using this trait for indirect assessment of GY. This study confirmed some of the major QTL for GY and drought related traits previously described in biparental mapping populations and highlighted a novel set of MTA for yield and drought-related traits that should be further validated and utilized in MAS.

Confirmation study

The confirmation study conducted in 2013-14 at two different sites (Greeley and Fort Collins) and with multiple water treatments, confirmed some of the results from the original study. In this study, only two traits were investigated, GY and T_c at different stages. Entries differed significantly (P<0.001) for GY in the WW treatment only (Table 2.19) at the Greeley site, and for both treatments at the Fort Collins site (P<0.0001, Table 2. 20). Grain yield at the Fort Collins site was higher again, confirming the previous two years and there was a very small yield reduction rate due to water stress at the Fort Collins site. This is due to the excessive rainfall that was received at this site. The negative association of GY with T_c at the vegetative

stage was confirmed in the FC site, where GY correlated significantly (P<0.05) and negatively with $T_c$vg in the WW treatment (r=-0.23) (Table 2.23), but this correlation was not repeated in other environments. Treatment effect was significant (P<0.0001) at both sites and the test of G × E showed that T_c has a high G × E interaction compared to yield in both sites (Tables 2.22 and 2.24) which confirms the previous study. The same subset was planted again this year (2014-15) in Dailey, CO and further conclusions can be made based on the repeated measurements.

Table 2.1 Distribution of markers across genomes and chromosome in the hard winter wheat association-mapping panel (HWWAMP), provided by M. Guttieri (University of Nebraska-Lincoln, personal communication).

Chromosome number	Genome		
	A	B	D
1	998	1188	420
2	915	1101	520
3	747	1065	230
4	713	425	52
5	754	1475	153
6	920	1182	117
7	924	808	122
Total SNPs per genome	5,971	7,244	1,614
Total mapped SNPs	14,829		
Total unmapped SNPs	1,223		

Table 2.2 Mean values for BLUPs, standard errors, and ranges of the hard winter wheat association mapping panel (HWWAMP) (n=299) for traits measured under well-watered (WW) and water-stressed (WS) treatments at Greeley, CO in 2011-2012.

	Environments									
	Greeley 2012 WW				**Greeley 2012 WS**				**Treatment**	
Traits†	Mean	SE	Range	Entry *P*-Value	Mean	SE	Range	Entry *P*-Value	Treatment *P*-Value	% Reduction
GY	4780	54.59	1711 to 6604	***	2539	65.57	1945 to 3104	***	**	47
TBM	477	22.16	187 to 604	***	234	7.74	232 to 236	ns	**	51
BGW	168	73.90	52 to 269	***	89	3.16	62 to 128	***	**	47
HI	0.36	0.13	0.11 to 0.59	***	0.38	0.01	0.25 to 0.55	***	**	-6
Ht	91	1.45	70 to 111	***	58	0.75	47 to 73	***	**	36
RWC	86	1.77	66 to 92	***	84	2.82	71 to 90	**	ns	2
$T_c$lh	28.1	13.18	27.6 to 28.5	ns	32.5	10.69	30.7 to 34.0	***	ns	-16
CID	27.07	0.54	-28.74 to -25.38	***						

*, **, ***, Significance for entries and treatment at 0.05, 0.001, and 0.0001 probability levels, respectively; ns; non-significant at 0.05 level.

† % reduction, percentage of reduction in trait value from WW to WS treatments; SE, standard error; GY, grain yield (kg ha^{-1}); TBM, total biomass weight at maturity (g); BGW, biomass grain weight (g); HI, harvest index; Ht, plant height (cm); RWC, relative water content (percentage); $T_c$lh, canopy temperature collected at late heading stage; CID, carbon isotope discrimination.

Table 2.3 Phenotypic correlations among agronomic and physiological traits at Greeley 2011-12 under well-watered (below diagonal) and water-stressed (above diagonal) treatments (n=299).

Traits†	$T_c$lh	TBM	BGW	HI	GY	Ht	DSI
$T_c$lh		-0.06ns	-0.10ns	-0.09ns	-0.08ns	-0.03ns	-0.06
TBM	-0.01ns		0.54***	-0.05ns	0.18**	0.19***	-0.15*
BGW	0.09ns	0.46***		0.57***	0.50***	-0.04ns	0.05
HI	0.11ns	-0.03ns	0.81***		0.61***	-0.29***	0.32***
GY	0.10ns	0.11ns	0.71***	0.76***		-0.17*	-0.02
Ht	-0.11ns	0.10ns	-0.46***	-0.61***	-0.60***		-0.38***
DSI	0.12*	0.05ns	0.57***	0.63***	0.81***	-0.48***	
CID	0.03ns	-0.02ns	0.13*	0.15**	0.08	-0.14*	0.07ns

*, **, *** Significant at *P*-values <0.05, 0.001, and 0.0001 probability levels, respectively; ns, non-significant at 0.05.

† $T_c$lh, canopy temperature at late heading stage (°C); TBM, total biomass weight at maturity (g); BGW, biomass grain weight (g); HI, harvest index; GY, grain yield (kg ha^{-1}); Ht, plant height (cm); DSI, drought susceptibility index; CID, carbon isotope discrimination.

Table 2.4 Phenotypic correlations between the same trait measured in both water treatments (well-watered and water-stressed) at Greeley, 2011-12 (n=299).

Traits†	*r*
$T_c$lh	0.03ns
TBM	0.14*
BGW	0.24***
HI	0.61***
GY	0.48***
Ht	0.48***

*, **, ***, significance level of correlation at P-value 0.05, 0.001, and 0.0001; ns, non-significant at 0.05 level.

† r, correlation coefficient; n, number of observations used; Tclh, canopy temperature at late heading stage (°C); TBM, total biomass weight at maturity (g); BGW, biomass grain weight (g); HI, harvest index; GY, grain yield (kg ha-1); Ht, plant height (cm).

Table 2.5 Mean values, standard errors, and ranges of the hard winter wheat association mapping panel (HWWAMP) (n=299) for traits measured under well-watered (WW) and water-stressed (WS) treatments at Fort Collins, CO in 2012-2013.

	Environments									
	Fort Collins 2013 WW				Fort Collins 2013 WS				Treatment	
Traits†	Mean	SE	Range	Entry P-Value	Traits†	Mean	SE	Range	Entry P-Value	Treatment P-Value	% Reduction
GY	4973	169.07	3959 to 6395	***	GY	2687	92.04	2318 to 3316	**	**	46
TBM	305	10.89	269 to 351	ns	TBM	229	7.88	196 to 274	*	**	25
BGW	122	4.74	97 to 156	*	BGW	77	3.07	65 to 96	*	**	37
HI	0.40	0.003	0.38 to 0.45	*	HI	0.36	0.005	0.28 to 0.37	*	**	10
Ht	79	1.23	66 to 100	***	Ht	62	1.87	42 to 75	***	**	22
RWC	82	3.03	74 to 88	*	RWC	61	36.03	34 to 87	***	ns	26
$T_c$bs	24	0.36	23 to 26	***	$T_c$bs	27	0.41	25 to 30	***	**	-13
$T_c$lgf	31	0.41	29 to 33	***	$T_c$egf	35	0.49	31 to 38	***	na	na

*, **, ***, Significance for entries and treatment at 0.05, 0.001, and 0.0001 probability levels, respectively; ns, non-significant at 0.05 level; na, not available.

† % reduction, percentage of reduction in trait value from WW to WS treatments; SE, standard error; GY, grain yield (kg ha^{-1}); TBM, total biomass weight at maturity (g); BGW, biomass grain weight (g); HI, harvest index; Ht, plant height (cm); RWC, relative water content (percentage); $T_c$bs, canopy temperature collected at booting stage; T_cLgf, canopy temperature collected at late grain fill stage; $T_c$egf, canopy temperature collected at early grain fill stage.

Table 2.6 Phenotypic correlations among agronomic and physiological traits at Fort Collins, 2012-13 under well-watered (below diagonal) and water-stressed (above diagonal) treatments for the hard winter wheat association mapping panel (n=299).

Traits†	$T_c$bs	TBM	BGW	HI	Ht	GY	DSI
$T_c$bs		-0.24***	-0.24***	-0.10ns	-0.28***	-0.38***	0.24***
TBM	-0.32***		0.88***	0.12*	0.22***	0.37***	-0.16**
BGW	-0.25***	0.87***		0.53***	0.21**	0.53***	-0.24***
HI	0.05ns	0.09ns	0.48***		0.06ns	0.51***	-0.24***
Ht	-0.14*	0.35***	0.18**	-0.32***		0.27***	-0.22***
GY	-0.35***	0.39***	0.52***	0.42***	0.13*		-0.36***
DSI	-0.25***	0.33***	0.40***	0.28***	0.14*	0.78***	

*, **, *** Significant at *P*-values <0.05, 0.001, and 0.0001 probability levels, respectively.
† $T_c$bs, canopy temperature at booting stage (°C); TBM, total biomass weight at maturity (g); BGW, biomass grain weight (g); HI, harvest index; GY, grain yield (kg ha^{-1}); Ht, plant height (cm); DSI, drought susceptibility index.

Table 2.7 Phenotypic correlations between the same trait measured in both water treatments (well-watered and water-stressed) at Fort Collins 2012-13 (n=299).

Trait†	**r**
$T_c$bs	0.03ns
TBM	0.07ns
BGW	0.11ns
HI	0.28***
Ht	0.59***
GY	0.24***

*, **, ***, significance level of correlation at P-value 0.05, 0.001, and 0.0001; ns, non-significant at 0.05 level.
† r, correlation coefficient; n, number of observations used; $T_c$bs, canopy temperature at booting stage (°C); TBM, total biomass weight at maturity (g); BGW, biomass grain weight (g); HI, harvest index; GY, grain yield (kg ha^{-1}); Ht, plant height (cm).

Table 2.8 Summary of marker–trait associations (P<0.001) detected for agronomic and drought-related traits detected in four environments (Greeley 2012 WW (Gr12 WW), Greeley 2012 WS (Gr12 WS), Fort Collins 2013 WW (FC13 WW), and Fort Collins 2013 WS (FC13 WS)).

Trait†	Environment					
	Gr12 WW	Gr12 WS	FC13 WW	FC13 WS	Total	$R^{2‡}$ (%) range
GY	15	16	21	5	57	3.4 to 5.6
TBM	3	5	5	21	34	3.7 to 4.9
BGW	3	3	4	8	18	3.6 to 5.0
HI	9	3	25	21	58	3.4 to 6.8
Ht	7	5	3	8	23	3.7 to 6.2
$T_c$bs	NA¶	NA	5	9	14	3.7 to 5.1
$T_c$lh	40	10	NA	NA	50	3.7 to 5.5
$T_c$gf	NA	NA	NA	21	21	3.7 to 7.3
CID	29	NA	NA	NA	29	3.8 to 5.0
DSI	19		8		27	3.7 to 5.2

† GY, grain yield (kg ha-1); TBM, total biomass weight at maturity (g); BGW, biomass grain weight (g); HI, harvest index; Ht, plant height (cm); Tcbs, canopy temperature at booting stage (°C); Tclh, canopy temperature at late heading stage (°C); Tcgf, canopy temperature at grain filling stage (°C); CID, carbon isotope discrimination; DSI, drought susceptibility index.

‡ R^2, range of phenotypic variation explained by markers.

¶ NA, the trait was not measured in this environments.

Table 2.9 Marker-trait associations detected for grain yield (GY) in four environments.

SNP name	Chromosome	Position (cM)	*P*-Value	maf†	R^2 (%) ‡
Greeley 2012 WW					
RAC875_c91464_170	2B	67.6	0.00069	0.18	3.8
BS00010698_51	2B	68.9	0.00028	0.21	4.3
tplb0060m15_257	2B	68.9	0.00045	0.2	4.0
wsnp_Ex_c31914_40647870	2B	70.3	0.00079	0.2	3.7
Ku_c8237_1721	2B	70.3	0.00081	0.19	3.7
BS00000365_51	2B	70.3	0.00087	0.20	3.6
BS00096758_51	2B	70.3	0.0009	0.20	3.6
Kukri_c29052_75	3D	129.1	0.00063	0.14	3.8
RAC875_c42356_237	4D	63.1	0.00096	0.12	3.6
Tdurum_contig46877_76	5B	55.6	0.00018	0.33	4.6
Tdurum_contig46877_262	5B	55.6	0.0004	0.34	4.1
Tdurum_contig46877_488	5B	55.6	0.00053	0.34	3.9
Tdurum_contig46877_84	5B	56.9	0.00031	0.34	4.3
BS00003726_51	5B	58.6	0.00096	0.23	3.6
Jagger_c7991_95	UM¶	.	0.00088	0.26	3.6
Greeley 2012 WS					
Kukri_c706_702	1B	151.3	0.00083	0.37	3.5
BobWhite_c14689_172	2B	98.9	0.00002	0.11	5.6
Ku_c21235_676	2B	98.9	0.00003	0.11	5.5
wsnp_Ex_c3772_6866645	2B	98.9	0.00007	0.12	4.9
IAAV8042	2B	98.9	0.00014	0.1	4.5
D_contig04922_546	2B	99.6	0.00025	0.15	4.2
RAC875_c40928_60	2B	99.6	0.00067	0.21	3.6
Excalibur_c44713_137	4A	69.7	0.00048	0.45	3.8
IACX3190	4A	69.7	0.00091	0.44	3.4
BS00043664_51	4A	71.7	0.00048	0.45	3.8
IACX7741	4A	73	0.00008	0.31	4.9
Tdurum_contig44013_164	4A	73	0.00015	0.31	4.5
BobWhite_rep_c64944_264	4A	73	0.00024	0.30	4.2
Excalibur_rep_c82081_188	4A	141.3	0.00063	0.06	3.6
BobWhite_c7400_334	4B	32.7	0.00053	0.19	3.7
Kukri_c706_702	1B	151.3	0.00083	0.37	3.5
Fort Collins 2013 WW					
BS00001478_51	1D	101	0.00008	0.2	5.4
BS00000445_51	1D	101	0.00024	0.2	4.6
wsnp_CD454173A_Ta_2_8	1D	101	0.00025	0.2	4.6
BS00061179_51	1D	101	0.00027	0.2	4.6

BS00093871_51	1D	101	0.00027	0.2	4.6
BS00080879_51	1D	101	0.00034	0.2	4.4
Excalibur_c39248_485	1D	101	0.00083	0.3	3.8
wsnp_Ex_c9483_15722127	1D	105.7	0.00019	0.2	4.8
wsnp_Ex_c8884_14841846	1D	105.7	0.00026	0.2	4.6
BS00061173_51	1D	105.7	0.00057	0.2	4.1
Tdurum_contig50698_601	2Dx	64.9	0.00083	0.1	3.8
IAAV151	2Dx	94.9	0.00059	0.3	4.1
Excalibur_rep_c104696_400	2Dx	94.9	0.00068	0.3	4.0
Kukri_c669_259	2Dx	94.9	0.00068	0.3	4.0
wsnp_Ex_c30689_39574415	5A	67.2	0.00077	0.2	3.9
RAC875_c18821_137	5A	69.6	0.00023	0.3	4.7
Excalibur_rep_c67196_143	5A	69.6	0.00088	0.3	3.8
Ra_c23874_338	5A	69.6	0.00088	0.3	3.8
RAC875_rep_c117576_74	5A	69.6	0.00098	0.3	3.7
wsnp_Ex_c6143_10747643	5A	69.8	0.001	0.3	3.7
TA001038.0975	UM	-	0.0002	0.2	4.8
Fort Collins 2013 WS					
Excalibur_s111479_146	5A	49.3	0.00065	0.17	3.9
BS00064715_51	5A	49.3	0.00079	0.17	3.8
RAC875_c494_436	6B	86.3	0.0001	0.20	5.1
BS00067293_51	UM	-	0.00058	0.17	3.9
GENE.0221_721	UM	-	0.00072	0.17	3.8

† maf, minor allele frequency.

‡ R^2 (%), percentage of phenotypic variation explained by the SNP.

¶ UM, unmapped marker.

Table 2.10 Marker-trait associations detected for total biomass (TBM) in four environments.

SNP name	Chromosome	Position (cM)	*P*-value	maf†	R^2 (%) ‡
Greeley 2012 WW					
BS00022525_51	4D	11.2	0.00042	0.48	4.3
BS00083715_51	4D	11.2	0.00063	0.48	4.0
RFL_Contig4911_357	4D	11.2	0.00097	0.47	3.7
Greeley 2012 WS					
Ku_c1485_980	1D	86.7	0.00078	0.1	3.9
Kukri_c17417_291	2A	114.5	0.00023	0.38	4.7
Excalibur_c14217_1260	2A	114.5	0.0006	0.28	4.1
Ra_c16330_1197	2A	114.5	0.0009	0.28	3.8
Kukri_c11467_993	5B	150.6	0.00098	0.15	3.7
Fort Collins 2013 WW					
wsnp_Ex_c10657_17376448	1A	13.7	0.00096	0.25	3.7
Kukri_c5766_550	2B	53.5	0.00062	0.43	4.0
BobWhite_c10343_320	2Dx	140.9	0.00048	0.19	4.2
Tdurum_contig81288_341	3A	45.2	0.00064	0.09	4.0
IAAV6015	6D	86.1	0.00015	0.08	4.9
Fort Collins 2013 WS					
BS00026777_51	1A	70.8	0.00077	0.27	3.9
BobWhite_c26212_208	1A	93.6	0.00019	0.43	4.8
BS00065430_51	1A	94.5	0.00023	0.36	4.7
BS00039378_51	1A	94.5	0.00064	0.35	4.0
wsnp_Ex_c3258_6004611	1A	95.2	0.00027	0.47	4.6
wsnp_Ex_c43228_49605281	1A	95.2	0.00029	0.47	4.5
Kukri_c67383_102	1A	95.2	0.00048	0.48	4.2
wsnp_Ex_rep_c68085_66839109	1A	95.2	0.00058	0.47	4.1
BS00009808_51	1A	95.3	0.00064	0.47	4.0
wsnp_Ex_rep_c68493_67320068	1A	95.6	0.0005	0.48	4.2
BS00034278_51	1A	95.6	0.00068	0.47	4.0
wsnp_Ex_c271_521429	1A	139.7	0.0007	0.34	3.9
BS00084995_51	1A	139.7	0.00078	0.34	3.9
BS00021990_51	1A	139.7	0.00087	0.34	3.8
BS00068508_51	1D	177.2	0.00036	0.37	4.4
Excalibur_c5438_274	3D	142.8	0.00066	0.06	4.0
BobWhite_c13030_406	7A	188.7	0.00061	0.48	4.0
Excalibur_c687_886	7A	194.9	0.00078	0.48	3.9
RAC875_c13169_459	7A	194.9	0.00095	0.49	3.8
D_GDS7LZN01CBWNE_99	7A	194.9	0.00096	0.49	3.7
D_GA8KES401AL4GG_122	7A	198.2	0.00074	0.49	3.9

† maf, minor allele frequency.

‡ R^2 (%), percentage of phenotypic variation explained by the SNP.

Table 2.11 Marker-trait associations detected for biomass grain weight (BGW) in four environments.

SNP name	Chromosome	Position (cM)	*P*-value	maf†	R^2 (%) ‡
Greeley 2012 WW					
RAC875_c42356_237	4D	63.1	0.00047	0.12	4.2
Excalibur_c35797_547	4D	69.2	0.00055	0.12	4.1
Excalibur_c38928_307	5A	57.1	0.00084	0.36	3.8
Greeley 2012 WS					
Kukri_c3582_87	1A	76.8	0.00065	0.29	4.0
Kukri_c8405_1394	5B	134.4	0.00074	0.43	3.9
GENE.0112_64	UM¶	-	0.00065	0.29	4.0
Fort Collins 2013 WW					
BS00062827_51	4A	73.1	0.00029	0.09	4.5
RAC875_c17182_600	5B	3.3	0.00044	0.41	4.2
Kukri_rep_c71778_644	5B	3.3	0.0006	0.42	4.0
RFL_Contig2424_2617	UM	-	0.00047	0.17	4.2
Fort Collins 2013 WS					
wsnp_CAP11_c951_572693	2B	38.9	0.0005	0.15	4.0
wsnp_Ex_c356_698872	2B	39.6	0.0001	0.16	5.0
wsnp_Ku_c4389_7970859	2B	39.6	0.0001	0.16	5.0
wsnp_Ex_c15046_23216392	2B	39.6	0.00014	0.16	4.8
RAC875_c19210_348	3D	97	0.00095	0.23	3.6
Excalibur_c5438_274	3D	142.8	0.00084	0.06	3.6
Kukri_c7972_1529	5A	64.1	0.00047	0.08	4.0
BobWhite_c13030_406	7A	188.7	0.00096	0.48	3.6

† maf, minor allele frequency.

‡ R^2 (%), percentage of phenotypic variation explained by the SNP.

¶ UM, unmapped marker.

Table 2.12 Marker-trait associations detected for harvest index (HI) in four environments.

SNP name	Chromosome	Position (cM)	*P*-value	maf†	R^2 (%) ‡
Greeley 2012 WW					
BS00022332_51	1B	79.3	0.00084	0.06	3.6
Tdurum_contig12246_456	1B	79.3	0.00086	0.06	3.6
Kukri_c29052_75	3D	129.1	0.00059	0.14	3.8
Tdurum_contig50954_1095	4A	134.8	0.00061	0.4	3.8
CAP12_c470_361	6B	97.7	0.0006	0.16	3.8
RAC875_c24641_720	6B	97.7	0.00087	0.15	3.6
wsnp_CAP7_rep_c5643_2537213	6B	97.7	0.0009	0.15	3.6
Kukri_c22857_496	6B	99.6	0.00053	0.16	3.9
Kukri_c19263_346	6B	99.6	0.00062	0.16	3.8
Greeley 2012 WS					
BS00022438_51	4D	161.3	0.0003	0.27	4.0
Excalibur_c92555_283	4D	161.3	0.0003	0.27	4.0
D_contig18193_134	UM¶	-	0.00007	0.21	4.9
Fort Collins 2013 WW					
Ex_c40210_281	2A	47.5	0.00037	0.1	4.4
BS00000615_51	2B	50.6	0.00026	0.26	4.6
CAP11_c3138_241	3A	118.4	0.00003	0.18	5.9
BS00046977_51	3A	118.4	0.00006	0.18	5.5
JD_c19177_1284	3A	118.4	0.00007	0.18	5.5
IAAV6043	3A	118.4	0.00007	0.18	5.5
JD_c19177_1462	3A	118.4	0.00008	0.19	5.4
BS00046976_51	3A	118.4	0.00012	0.18	5.1
Excalibur_c6196_668	3A	120.7	0.00013	0.11	5.0
RFL_Contig2200_1024	3A	120.7	0.00057	0.11	4.1
Ex_c6196_971	3A	121.4	0.00016	0.12	4.9
BS00040600_51	3A	122.9	0.00001	0.13	6.8
BS00040601_51	3A	122.9	0.00001	0.13	6.7
BS00034689_51	3A	122.9	0.00002	0.13	6.5
wsnp_JG_c227_167774	3A	122.9	0.00002	0.13	6.5
RAC875_c7988_1588	3A	122.9	0.00002	0.13	6.5
RAC875_c101928_381	3A	122.9	0.00002	0.13	6.4
wsnp_Ex_rep_c66524_64798744	3D	99.7	0.00069	0.05	3.9
wsnp_Ex_rep_c66524_64799194	3D	99.7	0.00069	0.05	3.9
RAC875_rep_c74271_414	4D	183.9	0.00069	0.21	3.9
Excalibur_c9619_1136	6A	100.6	0.00096	0.07	3.7
RFL_Contig4583_1909	6A	100.6	0.00097	0.08	3.7

GENE.3018_145	UM	-	0.00007	0.18	5.5
RAC875_rep_c110962_423	UM	-	0.00078	0.11	3.9
GENE.4587_50	UM	-	0.00079	0.16	3.9
	Fort Collins 2013 WS				
wsnp_Ex_c3963_7179957	1A	70.1	0.0000	0.21	6.7
Excalibur_c12980_2621	2A	19.6	0.00097	0.47	3.4
wsnp_Ex_c16175_24619793	2A	67	0.00023	0.13	4.2
RAC875_c18090_752	2A	67	0.00084	0.12	3.5
BS00030838_51	2A	67	0.00086	0.12	3.4
Excalibur_c40202_114	2A	67	0.00086	0.12	3.4
Kukri_c19216_123	2A	67	0.00086	0.12	3.4
IAAV3132	2A	67	0.00092	0.11	3.4
IAAV3480	2A	67	0.00092	0.11	3.4
Excalibur_c8658_335	2A	67	0.00092	0.11	3.4
Tdurum_contig14947_611	2A	67	0.00096	0.11	3.4
Ra_c23848_305	2A	69.2	0.00064	0.13	3.6
wsnp_Ex_c19207_28125072	2A	69.6	0.00068	0.16	3.6
wsnp_Ku_c3237_6024936	2A	70	0.00039	0.14	3.9
wsnp_Ex_c24474_33721784	2A	70	0.00059	0.15	3.7
Excalibur_c24511_1196	2A	74.5	0.00053	0.21	3.7
Excalibur_c46394_762	2A	74.5	0.00099	0.22	3.4
Excalibur_s111479_146	5A	49.3	0.00028	0.17	4.1
BS00064715_51	5A	49.3	0.00031	0.17	4.0
BS00067293_51	UM	-	0.00025	0.17	4.2
GENE.0221_721	UM	-	0.00036	0.17	4.0

† maf, minor allele frequency.

‡ R^2 (%), percentage of phenotypic variation explained by the SNP.

¶ UM, unmapped marker.

Table 2.13 Marker-trait associations detected for plant height (Ht) in four environments.

SNP name	Chromosome	Position (cM)	*P*-value	maf†	R^2 (%) ‡
Greeley 2012 WW					
Tdurum_contig69003_459	3A	42.1	0.00079	0.12	3.8
IAAV5863	3B	114.6	0.00039	0.44	4.3
Tdurum_contig15050_103	4A	123.4	0.00071	0.09	3.9
BS00015891_51	4A	123.4	0.00076	0.08	3.8
Tdurum_contig52980_116	4A	123.6	0.00074	0.12	3.8
Excalibur_c14461_1653	4D	58.2	0.00066	0.31	3.9
wsnp_Ex_c19364_28303089	6A	39.7	0.00035	0.07	4.3
Greeley 2012 WS					
wsnp_CAP7_c940_480745	3B	70.1	0.00082	0.37	3.8
wsnp_Ex_c3681_6716255	3B	70.1	0.00099	0.36	3.7
BS00012081_51	UM¶	-	0.00002	0.34	6.2
RAC875_c31358_214	UM	-	0.00002	0.35	6.2
RAC875_c15844_348	UM	-	0.00002	0.35	6.2
Fort Collins 2013 WW					
Tdurum_contig45588_730	4D	142.6	0.00077	0.32	3.9
RAC875_rep_c96433_140	4D	188.6	0.00099	0.05	3.7
wsnp_JD_c12221_12509984	4D	188.6	0.00099	0.05	3.7
Fort Collins 2013 WS					
wsnp_Ex_c55051_57706127	1D	47.2	0.00009	0.19	5.1
wsnp_Ra_rep_c75740_73183118	4A	144.7	0.00016	0.44	4.8
Excalibur_c6906_2385	4A	144.7	0.00033	0.47	4.3
IAAV8659	4A	144.7	0.00037	0.44	4.2
IAAV2271	4B	39.9	0.00058	0.46	3.9
RAC875_c12959_869	4B	39.9	0.00074	0.46	3.8
Excalibur_c16245_801	5B	142.7	0.0007	0.06	3.8

† maf, minor allele frequency.

‡ R^2 (%), percentage of phenotypic variation explained by the SNP.

¶ UM, unmapped marker.

Table 2.14 Marker-trait associations detected for canopy temperature at booting stage ($T_c$bs) in two environments.

SNP name	Chromosome	Position (cM)	*P*-value	maf†	R^2 (%) ‡
Fort Collins 2013 WW					
tplb0048g05_866	3D	114.1	0.00074	0.23	3.9
wsnp_Ra_rep_c74497_72390803	3D	119.1	0.00074	0.29	3.9
Tdurum_contig10240_88	4D	63.1	0.00012	0.16	5.1
Kukri_c12032_508	6A	77.8	0.00097	0.11	3.7
TA001900.1836	UM¶	-	0.00099	0.17	3.7
Fort Collins 2013 WS					
IACX3586	2Dx	84.7	0.00037	0.25	4.4
Excalibur_rep_c105491_144	2Dx	84.7	0.00041	0.26	4.3
wsnp_BE403818A_Ta_2_1	2Dx	84.7	0.00092	0.25	3.8
RAC875_c58425_331	5A	9.5	0.00096	0.26	3.7
RAC875_c46007_340	6A	43.4	0.00083	0.27	3.8
BS00067584_51	6A	43.7	0.00035	0.19	4.4
BS00079440_51	6A	43.7	0.00058	0.19	4.1
BS00021940_51	6A	44.3	0.00053	0.29	4.1
BS00083504_51	6A	45.3	0.0007	0.28	3.9

† maf, minor allele frequency.

‡ R^2 (%), percentage of phenotypic variation explained by the SNP.

¶ UM, unmapped marker.

Table 2.15 Marker-trait associations detected for canopy temperature at late heading stage ($T_c$lh) in two environments.

Greeley 2012 WW					
SNP name	**Chromosome**	**Position (cM)**	***P*-value**	**maf†**	***R^2* (%) ‡**
BS00025084_51	2Dx	41.1	0.00063	0.49	4.0
BS00011436_51	2Dx	41.5	0.00052	0.38	4.2
Kukri_c1192_825	2Dx	41.5	0.00059	0.37	4.1
Tdurum_contig75595_643	2Dx	41.5	0.00061	0.38	4.0
BS00009331_51	2Dx	41.5	0.00072	0.38	3.9
Tdurum_contig75595_1072	2Dx	41.5	0.00072	0.38	3.9
BS00085980_51	2Dx	41.5	0.00074	0.38	3.9
BS00074992_51	2Dx	41.5	0.00087	0.38	3.8
wsnp_JD_rep_c49357_33576509	3B	60.6	0.00034	0.22	4.4
RAC875_c30381_573	3B	62.6	0.0006	0.07	4.1
wsnp_JD_c4444_5575748	3B	62.8	0.0006	0.07	4.1
Excalibur_c56657_282	3B	62.8	0.0006	0.07	4.1
RAC875_c20775_540	3B	64.1	0.00036	0.06	4.4
BobWhite_rep_c66146_237	3B	64.3	0.00036	0.06	4.4
Tdurum_contig83763_107	3B	64.3	0.00096	0.08	3.8
Excalibur_c15885_1145	3B	64.3	0.00096	0.08	3.8
Kukri_c10593_1064	3B	64.9	0.00092	0.2	3.8
Ra_c21902_654	3B	64.9	0.00092	0.2	3.8
wsnp_Ex_c15934_24341135	3B	64.9	0.00092	0.2	3.8
Kukri_c49091_371	3B	65.4	0.00008	0.11	5.4
wsnp_Ex_c26419_35667216	3B	65.4	0.00014	0.23	5.0
wsnp_Ku_c16117_24917524	3B	65.4	0.00014	0.23	5.0
Ex_c34713_501	3B	65.4	0.00092	0.2	3.8
Kukri_c37856_492	3B	65.4	0.00092	0.2	3.8
Ra_c23839_884	3B	65.4	0.00092	0.2	3.8
wsnp_Ku_c9014_15193623	3B	65.4	0.00092	0.2	3.8
CAP7_rep_c7114_55	3B	65.4	0.00092	0.2	3.8
Kukri_c2297_181	3B	65.4	0.00092	0.2	3.8
Tdurum_contig10326_151	3B	66.1	0.00007	0.22	5.5
Tdurum_contig42856_1271	3B	66.1	0.00008	0.22	5.4
BS00052075_51	3B	66.1	0.00009	0.22	5.3
Tdurum_contig46780_203	3B	66.1	0.0001	0.22	5.3
BS00022670_51	3B	66.1	0.00015	0.08	5.0
CAP11_c1969_268	3B	66.1	0.00072	0.2	3.9
BobWhite_c1318_691	3B	66.7	0.0001	0.23	5.2
RAC875_c400_2722	3D	99.6	0.0006	0.08	4.1

RAC875_c400_1271	3D	99.6	0.0006	0.08	4.1
Kukri_c78742_122	UM¶	-	0.00036	0.06	4.4
TA015141.0717	UM	-	0.00061	0.07	4.0
GENE.1214_159	UM	-	0.00092	0.2	3.8
Greeley 2012 WS					
Kukri_rep_c102263_1262	2Dx	21.1	0.000142	0.094275	5.0
Ex_c21841_1883	2Dx	22	0.000175	0.097315	4.9
Excalibur_rep_c110867_88	2Dx	25.5	0.000142	0.09396	5.0
Kukri_c52953_562	2Dx	25.5	0.000142	0.09396	5.0
BobWhite_c22086_444	2Dx	25.5	0.000175	0.097315	4.9
Tdurum_contig10208_447	3D	16.9	0.00038	0.169486	4.4
Tdurum_contig10208_452	3D	16.9	0.000447	0.166659	4.3
RFL_Contig3158_598	6B	4	0.000956	0.198616	3.8
Kukri_c64744_1087	6D	17.7	0.000997	0.258389	3.7
BS00078603_51	7A	204.6	0.0005	0.054235	4.2

† maf, minor allele frequency.

‡ R^2 (%), percentage of phenotypic variation explained by the SNP.

¶ UM, unmapped marker.

Table 2.16 Marker-trait associations detected for canopy temperature at grain filling stage ($T_c$gf) in Fort Collins 2013 dry (FC13 WS).

SNP name	Chromosome	Position (cM)	*P*-value	maf†	R^2 (%) ‡
Excalibur_c55677_217	1A	48.4	0.00001	0.34	6.7
wsnp_Ex_c64327_63176640	1A	48.4	0.00015	0.34	5.0
BS00035122_51	2A	51.7	0	0.07	7.3
wsnp_BE442666A_Ta_2_1	2A	51.7	0.00012	0.08	5.1
BS00010925_51	2A	52	0.00001	0.07	7.2
BS00072629_51	2A	52	0.00001	0.07	7.2
BS00022596_51	2A	52	0.00001	0.07	7.2
BS00011273_51	2A	52	0.00001	0.07	7.2
Excalibur_c96303_224	2A	52.3	0.00004	0.08	5.8
Excalibur_c32735_603	2A	52.3	0.00014	0.08	5.0
RAC875_c7734_411	2A	127.1	0.0009	0.08	3.8
Ku_c47168_563	2B	57.1	0.0003	0.06	4.5
wsnp_Ex_c7168_12311649	2B	57.9	0.00004	0.06	5.8
BobWhite_c6759_365	2B	57.9	0.00006	0.06	5.5
CAP8_c1066_309	2B	57.9	0.00015	0.06	5.0
Excalibur_c33675_410	4D	94.9	0.00089	0.08	3.8
wsnp_Ex_c33675_42124657	4D	94.9	0.00097	0.08	3.7
wsnp_Ex_c4124_7455225	5A	72.3	0.00029	0.24	4.5
BobWhite_c45091_53	5A	72.3	0.00034	0.24	4.4
Tdurum_contig42414_1071	5A	72.3	0.00039	0.25	4.3
wsnp_Ex_c4124_7455032	5A	72.3	0.00039	0.24	4.3

† maf, minor allele frequency.

‡ R^2 (%), percentage of phenotypic variation explained by the SNP.

Table 2.17 Marker-trait associations detected for carbon isotope discrimination (CID) in Greeley 2012 wet (Gr12 WW).

SNP name	Chromosome	Position (cM)	*P*-value	maf†	R^2 (%) ‡
Tdurum_contig92819_647	2Dx	63.7	0.00034	0.15	4.4
Jagger_c2853_75	2Dx	63.7	0.0004	0.16	4.3
wsnp_Ex_c15378_23638822	2Dx	63.7	0.00081	0.43	3.8
Tdurum_contig50698_601	2Dx	64.9	0.00036	0.15	4.4
BS00063107_51	3D	47.5	0.00078	0.34	3.9
Excalibur_c41752_392	4A	66.8	0.0003	0.36	4.5
BS00057451_51	4A	66.8	0.00032	0.36	4.4
BS00003522_51	4A	66.8	0.00068	0.23	3.9
RAC875_c4024_112	4A	70.7	0.00084	0.18	3.8
RAC875_c53296_61	4A	71.3	0.00058	0.17	4.0
wsnp_Ex_c5547_9774453	4A	71.3	0.00072	0.16	3.9
RAC875_c53296_378	4A	71.3	0.00077	0.16	3.9
BS00073011_51	4A	71.3	0.00079	0.16	3.8
RAC875_c53296_529	4A	71.3	0.00084	0.17	3.8
Ku_c31046_525	4A	71.5	0.0008	0.16	3.8
Ku_c25346_503	4A	71.7	0.00074	0.27	3.9
Ku_c25346_508	4A	71.7	0.00079	0.27	3.8
Kukri_c25794_863	4A	71.9	0.00071	0.27	3.9
BobWhite_c40455_116	4A	72.2	0.00079	0.16	3.8
wsnp_BE445348B_Ta_2_1	4A	73.4	0.00031	0.15	4.4
BobWhite_rep_c64527_134	4D	95.5	0.00038	0.12	4.3
Tdurum_contig53072_1935	4D	95.5	0.00043	0.12	4.2
BS00061326_51	4D	96.7	0.00013	0.14	5.0
BobWhite_c34759_227	4D	96.7	0.00039	0.12	4.3
BS00109560_51	4D	96.7	0.00048	0.13	4.2
BS00105054_51	4D	98	0.0009	0.16	3.8
Tdurum_contig12995_722	4D	98.3	0.0008	0.15	3.8
Tdurum_contig12995_792	4D	98.3	0.00087	0.14	3.8
TA002756.0960	UM¶	-	0.00052	0.17	4.1

† maf, minor allele frequency.

‡ R^2 (%), percentage of phenotypic variation explained by the SNP.

¶ UM, unmapped marker.

Table 2.18 Marker-trait associations detected for drought susceptibility index (DSI) in two locations, Greeley 2012 and Fort Collins 2013.

Greeley 2012					
SNP name	**Chromosome**	**Position (cM)**	***P*-value**	**maf†**	***R^2* (%) ‡**
RAC875_c52195_324	1D	146.9	0.00071	0.26	3.9
Excalibur_c7034_692	2A	108.7	0.0001	0.29	5.2
RAC875_c917_442	2A	108.7	0.0001	0.29	5.2
RAC875_c29282_187	2A	108.7	0.0001	0.29	5.2
RAC875_c29282_216	2A	108.7	0.0001	0.29	5.2
RAC875_c29282_566	2A	108.7	0.0001	0.29	5.2
Excalibur_c53103_113	2A	108.7	0.00014	0.29	5.0
Excalibur_c17992_1071	2A	108.7	0.0002	0.28	4.8
BS00066891_51	2A	110.1	0.00025	0.28	4.6
wsnp_Ex_c1600_3051075	3B	74	0.00017	0.07	4.9
Excalibur_c29124_598	5B	92.5	0.00068	0.37	3.9
Excalibur_c11093_519	5B	92.5	0.00073	0.37	3.9
BS00029789_51	5B	92.5	0.0008	0.38	3.8
BS00066484_51	5B	92.5	0.00092	0.38	3.8
BS00035559_51	5B	93.2	0.00018	0.33	4.8
BS00029286_51	5B	94.8	0.00015	0.32	5.0
BS00029287_51	5B	94.8	0.00019	0.33	4.8
GENE.4618_413	UM¶	-	0.0001	0.29	5.2
TA004145.0795	UM	-	0.00092	0.38	3.8
Fort Collins 2013					
BS00004377_51	2D	74.2	0.00096	0.05	3.7
Ex_c66350_301	4D	51.2	0.00066	0.5	4.0
JD_c3499_551	4D	51.2	0.00081	0.5	3.8
Ex_c66350_302	4D	51.2	0.00083	0.49	3.8
wsnp_Ku_c3869_7094615	4D	52.1	0.00075	0.27	3.9
wsnp_Ex_c1130_2166731	5D	70.8	0.00045	0.14	4.2
wsnp_Ex_c16720_25268525	5D	70.8	0.00092	0.14	3.8
wsnp_Ex_rep_c108057_91436561	5D	71.9	0.00092	0.14	3.8

† maf, minor allele frequency.

‡ R^2 (%), percentage of phenotypic variation explained by the SNP.

¶ UM, unmapped marker.

Table 2.19 Analysis of variance and summary statistics for GY and T_c traits for the hard winter wheat association mapping panel (HWWAMP) confirmation subset at Greeley 2013-14 under well-watered (WW) and water-stressed (WS) treatments.

Traits†	Treatment	Entry P-value	Treatment p-value	Mean	Range	CV	R^2
GY	WW	**	***	5130	3016 to 6467	13.64	0.70
	WS	ns		3630	2965 to 4203	15.90	0.29
$T_c$vg	WW	ns	***	26.2	23 to 27.5	4.82	0.47
	WS	ns		27.7	26.8 to 28.8	4.09	0.24
$T_c$egf	WW	ns	***	18.4	14.5 to 20	11.86	0.43
	WS	ns		20.0	18.5 to 21.5	9.09	0.19
$T_c$lgf	WW	ns	ns	28.6	25 to 32.5	9.62	0.53
	WS	ns		28.7	26.3 to 32.3	10.42	0.26

*, **, ***, Significance for entries and treatment at 0.05, 0.001, and 0.0001 probability levels, respectively; ns; non-significant at 0.05 level.

†WW, well-watered; WS, water-stressed; CV, coefficient of variation %; GY, grain yield (kg ha^{-1}); $T_c$vg, canopy temperature at vegetative stage (°C); $T_c$egf, canopy temperature at early grain fill stage(°C); $T_c$lgf**,** canopy temperature at late grain fill stage(°C).

Table 2.20 Analysis of variance and summary statistics for GY and T_c traits for the hard winter wheat association mapping panel (HWWAMP) confirmation subset (n=50) at Fort Collins, CO 2013-14 WW (well-watered) and WS (water-stressed).

Traits†	Treatment	Entry P-value	Treatment p-value	Mean	Range	CV	R^2
GY	WW	***	***	6614	4168 to 8324	8.65	0.82
	WS	***		5360	3378 to 6718	8.32	0.80
$T_c$vg	WW	ns	***	21.253	19.3 to 23.0	5.88	0.56
	WS	ns		20.333	18.7 to 21.7	5.88	0.52
$T_c$gf	WW	ns	**	21.6	17.7 to 23.0	8.24	0.32
	WS	ns		20.8	18.7 to 23.0	8.77	0.42

*, **, ***, Significance for entries and treatment at 0.05, 0.001, and 0.0001 probability levels, respectively; ns; non-significant at 0.05 level.

† WW, well-watered; WS, water-stressed; CV, coefficient of variation%; GY, grain yield (kg ha^{-1}); $T_c$vg, canopy temperature at vegetative stage (°C); $T_c$gf, canopy temperature at grain fill stage (°C).

Table 2.21 Phenotypic correlations among GY and T_c traits for the hard winter wheat association mapping panel confirmation subset (n=50) at Greeley 2013-14 under well-watered (below diagonal) and water-stressed (above diagonal) treatments.

Traits†	GY	$T_c$vg	$T_c$egf	$T_c$lgf
GY		0.12ns	0.17ns	0.07ns
$T_c$vg	0.06ns		0.07ns	-0.06ns
$T_c$egf	-0.03ns	-0.04ns		-0.17ns
$T_c$lgf	0.17ns	-0.22ns	0.07ns	

ns, non-significant at *P*<0.05 probability level.
†GY, grain yield (kg ha^{-1}); $T_c$vg, canopy temperature at vegetative stage (°C); $T_c$egf, canopy temperature at early grain fill stage (°C); $T_c$lgf, canopy temperature at late grain fill stage (°C).

Table 2.22 Phenotypic correlations between the same trait measured in both water treatments (well-watered and water-stressed) at Greeley 2013-14 (n=50).

Traits†	r
GY	0.55***
$T_c$vg	0.11ns
$T_c$egf	0.25ns
$T_c$lgf	0.27ns

***, significant at *P*<0.0001 probability level; ns, non-significant at *P*<0.05 probability level.
† r, correlation coefficient; GY, grain yield (kg ha^{-1}); $T_c$vg, canopy temperature at vegetative stage (°C); $T_c$egf, canopy temperature at early grain fill stage (°C); $T_c$lgf, canopy temperature at late grain fill stage (°C).

Table 2.23 Phenotypic correlations among GY and Tc traits for the hard winter wheat association mapping panel confirmation subset (n-50) at Fort Collins 2013-14 under well-watered (below diagonal) and water-stressed (above diagonal) treatments.

Traits†	GY	$T_c$vg	$T_c$gf
GY		-0.03ns	-0.13ns
$T_c$vg	-0.23*		-0.38**
$T_c$gf	0.21ns	-0.01ns	

**, significant at *P*<0.001; ns, non-significant at *P*<0.05 probability level.
†GY, grain yield (kg ha^{-1}); $T_c$vg, canopy temperature at vegetative stage (°C); $T_c$gf, canopy temperature at grain fill stage (°C).

Table 2.24 Phenotypic correlations between the same trait measured in both water treatments (well-watered and water-stressed) at Fort Collins 2013-14 (n=50).

Traits†	*r*
GY	0.86***
$T_c$vg	0.05ns
$T_c$gf	0.21ns

***, significant at *P*<0.0001; ns, no-significance correlation at *P*<0.05.
† *r*, correlation coefficient; GY, grain yield (kg ha^{-1}); $T_c$vg, canopy temperature at vegetative stage (°C); $T_c$gf, canopy temperature at grain fill stage (°C).

CHAPTER 3: ROOT TRAIT DIVERSITY IN WINTER WHEAT UNDER DROUGHT STRESS

Summary

Drought is among the most serious environmental challenges farmers face. The development of a deep and extensive root system is a drought adaptation mechanism to allow water and nutrient extraction from the soil profile. We conducted two studies to investigate the variation in root architecture and related physiological and morphological traits in winter wheat (*Triticum aestivum* L.) under drought stress. The first study evaluated 30 entries (cultivars and advanced lines) primarily from Colorado, and the second study included 30 entries from seven Great Plains states. Entries were evaluated in a greenhouse in 2012 and 2013 in 1 m x 10 cm plastic tubes filled with a fritted clay medium. Drought stress was imposed by withholding water after the emergence of the fourth leaf. After three weeks without watering, above ground biomass was harvested and roots were separated from the growing medium, washed, scanned, and digitally analyzed. Colorado entries differed significantly ($P<0.05$) for estimated transpiration, above ground biomass, average root diameter, total root length for bottom, middle, and top sections, and root length in most diameter classes. Great Plains entries differed significantly ($P<0.05$) for above ground biomass, stomatal conductance, water use efficiency, total root length, and root length for several diameter classes. Total root length adjusted for above ground plant size of Colorado entries ranged from 5212 to 7279 cm and average diameter ranged from 0.33 to 0.40 mm. Total root length correlated positively ($P<0.05$) with leaf elongation rate and RWC for Colorado entries and total root length correlated negativly

with average root diameter for Colorado and Great Plains entries. No significant differences were observed for any root trait between entries with and without *Rht* semi-dwarf alleles. The variation in root traits among Colorado and Great Plains winter wheat entries can be exploited in breeding programs to help develop plants with the best adapted root systems to withstand drought stress.

Introduction

Drought is among the most serious environmental challenges farmers face around the globe and is the most important cause of yield reduction in crops in arid and semiarid areas (Cattivelli et al., 2008; Ehdaie et al., 2012). Due to climate change, water stress will likely lead to further crop losses (Kang et al., 2009).

The effects of drought occur at all levels of crop growth, from crop canopy to molecular levels, and are evident at all phenological stages of plant development at which the water shortage occurs (Farooq et al., 2009a). Responses to drought include a reduction in photosynthesis, decrease in leaf expansion, stomatal closure, enhanced formation of reactive oxygen species, premature leaf senescence, decreased assimilate translocation, and associated reduction in crop production (Farooq et al., 2009a). In addition, the stress imposed by drought conditions affects water relations, such as water use efficiency, relative water content, leaf water potential, stomatal resistance, rate of transpiration, and canopy temperature. Most of these physiological responses to water stress are related to root system function (Farooq et al., 2009b).

The development of a deep and extensive root system is a drought adaptation strategy that allows the plant to efficiently acquire water and nutrients from deep in the soil profile,

helping the plant to meet evapotranspiration demands. Root growth and distribution play an important role in plant response to water availability. They are central in determining the growth and yield of crops in water-limited environments, and are determined by both plant genotype and the soil environment (Hund et al., 2009).

There is evidence that under moderate stress, mechanisms that provide drought avoidance (such as water acquisition from deeper in the soil profile) and increased water use efficiency (WUE, defined as the ratio of dry biomass produced to the amount of water consumed) are effective in maintaining yield without detrimental effects on productivity under favorable conditions (Collins et al., 2008)). Improved water acquisition as a result of size, depth and architecture of roots has been related to increased drought tolerance in small grain cereals (Richards, 1991; Lopes and Reynolds, 2010). However, shallow root systems may also be advantageous for capturing rainfall that does not infiltrate to deeper in the soil profile (Ehdaie et al., 2012). An overview of root traits associated with drought tolerance is presented in Comas et al. (2013).

The semi-dwarfing genes *Rht-B1b* and *Rht-D1b* were introduced into wheat (*Triticum aestivum* L.) germplasm as a key part of the Green Revolution and led to significant increases in grain yields after the 1960s (Reynolds and Borlaug, 2006). More than 70% of wheat cultivars grown worldwide have a semi-dwarf phenotype controlled by the major genes *Rht-B1b*, *Rht-D1b*, and *Rht8c* (Guedira et al., 2010). There are contrasting reports about the effects of *Rht* semi-dwarf alleles on root system growth and architecture in wheat. Li *et al.* (2011) suggested that the dwarfing gene *Rht-B1b* has a pleiotropic effect of increasing longest root length and total root length. Waines and Ehdaie, (2007) reported that older wheat varieties and landraces

produced larger root systems relative to modern varieties. However, Cholick et al. (1977) evaluated the rooting patterns of two tall and three semi-dwarf winter wheat cultivars under dryland field conditions in eastern Colorado and found no significant relationships between cultivar height and rooting depth or moisture extraction patterns. Inconsistent results were reported by Wojciechowski et al. (2009) for the effect of *Rht* genes on root growth depending on the experimental design and growth medium used.

Most previous studies on root morphology have indicated that root evaluations are intrinsically difficult to perform, due to the plastic nature of root development, which shows a high interaction with the experimental environment (Manschadi et al., 2008; Walter et al., 2009) . In addition, because of their underground location, it is difficult to observe roots, especially in a non-destructive manner during the growing season. The majority of root studies have, therefore, relied on aboveground estimates of root characteristics (such as root pulling force, root lodging, and number of brace roots), or on the phenotype of root systems from early stages of plant development (e.g., Hund, 2010). These studies are mainly grown under artificial conditions such as hydroponics, artificial media or root tubes in the greenhouse. Evidence that supports the use of these artificial systems includes the identification of overlapping quantitative trait loci (QTL) for maize root traits measured in hydroponic systems and in the field under two different water regimens (Tuberosa et al., 2002). Tube rhizotrons have been proposed as a valuable tool for root phenotyping (Gregory et al., 2009; Ytting et al., 2014). Moreover, root tubes (or columns) were usefully implemented by wheat researchers to investigate genetic variability of roots in wheat populations (Narayanan et al., 2014; Narayanan

and Prasad, 2014). These findings support the exploration and implementation of artificial systems as an alternative to laborious and expensive field-based root phenotyping.

Root characteristics such as root length, root diameter, and root architecture are challenging to measure. However, software systems like WinRhizo (Regent Instruments Inc., Quebec, Canada) provide a detailed digital analysis of the root system and allow the evaluation of hundreds of root samples per week (Clark et al., 2013).

The overall goal of this research is to improve understanding of the role of root architecture in drought adaptation of winter wheat. The specific objectives of this study were 1) to investigate the variation in root architecture and drought tolerance related physiological and morphological traits among U.S. Great Plains winter wheat germplasm under drought stress conditions, 2) to examine correlations among the evaluated traits, and 3) to compare root traits of entries with and without *Rht* semi-dwarf alleles.

Materials and Methods

Two greenhouse studies (I and II) were conducted to study the variation in root architecture traits among U.S. Great Plains winter wheat entries (cultivars and advanced breeding lines).

Plant Materials

In Study I, 28 entries developed by Colorado State University (CSU), one cultivar from Kansas State University, and a cultivar released by AgriPro (Junction City, KS) were evaluated (Appendix 3). In Study II, we evaluated 30 entries from seven Great Plains states (Colorado, Kansas, Montana, Oklahoma, Nebraska, South Dakota, and Texas), which included four historical cultivars developed before 1945 (‘Kharkof’, ‘Wichita’, ‘Cheyenne’, and ‘Comanche’)

(Appendix 4). These four cultivars in addition to 'Parker 76', 'Harding', and 'Centura' do not contain *Rht* semi-dwarf alleles, but the other entries all contain either *Rht-Blb* or *Rht-D1b*. The two groups of entries were selected from the Hard Winter Wheat Association Mapping Panel of 299 entries adapted to the Great Plains region and developed by the Triticeae Coordinated Agricultural Project (http://www.triticeaecap.org/). Four entries from CSU ('Byrd', 'Ripper', 'Hatcher', and 'Vona') were common to both studies.

Experimental design and growing conditions

Study I

The experimental design of Study I was a randomized complete block with four replications. The whole experiment was conducted twice. Seeds of 30 entries were planted in a 5 × 24 array of 1 m high, 10 cm diameter polyvinyl chloride tubes in a CSU greenhouse, Fort Collins, CO on 16 Jan. 2012 and 13 April 2012. Each tube was lined with a 4 ml poly tube liner (Uline, Pleasant Prairie, WI) with two holes in the bottom for drainage. Each tube was filled with 5.3 kg of dry Greens Grade fritted clay media (Profile Products LLC, Buffalo Grove, IL) and packed to a height of 99 cm, resulting in a bulk density of approximately 0.74 g cm^{-3} after irrigation.

Greenhouse conditions consisted of 16/8 h of light/dark photoperiod at a temperature range from 18 to 25°C. Six days after planting, seedlings were thinned to a single plant per tube. Water was applied through drip irrigation with Peters Professional 15-16-17 fertilizer (The Scotts Company, Marysville, OH) at a concentration of 0.20 g L^{-1} with a 1:100 injector ratio. For the first 21 d (until 4^{th} leaf stage), all plants received 200 mL of water per day. Then irrigation

was removed, allowing natural dry down from evapotranspiration and surface evaporation throughout the remaining 21 d of the experiment.

Study II

Study II was planted on 20 Feb. 2013 and again on 20 May 2013. The total duration of each experiment was 2 d longer than Study I (the dry-down treatment began 1 d later and lasted an extra day). It followed the same procedures as Study I with slight differences, as explained below.

Physiological and biomass measurements

Root tubes were weighed with a hanging scale at the beginning of the dry-down treatment and at the end of the experiment to calculate estimated transpiration (Etr) in Study 1. In Study II, tubes were weighed every 3 d for six gravimetric water content measurements. To estimate the amount of water transpired, the water loss was adjusted by subtracting evaporation from the upper surface of the growth medium. This was done by measuring water loss for five tubes filled with Greens Grade medium but without plants. The tubes were saturated with water and left to dry down for the same number of days as the dry-down treatment. Adjusted water loss will henceforth be referred to as estimated transpiration (ETr). Above ground biomass (ABM) was collected, dried for approximately 48 h at 68°C, and weighed. Water use efficiency (WUE) was calculated as: above ground biomass (g)/estimated transpiration (g). In Study I, growth rate of the newest leaf after the initiation of the dry-down treatment (the fifth leaf) was calculated by dividing the final length of the leaf by the number of days from its emergence from the fourth leaf collar till the appearance of the fifth collar, similar

to Praba et al. (2009). In Study II, leaf length was measured every 3 d after the initiation of the dry treatment for six repeated measurements.

At the end of the dry-down treatment, the poly liner and its contents were pulled from the tubes, slit open, and roots were washed out from the soil media. Depth of longest root was measured in cm. Roots were then divided into three sections (Top, 0-33 cm tube depth; Middle, 33-66 cm tube depth; Bottom, 66-99 cm tube depth). In Study I, the washed root masses were dyed by soaking in a 1% methylene blue solution for approximately 5 min prior to scanning. In Study II, roots were not dyed before scanning. Individual root sections were floated in approximately 1 cm of water in a 30 cm x 40.5 cm plexiglass tray and scanned with a MicroTek Scanmaker 9800XL (Microtek, Santa Fe Springs, CA). Digital images were analyzed with WinRhizo Regular software (Regent Instruments Inc., Quebec, Canada), an image analysis system specifically designed for root measurements. Root measurements recorded by WinRhizo included total root length (overall and per section), total root diameter (overall and per section), and root length of the following diameter classes: 0.00 to 0.25 mm, 0.25 to 0.5 mm, 0.5 to 0.75 mm, 0.75 to 1.0 mm, and >1.0 mm. Root sections were collected after scanning and dried at 68° C for at least 48 h prior to weighing to obtain root biomass weight.

Stomatal conductance, the rate of passage of carbon dioxide entering, or water vapor exiting, through the stomata of a leaf in mmol m^{-2} s^{-1} was measured daily for the last 3 d of the experiment for both sides of the leaf. Conductance was recorded with a leaf porometer (Model SC-1, Decagon Devices, Inc. Pullman, WA).

Samples of the newest, fully emerged leaf were collected for measuring relative water content (RWC) at the end of the experiment. The complete leaf was divided into halves, the

proximal portion was used for RWC measurement. To measure the RWC, each leaf section was immediately weighed to obtain fresh weight (FW), and then sealed in a 50 mL tube containing 5 mL of de-ionized water. The leaf samples were allowed to saturate for 4 h and then re-weighed for the turgid weight (TW). Leaf samples were then placed in a dryer for about 48 h at 68°C. Dry weight (DW) was then obtained and RWC was calculated using the following formula from Barr and Weatherley (1962): RWC (%) = [(FW - DW) / (TW - DW)] * 100.

Statistical Analyses

All statistical analyses were conducted in SAS version 9.3 (SAS Institute, 2011) unless otherwise stated. Analysis of variance (ANOVA) for each trait was conducted with the GLM procedure, with entries and experiments considered fixed variables and replication a random variable. The REG procedure was used to adjust root length for above-ground plant size, where shoot biomass weight was used as a covariate in the analysis. The adjusted total root length and adjusted root length per section was used in the ANOVA with the GLM procedure. From this point, all results of root length refer to adjusted values. Spearman rank correlation coefficients were obtained for all pairs of traits using least squares means in the CORR procedure. Graphs were constructed using Microsoft Office Excel (Microsoft, 2013) and JMP Pro 9.0.2 (JMP, 2010). A contrast statement was used in the SAS GLM procedure to contrast entries with and without *Rht-B1b* and *Rht-D1b* alleles in Study II. *Rht* alleles were determined based on Kompetitive Allele Specific PCR (KASP) marker analysis kindly provided by Gina Brown-Guedira, USDA-ARS, Raleigh, NC.

Results

Study I

Variation in physiological and biomass traits

Colorado winter wheat entries differed significantly for ABM and ETr (P<0.01) (Table 3.1). Average ABM was 1.00 g and ranged from 0.76 to 1.23 g, with 'Avalanche' having the highest and 'Halt' the lowest values. Average ETr was 788 g and ranged from 701 to 872 g, with Duke having the highest and Lamar the lowest values (Table 3.2). Other morphological and physiological traits (TRBM, top tube section root biomass; MRBM, middle tube section root biomass; TotRBM, Total root biomass; BRBM, bottom tube section root biomass; LR, longest root depth; RWC, relative water content; WUE, water use efficiency and g_s, stomatal conductance), did not show significant variation (P>0.05) among the Colorado winter wheat entries studied (Table 3.1). Trait differences due to experiment (date of planting) effects were significant (P<0.01) for ABM, TRBM, TotRBM, BRBM, LR, and WUE (Table 3.1). The Entry x Experiment interaction effect was significant (P<0.01) only for ABM (Table 3.1).

Variation in root allocation and morphology traits

Roots of Colorado winter wheat entries differed significantly (P<0.001) for all measured traits (AD, average diameter in mm; ADBS, average diameter for bottom section; ADMS, average diameter for middle section; ADTS, average diameter for top section; TL, total length; TLBS, total length for bottom section; TLMS, total length for middle section; and TLTS, total length for top section) (Table 3.3). Trait differences due to experiment effects were significant (P<0.01) for all WinRhizo root traits measured (Table 3.3). The Entry x Experiment interaction effect was significant (P<0.05) only for AD (Table 3.3). Mean TL of the 30 entries ranged from

5239 to 7284 cm and mean average diameter ranged from 0.33 to 0.40 mm (Appendix 29). The greatest total root length was recorded for 'Bill Brown' (7284 cm), followed by Byrd (6782) and Above (6766), respectively (Figure 3.1 and Appendix 5). 'Prairie Red' had the smallest average root diameter followed by Byrd, C004499, and 'Halt' (Figure 3.2 & Appendix 5). RonL, Avalanche, Prowers, Lamar, and CO04499, respectively ranked highest for total root allocation percentage in the top section (from 35.1 to 40.4 %), and Thunder CL, Halt, Bond CL, Jules, and CO04393, respectively ranked highest for total root allocation percentage in the bottom section (from 41.8 to 44.7 %) (Appendix 5). Entries differed significantly (P<0.05) for root length in each of the five diameter classes (0.0-0.25, 0.25-0.50, 0.50-0.75, 0.75-1.00, and >1.00 mm) for the three root sections, except for three diameter classes in the middle section (0.50-0.75, 0.75-1.00, and >1.00 mm) (Table 3.3). Furthermore, most of the root length in the top section (50%) fell within the finest/first diameter class (from 0.00 to 0.25 mm) (Appendix 7), while in both middle and bottom root sections, the greatest proportion of root length (49 and 55% respectively) fell within the second diameter class (0.25-0.50 mm) (Appendix 7).

Correlations among traits

There were negative correlations (P<0.05) between adjusted total root length overall and per section and average diameter overall and per section (r ranged from -0.43 to -0.69) (Table 3.4). Adjusted total root length (TL) significantly correlated (P<0.05) with TotRBM, LER, and RWC (r=0.45, 0.49, and 0.43, respectively) (Table 3.4). Relative water content correlated significantly (P<0.05) and positively with three root length traits (TLTS, TLBS, and TL), r=0.38, 0.64, and 0.43, respectively); RWC also correlated significantly (P<0.05) with TotRBM, ABM,

LER, and LR (*r*=0.37, 0.63, 0.37, and -0.45, respectively). A complete correlation matrix between pairs of traits in Study I is contained in Table 3.4.

Study II

Variation in physiological and morphological traits

Three morphological or physiological traits showed significant differences among Great Plains winter wheat entries used in study II. Entries differed significantly ($P<0.05$) for ABM, g_s and WUE (Table 3.6). Average ABM was 1.35 g and ranged from 0.81 to 1.65 g, with TX04V075 having the highest and MT06103 the lowest values (Table 3.7). Average stomatal conductance was 73.51 mmol m^{-2} s^{-1} and ranged from 39.98 to 108.37 mmol m^{-2} s^{-1}; 'Arapahoe' had the highest and MT06103 the lowest g_s among all entries used in this study. Average WUE was 0.00134 and ranged from 0.00087 to 0.00164, with 'TAM111' having the highest and MT06103 the lowest values (Table 3.7).

The experiment effect (planting date) was significant for ABM, RWC and WUE while the Entry x Experiment interaction effect was significant ($P<0.05$) only for LR (Table 3.6).

Variation in root allocation and morphology

The 30 Great Plains winter wheat entries differed for three root allocation traits (TL, TLBS, and TLTS), $P<0.05$ (Table 3.8). There were no significant differences due to experiment or Entry x Experiment interaction for any of the root allocation and morphology traits ($P>0.05$) (Table 3.8). Average TL was 9584 cm and ranged from 8502 to 11,119 cm. 'Parker 76' had the lowest and 'Vona' had the greatest TL, followed by 'SD01237' and Colorado entries 'Byrd' and 'Hatcher', respectively (Figure 3.3 and Appendix 6). Hatcher, Parker, TX07A001, Darrell, and Ogallala, respectively ranked heist for total root allocation percentage in the top section (from

30.0 to 32.2 %), and MT85200, MT06103, Comanche, SD01237, and Arapahoe, respectively ranked highest for total root allocation percentage in the bottom section (from 41.4 to 44.3 %) (Appendix 6).

Although Great Plains winter wheat entries did not differ significantly for any of the root diameter traits (Figure 3.4), they did differ for many of the root length per diameter class traits (Table 3.9). Entries differed significantly (P<0.05) for root length for four diameter classes (0.0 - 0.25, 0.50 -0.75, 0.75 -1.00, and >1.00 mm) in the top section (0-33 cm tube depth), and for the 0.0 -0.25 mm diameter class in the middle (33-66 cm tube depth) and bottom (66-99 cm tube depth) sections. Moreover, the highest proportion of roots in the three sections fell within the finest/first diameter class (0.00 - 0.25 mm) followed by the second diameter class (Appendix 8). In the top section, 58% of the total root length fell within the first diameter class compared to 56% in the middle and 49% in the bottom root sections (Appendix 8).

Correlations among traits

Similar to Study I, there were negative (P<0.05) correlations between adjusted total root length overall and per section and average diameter overall and per section (r ranged from -0.35 to -0.40) (Table 3.10). Relative water content correlated negatively (P<0.05) with LR (r=-0.39) which is also similar to Study I. Moreover, ABM correlated positively with ETr (r=0.52) (Table 3.10). A complete correlation matrix between pairs of traits in Study II is contained in Table 3.10.

Comparison between tall and semi dwarf-entries

Seven tall entries (entries lacking the semi-dwarf alleles *Rht B1b* or *Rht D1b*) were contrasted with the other 23 entries, 21 possessing *Rht Blb* and two having *Rht D1b*. The two groups were not significantly different ($P>0.05$) for any of the root traits (data not shown).

Discussion

Study I

In this study, we investigated the variation in root and drought tolerance related morphological and physiological traits among Colorado winter wheat entries under drought stress conditions in the greenhouse, thus achieving a major objective of this study. Colorado winter wheat entries showed relatively little variation in physiological traits under drought stress. This may be due to the close genetic relationships among some of the entries used, and the fact that all were selected under moisture stress conditions typical of eastern Colorado. Apart from the root traits, two traits showed significant ($P<0.05$) variation among the 30 entries: ETr and ABM. These two traits are important indicators of a wheat plant's drought tolerance and adaptation. Estimated transpiration indicates the plant's ability to extract and use water and can be assessed through water loss from the tubes in our experimental set-up. In this study, we were able to account for evaporation from the soil surface, so that adjusted water loss was a reasonable estimate of transpiration through stomata (stomatal conductance to the atmosphere). Plant conservative water use can be beneficial in areas of limited soil moisture where plants depend on the underground water, especially later in the season, when there is a terminal drought (Zaman-Allah et al., 2011). On the other hand, plants with high water use are preferable in areas with available water, or when plants depend on frequent

ample rainfall (Palta et al., 2011). Estimated transpiration is an indicator of plant water uptake and use, which in turn is correlated with plant root system size. Bill Brown had the highest total root length and ranked third in respect to ETr (Table 3.2). Meanwhile, Snowmass had the smallest total root length (5239 cm) and ranked among the lowest five entries for ETr. This may explain why Snowmass is less drought stress tolerant than entries like Byrd and Ripper in field trials (S. Haley, personal observation).

Entries varied in regards to ABM, a key trait that is an indicator of productivity under drought stress. Under low moisture conditions, entries that can maintain a reasonable above ground biomass are considered drought tolerant (Huang et al., 2007). The correlation between ABM and TRBM and TotRBM was significant (P<0.05) (r=0.55 and 0.58, respectively). Since biomass production is tightly linked to transpiration, breeding for maximized soil moisture capture for transpiration through roots is the most important target for yield improvement under drought stress (Blum, 2009). Effective use of water, as described by Blum (2009), implies maximal soil moisture capture for transpiration, and involves reduced non-stomatal transpiration and minimal water loss by soil evaporation. Moreover, ABM is an indicator of plant size, which is often highly correlated with yield and productivity as well as drought tolerance. Small plant size, reflected here by ABM, is a function of drought sensitivity as it is a function of reduced leaf area, early maturity, prolonged stomatal closure, reduced evapotranspiration, and reduced photosynthesis, which in the end leads to reduced yield potential (Karamanos and Papatheohari, 1999). Entries differed significantly in respect to root diameter in this study (P<0.05, Table 3.2) and root diameter is known to be influential in regards to water uptake from the soil profile. Previous studies have shown that plants with

smaller root diameter are able to withdraw more water from deep in the soil profile (Palta et al., 2011). This is because smaller root diameters increase soil contact per biomass of root; also from an allocation perspective, a plant can produce more root length for the same biomass invested if those roots are thinner. This association was very clear in this study as adjusted total root length traits negatively correlated with root diameter traits (Table 3.4). WinRhizo was able to quantify the total root length in each of the pre-identified five diameter classes. This enabled us to calculate the percentage of total root length within each diameter class in the three tube sections. It is notable that the finer classes (class 1: 0-0.25 mm and class 2: 0.25-0.50 mm) represented most of the total root length in all sections = this is of special importance for the bottom root section, where the two finest classes made up 84 % of total root length. Byrd is well known to be a drought tolerant and high yielding cultivar (Becker, 2014 and S. Haley, personal observation) and ranked second for TL (6782 cm). In addition, Byrd ranked second for AD (second finest root system). This suggests that a combination of high total root length and small root diameter is beneficial for drought adaptation, which is in consistent with the conclusion of Wasson et al. (2012). Total root length calculated by WinRhizo software and adjusted for plant size can be used as an indicator of root system size. In this study, it was notable to see that the Colorado entries have a reasonable variation in regards to this trait in all root sections. This variation can be utilized in pre-breeding to screen entries for desired root traits.

The Study I entries did not show significant variation for many physiological traits, including relative water content and stomatal conductance. This may be because all the entries were developed in the same region - in this case, Colorado - and therefore, may be adapted

physiologically to drought stress in the same way. Another explanation may be that some of the entries share a major part of their pedigrees. However, RWC, an important indicator for water status in the plants, associated significantly (P<0.05) and positively with three root allocation traits (TLTS, TLBS, and TL), r=0.38, 0.64, and 0.43, respectively). This positive association suggests that plants with more root allocation are able to keep their water status under drying soil conditions and vice versa. Plants that were able to keep a high water status reflected in RWC were able to avoid drought stress and keep a relatively high LER and ABM. Correlation coefficients between RWC and LER and ABM were relatively high (r= 0.63, 0.37, respectively) in this study (Table 3.4).

Study II

This study investigated the variation in drought tolerance related traits among 30 entries, selected from a Great Plains winter wheat association mapping population to represent breeding programs in seven states. Most of the entries used in this study were recently developed, but we included four very old varieties and three others without *Rht* semi-dwarf alleles. The purpose was to compare the newer (post-Green Revolution) and older (pre-Green Revolution) wheat varieties to determine if root traits were modified in the recent entries due to introduction of semi-dwarf (*Rht-B1b* and *Rht-D1b*) genes.

Great Plains entries differed significantly (P<0.05) for three important morphological and physiological traits: ABM, g_s and WUE. Above ground biomass was also significant in Study I. MT06103 had the lowest ABM as well as the lowest below ground biomass (root biomass) among all entries. However, for the entire study above ground and below ground biomass were not significantly correlated, though they were significantly correlated in Study I. The negative

correlations between root allocation (root length) and average root diameter observed in Study I, was confirmed again in this Study. This confirms the interpretation that plants with fine roots are able to allocate more root length per the same unit of root biomass. This may result in more water extraction deep in the soil profile in drying soil conditions and is an advantageous trait for drought adaptation. Byrd is a new high yielding and drought tolerant cultivar; it ranked third for total root length and ranked very high for root allocation at depth (TLBS) as well as root biomass. This is in agreement with results from Study I, where Byrd was ranked second for total root length, which gives us confidence in the repeatability of these experiments. Byrd also had a very high leaf elongation rate, RWC percentage (ranked first), and a large proportion of roots with finer root diameter under drought stress. A larger root system (indicated by greater total root length allocation) with a fine diameter may contribute to Byrd's drought tolerance capacity, as was also suggested in Study I. This finding supports other research, which indicated that a larger root system is important for water uptake and maintenance of water status (Reynolds et al., 2007). Moreover, by increasing the relative size of root systems and consequently their absorptive surface area, plants may enhance the availability of the soil-limited resources, including water and nutrients (Fitter 1994; Rodrigues et al. 1995).

It was notable that old varieties like Kharkof, Comanche, and Wichita had a very low total root length (among the lowest five entries). Kharkof and Wichita had very high above ground biomass, probably because they do not have the reduced-height genes (*Rht-B1b* and *Rht-D1b*). This indicates that the older varieties did not have a favorable balance between below and above ground biomass, which is an important requirement for drought adaptation. Above ground biomass of the older varieties was very high compared to below ground biomass, which

makes such wheat germplasm more susceptible to lodging in the field. Meanwhile, new cultivars like Byrd and Hatcher had a more favorable balance between the below and above ground biomass. This trait is "root:shoot ratio" and indicates a plant's tendency to allocate resources among organs to optimize whole plant growth (Thornley, 1972; Blum et al., 1982). Plants may be adapted to produce a particular root:shoot ratio but this ratio will shift to balance resources limiting growth with a degree of plasticity, or responsiveness, which is a trait of interest (Shipley and Meziane, 2002). Root:shoot ratio changes with plant growth and development in addition to shifting in response to limiting resources above versus below ground (Comas et al., 2013).

In this study, root:shoot ratio ranged from 0.50 to 0.30, and Byrd (recent drought tolerant cultivar) ranked exactly in the middle (ratio of 0.36, 16 among 30 entries). On the other hand, Comanche, Wichita, Kharkof, and Cheyenne ranked number 20, 23, 24, and 18. Lower root: shoot ratio in the case of the four old varieties and intermediate ratio in the case of Byrd and Hatcher suggest the hypothesis that recent cultivars are able to adapt to a range of environmental conditions and improve grain yield by optimizing their root: shoot ratio. Comas et al. (2013) suggested that the size of a plant's root system which is a key trait for acquisition of soil resources is valid only when considered in relation to the size of the remainder of the plant, either relative to leaf area, shoot or whole plant size. Shifts in allometry (metrics of root to shoot relationships) and shoot stature can compensate for water shortage, and, along with shifts in stand densities, can maintain stomatal conductance under xeric conditions similar to levels under mesic conditions (Maseda and Fernández, 2006; Mencuccini, 2003). For a given population of target environments, there is likely an optimum root:shoot ratio above which

further increases in root size would provide limited benefits but would impose a cost on shoot growth by consuming biomass (Passioura, 1983).

The contrast analysis clearly revealed that the *Rht* semi-dwarf alleles that were introduced in wheat during the Green Revolution did not affect the root allocation and morphology traits analyzed within the Great Plains winter wheat entries in this study. This finding is in agreement with Wojciechowski et al (2009) in the UK, who concluded that there were no significant differences in root length between semi-dwarfing lines and the control lines. Similarly, Miralles et al. (1997) and Richards (1992) concluded that dwarfing genes *Rht-B1b* and *Rht-D1b* in wheat reduce height and shoot biomass but do not reduce root length or weight, root system depth or water uptake compared with tall near-isogenic lines. However, other studies suggested that semi-dwarf alleles may be associated with reduced root growth (Waines and Ehdaie, 2007). These contrasting reports may be due to differences in experimental methods or in the genetic background of the plant material. However, our results are consistent with the majority of reports, finding no root trait effects of *Rht* semi-dwarf alleles.

In summary, the tube rhizotron system was shown to be an effective method to phenotype wheat root systems. Based on this system, variation was observed in root and other drought related traits among Colorado and Great Plains winter wheat entries. There was no significant effect of the *Rht-B1b* or *Rht-D1b* genes on root system growth of the winter wheat germplasm used in this study. Variation in root allocation and morphology and correlations of these traits with other morphological and physiological traits can advance understanding of

drought adaptation mechanisms and help breeders select for plants with desirable root traits for drought stressed environments.

Table 3.1 Analysis of variance for physiological and morphological traits in Study I (Colorado winter wheat entries) under drought stress.

Source	ABM†	TRBM	MRBM	BRBM	TotRBM	LR	RWC	g_s	ETr	WUE
Entry (E)	**	NS	NS	NS	NS	NS	NS	NS	**	NS
Experiment (Exp)	***	**	NS	***	***	***	NS	NS	NS	**
E × Exp	**	NS	NS	NS	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† ABM, above-ground biomass; TRBM, top tube section root biomass; MRBM, middle tube section root biomass; TotRBM, Total root biomass; BRBM, bottom tube section root biomass; LR, longest root depth; RWC, relative water content; g_s, stomatal conductance; ETr, estimated transpiration; WUE, water use efficiency.

Table 3.2 Least squares means of physiological and morphological traits in greenhouse Study I (Colorado winter wheat entries) under drought stress.

Entry	ABM† g	TRBM g	MRBM g	BRBM g	LR cm	RWC %	g_s mmol m^{-2} s^{-1}	ETr kg	WUE NA	Root: Shoot Ratio
Above	0.85	0.125	0.040	0.064	120	89	224	0.701	0.00114	0.253
Akron	0.98	0.152	0.054	0.067	115	86	78	0.727	0.00132	0.272
Avalanche	1.14	0.139	0.039	0.064	122	88	145	0.734	0.00135	0.205
Bill Brown	0.95	0.143	0.033	0.089	125	89	222	0.748	0.00119	0.266
Bond CL	1.08	0.077	0.049	0.069	119	88	166	0.755	0.00139	0.201
Byrd	1.08	0.101	0.073	0.102	120	83	106	0.759	0.00134	0.258
Carson	1.12	0.097	0.032	0.138	117	72	72	0.763	0.00172	0.231
CO04393	1.04	0.210	0.059	0.106	124	87	91	0.769	0.00134	0.348
CO04499	0.92	0.104	0.032	0.063	115	87	102	0.769	0.00124	0.203
CO04W320	0.89	0.121	0.036	0.063	121	84	143	0.769	0.00116	0.223
CO940610	1.10	0.101	0.040	0.095	121	85	154	0.775	0.00137	0.198
Denali	0.97	0.102	0.062	0.085	118	88	99	0.776	0.00124	0.280
Duke	0.97	0.117	0.045	0.085	114	88	140	0.779	0.00110	0.293
Hail	1.07	0.108	0.060	0.093	118	81	108	0.783	0.00132	0.244
Halt	0.76	0.130	0.031	0.082	120	72	114	0.787	0.00110	0.320
Hatcher	1.04	0.116	0.064	0.116	117	78	106	0.790	0.00127	0.286
Jules	0.82	0.091	0.037	0.064	115	90	161	0.791	0.00108	0.209
Lamar	1.13	0.142	0.038	0.098	116	88	158	0.795	0.00176	0.273
Lindon	0.88	0.121	0.059	0.117	118	88	187	0.797	0.00120	0.330
Platte	1.09	0.104	0.042	0.084	116	81	116	0.799	0.00132	0.245
Prairie Red	0.92	0.099	0.055	0.080	119	88	129	0.804	0.00116	0.238
Prowers	1.05	0.099	0.032	0.042	116	89	116	0.811	0.00134	0.159
Ripper	1.02	0.105	0.058	0.108	116	90	162	0.812	0.00132	0.261
RonL	1.23	0.096	0.058	0.199	114	81	103	0.815	0.00167	0.344
Sandy	1.05	0.078	0.055	0.106	115	88	99	0.819	0.00139	0.230
Snowmass	0.90	0.085	0.045	0.102	111	86	96	0.826	0.00121	0.258
Thunder CL	0.91	0.107	0.073	0.107	125	75	118	0.827	0.00117	0.287
Vona	0.90	0.143	0.042	0.081	113	83	197	0.840	0.00117	0.324
Yuma	1.07	0.103	0.047	0.093	116	84	127	0.861	0.00132	0.249
Yumar	0.93	0.107	0.046	0.075	115	83	168	0.872	0.00118	0.236
Mean	1.00	0.114	0.048	0.091	118	85	134	0.788	0.00130	0.257

† ABM, above-ground biomass; TRBM, top tube section root biomass; MRBM, middle tube section root biomass; BRBM, bottom tube section root biomass; LR, longest root depth; RWC, relative water content; g_s, stomatal conductance; ETr, estimated transpiration; WUE, water use efficiency.

Table 3.3 Analysis of variance for scanned root traits in Study I (Colorado winter wheat entries) under drought stress

Source	AD†	ADTS	ADMS	ADBS	TL	TLTS	TLMS	TLBS
Entry (E)	**	**	**	*	***	***	**	**
Experiment (Exp)	***	***	***	***	***	*	***	***
E × Exp	*	NS	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† AD, average diameter in mm; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; TL, total length; TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section.

Table 3.4 Analysis of variance for total root length per root diameter class in Study I (Colorado winter wheat entries) under drought stress.

Source	Root Length per root diameter class (cm)														
	0-33 cm tube depth					33-66 cm tube depth					66-99 cm tube depth				
Root diameter class (mm)	0.0 - 0.25	0.25 - 0.50	0.50 - 0.75	0.75 - 1.00	>1.00	0.0 - 0.25	0.25 - 0.50	0.50 - 0.75	0.75 - 1.00	>1.00	0.0 - 0.25	0.25 - 0.50	0.50 - 0.75	0.75 - 1.00	>1.00
Entry (E)	***	*	***	***	***	**	*	NS	NS	NS	***	*	**	**	***
Experiment (Exp)	***	*	NS	NS	NS	***	NS	NS	NS	*	***	*	NS	NS	NS
E × Exp	NS	NS	*	*	*	NS	NS	NS	NS	NS	NS	NS	*	*	*

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 3.5 Phenotypic correlation coefficients among physiological, morphological and scanned root traits under drought stress among Colorado winter wheat entries (Study I) (n=30).

Traits†	TLMS	TLBS	TL	ADTS	ADMS	ADBS	AD	TRBM	MRBM	BRBM	TotRBM	ABM	LER	g_s	LR	RWC	ETr
TLTS	0.02	0.25	0.49*	-0.43*	-0.08	0.13	-0.15	0.27	0.05	-0.18	0.13	0.30	0.07	0.18	0.09	0.38*	0.12
TLMS		0.21	0.50*	-0.04	0.07	0.00	0.16	0.03	0.12	0.05	0.22	-0.03	0.21	-0.09	-0.30	0.08	0.03
TLBS			0.50*	-0.24	-0.58**	-0.17	0.02	-0.16	0.04	0.42*	0.50*	0.59**	0.34	0.16	-0.44*	0.64***	0.01
TL				-0.69***	-0.60**	0.16	-0.03	-0.09	-0.20	0.18	0.45*	0.18	0.49*	000.04	-0.43*	0.43*	-0.34
ADTS					0.65***	-0.28	0.12	0.17	0.56**	0.05	-0.20	-0.19	-0.43*	0.12	0.35	-0.30	0.42*
ADMS						0.09	0.24	0.34	0.30	-0.31	-0.43*	-0.35	-0.34	-0.02	0.42*	-0.47*	0.40*
ADBS							0.55*	0.05	-0.53*	-0.30	-0.10	0.05	0.43	-0.02	0.04	0.09	-0.13
AD								-0.34	-0.34	0.29	0.29	0.46*	0.39*	-0.40*	-0.18	0.32	-0.04
TRBM									0.34	-0.43*	-0.14	-0.48*	-0.16	0.51*	0.34	-0.43*	-0.02
MRBM										0.11	0.06	-0.24	-0.47*	0.31	0.30	-0.12	0.37*
BRBM											0.82***	0.55*	0.09	-0.29	-0.49*	0.27	-0.04
TotRBM												0.58**	0.22	-0.22	-0.51*	0.37*	-0.19
ABM													0.16	-0.22	-0.50*	0.63**	0.17
LER														-0.24	-0.13	0.37*	-0.47*
g_s															0.26	-0.24	0.05
LR																-0.45*	-0.02
RWC																	0.00

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† TLTS, total length for top root section; TLMS, total length for middle root section ; TLBS, total length for bottom root section; TL, total root length; ADTS, average diameter for top root section; ADMS, average diameter for middle root section; ADBS, average diameter for bottom root section ; AD, average root diameter; TRBM, top root section biomass; MRBM, middle root section biomass; BRBM, bottom root section biomass; TotRBM, Total root biomass; ABM, above-ground biomass; LER, leaf elongation rate; g_s, stomatal conductance; LR, longest root depth; RWC, relative water content; ETr, estimated transpiration.

Table 3.6 Analysis of variance for physiological and morphological traits of Study II (Great Plains winter wheat entries) under drought stress.

Source	ABM†	TRBM	MRBM	BRBM	TotRBM	LR	RWC	g_s	ETr	WUE
Entry (E)	***	NS	NS	NS	NS	NS	NS	**	NS	***
Experiment (Exp)	***	NS	NS	NS	NS	NS	**	NS	**	***
E × Exp	NS	NS	NS	NS	NS	*	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† ABM, above-ground biomass; TRBM, top tube section root biomass; MRBM, middle tube section root biomass; BRBM, bottom tube section root biomass; TotRBM, Total root biomass; LR, longest root depth; RWC, relative water content; g_s, stomatal conductance; ETr, estimated transpiration WUE, water use efficiency.

Table 3.7 Least squares means of physiological and morphological traits in greenhouse Study II (Great Plains winter wheat entries) under drought stress.

Entry	ABM† g	TRBM g	MRBM g	BRBM g	LR cm	RWC %	g_s mmol m^{-2} s^{-1}	ETr kg	WUE NA	Root: Shoot Ratio	LER cm/d
Arapahoe	1.351	0.166	0.114	0.183	122	77	108	0.978	0.00139	0.285	0.541
Byrd	1.494	0.174	0.147	0.217	124	87	79	1.099	0.00137	0.340	0.817
Centura	1.502	0.169	0.126	0.182	123	78	77	0.953	0.00160	0.298	0.841
Cheyenne	1.221	0.136	0.120	0.180	122	78	70	0.989	0.00123	0.286	0.650
Comanche	1.268	0.133	0.134	0.172	124	77	72	0.956	0.00132	0.317	0.533
Darrell	1.504	0.209	0.254	0.151	126	75	77	1.014	0.00150	0.360	0.279
Hatcher	1.449	0.201	0.158	0.225	125	71	72	1.113	0.00133	0.353	0.717
Harding	1.190	0.150	0.109	0.165	126	82	63	0.916	0.00127	0.310	0.575
Judee	1.238	0.185	0.149	0.165	123	80	94	1.028	0.00123	0.343	0.573
MT06103	0.809	0.140	0.109	0.145	125	73	39	0.929	0.00087	0.410	0.646
MT85200	1.272	0.160	0.115	0.189	125	77	68	1.010	0.00127	0.308	0.443
Nusky	1.154	0.201	0.129	0.194	122	74	81	0.950	0.00125	0.383	0.601
OK05204	1.434	0.178	0.135	0.204	123	69	86	1.053	0.00137	0.290	0.476
OK05526	1.319	0.213	0.095	0.179	124	71	60	1.008	0.00134	0.310	0.504
OK06114	1.514	0.169	0.127	0.223	127	73	72	1.053	0.00145	0.310	0.621
OK101	1.286	0.224	0.136	0.161	126	70	96	1.017	0.00126	0.320	0.420
Ogallala	1.226	0.207	0.118	0.157	121	82	63	0.967	0.00125	0.305	0.705
Overland	1.473	0.160	0.126	0.193	125	84	89	1.063	0.00142	0.285	0.640
Parker	1.476	0.191	0.117	0.175	129	73	61	1.045	0.00143	0.293	0.811
Parker 76	1.557	0.147	0.131	0.186	125	74	69	1.013	0.00158	0.293	0.718
Ripper	1.305	0.141	0.115	0.160	126	68	76	1.045	0.00126	0.276	0.539
SD01237	1.338	0.182	0.126	0.202	121	79	60	1.063	0.00127	0.330	0.574
TAM 111	.	0.198	0.142	0.211	129	75	76	0.996	0.00164	0.005	0.412
TX04V075	1.652	0.205	0.121	0.203	127	73	71	1.033	0.00136	0.273	0.559
TX05A001	1.398	0.172	0.138	0.181	118	78	50	1.038	0.00136	0.260	0.706
TX07A001	1.398	0.182	0.124	0.222	121	75	70	1.030	0.00108	0.312	0.595
Vona	1.117	0.181	0.137	0.226	127	74	53	1.029	0.00130	0.397	0.675
Wendy	1.354	0.217	0.174	0.190	120	75	61	1.063	0.00139	0.355	0.562
Kharkof	1.511	0.164	0.131	0.211	124	79	99	1.109	0.00152	0.290	0.675
Wichita	1.473	0.174	0.117	0.210	125	78	77	0.982	0.00139	0.304	0.481
Mean	1.355	0.178	0.132	0.189	124	76	73	1.018	0.00134	0.307	0.596

† ABM, above-ground biomass; TRBM, top tube section root biomass; MRBM, middle tube section root biomass; BRBM, bottom tube section root biomass; LR, longest root depth; RWC, relative water content; g_s, stomatal conductance; ETr, estimated transpiration; WUE, water use efficiency; LER, leaf elongation rate.

Table 3.8 Analysis of variance for scanned root traits in Study II (Great Plains winter wheat entries) under drought stress

Source	AD†	ADTS	ADMS	ADBS	TL	TLTS	TLMS	TLBS
Entry (E)	NS	NS	NS	NS	*	*	NS	*
Experiment (Exp)	NS	NS	NS	NS	NS	NS	NS	NS
E × Exp	NS	NS	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† AD, average diameter; ADTS, average diameter for top section; ADMS, average diameter for middle section ; ADBS, average diameter for bottom section; TL, total length; TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section.

Table 3.9 Analysis of variance for root traits (root length per root diameter) in Study II (Great Plains winter wheat entries) under drought stress.

Source	Root Length per root diameter class (cm)														
	0-33 cm tube depth					33-66 cm tube depth					66-99 cm tube depth				
Root diameter class (mm)	0.0 - 0.25	0.25 - 0.50	0.50 - 0.75	0.75 - 1.00	>1.00	0.0 - 0.25	0.25 - 0.50	0.50 - 0.75	0.75 - 1.00	>1.00	0.0 - 0.25	0.25 - 0.50	0.50 - 0.75	0.75 - 1.00	>1.00
Entry (E)	***	NS	*	**	**	**	NS	NS	NS	NS	**	NS	NS	NS	NS
Experiment (Exp)	**	***	***	***	***	***	**	**	**	*	*	NS	NS	*	NS
E × Exp	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 3.10 Phenotypic correlation coefficients among physiological, morphological and scanned root traits under drought stress in Study II (Great Plains winter wheat entries) (n=30).

Traits†	TLMS	TLBS	TL	ADTS	ADMS	ADBS	AD	TRBM	MRBM	BRBM	TotRBM	ABM	LER	g_s	LR	RWC	ETr
TLTS	0.73***	0.11	0.82***	-0.10	-0.35*	-0.15	-0.39*	0.51*	0.08	0.12	0.42*	0.11	0.01	-0.10	0.15	0.12	0.13
TLMS		0.49*	0.71***	-0.09	-0.19	-0.19	-0.25	0.34	0.15	0.28	0.38*	-0.04	0.30	-0.27	0.01	0.22	0.16
TLBS			0.22	0.27	0.02	-0.15	0.19	0.01	0.14	0.26	0.13	0.06	0.16	-0.09	0.05	-0.20	0.34
TL				-0.13	-0.32	-0.36*	-0.40*	0.33	0.09	0.16	0.30	0.04	0.11	-0.14	0.02	0.21	-0.05
ADTS					0.12	-0.20	0.61	-0.02	-0.03	-0.08	-0.06	-0.06	-0.18	0.33	0.13	-0.05	0.03
ADMS						0.20	0.74***	-0.12	0.06	-0.33	-0.18	-0.09	0.04	-0.09	0.19	0.11	0.13
ADBS							0.33	0.05	-0.20	-0.27	-0.13	0.14	0.20	-0.15	0.07	-0.11	0.30
AD								-0.09	-0.04	-0.32	-0.18	-0.11	-0.06	0.10	0.17	-0.05	0.11
TRBM									0.37*	0.09	0.75***	-0.23	-0.26	-0.20	0.15	0.15	-0.07
MRBM										0.30	0.71***	-0.12	-0.24	-0.14	0.11	0.01	0.01
BRBM											0.58**	-0.31	-0.11	-0.19	-0.22	0.08	-0.03
TotRBM												-0.23	-0.34	-0.22	0.02	0.13	0.02
ABM													0.24	0.26	0.08	-0.10	0.52*
LER														-0.27	-0.18	0.26	0.18
g_s															0.01	0.10	0.10
LR																-0.39*	0.01
RWC																	-0.11

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† TLTS, total length for top root section; TLMS, total length for middle root section ; TLBS, total length for bottom root section; TL, total root length; ADTS, average diameter for top root section; ADMS, average diameter for middle root section; ADBS, average diameter for bottom root section ; AD, average root diameter; TRBM, top root section biomass; MRBM, middle root section biomass; BRBM, bottom root section biomass; TotRBM, Total root biomass; ABM, above-ground biomass; LER, leaf elongation rate; g_s, stomatal conductance; LR, longest root depth; RWC, relative water content; ETr, estimated transpiration.

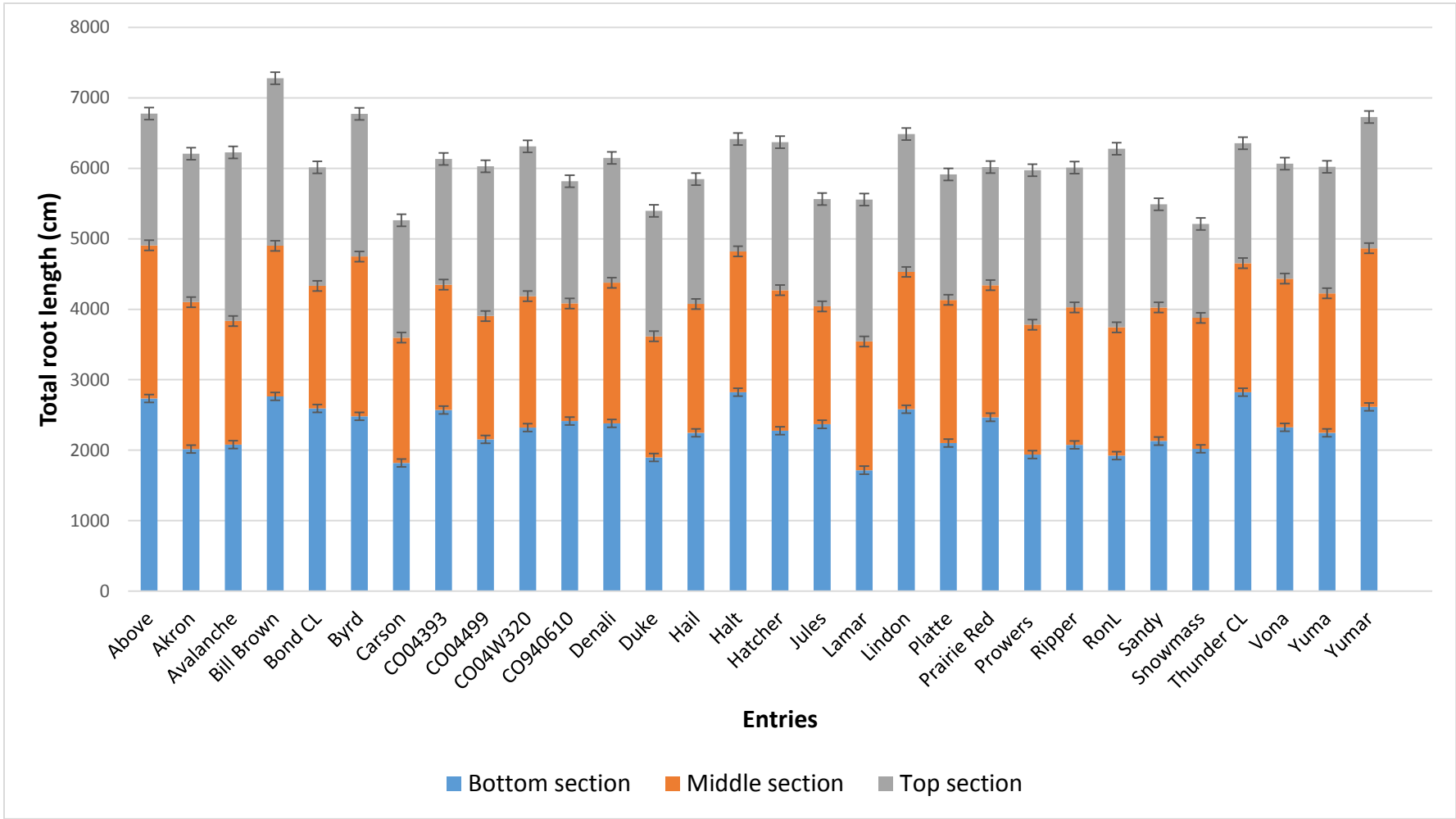

Figure 3.1 Adjusted total root length (cm) for above ground plant size in the three root sections (bottom, middle and top) of the tubes for entries in Study I (Colorado winter wheat entries) under drought stress.

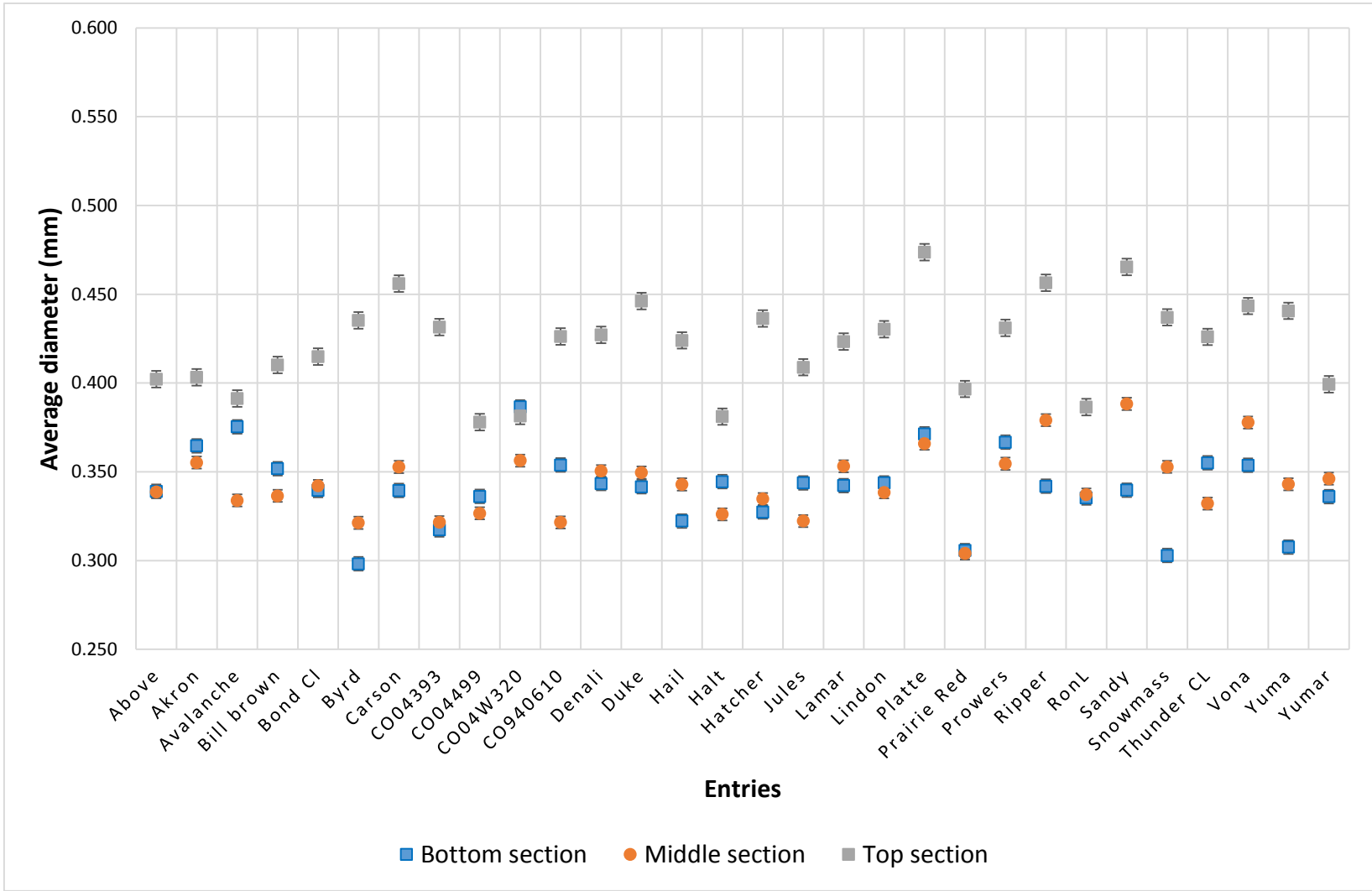

Figure 3.2 Root average diameter in mm for all roots in the three root sections (bottom, middle and top) of the tubes for entries in Study I (Colorado winter wheat entries) under drought stress.

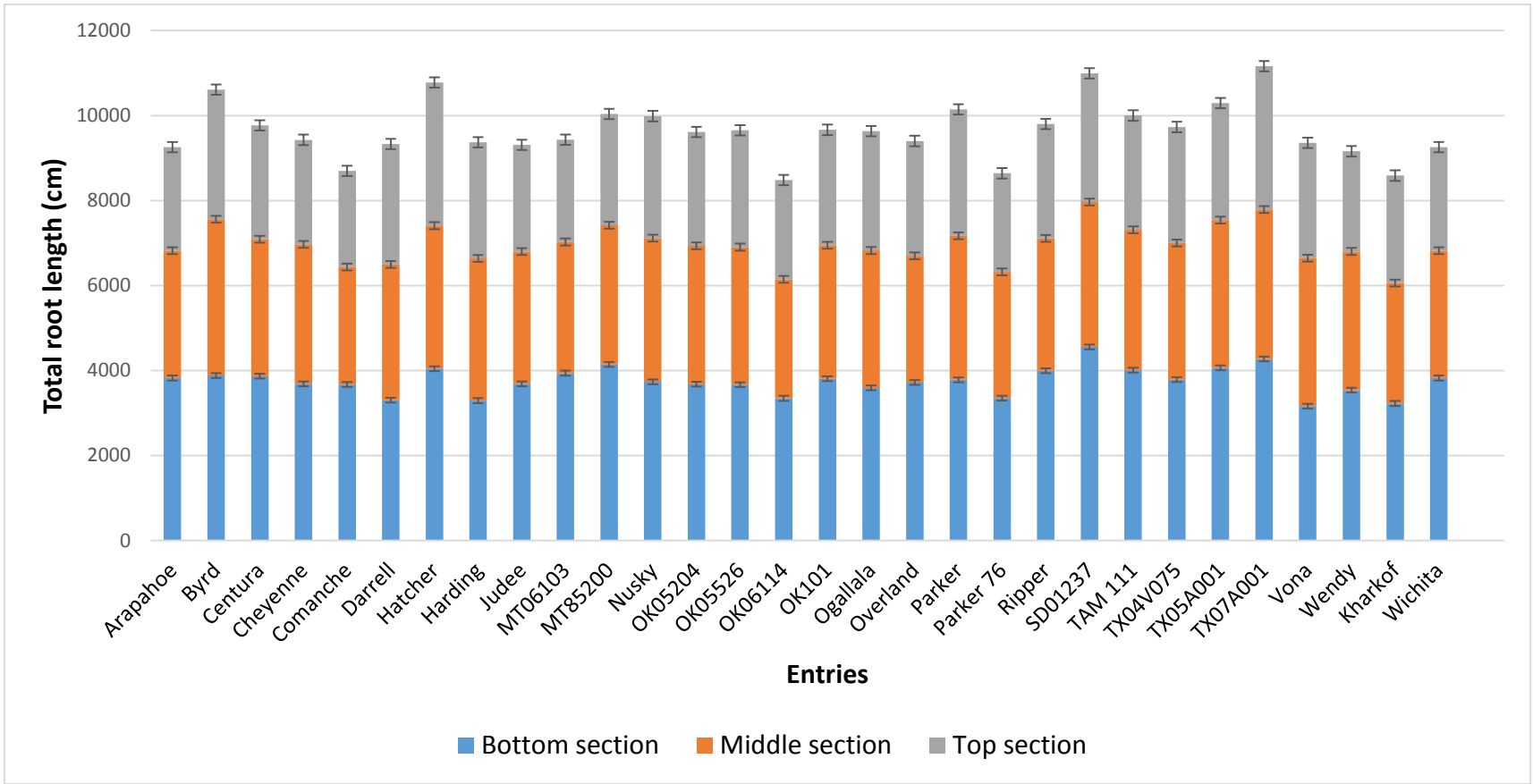

Figure 3.3 Adjusted total root length (cm) for above ground plant size in the three root sections (bottom, middle and top) of the tubes for entries in Study II (Great Plains entries) under drought stress.

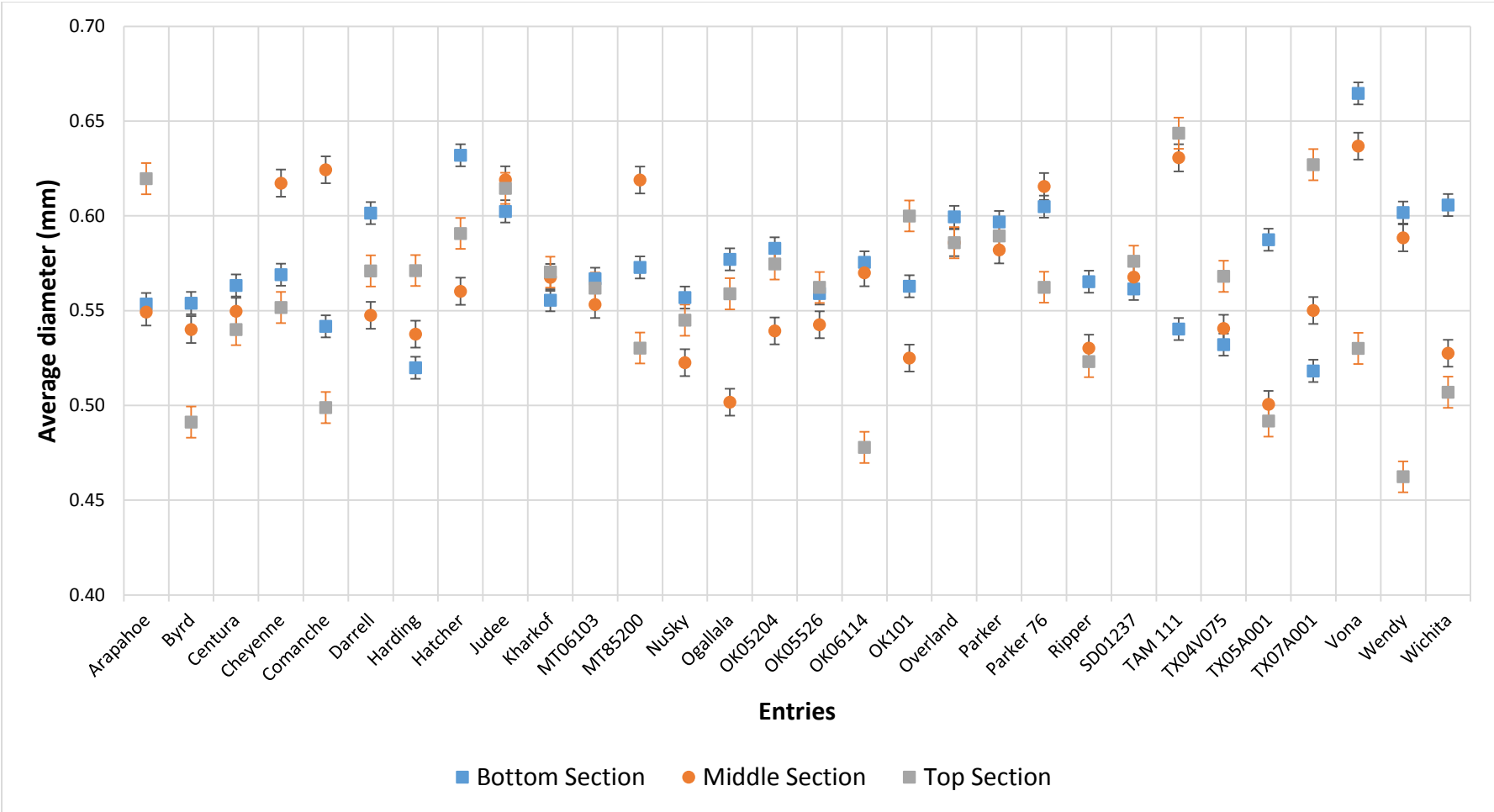

Figure 3.4 Root average diameter in mm for all roots in the three root sections (bottom, middle, and top) of the tubes for entries in Study II (Great Plains entries) under drought stress.

CHAPTER 4: ROOT SYSTEM EVALUATION BY SOIL CORING IN MULTIPLE FIELD ENVIRONMENTS

Summary

Root system traits make major contribution to wheat performance in semi-arid regions. It is very important to evaluate root traits of wheat germplasm in relevant field environments. Soil coring in multiple environments can directly quantify variation in root traits. Therefore, the objectives of this study were 1) to determine the variation in root architecture traits among US Great Plains winter wheat germplasm in the field under water-stressed conditions, 2) to examine correlations among the evaluated root traits and yield, canopy temperature, carbon isotope discrimination, plant height and harvest index, and 3) to examine correlations between root traits in field and greenhouse evaluations. This study was conducted in three location-year environments, each with a different set of entries. In Greeley 2011-12, we collected soil cores of 21 entries that were used in the greenhouse root tube Study I as discussed in Chapter 3. In Fort Collins 2012-13, we collected soil cores of 25 entries that were used in the greenhouse root study II. In Fort Collins 2013-14, we collected soil cores for 12 entries that were part of the confirmation study described in Chapter 2. All entries were included in the Hard Winter Wheat Association Mapping Panel (HWWAMP) (n=299), that was described in Chapter 2. In all three environments, soil cores were collected from the water stressed-treatment (WS) using a 1 m high, 5 cm diameter truck or tractor-mounted hydraulic soil probe at three depths. Roots from the soil cores were washed free of soil, scanned and digital images were analyzed with WinRhizo Regular software. Entries in the Greeley 2011-12 WS trial differed significantly for total length in the

middle section (TLMS), average diameter at the top section (ADTS), average diameter at the middle section (ADMS), total root length (TL), average diameter (AD) (P<0.05) and average diameter at the bottom section (ADBS) (P<0.001). Harvest index (HI) correlated significantly with TLMS (r=0.50, P<0.05), ADMS (r=0.75, P<0.0001), AD (r=0.60), and TL (r=0.40). Moreover, TL correlated negatively with canopy temperature at late heading stage ($T_c$lh) (r=-0.51, P<0.05). The 25 entries at Fort Collins 2012-13 WS trial differed significantly for TLTS, TLBS, and TL (P<0.05), TLMS (P<0.01), ADTS and AD (P<0.001). There was significant (P<0.05) negative correlation between canopy temperature at booting stage ($T_c$bs) and three root traits, TLTS, TLMS, and TL (r=-0.42, -0.24, and -0.36, respectively). The 12 entries in the Fort Collins 2013-14 WS trial differed significantly for TL (P<0.05) and TLMS (P<0.01). There were two interesting correlations, the first was a significant negative correlation between TL and $T_c$vg (r=-0.23, P<0.05), as well as a significant negative correlation between TLMS and canopy temperature at grain filling stage (r=-0.40, P<0.05). In this study plants with larger root systems, reflected by TL, were able to cool down under water stress conditions as T_c was negatively correlated with TL in most cases. In addition, TL was positively correlated with HI in some environments, which may suggest that this trait (TL) is an important trait for productivity of wheat under water stress. When a pair–wise correlation analysis was conducted between root traits collected from the soil coring in the field and the greenhouse studies, only three traits had a significant correlation (TLMS at Gr12, r=0.39, TLBS, and TL at FC14, r=0.64 and 0.70, respectively). These results confirms other studies about root system plasticity.

Introduction

The conceptual framework for yield under drought stress suggested by Passioura (1977) has three important drivers: (1) water uptake (WU), (2) water-use efficiency (WUE) and (3)

harvest index (HI). These drivers stimulate trait-based breeding and genetic dissection of drought-adaptive mechanisms. Several traits have been found to be associated with the above yield component drivers. For WU, when direct selection for variation in root characteristics is unfeasible, measurements associated with stomatal conductance like that of canopy temperature (T_c) provide indirect indicators of water uptake by roots (Reynolds and Tuberosa, 2008). Canopy temperature has been long used as an indirect indicator of root performance and especially rooting depth. Canopy temperature reduction is often an indicator of increased soil moisture extraction capabilities due to increased soil exploration by roots (Lopes and Reynolds, 2010). In addition, studies indicated that T_c during peak stress periods was associated with 50 % of the variation in water extraction in deep soil profiles and also with root length density (Reynolds et al., 2007). For water use efficiency (WUE), carbon isotope discrimination seems to be the best estimate and is based on higher affinity of the carbon-fixing enzyme (Rubisco) for the more common ^{12}C isotope over the less common ^{13}C. A lower discrimination value indicates higher WUE (Mir et al., 2012).

Reynolds et al. (2005) at CIMMYT developed a general model for drought adaptation of wheat that encompasses traits with potential role in dry environments. In their model, some of the important traits included: (1) access to water as a result of rooting depth or density that could be expressed by a relatively cool canopy (Reynolds et al. 2005), (2) water-use efficiency (WUE) as indicated by relatively higher biomass/mm of water extracted from the soil, transpiration efficiency of growth (TE = biomass/mm water transpired) indicated by carbon isotope discrimination of leaves. The model is used to assist in taking breeding decisions by permitting a strategic approach of accumulating drought adaptive alleles by crossing parents with contrasting drought-adaptive mechanisms. Root architecture that helps the plant to have better access to soil moisture under drought enables heat-stressed

crop canopies to meet high evaporative demand associated with hot, low-relative humidity environments, thus resulting in cooler canopies (Reynolds et al., 2005).

There is an increasing number of publications dealing with crop root systems, which indicates the growing awareness regarding their importance for crop productivity, especially in dry environments (Zhu, 2011). Due to the lack of high throughput phenotyping systems and ignorance of trait utility, root traits have not been directly selected so far (Passioura, 2012). For the incorporation of root traits into breeding programs, there is a need for high throughput phenotyping approaches to enable evaluation of mature root systems in the field (Passioura, 2012; Zhu et al., 2011).

'Shovelomics' has been used in maize for field excavation of mature root crowns, followed by root separation and digital imaging which enables a relatively high throughput analysis needed for breeding and quantitative genetics (Colombi et al., 2015b; Trachsel et al., 2010; Wasson et al., 2012b). Soil coring at multiple field environments as well can directly quantify variation in root traits. Increasing the moisture extraction during the grain-filling period by 1 mm may contribute to a 55 kg/ha increase in grain yield for rainfed wheat, according to research in Australia (Manschadi et al., 2006). Water extraction from deep in the soil profile can be achieved by a deep and extensive root system. Depending on the specific method used, root trait screening in controlled environments may offer the advantage of examining a relatively large number of genotypes with reduced environmental variation. However, it is unclear if these controlled environment screens, generally conducted on seedling root systems, translate to larger root systems in the field at the time of grain development (Wasson et al., 2014). Screening for deep roots in the field may have an advantage over controlled environments for breeding programs as an appropriate field

site better represents the target environment. Therefore, the specific objectives of this study were as follows:

I. To determine the variation in root architecture traits among U.S. Great Plains winter wheat germplasm under field drought stress conditions;

II. To assess the association between the evaluated root traits using direct soil coring and canopy temperature (indirect measure of root performance) at different growth stages, as well as other agronomic traits (grain yield, total biomass weight, biomass grain weight, plant height and harvest index);

III. To compare the root traits evaluated in the field with the root traits evaluated in the greenhouse for the same entries.

Materials and Methods

The study was conducted in three location-year environments, each with a different set of entries, as described below.

Greeley 2011-12

Plant materials

In this study, the target was to collect soil cores for the 30 entries that were used in the greenhouse root tube Study I as described in Chapter 3 (Table 3.1). These entries were part of the Hard Winter Wheat Association Mapping Panel (HWWAMP) (n=299), consisting of cultivars and advanced lines developed by the Triticeae Coordinated Agricultural Project (TCAP). We were successful in collecting soil cores for 21 entries in total. Some plots were very difficult to core due to the existence of a hard-pan layer in the soil; these plots were excluded from the analysis.

Experimental design and growing conditions

The soil cores were collected from the dry treatment of the field trial conducted at the USDA-Agricultural Research Service Limited Irrigation Research Farm in Greeley, CO. Details of the experimental design, growing conditions, and agronomic trait measurements are described in Chapter 2.

Soil coring and root analysis

Soil cores were collected from the water stressed treatment only using a 1 m high, 5 cm diameter truck-mounted hydraulic soil probe (#15-SCS / Model GSRPS, Giddings Machine Company Inc., Windsor, CO, USA) at three depths (Top, 0-33 cm probe depth; Middle, 33-66 cm probe depth; Bottom, 66-99 cm probe depth), with three cores per plot. The number of root samples analyzed was 189 total. Roots from the soil cores were washed free of soil using a 500 µm sieve to recover the roots, where large plant material and debris were removed by hand, and roots were picked by tweezers/forceps. Individual root sections were floated in approximately 1 cm of water in a 30 cm x 40.5 cm plexiglass tray and scanned with a MicroTek Scanmaker 9800XL (Microtek, Santa Fe Springs, CA). Digital images were analyzed with WinRhizo Regular software (Regent Instruments Inc., Quebec, Canada), an image analysis system specifically designed for root measurements. Root morphology measurements recorded by WinRhizo included TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter; and root length of the following diameter classes: 0.00 to 0.25 mm, 0.25 to 0.5 mm, 0.5 to 0.75 mm, 0.75 to 1.0 mm, and >1.0 mm.

Fort Collins 2012-13

Plant materials

In this study, the target was to collect soil cores for the 30 entries (part of the HWWAMP) that were used in the greenhouse root tube Study II as described in Chapter 3 (Table 3.2). We were successful in collecting soil cores for only 25 of these entries for the same reasons described in Greeley 2011-12.

Experimental design and growing conditions

The soil cores were collected from the WS trial. Details of the experimental design, growing conditions, and agronomic traits measurements are described in Chapter 2.

Soil coring and root analysis

Soil cores were collected using a 1 m high, 5 cm diameter tractor-mounted hydraulic soil probe at three depths (Top, 0-33 cm probe depth; Middle, 33-66 cm probe depth; Bottom, 66-99 cm probe depth), with four replicates per plot. The total number of samples was 300. Roots were separated from soil and analyzed as described for Greeley 2011-12.

Fort Collins 2013-14

Plant materials

In this study, we collected soil cores for 12 entries (Table 4.1) that were part of a confirmation subset of 50 cultivars and lines of the HWWAMP (as described in Chapter 2).

Experimental design and growing conditions

The soil cores were collected from the dry treatment of the filed trial conducted at Fort Collins, CO 2013-14. For details about experimental design and growing conditions, refer to Chapter 2 confirmation study.

Soil coring and root analysis

Soil cores were collected from reps 1 and 2 in the water stressed treatment using a 1 m high, 5 cm diameter tractor-mounted hydraulic soil probe at three depths (Top, 0-33 cm probe depth; Middle, 33-66 cm probe depth; Bottom, 66-99 cm probe depth), with three cores per plot. For each sample depth, the three soil cores per plot were bulked together. Therefore, the number of samples analyzed was 72. Roots were washed free of soil, scanned and analyzed for root traits with WinRhizo software as described in Greeley 2011-12. Values of root length were divided by three to get the root length per soil core.

Statistical analyses

All statistical analyses were conducted in SAS version 9.3 (SAS Institute, 2011) unless otherwise stated. Analysis of variance (ANOVA) for each trait was conducted with the GLM procedure, with entries considered a fixed variable. Correlation coefficients were obtained for all pairs of traits using least squares means in the CORR procedure. Graphs were constructed using Microsoft Office Excel (Microsoft, 2013) and JMP Pro 9.0.2 (JMP, 2010).

Results

Greeley 2011-12

Variation in root traits

Roots of this group of winter wheat entries, primarily from Colorado, differed significantly for TL, TLMS, AD, ADTS, ADMS, and ADBS ($P<0.05$) (Table 4.2). Mean TL of the 21 entries investigated was 2307 cm and mean average diameter was 0.56 mm (Table 4.2). The greatest total root length was recorded for Byrd (4673 cm), followed by Bond CL (3267) and CO04499 (3251), respectively. For TLBS, Jules ranked highest with 653 cm, followed by Duke and CO04393, with 648 and 592 cm, respectively (Table 4.3 & Figure 4.1). Three entries did not have any roots in the bottom section (Lindon, CO04W320 and Hatcher).

CO940610 had the smallest average root diameter followed by Avalanche, Akron and Prowers (Table 4.3 & Figure 4.2). A complete list of *P*-values, means, ranges, coefficient of variation (CV%), and coefficient of determination (R^2) per trait is included in Table 4.2 and the complete ranking of entries with respect to all measured root traits, is contained in Table 4.3.

Trait correlations

There were some significant correlations, where HI correlated significantly with TLMS (*r*=0.50, P<0.05), ADMS (*r*=0.75, P<0.0001), AD (*r*=0.60, *P*<0.5), and TL (*r*=0.40, *P*<0.05). Moreover, Total root length (TL) correlated negatively with $T_c$lh (*r*=-0.51, *P*<0.05, Table 4.4).

Fort Collins 2012-13

Variation in root traits

Scanned roots of this subset of Great Plains winter wheat entries differed significantly for TL, TLTS, TLMS, TLBS, AD, and ADTS (*P*<0.05) (Table 4.5). Mean TL of the 25 entries studied was 1619 cm and mean average diameter was 0.88 mm (Table 4.5). The highest total root length was recorded for Ripper (2643 cm), followed by Byrd (2417) and Cheyenne (2242), respectively (Figure 4.3). For TLBS, Wichita ranked highest with 626 cm, followed by Judee and Cheyenne with 923 and 843 cm, respectively (Table 4.6). OK05526 had the smallest average root diameter followed by SD01237, TX07A001, and TAM 111 (Table 4.5 & Figure 4.4). A complete list of *P*-values, means, ranges, CV, and R^2 per trait, refer to Table 4.4 and for the full rank of entries in respect to all measured root traits, refer to Table 4.6.

Trait Correlations

There was significant (*P*<0.05) negative correlation between canopy temperature at booting stage and three root traits, TLTS, TLMS, and TL (*r*=-0.42, -0.24, and -0.36, respectively) (Table 2.7). Moreover, there were a significant positive correlation between

ADTS and BGW (r=0.43, P<0.05) and between HI and AD (r=0.45, P<0.05, Table 2.7). There were also significant correlations between agronomic traits where GY correlated with TBM, BGW, and HI (r=0.59, 0.72, and 0.63, P<0.0001).

Fort Collins 2013-14

Variation in root traits

Roots of this subset of 12 Great Plains winter wheat entries differed significantly for TL and TLMS (P<0.05) (Table 4.8). Moreover, trait differences due to replication effects were significant for ADTS and ADMS (P<0.05). Mean for ADMS was 0.91 and 0.75 in rep 1 and 2, respectively and mean for ADTS was 0.90 and 0.78 in rep 1 and 2, respectively. Mean TL of the 12 entries studied was 1782 cm (bulk of three cores divided by 3) and mean average diameter was 1.21 mm (Table 4.8). The greatest total root length was recorded for Hatcher (2561 cm), followed closely by Byrd (2522) and Kharkof (2254), respectively (Table 4.8 & Figure 4.5). For TLBS, Hatcher ranked highest with 955 cm, followed by RonL, Kharkof and Byrd with 930, 911 and 858 cm, respectively (Table 4.8 & Figure 4.5). Prairie Red had the smallest average root diameter followed by Hatcher, Byrd and Kharkof (Table 4.8 & Figure 4.6). A complete list of P-values, means, ranges, CV, and R^2 per trait, refer to Table 4.8 and for the full rank of entries in respect to all measured root traits, refer to Table 4.9.

Trait correlations

There were only two significant correlations, the first is a significant negative correlation between TL and $T_c$vg (r=-0.23, P<0.05), as well as a significant negative correlation between TLMS and $T_c$gf (r=-0.40, P<0.05) (Table 2.10).

Correlation between field soil cores and greenhouse root traits

There was a significant (P<0.05) correlation between TLMS collected from greenhouse Study I and Greeley 2012 (r=0.39) (Table 4.11). There was also a significant (P<0.05)

correlation between two root traits collected from greenhouse studies (I and II) and field study at Fort Collins 2014, these two traits were TLBS and TL (r=0.64 and 0.70, respectively) (Table 4.11).

Discussion

The most common strategy for drought adaptation exploited in crop breeding is drought escape, which refers to plants that complete their life cycle prior to the onset of drought, thus avoiding moisture stress. An alternative strategy, dehydration avoidance, is the sustaining of internal water status during dry external conditions by minimizing water loss and/or maximizing water uptake from the soil profile. Compared to drought escape, drought avoidance is less well studied. Mechanisms of drought avoidance include reduced stomatal conductance, increased water uptake by roots and maintenance of high leaf water potential. Canopy temperature is considered as a fast comparative assay of dehydration avoidance (maintenance of higher leaf water potential) in wheat breeding materials, subjected to soil moisture stress.

Associations between drought adaptation and increased root system size and rooting depth have been drawn across many species (e.g. Kirkegaard and Lilley, 2007; Lopes and Reynolds, 2010). However, the adaptive value of large or deep root systems varies by environment and an applied breeding strategy must consider the climatic trends of the target environment (Palta et al., 2011).

Entries tested varied significantly for most of the root traits in different environments. More variation was noticed in Gr12 and FC13 compared to FC14. Actually, in FC14, entries differed significantly (P<0.001) for TL and TLMS only (Table 4.8). The lack of variation may be because this group of entries was relatively small (12 entries) which may have influenced the statistical power to detect significant differences. The widest range of TL, which is an

indicator of the root size, was observed in the Gr12 site (3539 to 7684 cm with an overall mean of 2307 cm) (Table 4.2). The range of AD at this site (Gr12) was the lowest among the three environments (0.49 to 0.70 mm). Even though Gr12 WS experienced the most severe drought and high temperatures compared to the other two environments, the entries on average had the highest TL and lowest AD. These two traits were suggested as adaptation mechanisms for plants under water stress (Wasson et al., 2012).

Research in Australia by Manschadi et al. (2006) suggests that Increasing the moisture extraction during the grain-filling period by 1 mm may contribute to a 55 kg/ha increase in grain yield for rainfed wheat. Root traits correlations with GY were examined and GY was found to be associated with ADTS (r=0.46) at Gr12 WS. However, HI correlated significantly with several root traits (TLMS, TL, ADMS, and AD) (r=0.50, 0.40, 0.75, and 0.60, respectively). In the FC13 WS trial, only one yield related trait (BGW) was significantly (P<0.05) associated with a root trait (ADTS) (r=0.43, Table 4.7).

At Gr12 WS, $T_c$lh was highly negatively associated with TL (r=-0.51). Similarly, at FC13 WS, $T_c$bs associated negatively (P<0.05) with three roots trait (TLTS, TLMS, and TL, r=-0.42, -0.24, and -0.36, respectively, Table 4. 7). At FC14 WS, $T_c$gf correlated negatively with TLMS (r=-0.40). These associations suggest that a root system that is able to access deep soil water would result in more open stomates and lower leaf temperature. Canopy temperature measurements serve as a proxy for open transpiring stomata indicating continued access to water (Wasson et al., 2014). Canopy temperature can be an indirect measure of plant water status, although it is not the only factor that may cause stomata to close (Rebetzke et al., 2013). As a proxy for root traits, Lopes and Reynolds (2010) reported that wheat genotypes with more root growth at depth had cooler canopies, from more green leaf area and/or transpiration.

The cultivar Byrd was included in all three environments and ranked highest in respect to TL in all of them. Byrd also ranked highest for TL in the greenhouse root study (as discussed in Chapter 3). When a pair–wise correlation analysis was conducted between root traits collected from the soil coring in the field and the greenhouse studies, only three traits had a significant correlation (TLMS at Gr12, *r*=0.39, TLBS, and TL at FC14, *r*=0.64 and 0.70, respectively, Table 4.11). These results confirm other studies about root system plasticity. Most of the previous studies on root morphology have indicated the plastic nature of root development, which shows a high interaction with the experimental environment (Gregory et al., 2009; Kato et al., 2006; McKenzie et al., 2009; Passioura, 1983; Richards and Passioura, 1989; Smith and De Smet, 2012). However, through its negative association with T_c and positive association with GY and/or HI, root traits collected from this study should be regarded as a useful resource to gain insights about wheat adaptation to water stress in the field. Soil coring as a root evaluation method in the field is labor intensive and time consuming as it requires multiple replications. This method requires improvements to increase the throughput potential. However, this study suggests that field soil coring can directly identify variation in root traits to aid selection of genotypes for breeding under dry conditions.

Table 4.1 Wheat cultivars used in Fort Collins 2013-14 soil coring with their pedigrees, accession numbers, year of release, and origin.

Entry Name	Pedigree	Accession Number	Release Year	Origin
Bill Brown	Yumar/Arlin	PI 653260	2007	Colorado
Byrd	TAM 112/CO970547-7	PI 664257	2011	Colorado
Antero	KS01HW152-1/TAM111	PI 667743	2012	Colorado
Comanche	Oro/Tenmarq	CItr 11673	1942	Kansas
Hatcher	Yuma/PI372129//Tam-200/3/4*Yuma/4/KS91H184/Vista	PI 638512	2004	Colorado
Jules	Warrior*5/Agent//Agate-sib(NE76667)/3/Hawk	PI 564851	1994	Colorado
Kharkof	NA*	PI 5641	1900	Ukraine
Prairie Red	CO850034/PI372129//5*TAM 107CSU	PI 605390	1998	Colorado
Ripper	CO940606/TAM107R-2	PI 644222	2006	Colorado
RonL	Trego/3/(CO9600293) PI222668/TAM107//CO850034	Not available	2006	Kansas
TAM 112	TAM200/TA2460 (U1254-7-9-2-1)//(TXGH10440) TAM107*5/Largo	PI 643143	2005	Texas
Wichita	Early Blackhull/Tenmarq	CItr 11952	1944	Kansas

Table 4.2 Analysis of variance and summary statistics for root traits of 21 wheat cultivars and advanced lines grown in the Greeley 2011-12 dry treatment.

Trait[†] (unit)	Significance of entries	Mean	Range	CV[‡]	R^2[¶]
TLTS (cm)	NS	1482	681 to 3042	47.05	0.48
TLMS (cm)	***	684	49 to 1619	54.91	0.68
TLBS (cm)	NS	237	5 to 653[#]	106.25	0.58
TL (cm)	**	2307	820 to 4673	33.46	0.61
ADTS (mm)	***	0.57	0.47 to 0.68	8.50	0.64
ADMS (mm)	***	0.56	0.45 to 0.76	13.43	0.61
ADBS (mm)	*	0.53	0.42 to 0.71[#]	11.60	0.71
AD (mm)	***	0.56	0.49 to 0.70	8.31	0.73

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
[†] TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter.
[‡] CV, coefficient of variation.
[¶] R^2, coefficient of determination.
Range of values when roots were present in the bottom section.

Table 4.3 Least squares means of scanned root traits of 21 wheat cultivars and advanced lines grown in the Greeley 2011-12 dry treatment.

Entry	TLTS†	TLMS	TLBS	TL	ADTS	ADMS	ADBS	AD
	cm	cm	cm	cm	mm	mm	mm	mm
Above	2263 ba‡	354 ef	506 ba	2786 bdc	0.55 fedc	0.76 a	0.48 bdc	0.54 efd
Akron	1022 dc	1110 bdac	61 b	2194 fbedc	0.55 fedc	0.73 ba	0.46 dc	0.50 ef
Avalanche	770 dc	329 ef	9 b	1104 fe	0.46 f	0.62 bc	0.66 ba	0.53 efd
Bill brown	1417 bdc	1273 ba	410 ba	2827 bdc	0.57 edc	0.62 bc	0.61 bac	0.57 ecd
Bond CL	1818 bdac	1228 bac	220 ba	3267 ba	0.55 fedc	0.60 dc	0.51 bdc	0.54 efd
Byrd	3041 5a	1619 a	25 b	4673 a	0.62 bac	0.58 dce	0.55 bdac	0.61 bcd
CO04393	1419 bdc	223 f	591 ba	2036 fbedc	0.54 fedc	0.56 dce	0.51 bdc	0.53 efd
CO04499	2438 ba	684 ebdfc	127 ba	3250 bac	0.58 bedc	0.56 dce	0.45 d	0.51 ef
CO04W320	1878 bac	94 f	0	1973 fedc	0.54 fedc	0.56 dce	0	0.50 ef
CO940610	768 dc	49 f	5 b	819 f	0.49 fe	0.55 dce	0.42 d	0.47 f
Duke	681 d	1002 ebdac	648 ba	1899 fedc	0.66 ba	0.54 dce	0.69 a	0.70 ba
Halt	1765 bdac	600 edf	156 ba	2469 bedc	0.53 fed	0.54 dce	0.63 ba	0.57 ecd
Hatcher	883 dc	151 f	0	1034 f	0.67 a	0.54 dce	0	0.72 a
Jules	1606 bdc	329 f	653 a	2589 bdc	0.55 fedc	0.53 dce	0.51 bdc	0.52 ef
Lindon	1502 bdc	1222 bac	0	2724 bdc	0.55 fedc	0.51 dce	0	0.56 ecd
Platte	1433 bdc	1019 ebdac	223 ba	2676 bdc	0.54 fedc	0.51 dce	0.46 dc	0.54 efd
Prairie Red	1436 bdc	1146 bdac	324 ba	2798 bdc	0.53 fedc	0.50 dce	0.53 bdc	0.54 efd
Prowers	960 dc	628 edfc	84 ba	1673 fed	0.54 fedc	0.50 dce	0.56 bdac	0.53 efd
RonL	1243 bdc	542 edf	84 ba	1842 fedc	0.55 fedc	0.48 de	0.56 bdac	0.56 ecd
Sandy	1849 bac	210 f	64 ba	2080 fbedc	0.58 bdc	0.46 e	0.45 dc	0.54 efd
Thunder CL	1287 bdc	527 edf	121 ba	1936 fedc	0.68 a	0.45 e	0.60 bac	0.63 bc
Mean	**1499**	**683**	**206**	**2317**	**0.56**	**0.56**	**0.46**	**0.56**

† TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter.

‡ Values with similar letters within a treatment are not statistically different based on $LSD_{0.05}$ values.

Table 4.4 Phenotypic correlations among root and agronomic traits in the Greeley 2011-12 dry treatment (n=21).

Traits†	TLTS	TLMS	TLBS	TL	ADTS	ADMS	ADBS	AD	$T_c$lh	TBM	GBW	HI	GY
TLMS	0.19ns												
TLBS	-0.04ns	0.17ns											
TL	0.76***	0.71***	0.33ns										
ADTS	0.13ns	0.19ns	0.02ns	0.19ns									
ADMS	-0.03ns	0.41ns	0.45ns	0.20ns	0.46*								
ADBS	-0.36ns	0.24ns	0.27ns	-0.21ns	0.05ns	0.55*							
AD	0.15ns	0.29ns	0.06ns	0.22ns	0.80***	0.76***	0.26ns						
$T_c$lh	-0.41ns	-0.26ns	0.07ns	-0.51*	-0.29ns	0.11ns	0.61**	-0.01ns					
TBM	-0.14ns	-0.32ns	-0.35ns	-0.38ns	0.25ns	-0.14ns	0.02ns	0.08ns	0.11ns				
GBW	0.21ns	-0.08ns	-0.25ns	-0.04ns	0.37ns	0.13ns	0.13ns	0.32ns	0.15ns	0.58**			
HI	0.25ns	0.50*	-0.01ns	0.40*	0.36ns	0.75***	0.34ns	0.60*	-0.03ns	-0.22ns	0.21ns		
GY	0.09ns	0.32ns	0.08ns	0.33ns	0.46*	0.22ns	0.00ns	0.37ns	-0.19ns	0.03ns	0.06ns	0.38ns	
Ht	0.12ns	-0.01ns	-0.04ns	0.00ns	0.44*	0.09ns	0.32ns	0.44*	0.34ns	0.38ns	0.50*	-0.01ns	0.07ns

*, **, ***, significance level of correlation at *P*<0.05, <0.001, and <0.0001, ns; non-significant correlation and *P*<0.05.

† TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter; $T_c$lh, canopy temperature at late heading stage (°C); TBM, total biomass weight at maturity (g); BGW, biomass grain weight (g); HI, harvest index; GY, grain yield (kg ha^{-1}); Ht, plant height (cm).

Table 4.5 Analysis of variance and summary statistics for scanned root traits in 25 wheat cultivars and advanced lines grown in the Fort Collins 2012-13 dry treatment.

Trait† (unit)	Significance of entries	Mean	Range	CV ‡	R^2 ¶
TLTS (cm)	***	526	251 to 1028	50.90	0.46
TLMS (cm)	**	618	317 to 1004	44.19	0.41
TLBS (cm)	***	473	194 to 926	48.23	0.50
TL (cm)	***	1619	994 to 2643	29.63	0.56
ADTS (mm)	*	0.88	0.72 to 1.18	18.32	0.36
ADMS (mm)	NS	0.87	0.76 to 1.02	13.01	0.27
ADBS (mm)	NS	0.88	0.75 to 1.01	15.43	0.28
AD (mm)	*	0.88	0.76 to 1.01	10.52	0.37

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter.
‡ CV, coefficient of variation.
¶ R^2, coefficient of determination.

Table 4.6 Least squares means of scanned root traits in 25 wheat cultivars and advanced lines grown in the Fort Collins 2012-13 dry treatment.

Entry	TLTS†	TLMS	TLBS	TL	ADTS	ADMS	ADBS	AD
	cm	cm	cm	cm	mm	mm	mm	mm
Byrd	868 bac	1004 a‡	544 bedc	2416 ba	0.89 bdc	0.95 ba	1.01 a	0.95 ba
Centura	868 bac	681 ebdacf	508 fedc	2057 ebdacf	1.00 ba	0.91 bac	0.91 ebdac	0.94 bac
Cheyenne	485 de	913 bdac	843 ba	2241 bac	0.72 d	0.85 bc	0.94 bdac	0.83 ebdc
Comanche	313 e	424 ef	478 fedc	1216 hgi	1.18 a	0.87 bac	0.95 bdac	1.00 a
Darrell	363 e	424 ef	249 fe	1036 i	0.99 ba	0.82 bc	0.90 ebdac	0.90 bdac
Hatcher	985 ba	533 edf	332 fedc	1851 ebdgcf	0.86 bdc	0.82 bc	0.96 bac	0.88 ebdac
Judee	418 de	802 ebdac	923 a	2144 bdac	0.74 dc	0.86 bc	0.85 ebdac	0.82 edc
Kharkof	389 de	701 ebdacf	552 bedc	1643 ehdgicf	0.96 bac	0.92 bac	0.88 ebdac	0.92 bdac
MT06103	307 e	437 ef	308 fedc	1053 hi	0.82 bdc	0.88 bac	0.89 ebdac	0.86 ebdc
MT85200	408 de	615 ebdcf	386 fedc	1409 hgif	0.82 bdc	0.82bc	0.88 ebdac	0.84 ebdc
Nusky	451 de	465 ef	252 fed	1169 hgi	0.87 bdc	0.85 bc	0.86 ebdac	0.86 ebdc
Ogallala	269 e	423 ef	301 fedc	993 i	0.78 bdc	0.87 bac	0.90 ebdac	0.85 ebdc
OK05204	452 de	676 ebdacf	584 bdc	1713 ehdgcf	0.87 bdc	0.87 bac	0.86 ebdac	0.87 ebdc
OK05526	742 bdac	451 ef	393 fedc	1587 ehdgicf	0.74 dc	0.76 c	0.75 e	0.75 e
Ok06114	409 de	419 ef	536 fbedc	1365 hgif	0.86 bdc	0.82 bc	0.84 ebdac	0.84 ebdc
Overland	605 dec	434 ef	461 fedc	1501 ehdgif	0.92 bdc	0.90 bac	0.95 bdac	0.93 bdac
Parker	586 dec	952 bac	592 bdc	2130 ebdac	0.75 dc	0.90 bac	0.95 bdac	0.87 ebdc
Ripper	1028 a	998 ba	616 bac	2643 a	1.01 ba	1.02 a	0.99 ba	1.01 a

SD01237	250 e	663 ebdacf	328 fedc	1242 hgi	0.86 bdc	0.76 c	0.75 ed	0.79 ed
TAM 111	617 bdec	602 edcf	340 fedc	1559 ehdgif	0.84 bdc	0.83 bc	0.81 ebdc	0.83 ebdc
TX04v075	347 e	691 ebdacf	283 fed	1321 hgi	0.82 bdc	0.96 ba	0.78 edc	0.85 ebdc
TX05a001	466 de	476 ef	395 fedc	1339 hgi	0.85 bdc	0.86 bc	0.80 ebdc	0.83 ebdc
TX07a001	428 de	607 edcf	427 fedc	1463 ehgif	0.83 bdc	0.83 bc	0.83 ebdac	0.83 ebdc
Vona	522 dec	317 f	193 f	1033 i	0.98 ba	0.87 bac	0.80 ebdc	0.88 ebdac
Wichita	467 de	679 ebdacf	925 a	2072 ebdacf	0.93 bdc	0.94 ba	0.99 ba	0.95 ba
Mean	522	616	471	1608	0.88	0.87	0.88	0.88

† TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter.

‡ Values with similar letters within a treatment are not statistically different based on $LSD_{0.05}$ values.

Table 4.7 Phenotypic correlations among root and agronomic traits in the Fort Collins 2012-13 dry treatment (n=25).

Traits†	TLTS	TLMS	TLBS	TL	ADTS	ADMS	ADBS	AD	$T_c$bs	$T_c$egf	Ht	TBM	BGW	GY
TLMS	0.33ns													
TLBS	0.34ns	0.64***												
TL	0.67***	0.82***	0.84***											
ADTS	0.16ns	-0.14 ns	-0.02 ns	-0.07 ns										
ADMS	0.20ns	0.46*	0.36 ns	0.33 ns	0.33 ns									
ADBS	0.35 ns	0.29 ns	0.45 ns	0.46*	0.36 ns	0.49*								
AD	0.24 ns	0.17 ns	0.22 ns	0.19 ns	0.73***	0.77***	0.72***							
$T_c$bs	-0.42*	-0.24*	-0.20 ns	-0.36*	-0.11 ns	-0.08 ns	-0.17 ns	-0.14 ns						
$T_c$egf	0.04 ns	0.30 ns	0.12 ns	0.16 ns	-0.11 ns	0.13 ns	-0.07 ns	0.05 ns	0.06ns					
Ht	0.06 ns	0.12 ns	0.29 ns	0.13 ns	0.30 ns	0.13 ns	0.23 ns	0.27 ns	0.11ns	-0.14ns				
TBM	0.15 ns	-0.25 ns	-0.28 ns	-0.23 ns	0.15 ns	-0.08 ns	-0.10 ns	-0.02 ns	0.16ns	-0.35ns	0.06ns			
BGW	0.17 ns	-0.09 ns	-0.09 ns	-0.07 ns	0.43*	0.07 ns	0.12 ns	0.23 ns	0.07ns	-0.30ns	0.18ns	0.86***		
GY	0.30 ns	-0.13 ns	-0.08 ns	0.04 ns	0.22 ns	-0.23 ns	0.15 ns	0.02 ns	0.37ns	-0.25ns	0.04ns	0.59**	0.72***	
HI	0.24 ns	0.08 ns	0.05 ns	0.12 ns	0.70 ns	0.13 ns	0.32 ns	0.45*	0.14ns	-0.01ns	0.21ns	0.32ns	0.70***	0.63***

*, **, ***, significance level of correlation at P<0.05, <0.001, and <0.0001, ns; non-significant correlation and P<0.05.

† TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter; $T_c$bs, canopy temperature at booting stage (°C); $T_c$egf, canopy temperature at early grain fill stage (°C); Ht, plant height (cm); TBM, total biomass weight at maturity (g); BGW, biomass grain weight (g); HI, harvest index; GY, grain yield (kg ha^{-1}).

Table 4.8 Analysis of variance and summary statistics for scanned root traits in 12 wheat cultivars and advanced lines grown in the Fort Collins 2013-14 dry treatment.

Trait† (unit)	Entry *P*-value	Rep *P*-value	Mean	Range	CV‡	R^{2}¶
TLTS (cm)	NS	NS	1447	731 to 2070	35.66	0.58
TLMS (cm)	**	NS	1759	690 to 2936	27.45	0.83
TLBS (cm)	NS	NS	2142	1027 to 2867	23.83	0.73
TL (cm)	***	NS	5347	3539 to 7684	15.75	0.85
ADTS (cm)	NS	*	0.84	0.67 to 1.08	13.61	0.73
ADMS (mm)	NS	***	0.83	0.70 to 1.10	13.85	0.76
ADBS (mm)	NS	NS	1.73	0.72 to 2.70	58.38	0.52
AD (mm)	NS	NS	1.21	0.75 to 1.75	29.46	0.58

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter.
‡ CV, coefficient of variation.
¶ R^2, coefficient of determination.

Table 4.9 Least squares means of scanned root traits in 12 wheat cultivars and advanced lines grown in the Fort Collins 2013-14 dry treatment.

Entry	TLTS†	TLMS	TLBS	TL	ADTS	ADMS	ADBS	AD
	cm	cm	cm	cm	mm	mm	mm	mm
Bill Brown	419 ba	230 d	701 bac	1350 de	0.95 bac	0.91 ba	1.64 a	1.17 ba
Byrd	690 a	974 a	858 ba	2522 a	0.86 ac	0.81 ba	0.95 a	0.87 b
CO07W245	397 ba	514 bdc	554 bac	1464 dec	0.89 bac	0.91 ba	2.43 a	1.41 ba
Comanche	500 ba	452 dc	676 bac	1628 dec	0.75 bc	0.70 b	1.73 a	1.06 ba
Hatcher	627 a	978 a	955 a	2561 a	0.78 bc	0.84 ba	0.84 a	0.82 b
Jules	430 ba	553 bdc	342 c	1324 e	0.98 ba	0.94 ba	2.12 a	1.35 ba
Kharkof	674 a	669 bac	911 a	2254 ba	0.73 bc	0.75 ba	1.21 a	0.90 b
Prairie Red	458 ba	820 ba	724 bac	2003 bac	0.77 bc	0.76 ba	0.71 a	0.74 b
Ripper	244 b	452 dc	483 bc	1179 e	1.07 a	1.10 a	2.69 a	1.62 a
RonL	599 ba	426 dc	930 a	1955 bdac	0.80 bac	0.73 b	2.39 a	1.31 ba
TAM 112	392 ba	285 d	689 bac	1365 de	0.81 bac	0.79 ba	2.40 a	1.33 ba
Wichita	356 ba	682 bac	742 bac	1780 bdec	0.66 c	0.72 b	1.60 a	0.99 ba
Mean	482	586	714	1782	0.84	0.83	1.73	1.21

† TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter.

‡ Values with similar letters within a treatment are not statistically different based on LSD0.05 values.

Table 4.10 Phenotypic correlations among root and agronomic traits in the Fort Collins 2013-14 dry treatment (n=12).

Traits†	TLTS	TLMS	TLBS	TL	ADTS	ADMS	ADBS	AD	GY	$T_c$vg
TLMS	0.30ns									
TLBS	0.58*	0.37ns								
TL	0.77*	0.70*	0.85**							
ADTS	-0.12ns	-0.13ns	-0.47ns	-0.30ns						
ADMS	-0.13ns	0.34ns	-0.37ns	-0.09ns	0.83***					
ADBS	-0.32ns	-0.77*	-0.61ns	-0.66*	-0.14ns	-0.36ns				
AD	-0.60ns	-0.83**	-0.73*	-0.83*	0.47ns	0.42ns	0.96***			
GY	0.07ns	-0.12ns	0.32ns	0.07ns	0.64ns	0.64ns	-0.40ns	0.14ns		
$T_c$vg	-0.25ns	-0.10ns	0.09ns	-0.23*	-0.24ns	-0.47ns	0.22ns	-0.06ns	-0.22ns	
$T_c$gf	0.13ns	-0.40*	0.20ns	-0.09ns	-0.04ns	-0.27ns	0.23ns	0.12ns	-0.15ns	0.66*

*, **, ***, significance level of correlation at *P*<0.05, <0.001, and <0.0001, ns; non-significant correlation and *P*<0.05.

† TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter; GY, grain yield (kg ha^{-1}); $T_c$vg, canopy temperature at vegetative stage (°C); $T_c$gf, canopy temperature at grain fill stage (°C).

Table 4.11 Correlation between root traits from the soil cores in the field and same traits from the greenhouse studies.

Greeley 2012 and GH study I (n=21)		Fort Collins 2013 and GH study II (n=25)		Fort Collins 2014 and both GH studies (n=12)	
Trait†	*r*‡	Trait	*r*	Trait	*r*
TLTS	-0.11ns	TLTS	0.11ns	TLTS	0.57ns
TLMS	0.39*	TLMS	0.39ns	TLMS	0.50ns
TLBS	0.06ns	TLBS	-0.06ns	TLBS	0.64*
TL	0.09ns	TL	0.09ns	TL	0.70*
ADTS	0.40ns	ADTS	0.40ns	ADTS	0.45ns
ADMS	0.11ns	ADMS	-0.11ns	ADMS	0.48ns
ADBS	-0.12ns	ADBS	0.12ns	ADBS	-0.37ns
AD	0.10ns	AD	0.10ns	AD	0.42ns

*, **, ***, significance level of correlation at P<0.05, <0.001, and <0.0001, ns; non-significant correlation and P<0.05.

† TLTS, total length for top section; TLMS, total length for middle section; TLBS, total length for bottom section; TL, total length; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section; AD, average diameter.

‡ *r*, coefficient of correlation.

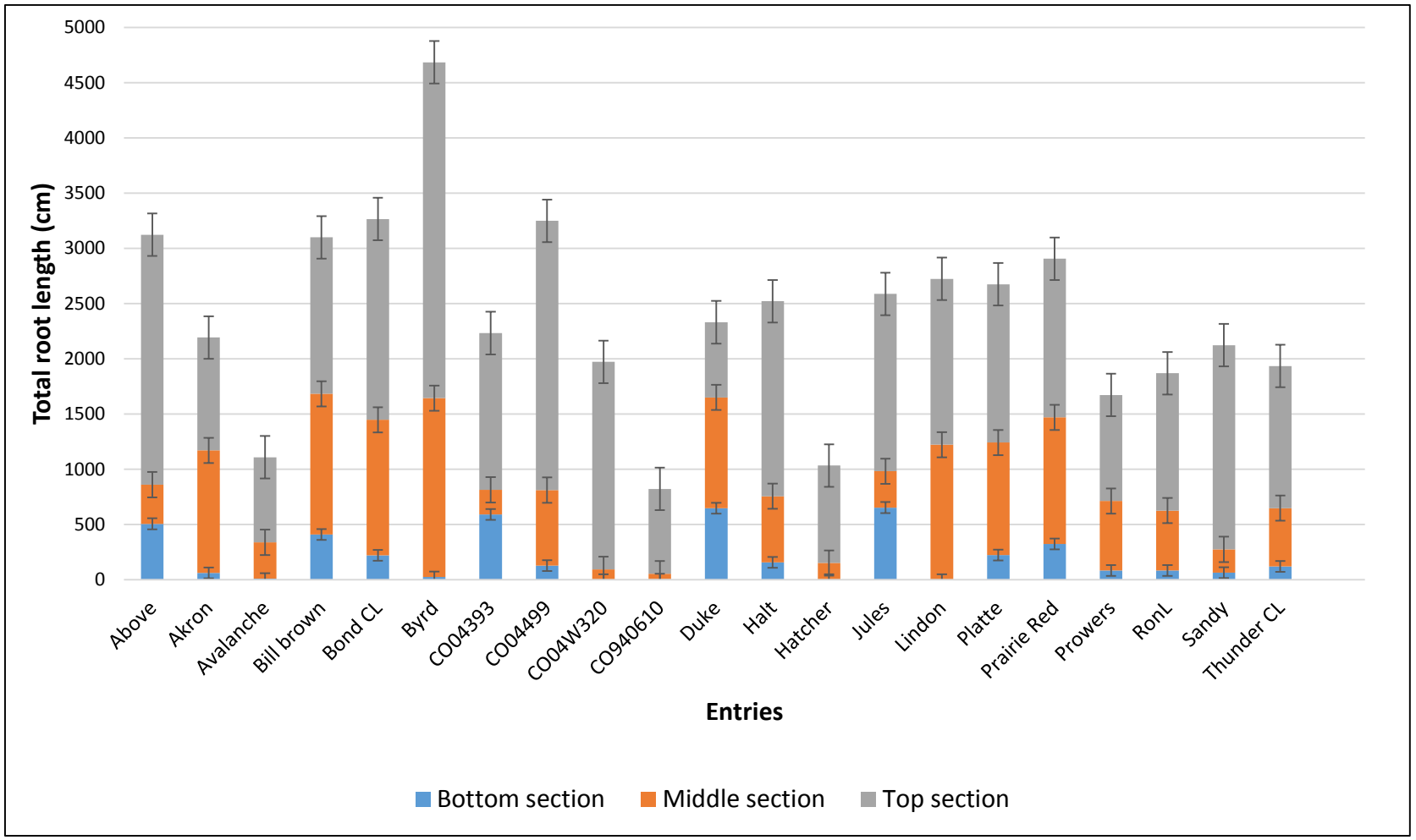

Figure 4.1 Total root length in cm in the three root sections (bottom, middle and top) for the Greeley 2011-12 dry treatment entries

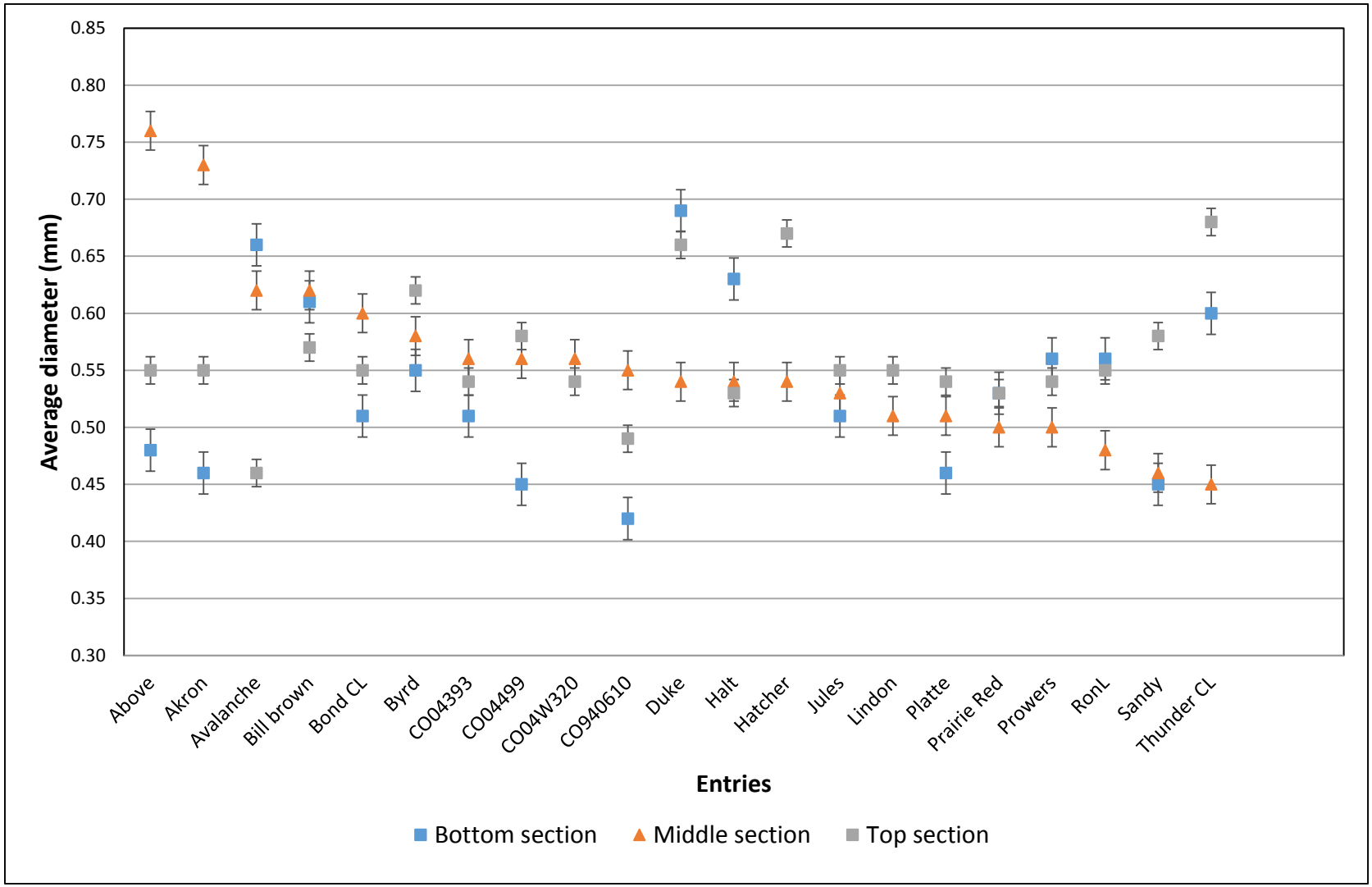

Figure 4.2 Root average diameter in mm for the three root sctions (bottom, middle, and top) for the Greeley 2011-12 dry treatment entries.

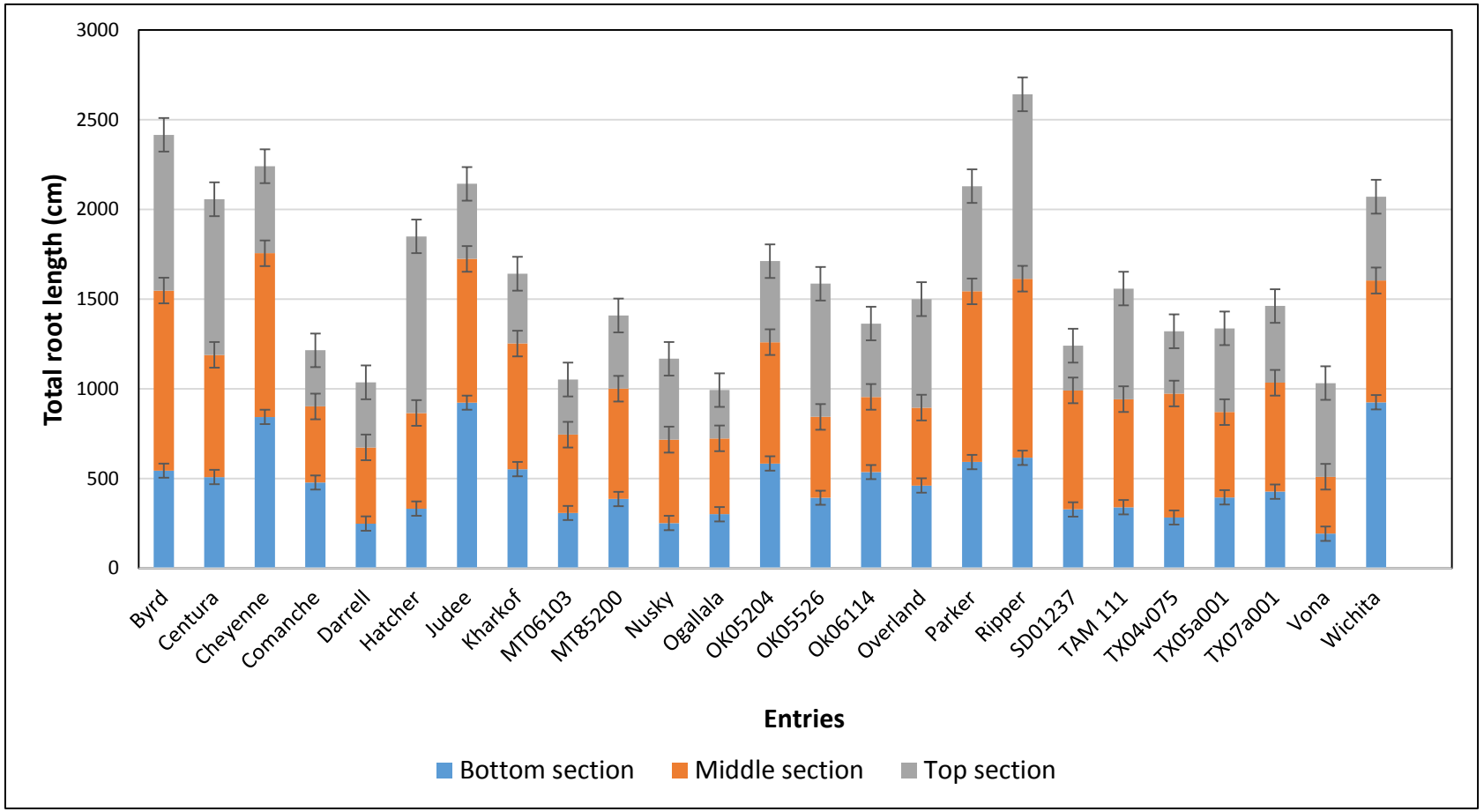

Figure 4.3 Total root length in cm in the three root sections (bottom, middle, and top) for the Fort Collins 2012-13 dry treatment entries.

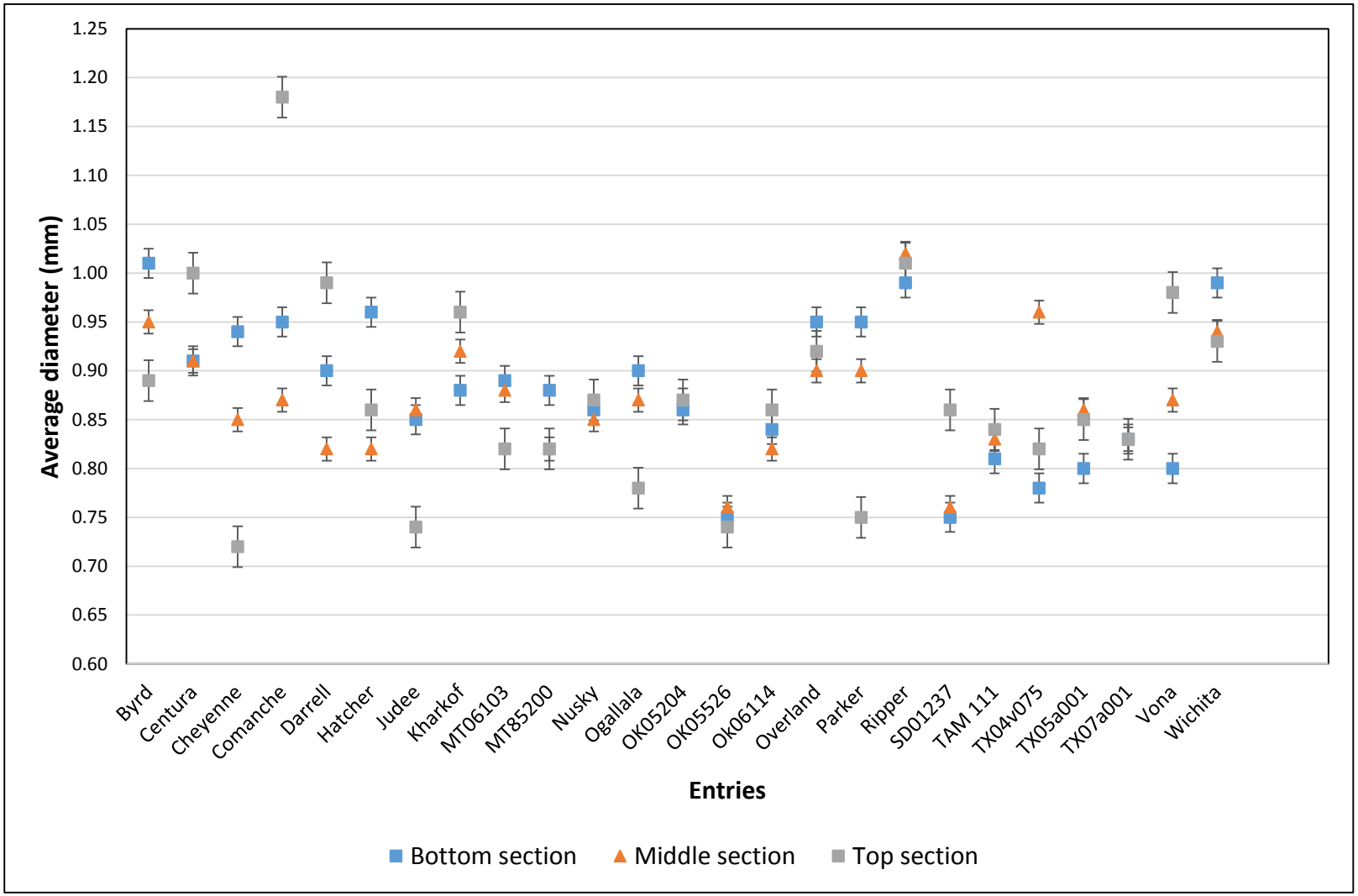

Figure 4.4 Root average diameter in mm for the three root sections (bottom, middle, and top) for the Fort Collins 2012-13 dry treatment entries.

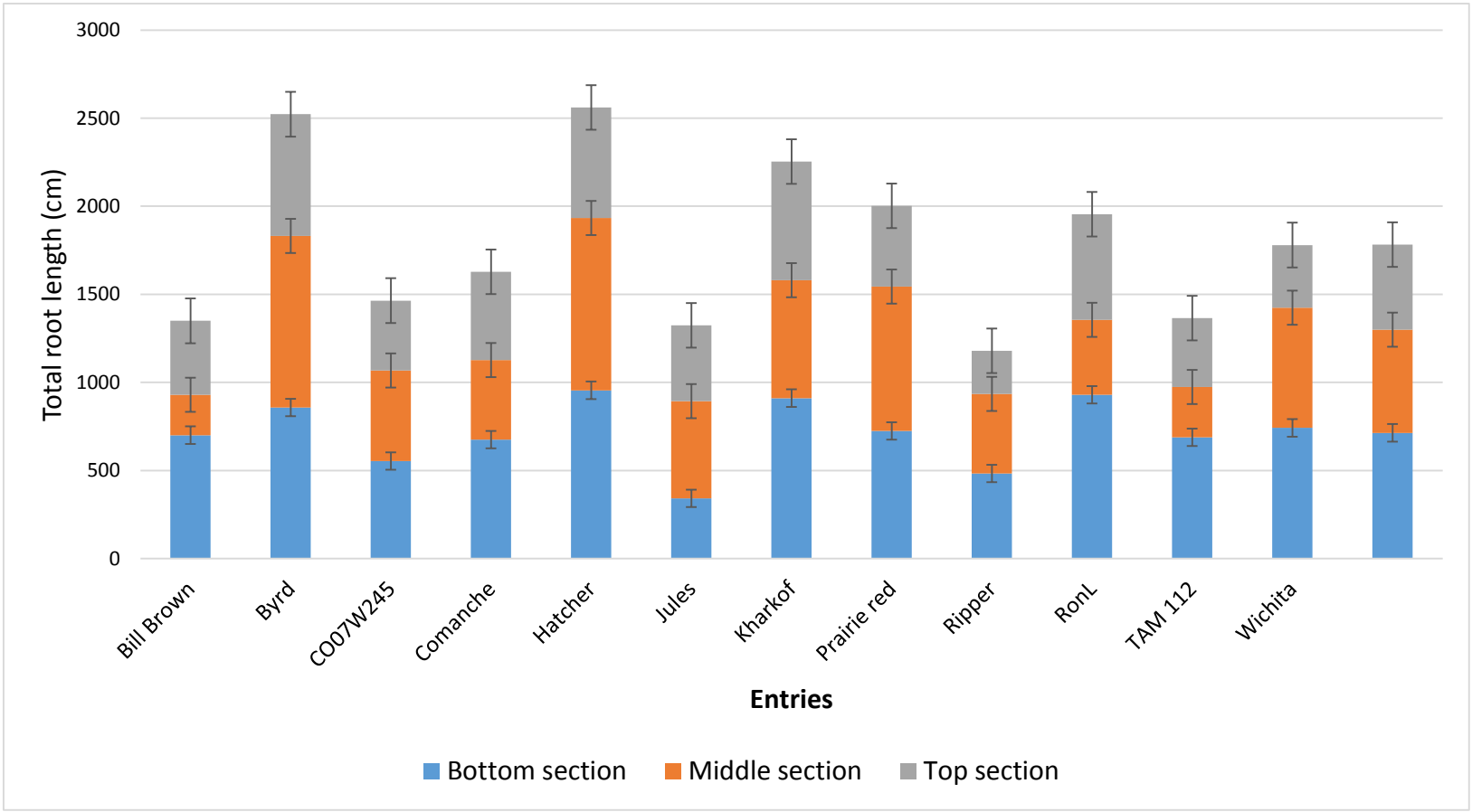

Figure 4.5 Total root length in cm in the three root sections (bottom, middle, and top) for the Fort Collins 2013-14 dry treatment entries.

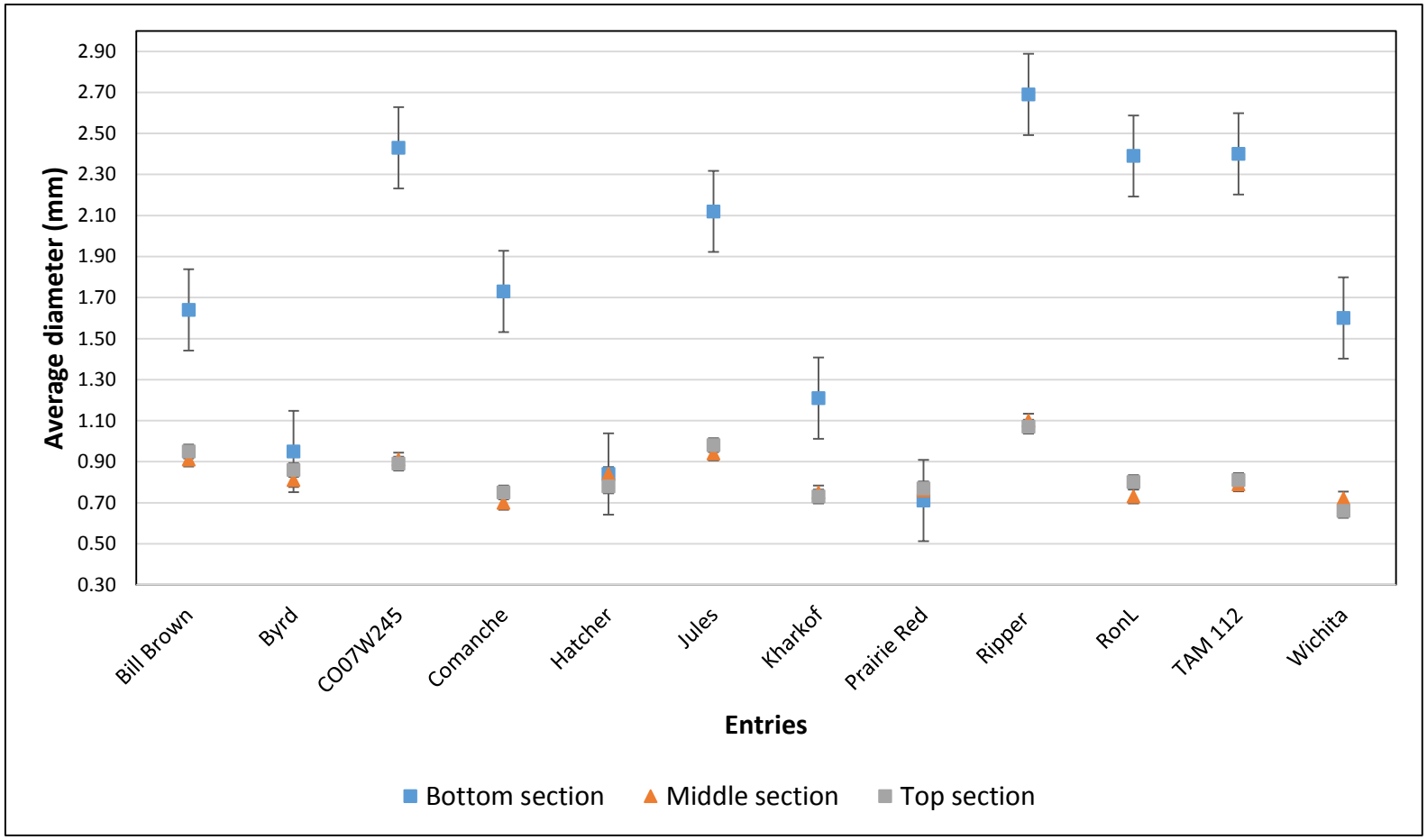

Figure 4.6 Root average diameter in mm for the three root sections (bottom, middle, and top) for the Fort Collins 2013-14 dry treatment entries.

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APPENDIX

Appendix 1. List of entries in the hard winter wheat association mapping panel (HWWAMP) used in this study with their names, year of release, type (C, cultivar; L, landrace; B, breeding line), origin, NSGC accession number, and pedigree[2].

ENTRY	NAME	Year	Type†	Origin	NSGC Accession	Pedigree
142	CO03064	.	B	CO	.	CO970547/Prowers 99
125	CO03W043	.	B	CO	.	KS96HW94/CO980352
126	CO03W054	.	B	CO	.	Arlin/KS89H20 (KS96HW94)/6/Trego/5/(CO960293) PI 222668 / TAM 107 /4/(CO0850034) Novi Sad 14 / Novi Sad 603 // Newton /3/ Probrand 835
128	CO04025	.	B	CO	.	CO940610/CO960293//CO99W189
129	CO04393	.	B	CO	.	Stanton/CO950043
130	CO04499	.	B	CO	.	Above/Stanton
131	CO04W320	.	B	CO	.	CO950635/CO99W1126
283	CO050337-2	.	B	CO	.	CO980829/TAM 111
120	CO940610	.	B	CO	GSTR10702	H15A13333 /5* Larned // Eagle / Sage /3/ TAM 105 (KS87H22) /4/ (MW09) Clark's Cream/5*KS75216 (Newton Sib)
84	TAM107-R7	.	B	CO	GSTR11601	CO850034 / PI372129 //5* TAM107
141	ABOVE	2001	C	CO	PI631449	TAM 110*4/FS2
146	AKRON	1994	C	CO	PI584504	TAM 107 / Hail
285	ANTERO	2013	C	CO	PI 667743	Trego/Betty sib (KS01HW152-1)//TAM 111

[2] Pedigree source is Guttieri et al (2015) and T3 database.

121	AVALANCHE	2001	C	CO	PI620766	RL6005 / RL6008 // Larned /3/ Cheney / Larned /4/ Bennett sib /5/ TAM107 (KS87H325) /6/ Rio Blanco
143	BILL BROWN	2007	C	CO	PI653260	Yumar/Arlin
122	BOND CL	2004	C	CO	PI639924	Yumar//TXGH12588-120*4/FS2
284	BYRD	2011	C	CO	PI 664257	TAM112//(CO970547-7) Ike/Halt
133	CARSON	1986	C	CO	PI501534	Anza / Scout // Centurk
100	DAWN	1982	C	CO	CItr17801	II 21031 / Trapper /4/(CO 652363) Warrior // Kenya 58 / Newthatch /2*(Cheyenne / Tenmarq / Mediterranean)/ Hope /3/ Parker
282	DENALI	2011	C	CO	PI 664256	Yuma/T-57//CO850034/3/4*Yuma/4/NEWS12 (CO980829)/5/Tam 111
136	DUKE	1981	C	CO	CItr17856	3* Sonora 64 / Warrior // Selkirk /2* Cheyenne /5/ Scout /4/ Quivera /3/ Tenmark // Marquis 1 / Oro
134	HAIL	1982	C	CO	PI470927	Mexican / USA // Scout /3/ Mara /4/ Scout /5/ Ciano /6/ Trapper /7/ Parker
137	HALT	1994	C	CO	PI584505	Sumner / CO820026 // PI372129 /3/ TAM 107
138	HATCHER	2004	C	CO	PI638512	Yuma / PI 372129 // TAM 200 /3/4* Yuma /4/ KS91H184 / Vista
147	JULES	1993	C	CO	PI564851	Warrior *5/ Agent // Agate sib (NE76667)/3/ Hawk
132	LAMAR	1988	C	CO	PI559719	74 F878 (Mexican dwarf)/ Wings // Vona
124	LINDON	1975	C	CO	CItr17440	Andes 64A / Sonora 64 // Tacuari (II21183)/4/(CO 652363) Warrior 2 / Kenya 58 / Newthatch // Cheyenne / Tenmark / Mediterranean / Hope /3/ Parker/5/ Lancer /3/(KS 62136) Norin 16 / CI 12500 // Kaw
234	OGALLALA	1993	C	CO	PI573037	TX81V6187 / Abilene
139	PRAIRIE RED	2000	C	CO	PI605390	CO850034 / PI 372129 //5* TAM 107
145	PROWERS	1997	C	CO	PI605389	CO850060 / PI 372129 //5* Lamar
144	RIPPER	2006	C	CO	PI644222	PI 220127/P5//TAM-200/KS87H66 (CO940606)/3/(TAM107R-2) CO850034/PI 372129//5*TAM 107
135	SANDY	1981	C	CO	CItr17857	Sonora 64A / Tezanos Pintos Precoz / Yaqui 54 //(Frontana / Kenya 58 / Newthatch)/ Norin 10 / Brevor / Gabo 55B / Trapper // Centurk
127	THUNDER CL	2008	C	CO	PI655528	FS2/KS97HW150//KS97HW349 (KS01-5539)/3/(CO99W165) KS92WGRC25/Halt
119	VONA	1976	C	CO	CItr17441	Andes 64A / Sonora 64 // Tacuari (II 21183) /4/ (CO 652363) Warrior // Kenya 58 / Newthatch /2*(Cheyenne / Tenmarq / Mediterranean / Hope /3/ Parker /5/ Lancer /4/ KS 62136
148	YUMA	1992	C	CO	PI559720	NS14 / NS25 //2* Vona
140	YUMAR	2000	C	CO	PI605388	Yuma / PI 372129 , F1 // CO850034 /3/4* Yuma
115	HV906-865	.	B	KS	.	G980039/Onaga

110	HV9W03-1379R	.	B	KS	.	B1127/3/B1551W//ROWDY/RWA 671 MONT
108	HV9W03-1551WP	.	B	KS	.	B1043/PL2180
111	HV9W03-1596R	.	B	KS	.	B1397-1/WGRC33
112	HV9W05-1280R	.	B	KS	.	SPARTANKA/G980761
113	HV9W06-504	.	B	KS	.	G982231/G982159//KS920709W
244	KS00F5-20-3	.	B	KS	.	0
237	W04-417	.	B	KS	.	BULK POPULATION
251	WB411W	.	B	KS	.	G3006/ARLIN
242	2145	2002	C	KS	PI 631087	HBA142A/HBZ621A//Abilene
183	2180	1989	C	KS	PI532912	TAM W-101 / Pioneer W603 // Pioneer W558
85	ARLIN	1992	C	KS	PI564246	Selection from population of intercrossed hard red winter wheat and hard red spring wheat genotypes
226	BAKER'S WHITE	2004	C	KS	PI 633865	Ponderosa/Jagger
222	BISON	1956	C	KS	CItr12518	Chiefkan // Oro / Tenmarq
227	BURCHETT	2004	C	KS	PI 633863	W91-126/WI88-052-05
210	CHENEY	1978	C	KS	CItr17765	Scout / Tascosa
225	COMANCHE	1942	C	KS	CItr11673	Oro / Tenmarq
247	COSSACK	1998	C	KS	PI 606780	BCD1828/83
228	CUTTER	2002	C	KS	PI 631389	JAGGER//(WI89-189-14)Tam200/Stallion sib
280	DANBY	2007	C	KS	PI 648010	Trego/Jagger 'S'
208	DODGE	1986	C	KS	PI506344	KS73H530 (Newton sib)/ KS76HN1978-1 (Arkan sib)
229	DUMAS	2001	C	KS	PI 619199	WI90-425/WI89-483
217	EAGLE	1970	C	KS	CItr15068	Selection from Scout
248	ENHANCER	1998	C	KS	PI606779	1992 Nebraska bulk selection
246	FULLER	2007	C	KS	PI 653521	Ogallala/KS95WGRC33//Jagger
109	G1878	1995	C	KS	PI 591622	Hawk/Sturdy//Plainsman V
243	HEYNE	2001	C	KS	PI612577	KS82W422 / SWM754308 / KS831182 / KS82W422
230	HONDO	1999	C	KS	PI 603958	W84-179/W81-171/5/Sturdy/Hawk/4/Vona/3/NDD63/CO652643//Centurk
231	JAGALENE	2002	C	KS	PI 631376	JAGGER/ABILENE
78	JAGGER	1994	C	KS	PI593688	KS82W418 / Stephens

207	KARL 92	1992	C	KS	PI564245	Selection from Karl = Plainsman V /3/ Kaw / Atlas 50 // Parker *5/ Agent
220	KAW61	1960	C	KS	CItr12871	purification and re - release of Kaw = Oro // Mediterranean / Hope /3/ Early Blackhull / Tenmarq
252	KEOTA	2007	C	KS	PI 648007	CUSTER/JAGGER
223	KIOWA	1950	C	KS	CItr12133	Chiefkan // Oro / Tenmarq
214	KIRWIN	1973	C	KS	CItr17275	Parker *3/ Bison
204	LAKIN	2002	C	KS	PI617032	KS89H130 / Arlin
212	LARNED	1976	C	KS	CItr17650	Ottawa /5* Scout
232	LONGHORN	1991	C	KS	PI552813	NS2630-1 / Thunderbird
233	NEOSHO	2006	C	KS	PI 639739	W91-376-20/W95-084
211	NEWTON	1978	C	KS	CItr17715	Pitic 62 / Chris sib //2* Sonora 64 /3/ Klein Rendidor /4/ Scout
209	NORKAN	1986	C	KS	PI506345	Plainsman V /3/2*(KS76H3705) Larned / Eagle // Sage
238	NUFRONTIER	2002	C	KS	PI 619089	2180/HBZ356A//Mesa
239	NUHORIZON	2001	C	KS	PI 619198	WI89-282/Arlin
240	ONAGA	1998	C	KS	.	HT43-231-19 (Pioneer bulk)
245	OVERLEY	2004	C	KS	PI 634974	TAM-107 *3/TA 2460 (U1275-1-4-2-2)//Heyne 'S'/3/Jagger
219	PARKER	1966	C	KS	CItr13285	Quivira /3/ Kanred / Hard Federation // Prelude / Kanred /4/ Kawvale / Marquillo // Kawvale / Tenmarq
213	PARKER 76	1976	C	KS	CItr17685	Parker *5/ Agent
123	PLATTE	1997	C	KS	PI 596297	Tesia 79 / Chat'S' // Abilene
235	POSTROCK	2006	C	KS	PI 643093	Ogallala/KSU94U261//Jagger
241	RONL	2007	C	KS	PI 648020	Trego/3/(CO9600293) PI222668/TAM 107//CO850034
215	SAGE	1973	C	KS	CItr17277	Agent /4* Scout
249	SANTA FE	2006	C	KS	PI 641772	G1878/Jagger
218	SHAWNEE	1967	C	KS	CItr14157	Mediterranean / Hope // Pawnee /3/ Oro / Illinois No. 1// Comanche
118	SHOCKER	2006	C	KS	PI 646185	FREEDOM/TOMAHAWK//JAGGER
117	SMOKYHILL	2006	C	KS	PI 646184	97 8/64 MASA (Population developed by combining several crosses with a common female "G2500")
114	SPARTAN	2007	C	KS	.	RL8400193/PL2180
205	STANTON	2002	C	KS	PI617033	PI 220350 / KS87H57 // TAM200 / KS87H66 /3/ KS87H325
116	TARKIO	2006	C	KS	.	OK90604/KSSB-369-7//SnowWhite
236	THUNDERBOLT	2000	C	KS	PI 608000	ABILENE/KS90WGRC10

206	TREGO	1999	C	KS	PI612576	RL6005 / RL6008 // Larned /3/ Cheney / Larned /4/ Bennet sib /5/ TAM107 (KS87H325)/6/ Rio Blanco
216	TRISON	1973	C	KS	CItr17278	Triumph / Bison
250	VENANGO	2000	C	KS	.	HBE1066-105/HBF0551-137
76	WICHITA	1944	C	KS	CItr11952	Early Blackhull / Tenmarq
224	WICHITA	1944	C	KS	CItr11952	Early Blackhull / Tenmarq
281	E2041	.	B	MI	.	Pioneer Brand 2552/Pioneer Brand 2737W
199	MT0495	.	B	MT	.	MT9640/NB1133
202	MT06103	.	B	MT	.	Composite cross
191	MT85200	.	B	MT	.	Froid/Winoka/3/TX55-391-56-D8/Westmont//Trader
193	MT9513	.	B	MT	.	NuWest/MT8030
194	MT9904	.	B	MT	.	MT85200/Tiber
195	MT9982	.	B	MT	.	Promontory/Judith
200	MTS0531	.	B	MT	.	L'Govskaya167/Rampart//MT9409
279	BIG SKY	2001	C	MT	PI619166	NuWest / Tiber
188	CREST	1967	C	MT	CItr13880	Westmont *2/ PI 178383
201	DECADE	2010	C	MT	PI660291	Composite
196	GENOU	2004	C	MT	PI640424	Lew/Tiber//Redwin (MTS92015)/3/Vanguard/Norstar
203	JUDEE	2011	C	MT	PI 665227	Vanguard/Norstar//Judith/3/NuHorizon
190	JUDITH	1989	C	MT	PI584526	Lancota / Froid // NE69559 / Winoka
197	NORRIS	2005	C	MT	PI643430	BigSky//TAM110sib*4/FS2
192	NUSKY	2001	C	MT	PI619167	NuWest / Tiber
189	ROSEBUD	1981	C	MT	PI473570	Lancer /2* BWH 1376-8
198	YELLOWSTONE	2005	C	MT	PI643428	Selected from a composite of F2 seed from two closely related populations: Promontory/Judith and Judith-phenotypic dwarf selection/Promontory
83	JERRY	2001	C	ND	PI632433	Roughrider // Winoka / NB66425 /3/ Arapahoe
287	NE02558	.	B	NE	.	JAGGER/ALLIANCE
289	NE04490	.	B	NE	.	NE95589/3/(NE94632) ABILENE/NORKAN//RAWHIDE/4/(NE95510)ABILENE/ARAPAHOE
290	NE05430	.	B	NE	.	IN92823A1-1-4-5/NE92458
291	NE05496	.	B	NE	.	KS87H325/RIO BLANCO (KS95HW62-6)//HALLAM
294	NE06607	.	B	NE	.	KS89H50-4/3/(NE90518)BRL//SXL/BENN (NE98466)/4/WESLEY

63	NE99495	.	B	NE	.	ALLIANCE/KARL 92
296	NI06736	.	B	NE	.	KM602-90/NE89657//ARLIN (NW97S312)/3/(KS96HW10-3) KS91HW29// RIO BLANCO/KS91H184
297	NI06737	.	B	NE	.	KM602-90/NE89657//ARLIN (NW97S312)/3/(KS96HW10-3) KS91HW29// RIO BLANCO/KS91H184
298	NI07703	.	B	NE	.	919021/B725//K92 (G97343, R-148)/5/(NI00436) BEZ 1/CTK78//ARTHUR/CTK78/3/BENNET/4/NORKAN
299	NI08707	.	B	NE	.	Yuma/T-57//CO850034/3/4*Yuma/4/NEWS1 (CO980829)/5/Wesley
300	NI08708	.	B	NE	.	Yuma/T-57//CO850034/3/4*Yuma/4/NEWS1 (CO980829)/5/Wesley
288	NW03666	.	B	NE	.	N94S097KS/NE93459
41	AGATE	1979	C	NE	CI17463	Ponca /3* Cheyenne // Kenya 58 / Newthatch //2*(Cheyenne / Tenmarq / Mediterranean / Hope)/3/ Scout
42	ALLIANCE	1993	C	NE	PI573096	Arkan/Colt//Chisholm (sib)
43	ANTELOPE	2005	C	NE	PI633910	Pronghorn / Arlin
81	ANTON	2007	C	NE	PI651044	WA691213-27 / PI 559717 // Platte
44	ARAPAHOE	1988	C	NE	PI518591	Brule /3/ Parker *4/ Agent // Belocerkovskaja 198 / Lancer
45	BENNETT	1978	C	NE	CI17723	Scout /3/ Quivira / Tenmarq // Marquillo / Oro /4/ Homestead
46	BUCKSKIN	1973	C	NE	CI17263	Scout/3/Quivera/Tenmarq//Marquillo/Oro
61	CAMELOT	2008	C	NE	PI653832	KS91H184/ARLIN SIB//KS91HW29/3/NE82761/REDLAND (NE91631)//VBFO168
47	CENTURA	1983	C	NE	PI476974	Warrior*5/Agent/NE68457/3/Centurk78
48	CENTURK 78	1978	C	NE	CItr17724	Selection from Centurk
50	COLT	1983	C	NE	PI476975	Agate sib (NE69441)// (Tx65A1503-1) 391-56-D8 / Kaw
51	COUGAR	2000	C	NE	PI613098	Warrior *5/ Agent // Kavkaz /4/ NE63218 / Kenya 58 /3/ Newthatch /2* CTMH // Ponca /* 2 Cheyenne (NE85707)/5/ Thunderbird (CTMH = Cheyenne / Tenmarq / Mediterranean / Hope)
52	CULVER	1999	C	NE	PI606726	NE82419/Arapahoe
293	FREEMAN	2013	C	NE	PI 667038	ABI86*3414/Jagger//Karl 92 (KS92-946-B-15-1)/3/ALLIANCE
53	GAGE	1963	C	NE	CItr13532	Ponca /3/ Mediterranean / Hope // Pawnee
54	GOODSTREAK	2002	C	NE	PI632434	Len // Butte / ND526 (ND604) /6/ (SD2971) Agent /3/ ND441 // Waldron / Bluebird /4/ Butte /5/ Len (SD3055) /7/ KS88H164 /8/ NE89646
55	HALLAM	2006	C	NE	PI638790	Brule / Bennett // Niobrara
56	HARRY	2002	C	NE	PI632435	Brule /4/ Parker *4/ Agent // Beloterkovskaia 198 / Lancer /3/ Newton / Brule (NE90614) /5/ (NE87612) Newton // Warrior *5/ Agent /3/ Agate sib

57	HOMESTEAD	1973	C	NE	CI17264	Scout /4/ Kenya / Newthatch // Cheyenne / Tenmarq / Mediterranean / Hope /3/ Pawnee / Cheyenne
58	INFINITY CL	2006	C	NE	PI639922	Windstar//Millennium sib/Above sib
79	LANCER	1963	C	NE	CItr13547	Turkey Red / Cheyenne // Hope /2* Cheyenne
82	MACE	2007	C	NE	PI651043	Yuma//PI 372129/3/CO850034/4/4*Yuma/5/KS91H184/Arlin S//KS91HW29/3/NE89526
286	MCGILL	2010	C	NE	PI659689	Vona // Chisholm / PlainsmanV (OK83201)/3/Redland (NE92458)/4/ Ike
60	MILLENNIUM	2000	C	NE	PI613099	Arapahoe / Abilene /4/ Colt /3/ Warrior *5/ Agent // Kavkaz
96	NEKOTA	1994	C	NE	PI584997	Bennett/TAM 107
64	NIOBRARA	1994	C	NE	PI584996	TAM 105*5/AMIGO//Brule
65	NUPLAINS	1998	C	NE	PI605741	Abilene / KS831872 = Abilene /3/ Plainsman V // Newton / Arthur 71
62	OVERLAND	2007	C	NE	PI647959	Millennium sib//(ND8974) Seward/Archer
292	PANHANDLE	2014	C	NE	.	BRIGANTINA/2*ARAPAHOE (NE97426)//NE98574
66	PRONGHORN	1996	C	NE	PI593047	Centura/Dawn//Colt
67	RAWHIDE	1990	C	NE	PI543893	Warrior *5/ Agent // Kavkaz /4/ Parker *4/ Agent // Belocerkovskaja 198 / Lancer /3/ Vona
68	REDLAND	1986	C	NE	PI502907	Selection from Brule
295	ROBIDOUX	2010	C	NE	PI659690	Odesskaya P / Cody // Pavon 76 /3* Scout 66 (NE96644)/3/ Wahoo sib
69	SCOUT 66	1967	C	NE	CI13996	composite of 85 selections from Scout, CItr 13546 (Scout = Nebred // Hope / Turkey /3/ Cheyenne / Ponca)
80	SETTLER CL	2009	C	NE	PI653833	Wesley sib // Millennium sib / Above sib
70	SIOUXLAND	1984	C	NE	PI483469	Warrior*5/Agent*2//Kavkaz
72	VISTA	1992	C	NE	PI562653	Warrior // Atlas 66 / Comanche /3/ Comanche / Ottawa (NE68513)/5/(NE68457) Ponca /2* Cheyenne /3/ Illinois No. 1//2* Chinese Spring /T. timopheevii /4/ Cheyenne / Tenmarq // Mediterranean / Hope /3/ Sando 60 /6/ Centurk / Brule
73	WAHOO	2000	C	NE	PI619098	Arapahoe *2/ Abilene
74	WARRIOR	1960	C	NE	CItr13190	Pawnee / Cheyenne
75	WESLEY	1998	C	NE	PI605742	KS831936-3 / NE86501 = Sumner sib (Plainsman V / Odesskaya 51)// Colt / Cody
77	WINDSTAR	1996	C	NE	PI597379	TAM103 / Newton sib (TX79A2729)// Caldwell / Brule field sel .6/3/ Siouxland
49	CHEYENNE	1933	L	NE	CI8885	selection from Crimean, CI 1435

71	TURKEY	1874	L	NE	CI 12137	The original Turkey (Nebr. No. 1) grown at Lincoln since 1897. From it were selected Nebr. 6, 60, etc.
16	OK02405	.	B	OK	.	Tonkawa/GK50
23	OK04111	.	B	OK	.	2174*2/Jagger
24	OK04415	.	B	OK	.	N563/OK98G508W
19	OK04505	.	B	OK	.	OK91724/2*Jagger
21	OK04507	.	B	OK	.	OK95593/Jagger//2174
20	OK04525	.	B	OK	.	FFR525W/Hickok//Coronado
27	OK05108	.	B	OK	.	Lut 13686/2174//Jagger
28	OK05122	.	B	OK	.	KS94U337/NE93427
30	OK05134	.	B	OK	.	OK97411/TX91D6825
34	OK05204	.	B	OK	.	SWM866442/OK95548
31	OK05303	.	B	OK	.	OK95548/TXHBG0358
32	OK05312	.	B	OK	.	TX93V5919/WGRC40//OK94P549/WGRC34
33	OK05511	.	B	OK	.	TAM 110/2174
25	OK05711W	.	B	OK	.	G1878/OK98G508W
26	OK05723W	.	B	OK	.	SWM866442/Betty
22	OK05830	.	B	OK	.	OK93617/Jagger
36	OK06114	.	B	OK	.	KS97P0630-4-5/CM95560//X920879-C15-1/3/X84WO63-9-18/U1324-25-1-4
37	OK06210	.	B	OK	.	KS90175-1-2/CMSW89Y271//K92/3/ABI 86*3414/X86035*-BB-34//HBC 302E
39	OK06318	.	B	OK	.	HBG0358/2174//2145
38	OK06319	.	B	OK	.	Enhancer/2174
40	OK06336	.	B	OK	.	Magvars/2174//Enhancer
276	OK07231	.	B	OK	.	OK92P577-RMH 3099/Duster
277	OK07S117	.	B	OK	.	[ALTAR84/AE.SQ//OPATA]/OK98G508W
278	OK08328	.	B	OK	.	GK Keve/Ok101//OK93P656-RMH3299
273	OK09634	.	B	OK	.	OK95616-98-6756/Overley
274	OK10119	.	B	OK	.	JEI 110/Overley
265	OK1067071	.	B	OK	.	TX98V9437/OK00316//Farmec
266	OK1067274	.	B	OK	.	GA961912-8-4-5/OK02129//Kristi-K.K

267	OK1068002	.	B	OK	.	EFECT/Jagalene//Deliver
268	OK1068009	.	B	OK	.	LADA/Jagalene//G980122
269	OK1068026	.	B	OK	.	ERYTHROSPERMUM 270/TAM 111//OK99212
270	OK1068112	.	B	OK	.	Farmec/Jagalene
272	OK1070267	.	B	OK	.	VI.9/Guymon//G980411W
271	OK1070275	.	B	OK	.	KNJAZHNA/KS00HW175-4//OK00611W
5	2174-05	1998	C	OK	PI602595	IL71-5662/PL145(Newton sib)//2165
18	BILLINGS	2009	C	OK	PI656843	N566/OK94P597
12	CENTERFIELD	2006	C	OK	PI644017	TXGH12588-105*4/FS4//2*2174
3	CENTURY	1986	C	OK	PI502912	Payne // TAM W-101 / Amigo
2	CHISHOLM	1983	C	OK	PI486219	Sturdy sib / Nicoma
4	CUSTER	1994	C	OK	.	F-29-76/TAM-105//Chisholm
10	DELIVER	2004	C	OK	PI639232	Yantar/2*Chisholm (OK91724)//Karl
14	DUSTER	2006	C	OK	PI644016	W0405D/NE78488//W7469C/TX81V6187
9	ENDURANCE	2004	C	OK	PI639233	HBY756A/Siouxland//2180
275	GALLAGHER	2013	C	OK	PI 667569	OK99711/Duster
35	GARRISON	2011	C	OK	PI661992	OK95616-1/Hickok//Betty
13	GUYMON	2005	C	OK	PI643133	Intrada/Platte
6	INTRADA	2000	C	OK	PI631402	Rio Blanco / TAM 200
11	OK BULLET	2005	C	OK	PI642415	KS96WGRC39/Jagger
15	OK RISING	2009	C	OK	PI656382	KS96WGRC39/Jagger
7	OK101	2001	C	OK	PI631493	OK87W663/Mesa//2180
8	OK102	2002	C	OK	PI632635	2174/Cimarron
17	PETE	2009	C	OK	PI656844	N40/OK94P455
29	RUBY LEE	2011	C	OK	PI661991	KS94U275/OK94P549
1	TRIUMPH 64	1964	C	OK	CItr13679	Danne Beardless Blackhull /3/ Kanred / Blackhull // Florence /4/ Kanred / Blackhull // Triumph
92	SD01058	.	B	SD	.	XH1877/NE967430
91	SD01237	.	B	SD	.	UNKNOWN
93	SD05118	.	B	SD	.	Wesley/NE93613
94	SD05210	.	B	SD	.	SD98444/SD97060

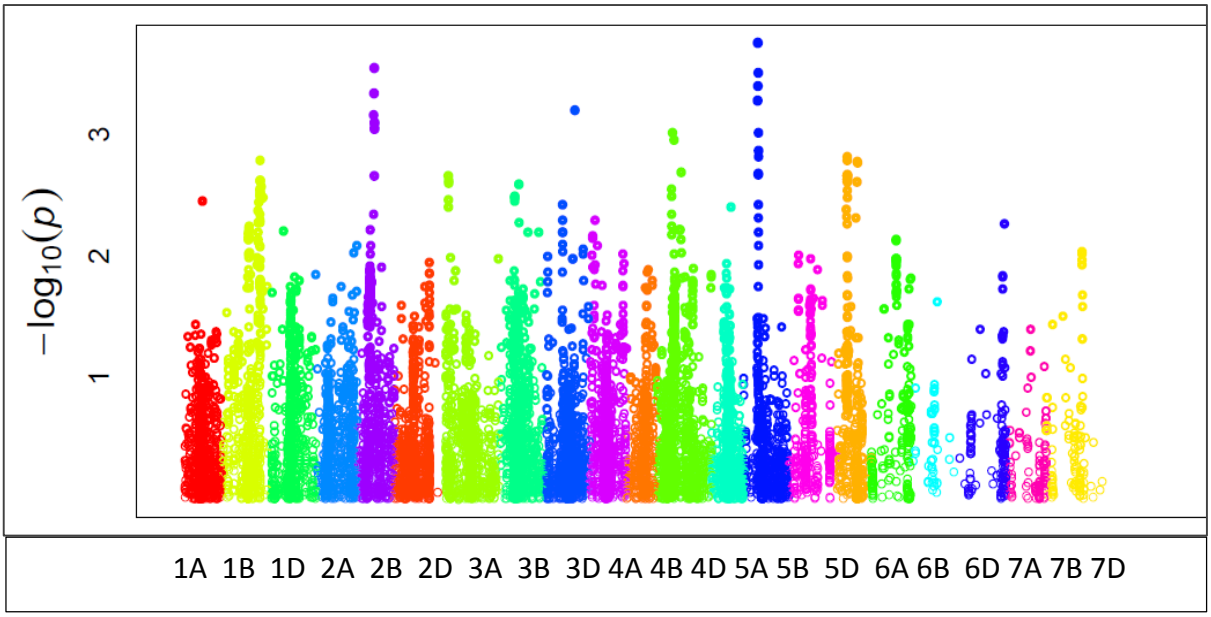
95	SD05W018	.	B	SD	.	SD98W302/SD98W175
86	ALICE	2006	C	SD	PI644223	Abilene/Karl.
104	BRONZE	1974	C	SD	CItr14013	Hume / Gage /4/ Hume /3/ NE61943 , Mida / Kenya 117A //2* Hope /2* Turkey Red
98	CRIMSON	1997	C	SD	PI601818	TAM-105 / Winoka
87	DARRELL	2006	C	SD	PI644224	2076-W12-11/Karl92
88	EXPEDITION	2002	C	SD	PI629060	Tomahawk / Bennett
106	GENT	1974	C	SD	CItr17293	Agent /4* Scout
107	HARDING	1999	C	SD	PI608049	Brule // Bennett / Chisholm /3/ Arapahoe
105	HUME	1965	C	SD	CItr13526	crosses involving: Minter, Kharkof, Wichita, Nebred, Cheyenne, and others
90	LYMAN	2009	C	SD	PI 658067	KS93U134/Arapahoe
102	NELL	1981	C	SD	CItr17803	Scout selection / Capitan
103	RITA	1980	C	SD	CItr17799	Seu Seun / Denton 8 // Westmont /3/ (SD 6689) Ponca //3* Cheyenne / Kenya58 / Newthatch //2*(Cheyenne / Tenmarq // Mediterranean / Hope)
99	ROSE	1979	C	SD	CItr17795	Seu Seun / Denton 8 // Westmont /4/ Hume /3/ NE 63265
97	TANDEM	1997	C	SD	PI601817	Brule / Agate
89	WENDY	2004	C	SD	PI638521	Gent/Siouxland (SD89333) // Abilene
101	WINOKA	1969	C	SD	CItr14000	Selection from Winalta
180	TX00V1131	.	B	TX	.	TX87V1613/KS91WGRC11
168	TX01A5936	.	B	TX	.	JAGGER/3/PSN 'S'/BOW 'S'//T200
179	TX01M5009-28	.	B	TX	.	MASON/JAGGER//PECOS
174	TX01V5134RC-3	.	B	TX	.	TAM-200/JAGGER
171	TX03A0148	.	B	TX	.	TX89A7137/TIPACNA
172	TX03A0563	.	B	TX	.	X96V107/OGALLALA
173	TX04A001246	.	B	TX	.	TX95V4339/TX94VT938-6
175	TX04M410164	.	B	TX	.	MIT/TX93V5722//W95-301
176	TX04M410211	.	B	TX	.	MASON/JAGGER//OGALLALA
177	TX04V075080	.	B	TX	.	JAGGER/TX93V5722//TX95D8905
260	TX05A001188	.	B	TX	.	T107//TX98V3620/Ctk78/3/TX87V1233/4/N87V106//TX86V1540/T200
253	TX05A001822	.	B	TX	.	2145/X940786-6-7
258	TX05V7259	.	B	TX	.	T107//TX78V3620/Ctk78/3/TX87V1233/4/Arap//TX86V1540/T200

259	TX05V7269	.	B	TX	.	HBG0358/4/T107//TX78V3620/Ctk78/3/TX87V1233
255	TX06A001132	.	B	TX	.	HBG0358/4/T107//TX78V3620/Ctk78/3/TX87V1233
254	TX06A001263	.	B	TX	.	UNKNOWN
256	TX06A001281	.	B	TX	.	TX98VR8422/U3704A-7-7
257	TX06A001386	.	B	TX	.	TX99A6030/CUSTER
264	TX06V7266	.	B	TX	.	TX99U8617/TX97U2001
261	TX07A001279	.	B	TX	.	X930332-4-1/TX97V2838
262	TX07A001318	.	B	TX	.	TX98VR8431/TX95A3091
263	TX07A001420	.	B	TX	.	U1254-1-5-2-1/TX81V6582//DESCONOCIDO
185	TX86A5606	.	B	TX	.	TAM 105*4/AMI*4//LGO
186	TX86A6880	.	B	TX	.	TAM 105*4/AMI*4//LGO
187	TX86A8072	.	B	TX	.	TAM 105*4/AMI*4//LGO
182	TX96D1073	.	B	TX	.	TX86D1310/Kavkaz//TX86D1308 (=WX87D144-10-99-12-18)
178	TX99A0153-1	.	B	TX	.	OGALLALA/TAM-202
181	TX99U8618	.	B	TX	.	TX84V1237/TX71C8130R
167	CAPROCK	1969	C	TX	CItr14516	Sinvalocho / Wichita // Hope / Cheyenne /3/ Wichita /4/ Seu Seun 27
184	HG-9	2000	C	TX	PI614118	TAM 200 outcross selection
163	LOCKETT	2001	C	TX	PI604245	TX86V1540 / TX78V2430-4
166	MIT	1980	C	TX	CItr17896	Sinvalocho / Wichita // Hope / Cheyenne /3/ Wichita /4/ Seu Seun 27 (TX391-56-D1 - 24)/6/T. dicoccoides / Aeg. speltoides , amphiploid //2* Austin /3/ Supremo (TX55C907)/4/ Bison /5/ Caddo/7/ Frontana / Westar
164	STURDY	1966	C	TX	CItr13684	Sinvalocho / Wichita // Hope / Cheyenne /3/2* Wichita /4/ Seu Seun 27
165	STURDY 2K	2005	C	TX	PI636307	Sturdy Resel.
150	TAM 105	1979	C	TX	CItr17826	' short wheat' / Sturdy composite bulk selection
151	TAM 107	1984	C	TX	PI495594	TAM 105 *4/ Amigo
152	TAM 109	1991	C	TX	PI554606	TAMW-101 *5/ CI9321
153	TAM 110	1996	C	TX	PI595757	TAM 107*5/Largo
154	TAM 111	2002	C	TX	PI631352	TAM 107 // TX78V3630 / Centurk 78 /3/ TX87V1233 = TAM 107 /4/ Sturdy sib / Kaw // Centurk /3/ Centurk 78 /5/ Sturdy sub / Kaw // Centurk /3/ Jupetaco / Bluejay
155	TAM 112	2007	C	TX	PI643143	TAM 200/TA2460 (U1254-7-9-2-1)//(TXGH10440) TAM 107*5/Largo
170	TAM 113	2013	C	TX	PI 666125	TX90V6313/TX94V3724

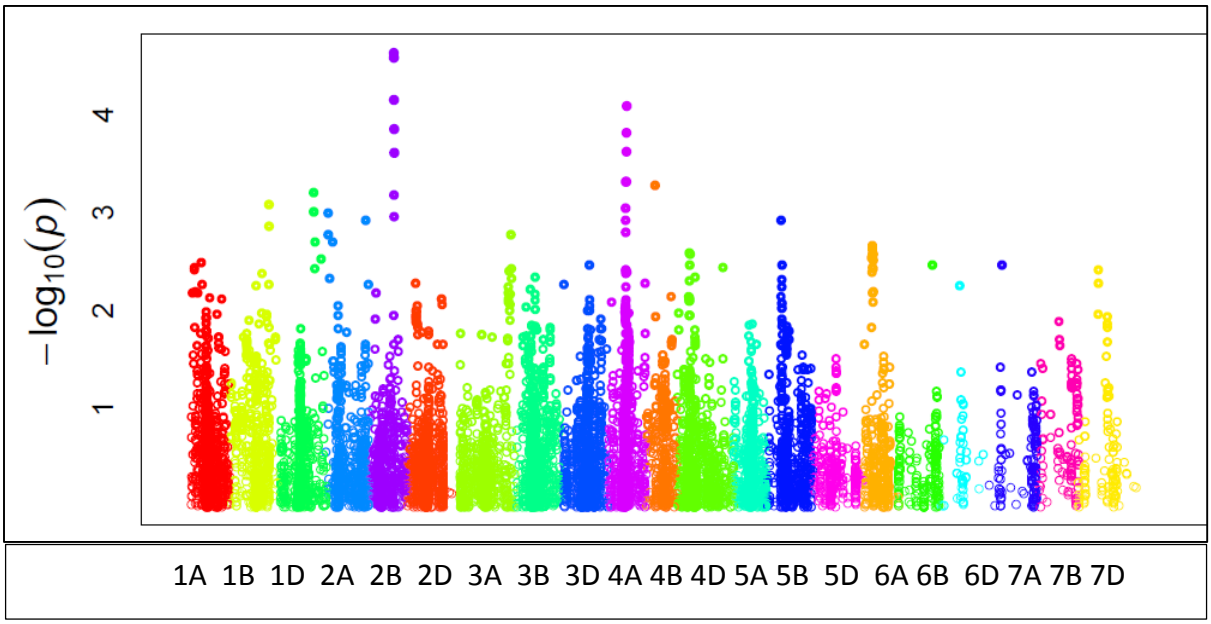
156	TAM 200	1986	C	TX	PI578255	Sturdy sib / Tascosa // Centurk *3/3/ Amigo
157	TAM 202	1992	C	TX	PI561933	Siouxland outcross
158	TAM 203	2009	C	TX	PI655960	TX89V4132/704 L I-2221
159	TAM 302	1998	C	TX	PI605910	Probrand 812 / Caldwell // (TX86D1310) TAM300 sib
160	TAM 303	2006	C	TX	.	TX89D1253*2/TTCC404 (=WX93D208-9-1-2)
161	TAM 304	2009	C	TX	PI655234	TX92U3060/TX91D6564
162	TAM 400	2001	C	TX	PI614876	TAM-200//(TX82D5668) Era/TAMW-101
169	TAM 401	2010	C	TX	PI658500	Mason/Jagger
149	TAM W-101	1971	C	TX	CItr15324	Norin 10 /3/ Nebraska 60 // Mediterranean / Hope /4/ Bison
221	TASCOSA	1959	C	TX	CItr13023	Kanred / Hard Federation // Tenmarq /3/ Mediterranean / Hope /4/ Cimarron
59	KHARKOF	1900	L	Ukraine	PI5641	KHARKOF

† C, cultivar; L, landrace; B, breeding line.

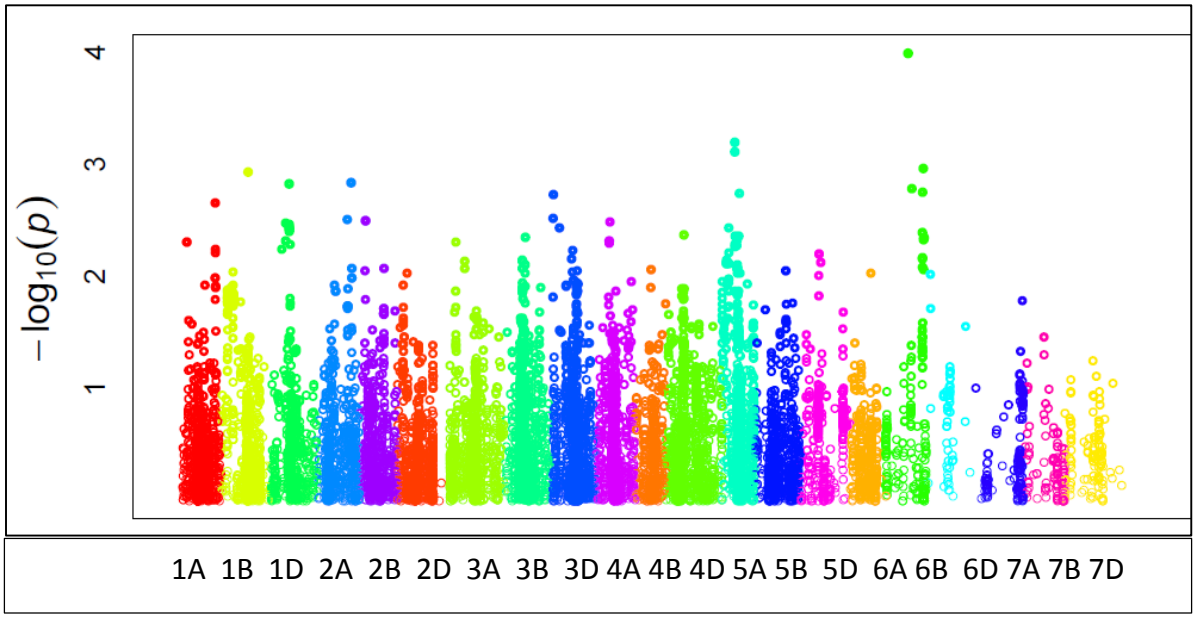
Appendix 2. Manhattan plots for all traits in different environments. The X-axis is the genomic position of each SNP; the Y-axis is the negative logarithm of the P-value obtained from the GWAS model.



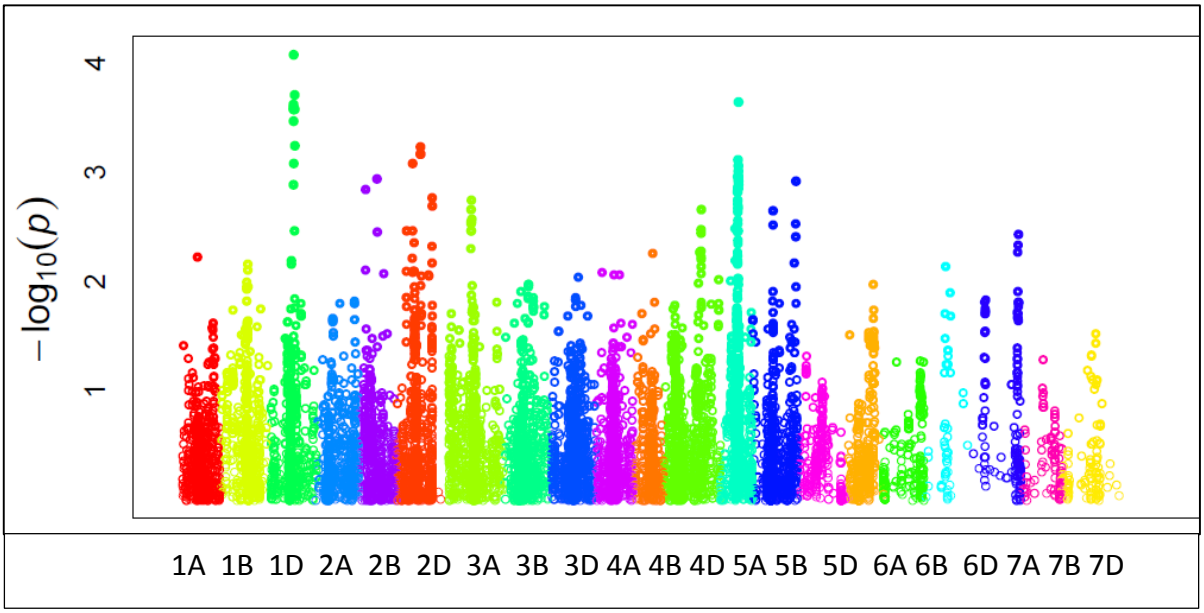
A 2.1 Manhattan plot of grain yield in Greeley 2012 well-watered trial



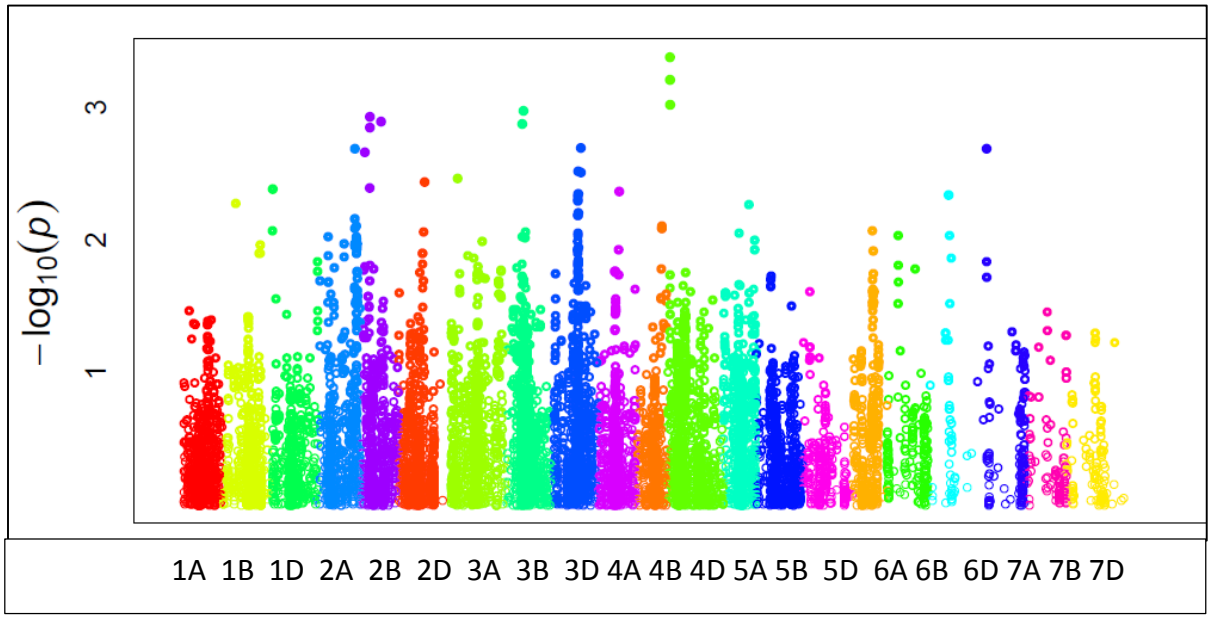
A 2.2 Manhattan plot of grain yield in Greeley 2012 water-stressed trial.



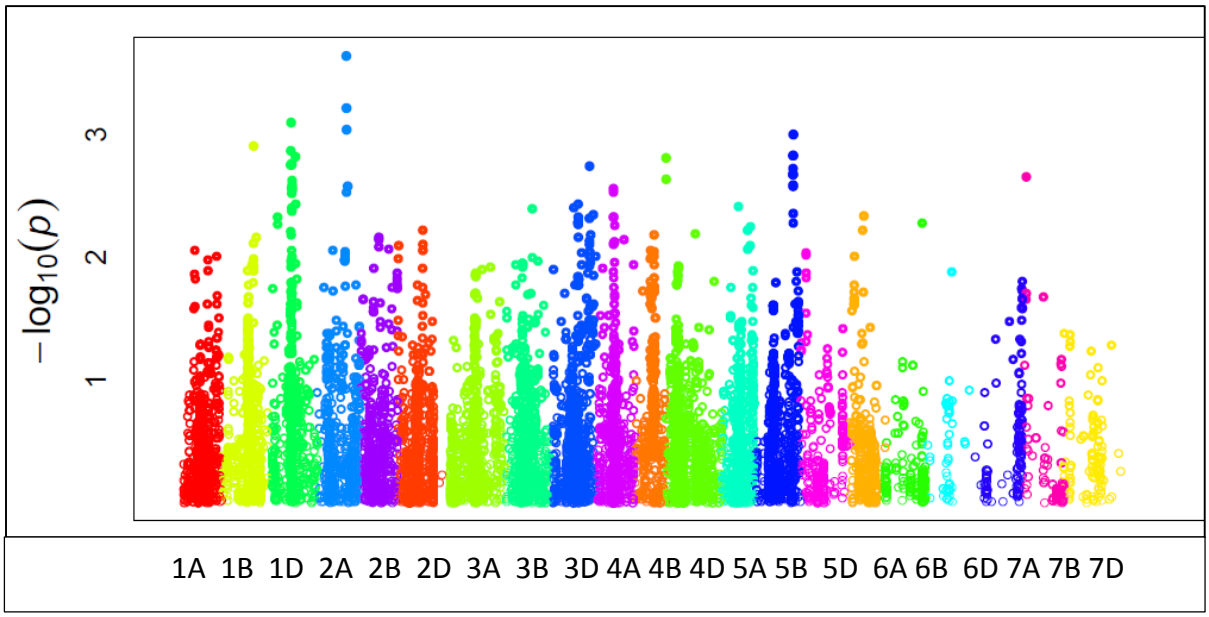
A 2.3 Manhattan plot of grain yield in Fort Collins 2013 well-watered trial.



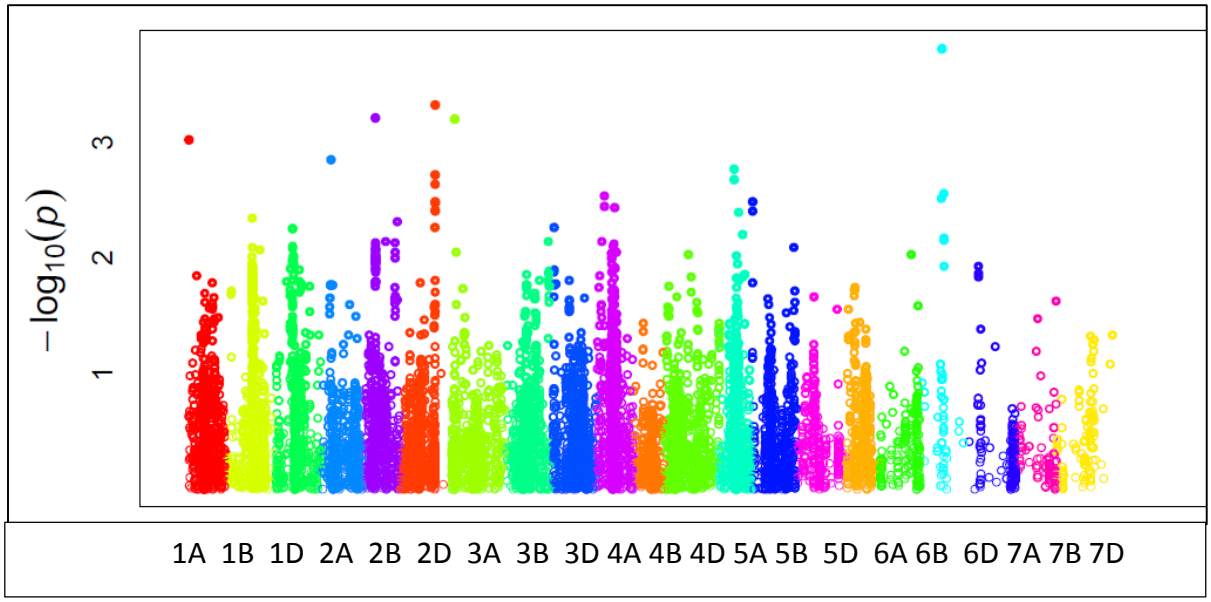
A 2.4 Manhattan plot of grain yield in Fort Collins 2013 water-stressed trial.



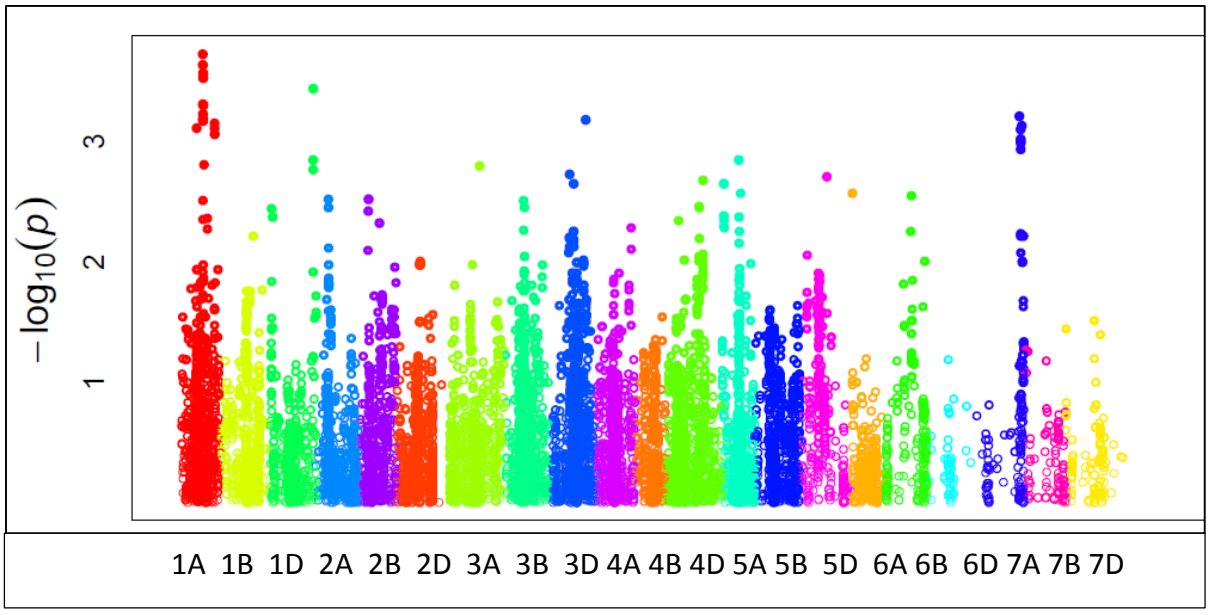
A 2.5 Manhattan plot of total biomass in Greeley 2012 well-watered trial.



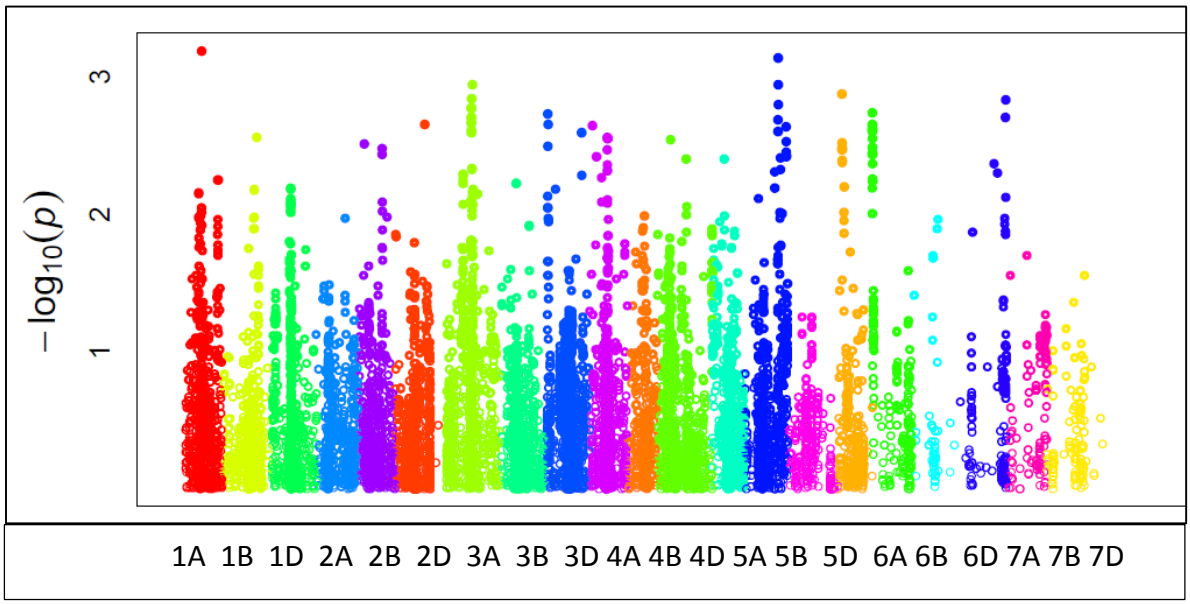
A 2.6 Manhattan plot of total biomass in Greeley 2012 water-stressed trial.



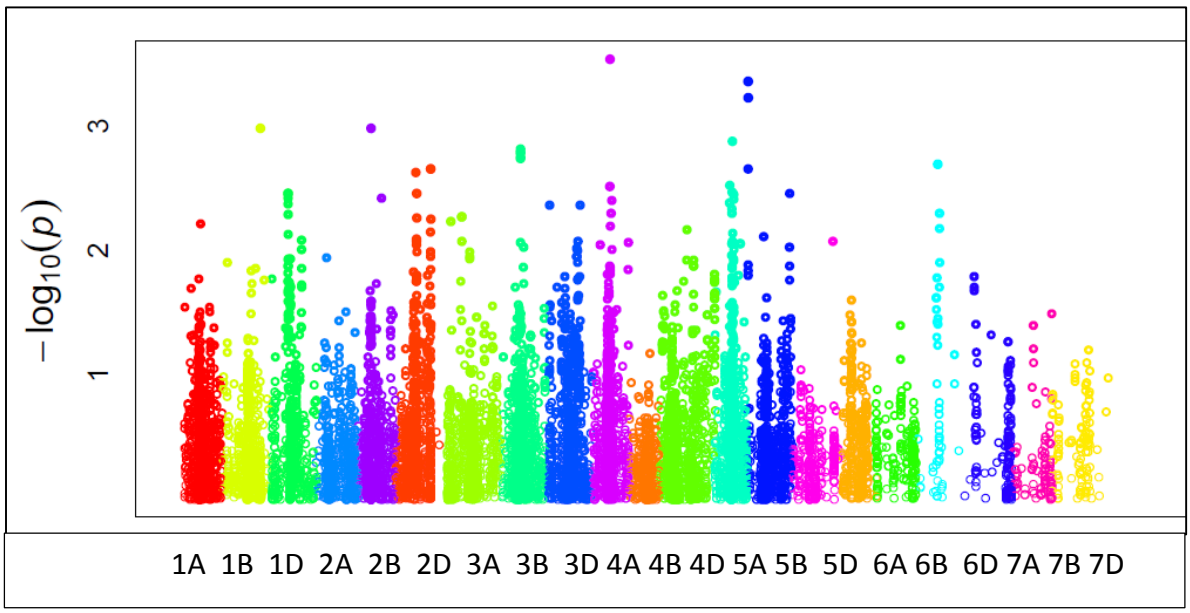
A 2.7 Manhattan plot of total biomass in Fort Collins 2013 well-watered trial.



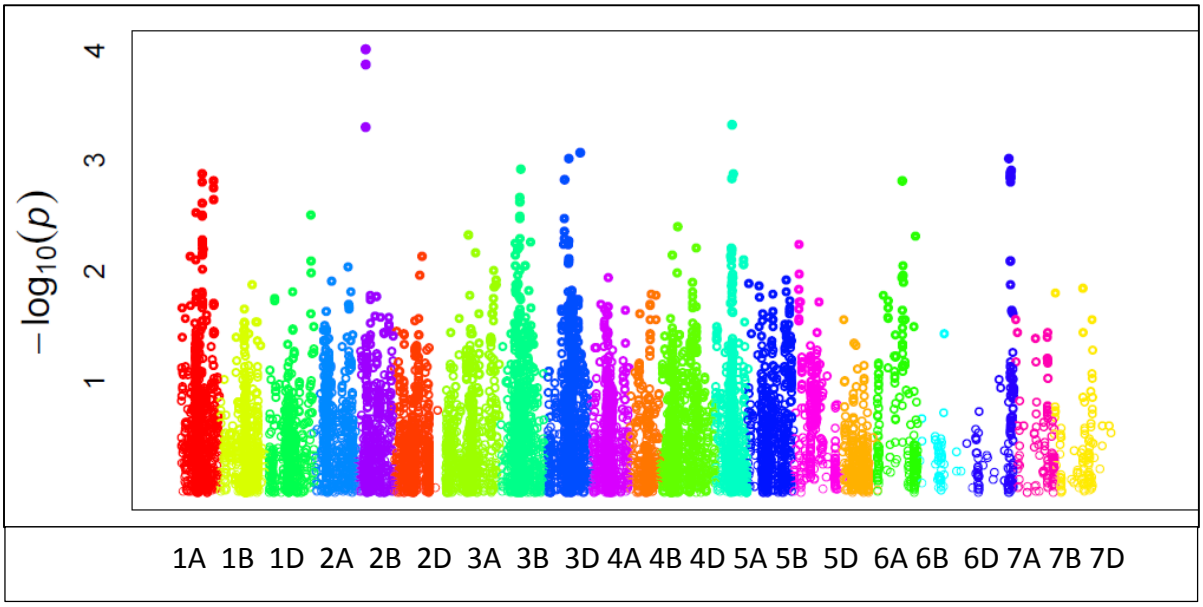
A 2.8 Manhattan plot of total biomass in Fort Collins 2013 water-stressed trial.



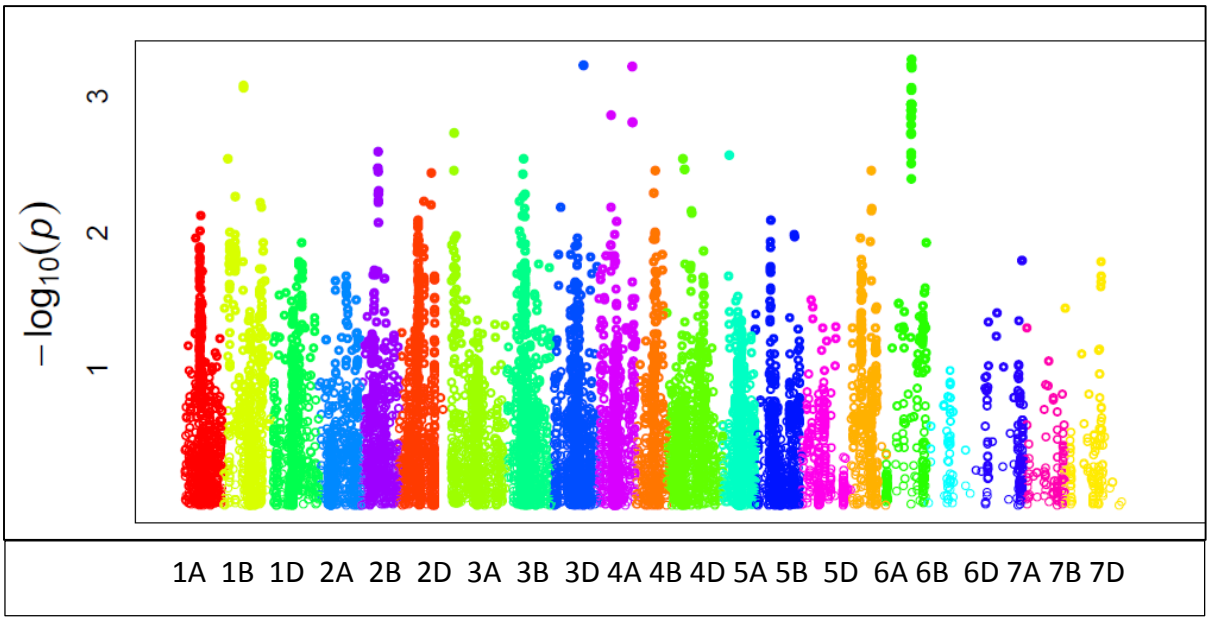
A 2.9 Manhattan plot of biomass grain weight in Greeley 2012 well-watered trial.



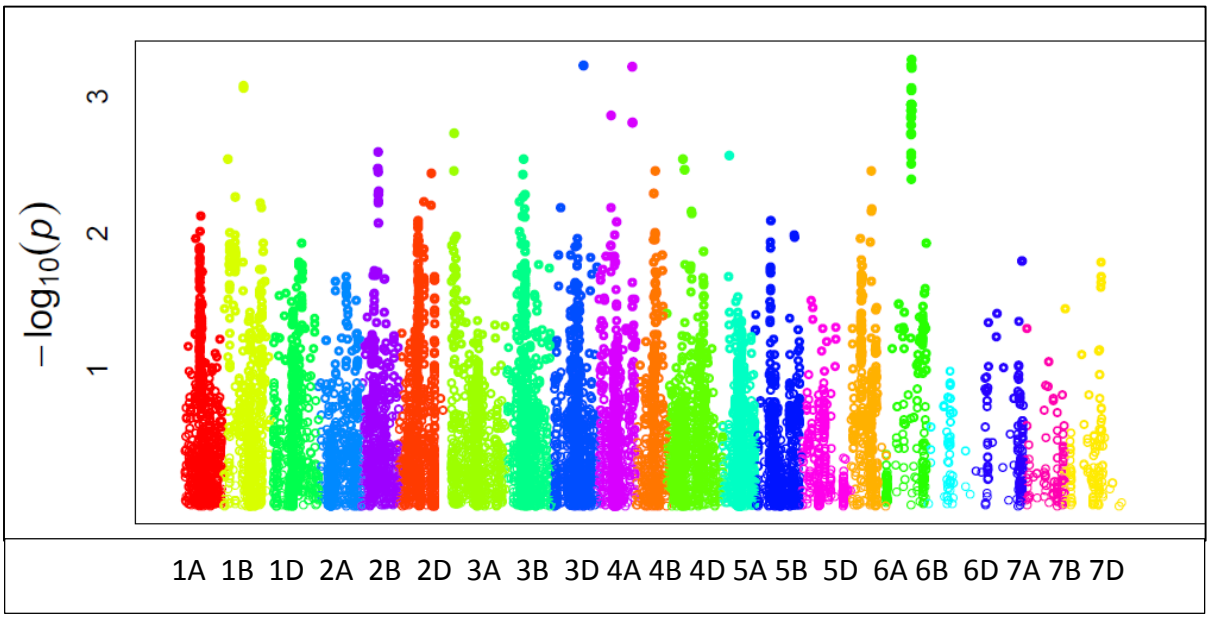
A 2.10 Manhattan plot of biomass grain weight in Greeley 2012 water-stressed trial.



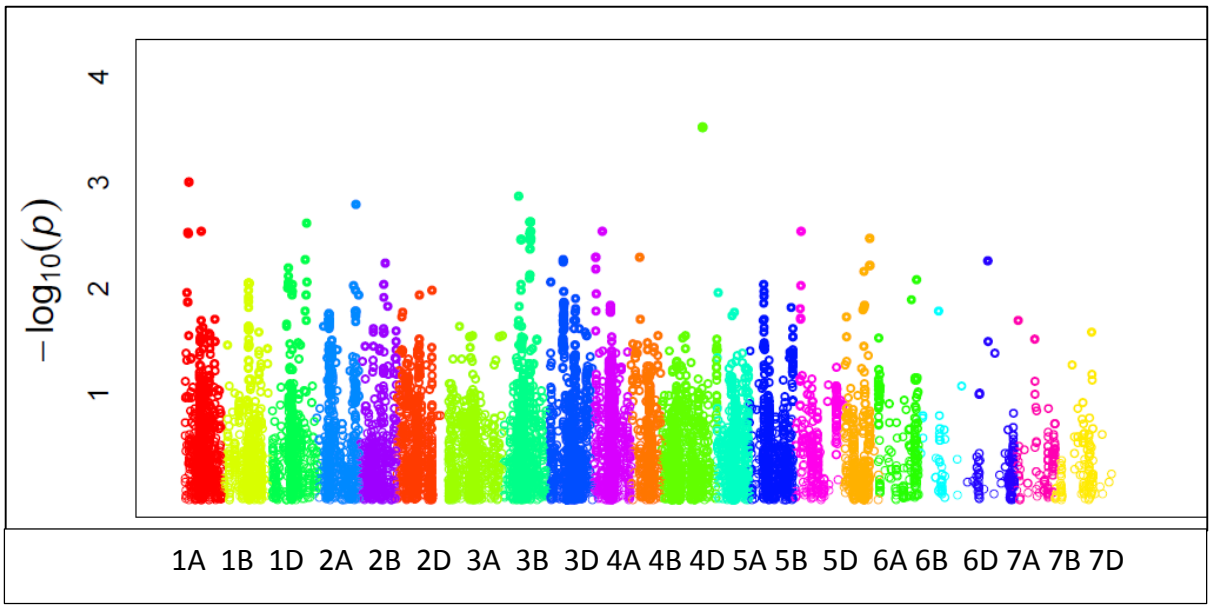
A 2.11 Manhattan plot of biomass grain weight in Fort Collins 2013 well-watered trial.



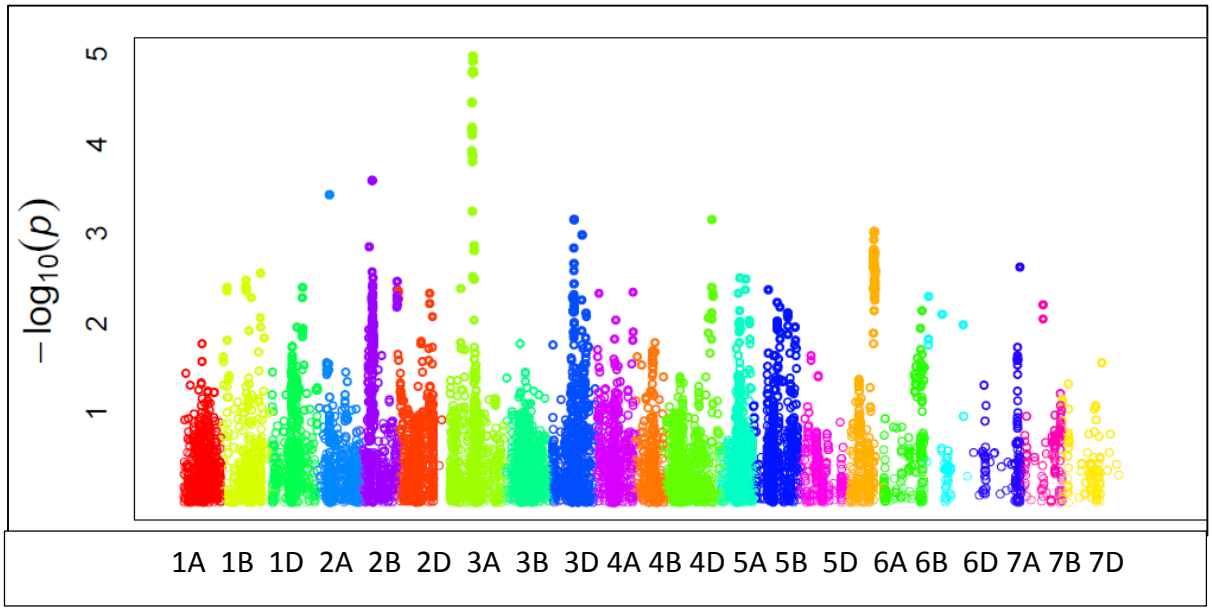
A 2.12 Manhattan plot of biomass grain weight in Fort Collins 2013 water-stressed trial.



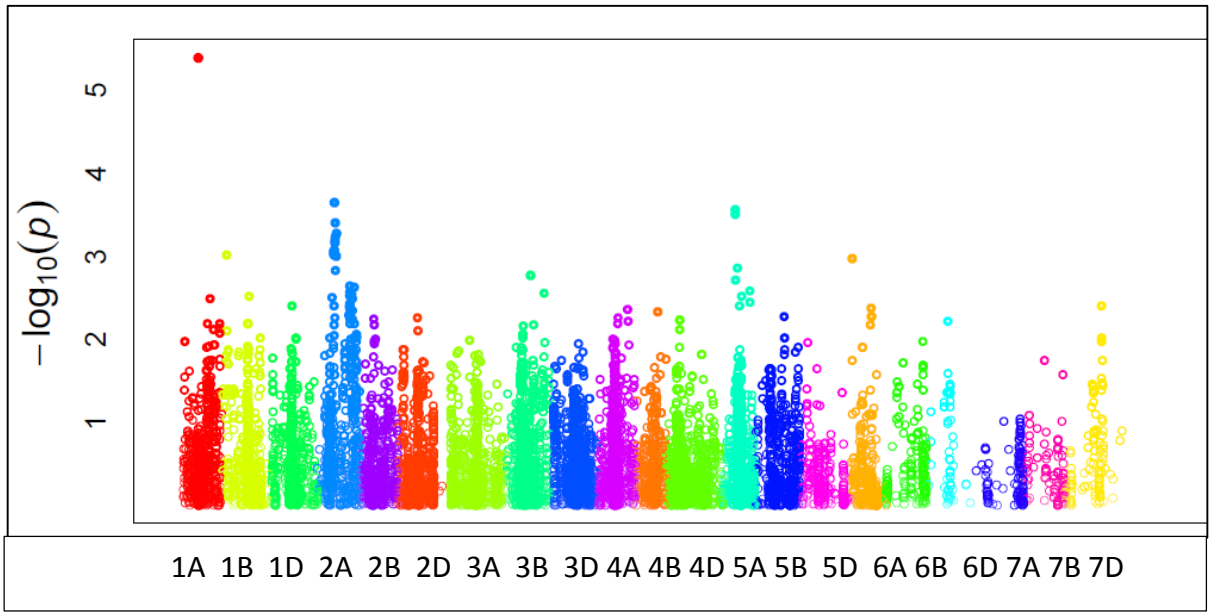
A 2.13 Manhattan plot of harvest index in Greeley 2012 well-watered trial.



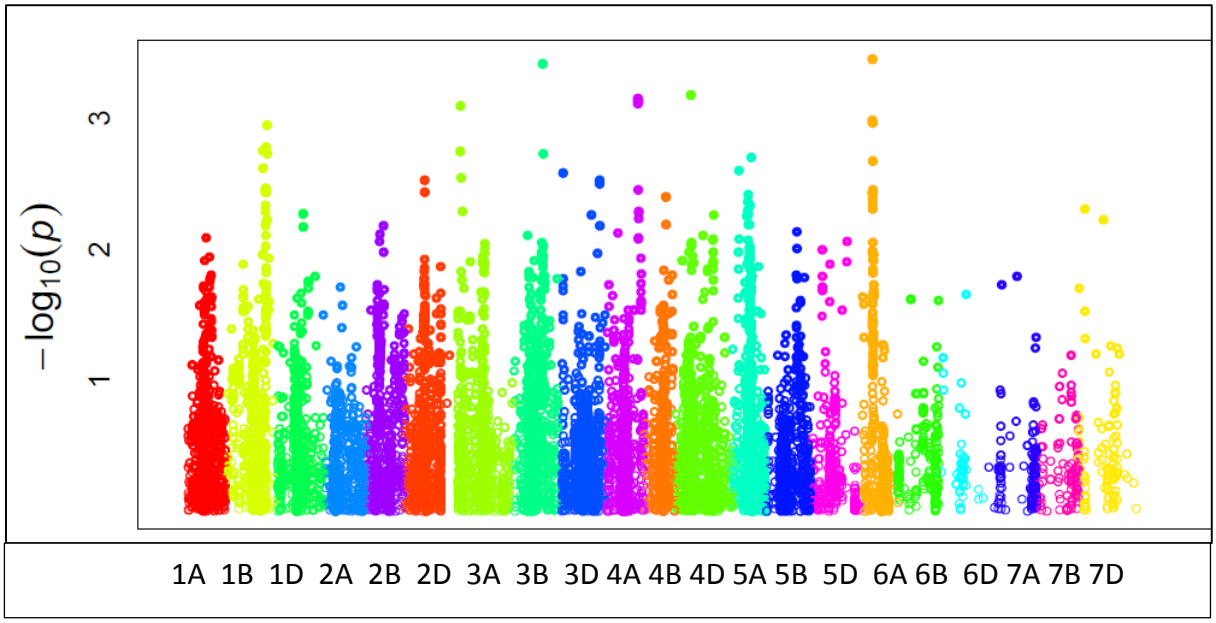
A 2.14 Manhattan plot of harvest index in Greeley 2012 water-stressed trial.



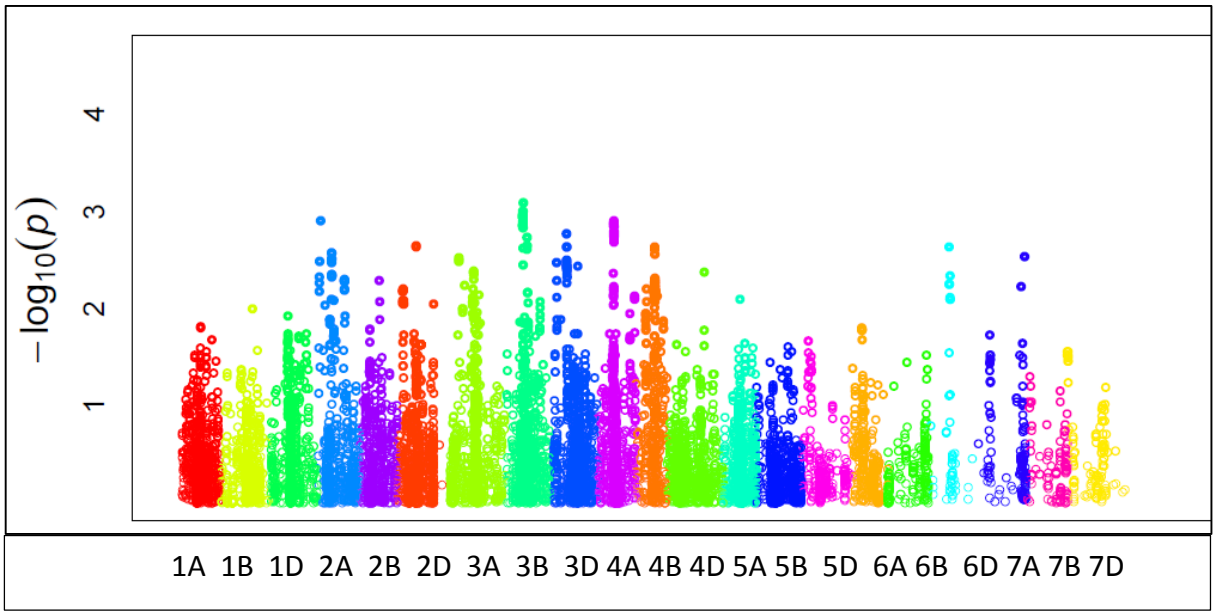
A 2.15 Manhattan plot of harvest index in Fort Collins 2013 well-watered trial.



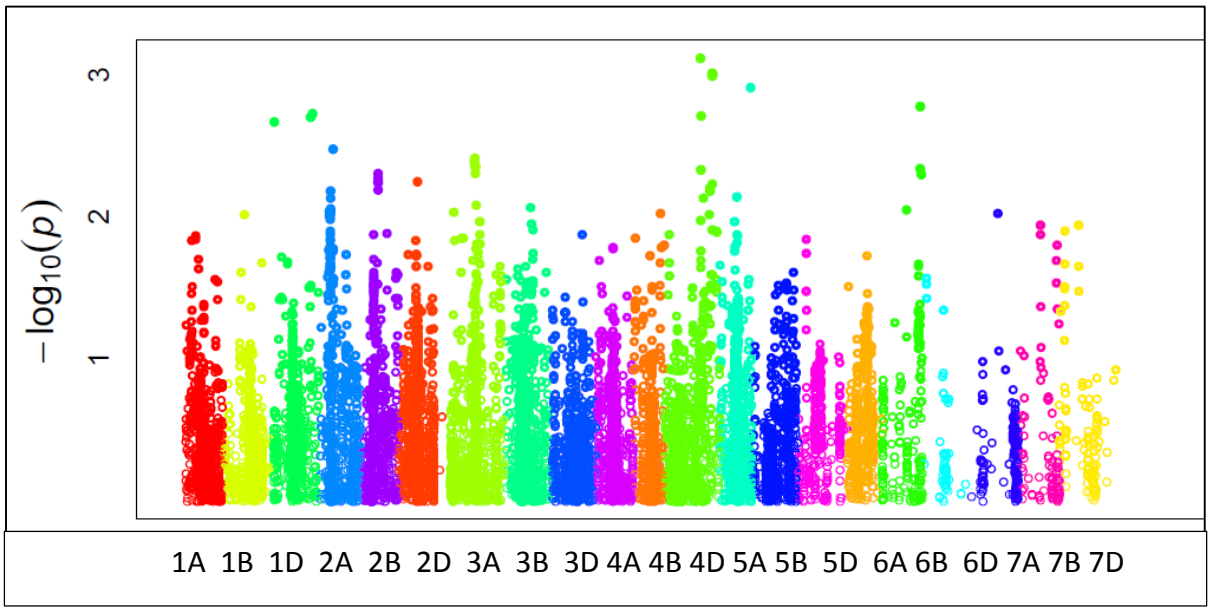
A 2.16 Manhattan plot of harvest index in Fort Collins 2013 water-stressed trial.



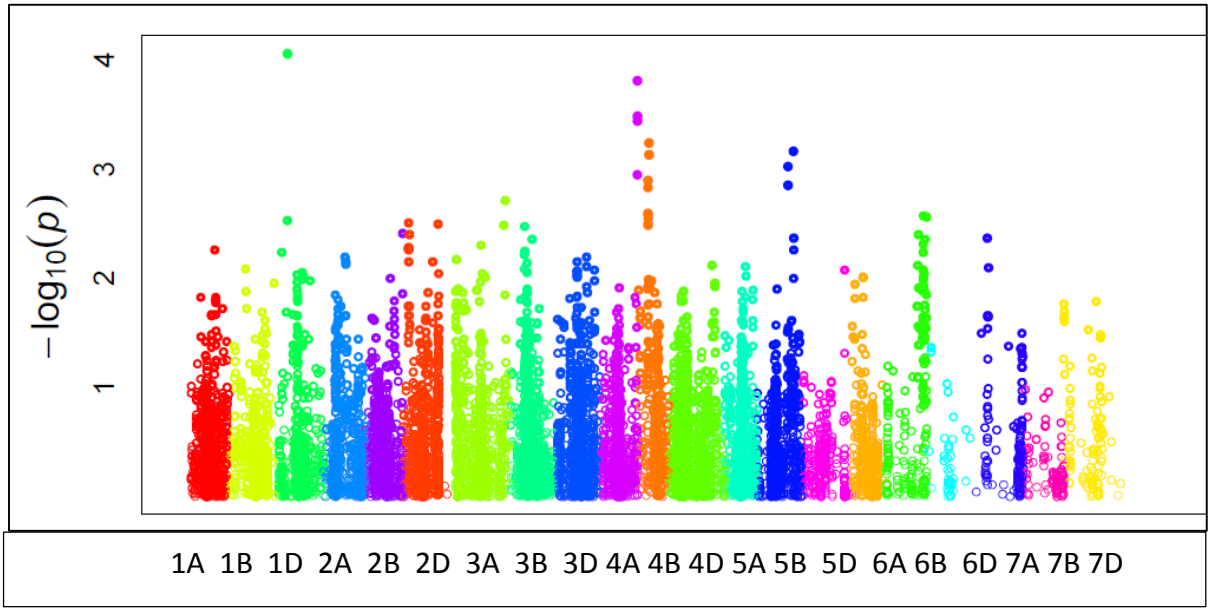
A 2.17 Manhattan plot of plant height in Greeley 2012 well-watered trial.



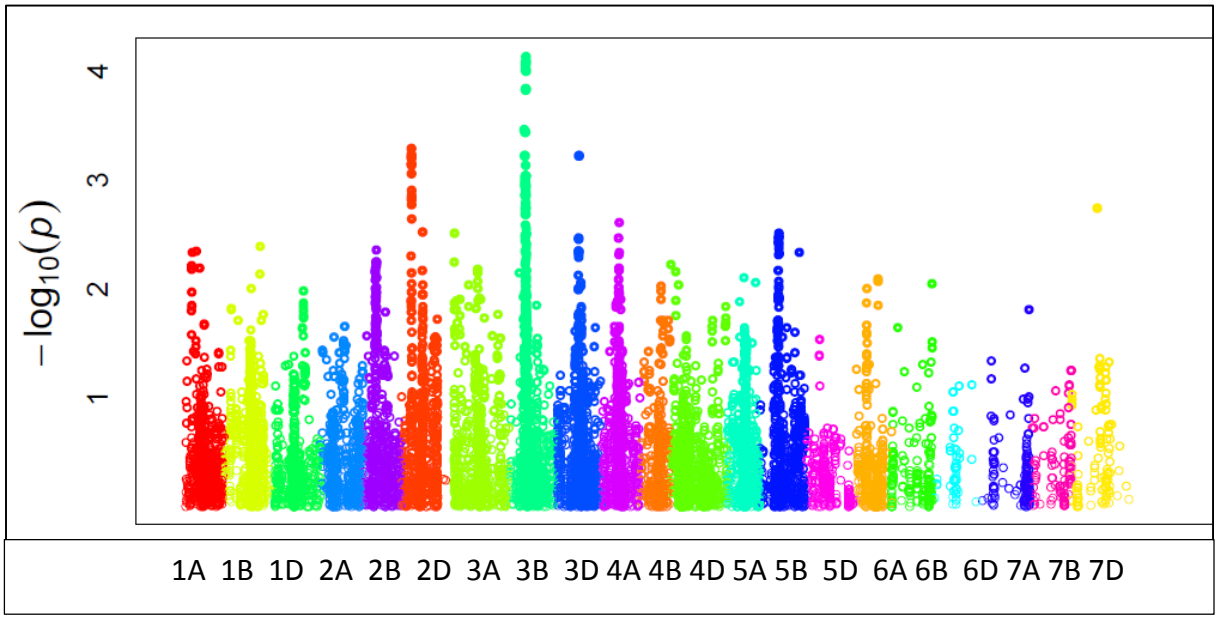
A 2.18 Manhattan plot of plant height in Greeley 2012 water-stressed trial.



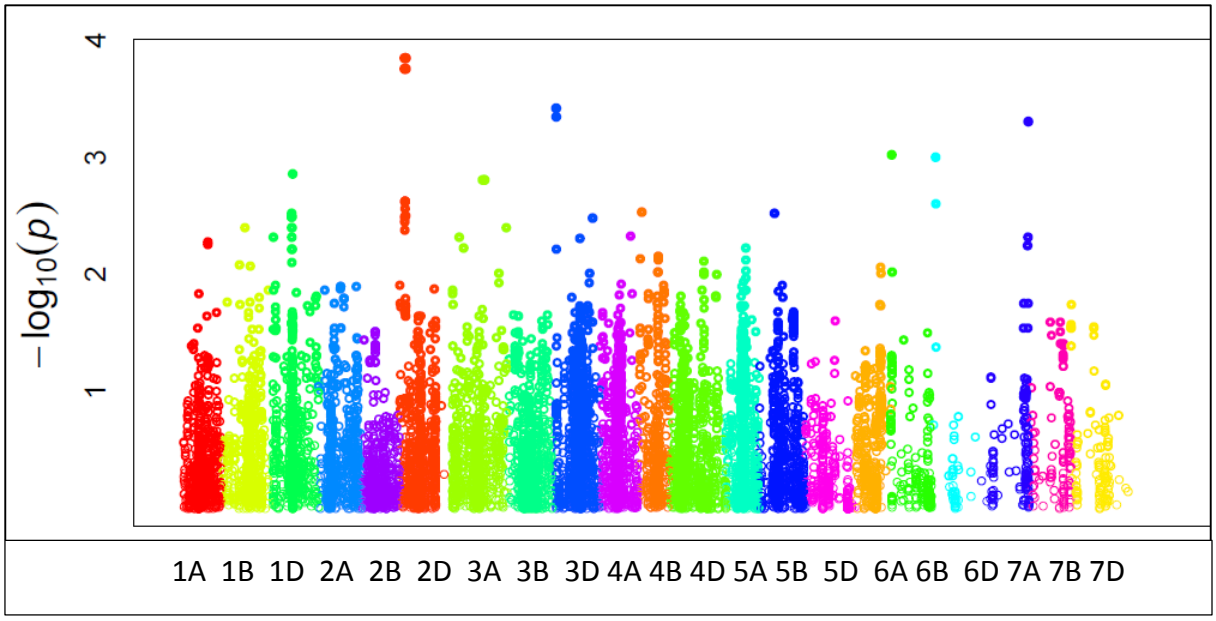
A 2.19 Manhattan plot of plant height in Fort Collins 2013 well-watered trial.



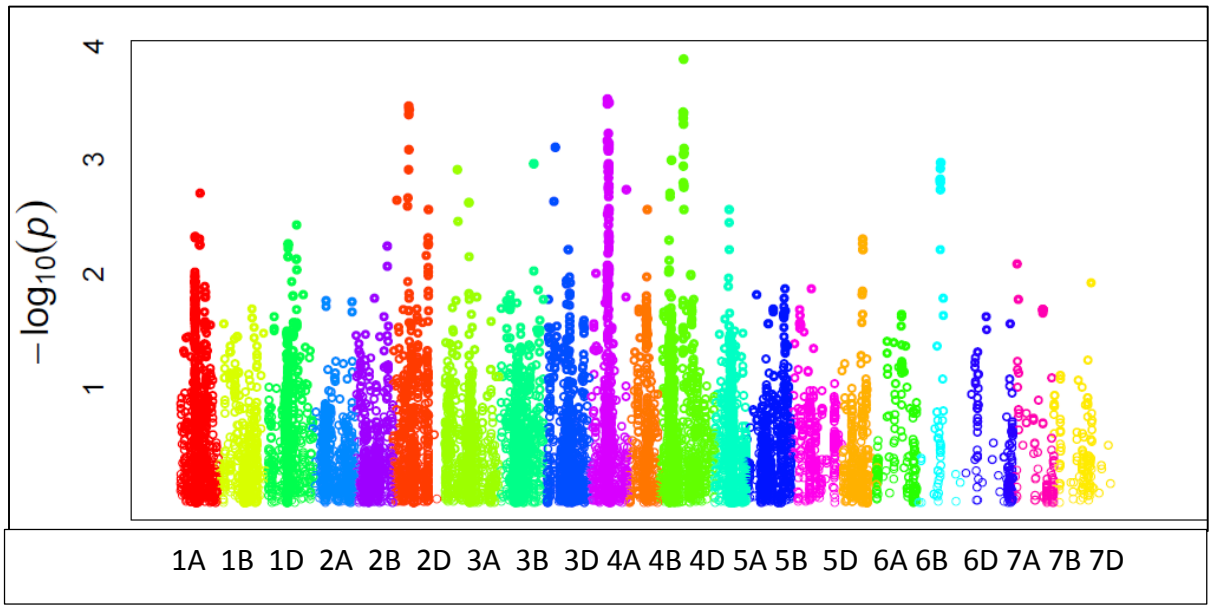
A 2.20 Manhattan plot of plant height in Fort Collins 2013 water-stressed trial.



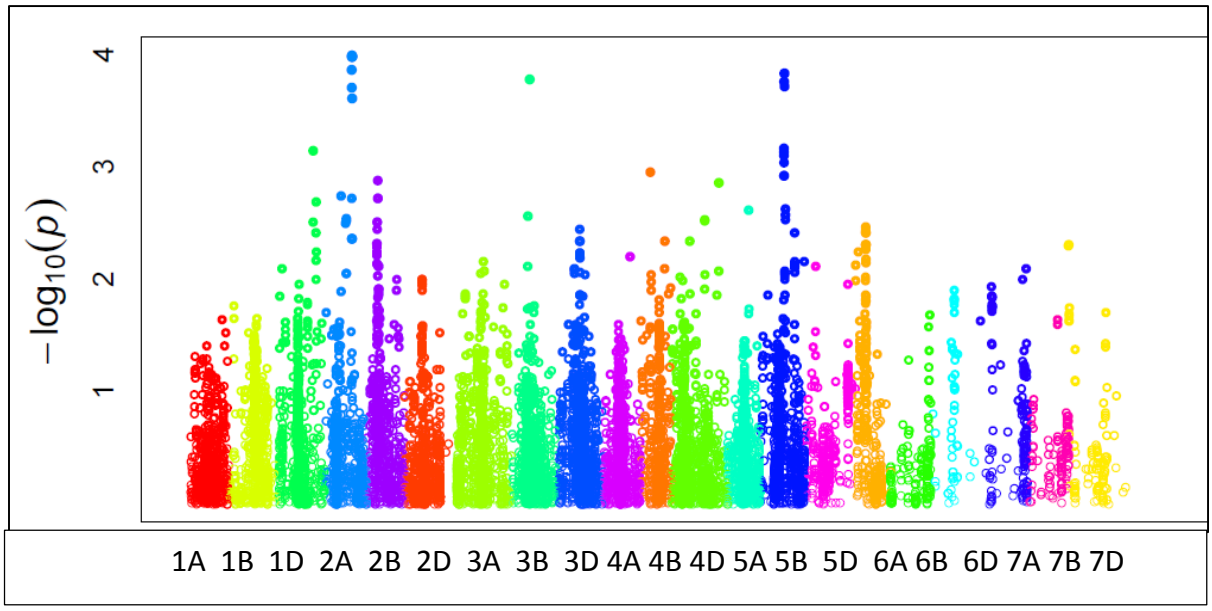
A 2.21 Manhattan plot of canopy temperature at late heading stage in Greeley 2012 well-watered trial.



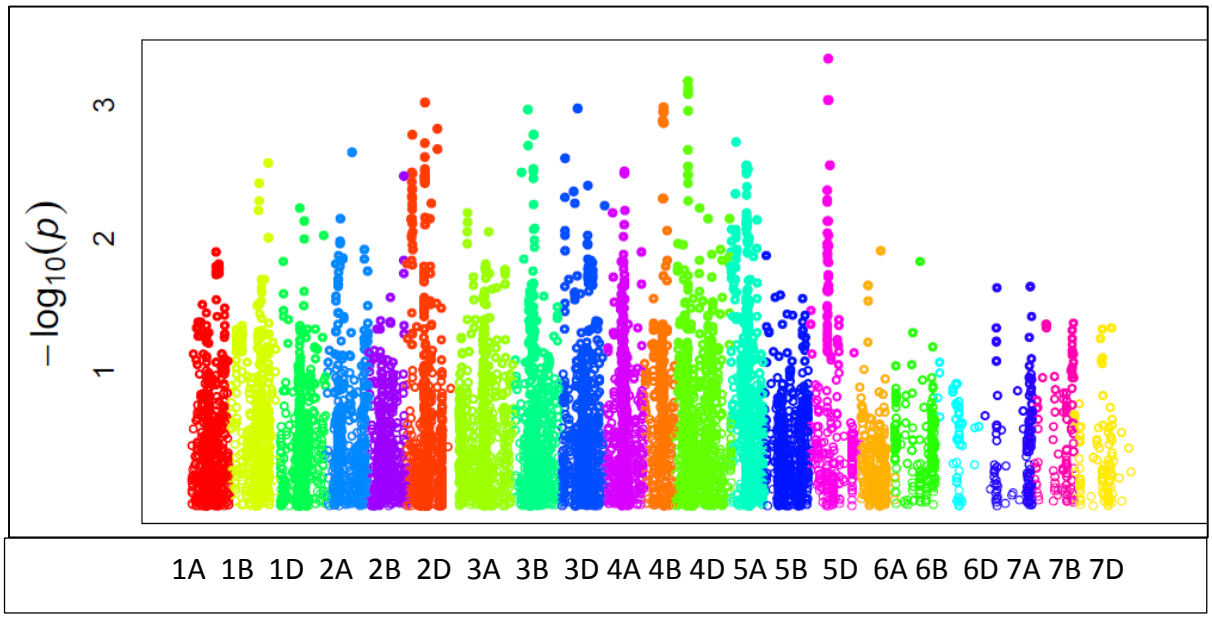
A 2.22 Manhattan plot of canopy temperature at late heading stage in Greeley 2012 water-stressed trial.



A 2.23 Manhattan plot of carbon isotope discrimination in Greeley 2012 well-watered trial.



A 2.24 Manhattan plot of drought susceptibility index in Greeley 2012.



A 2.25 Manhattan plot of drought susceptibility index in Fort Collins 2013.

Appendix 3. Entries included in Chapter 3, greenhouse Study I with their pedigrees, accession numbers, year of release and origin.

Entry Name	Pedigree	Accession Number	release year	Origin
Above	TAM110*4/FS2	PI 631449	2001	Colorado
Akron	TAM107/Hail	PI 584504	1994	Colorado
Avalanche	Bennett-sib/5/TAM107(KS87H325)/6/RioBlanco	PI 620766	2001	Colorado
Bill Brown	Yumar/Arlin	PI 653260	2007	Colorado
Bond CL	Yumar//TXGH12588-120*4/FS2	PI 639924	2004	Colorado
Byrd	TAM 112/CO970547-7	PI 664257	2011	Colorado
Carson	Anza/Scout//Centurk	PI 501534	1986	Colorado
CO04393	Stanton/CO950043		Exp†	Colorado
CO04499	Above/Stanton		Exp	Colorado
CO04W320	CO950635/CO99W1126		Exp	Colorado
CO940610	KS87H22/MWO9	GSTR 10702	Exp	Colorado
Denali	CO980829/TAM111		2011	Colorado
Duke	3*Sonora64/Warrior//Selkirk/2*Cheyenne/5/Scout/4/Quivera/3/Tenmark//Marquis1/Oro	CItr 17856	1981	Colorado
Hail	Mexican/USA//Scout/3/Mara/4/Scout/5/Ciano/6/Trapper/7/Parker	PI 470927	1982	Colorado
Halt	Sumner/CO820026//PI372129/3/TAM107	PI 584505	1994	Colorado
Hatcher	Yuma/PI372129//Tam-200/3/4*Yuma/4/KS91H184/Vista	PI 638512	2004	Colorado
Jules	Warrior*5/Agent//Agate-sib(NE76667)/3/Hawk	PI 564851	1994	Colorado
Lamar	74F878 (Mexicandwarf)/Wings//Vona	PI 559719	1988	Colorado
Lindon	Andes64A/Sonora64//Tacuari (I21183)/4/(CO652363) Warrio*r2*/Kenya58/Newthatch//Cheyenne/Tenmark/Mediterranean/Hope/3/Parker/5/Lancer/3/(KS 62136) Norin16/CI12500//Kaw	CItr 17440	1977	Colorado
Platte	Tesia79/Chat'S'//Abilene	PI 596297	1995	AgriPro
Prairie Red	CO850034/PI372129//5*TAM 107CSU	PI 605390	1998	Colorado
Prowers	CO850060/PI372129//5*Lamar	PI 605389	1997	Colorado
Ripper	CO940606/TAM107R-2	PI 644222	2006	Colorado
Ronl	Trego/3/(CO9600293) PI222668/TAM107//CO850034		2006	Kansas
Sandy	434Mexican spring semidwarf/Trapper//CenturkCSU	CItr 17857	1981	Colorado

Snowmass	KS96HW94//Trego/CO960293	PI 658597	2009	Colorado
Thunder CL	KS01-5539/CO99W165	PI 655528	2008	Colorado
Vona	Andes64A/Sonora64//Tacuari (II21183)/4/(CO652363) Warrior//Kenya58/Newthatch/2*(Cheyenne/Tenmarq/Mediterranean/Hope)/3/Parker/5/Lancer/4/KS62136	CItr 17441	1976	Colorado
Yuma	NS14/NS25//2*Vona	PI 559720	1991	Colorado
Yumar	Yuma/PI372129,F1//CO850034/3/4*Yuma	PI 605388	1997	Colorado

† Exp=experimental line

Appendix 4. Entries used in Chapter 3, greenhouse Study II with their pedigrees, accession numbers, year of release and origin.

Entry Name	pedigree	Accession Number	Release year	Origin
Ogallala	TX81V6187/Abilene	PI 573037	1993	AgriPro
Byrd	TAM 112/CO970547-7	PI 664257	2011	Colorado
Hatcher	Yuma/PI372129//Tam-200/3/4*Yuma/4/KS91H184/Vista	PI 638512	2004	Colorado
Ripper	CO940606/TAM107R-2	PI 644222	2006	Colorado
Vona	II-21183/3/CO-652363//LANCER/KS-62136	CItr 17441	1976	Colorado
Comanche	Oro/Tenmarq	CItr 11673	1942	Kansas
Parker	Quivira/3/Kanred/HardFederation//Prelude/Kanred/4/Kawvale/Marquillo//Kawvale/Tenmarq	CItr 13285	1966	Kansas
Parker 76	Parker*5/Agent	CItr 17685	1976	Kansas
Wichita	Early Blackhull/Tenmarq	CItr 11952	1944	Kansas
Judee	Vanguard/Norstar//Judith/3/NuHorizon	PI 665227	2011	Montana
MT06103	Composite cross		Exp†	Montana
MT85200	Froid/Winoka/3/TX55-391-56-D8/Westmont//Trader		Exp	Montana
Nusky	NuWest/Tiber	PI 619167	2001	Montana
Arapahoe	Brule/3/Parker*4/Agent//Belocerkovskaja198/Lancer	PI 518591	1988	Nebraska
Centura	Warrior*5/Agent/NE68457/3/Centurk78	PI 476974	1983	Nebraska
Cheyenne	CI8885	CItr 8885	1933	Nebraska
Darrell	2076-W12-11/Karl92	PI 644224	2006	Nebraska
Overland	758Millennium ‘S’/ND8974Nebraska	PI 647959	2007	Nebraska
OK05204	SWM866442/OK95548		Exp	Oklahoma
OK05526	KS94U275/OK94P549	PI 661991	2012	Oklahoma

OK06114	KS97P0630-4-5/CM95560//X920879-C15-1/3/X84WO63-9-18/U1324-25-1-4		Exp	Oklahoma
OK101	OK87W663/Mesa//2180	PI 631493	2001	Oklahoma
Harding	Brule//Bennett/Chisholm/3/Arapahoe	PI 608049	2000	South Dakota
SD01237	Not available		Exp	South Dakota
Wendy	SD89333/Abilene	PI 638521	2006	South Dakota
TAM111	TAM-107//TX78V3630/CTK78/3/TX87V1233	PI 631352	2002	Texas
TX04V075080	JAGGER/TX93V5722//TX95D8905		Exp	Texas
TX05A001822	2145/X940786-6-7		Exp	Texas
TX07A001279	X930332-4-1/TX97V2838		Exp	Texas
Kharkof	Not available	PI 5641	1900	Ukraine

† Exp=experimental line.

Appendix 5. Least squares means for root traits in Chapter 3, greenhouse Study I (Colorado winter wheat entries) under drought stress.

Entry	TL†	TLTS	RAT	TLMS	RAM	TLBS	RAB	AD	ADTS	ADMS	ADBS
	cm	cm	%	cm	%	cm	%	mm	mm	mm	mm
Above	6766	1869	28	2172	32	2735	40	0.36	0.40	0.34	0.34
Akron	6133	2107	34	2085	34	2016	33	0.37	0.40	0.36	0.37
Avalanche	6197	2391	39	1753	28	2081	34	0.37	0.39	0.33	0.38
Bill Brown	7284	2378	33	2137	29	2764	38	0.37	0.41	0.34	0.35
Bond CL	6000	1681	28	1739	29	2593	43	0.36	0.42	0.34	0.34
Byrd	6782	2024	30	2267	33	2481	37	0.34	0.44	0.32	0.30
Carson	5279	1666	32	1777	34	1821	34	0.38	0.46	0.35	0.34
CO04393	6158	1786	29	1777	29	2571	42	0.35	0.43	0.32	0.32
CO04499	6041	2123	35	1752	29	2153	36	0.35	0.38	0.33	0.34
CO04W320	6318	2127	34	1863	29	2322	37	0.38	0.38	0.36	0.39
CO940610	5785	1733	30	1668	29	2414	42	0.36	0.43	0.32	0.35
Denali	6189	1770	29	1995	32	2382	38	0.37	0.43	0.35	0.34
Duke	5445	1778	33	1720	32	1897	35	0.38	0.45	0.35	0.34
Hail	5858	1771	30	1830	31	2246	38	0.36	0.42	0.34	0.32
Halt	6436	1595	25	1998	31	2823	44	0.35	0.38	0.33	0.34
Hatcher	6155	2098	34	1993	32	2278	37	0.36	0.44	0.34	0.33
Jules	5580	1523	27	1674	30	2368	42	0.36	0.41	0.32	0.34
Lamar	5568	2013	36	1827	33	1717	31	0.37	0.42	0.35	0.34
Lindon	6505	1957	30	1948	30	2582	40	0.37	0.43	0.34	0.34
Platte	5882	1780	30	2032	35	2101	36	0.40	0.47	0.37	0.37
Prairie Red	6015	1676	28	1873	31	2468	41	0.33	0.40	0.30	0.31
Prowers	5959	2190	37	1843	31	1939	33	0.39	0.43	0.36	0.37
Ripper	5978	1981	33	1952	33	2075	35	0.39	0.46	0.38	0.34
RonL	6273	2534	40	1820	29	1924	31	0.35	0.39	0.34	0.34
Sandy	5488	1465	27	1895	35	2130	39	0.39	0.47	0.39	0.34
Snowmass	5239	1333	25	1858	35	2021	39	0.36	0.44	0.35	0.30
Thunder CL	6324	1703	27	1829	29	2824	45	0.37	0.43	0.33	0.36
Vona	6031	1633	27	2109	35	2325	39	0.39	0.44	0.38	0.35

Yuma	6033	1796	30	1980	33	2246	37	0.36	0.44	0.34	0.31
Yumar	6427	1860	29	2251	35	2615	41	0.36	0.40	0.35	0.34
Mean	6071	1878	31	1914	32	2297	38	0.37	0.42	0.34	0.34

† TL, total length; TLTS, total length for top section; RAT, root allocation in the top section; TLMS, total length for middle section; RAM, root allocation in the middle section; TLBS, total length for bottom section; RAB, roto allocation in the bottom section; AD, average diameter; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section.

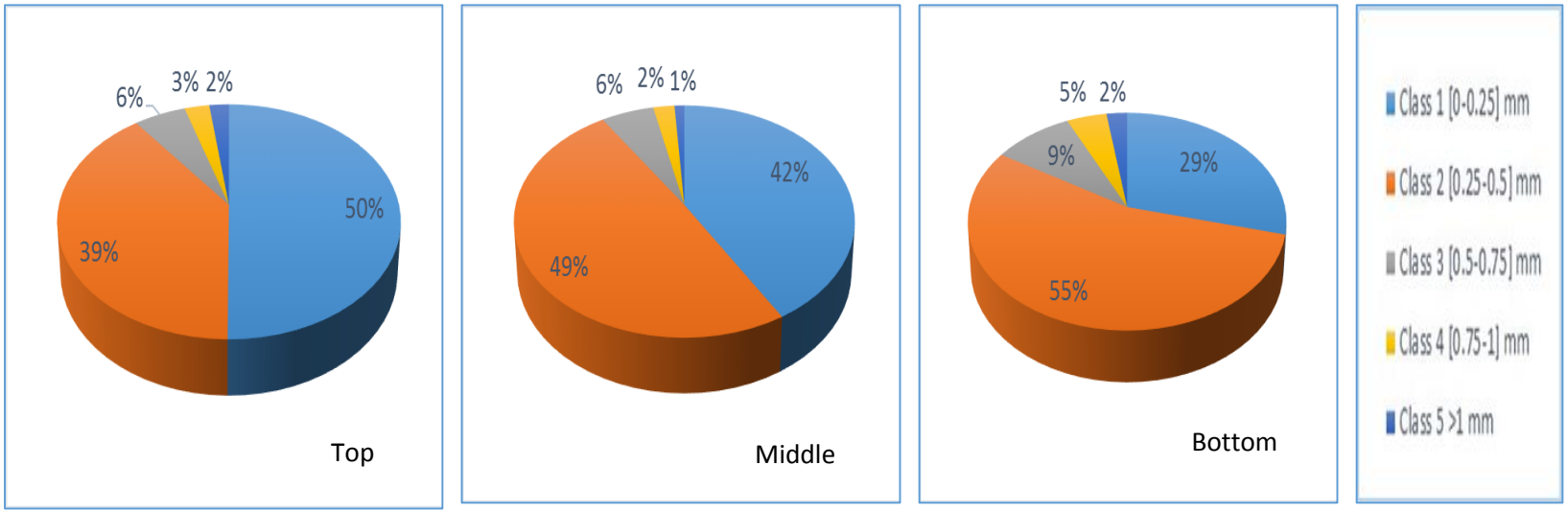
Appendix 6. Least squares means for root traits in Chapter 3, greenhouse Study II (Great Plains winter wheat entries) under drought stress.

Entry	TL†	TLTS	RAT	TLMS	RAM	TLBS	RAB	AD	ADTS	ADMS	ADBS
	cm	cm	%	cm	%	cm	%	mm	mm	mm	mm
Arapahoe	9246	2436	26	2998	32	3824	41	0.57	0.62	0.55	0.55
Byrd	10639	3051	29	3678	35	3882	36	0.53	0.49	0.54	0.55
Centura	9799	2683	27	3223	33	3865	39	0.55	0.54	0.55	0.56
Cheyenne	9394	2461	26	3281	35	3686	39	0.58	0.55	0.62	0.57
Comanche	8691	2263	26	2765	32	3670	42	0.56	0.50	0.62	0.54
Darrell	9382	2831	30	3196	34	3301	35	0.57	0.57	0.55	0.60
Hatcher	10448	3369	32	3365	32	4044	39	0.59	0.57	0.56	0.63
Harding	9324	2735	29	3345	36	3291	35	0.55	0.59	0.54	0.52
Judee	9303	2513	27	3111	33	3686	40	0.61	0.62	0.62	0.60
MT06103	9319	2408	26	3087	33	3938	42	0.56	0.57	0.55	0.57
MT85200	9345	2620	28	3277	35	4143	44	0.59	0.56	0.62	0.57
Nusky	9962	2872	29	3381	34	3734	37	0.54	0.53	0.52	0.56
OK05204	9628	2679	28	3255	34	3678	38	0.56	0.55	0.54	0.58
OK05526	9636	2748	29	3236	34	3667	38	0.55	0.56	0.54	0.56
OK06114	8502	2336	27	2802	33	3346	39	0.57	0.58	0.57	0.58
OK101	9675	2717	28	3142	32	3805	39	0.55	0.56	0.53	0.56
Ogallala	9377	2810	30	3233	34	3590	38	0.52	0.48	0.50	0.58
Overland	9424	2702	29	2980	32	3719	39	0.60	0.60	0.59	0.60
Parker	9764	2975	30	3389	35	3782	39	0.59	0.59	0.58	0.60
Parker 76	8502	2319	27	2972	35	3350	39	0.60	0.59	0.62	0.61
Ripper	9802	2692	27	3111	32	3997	41	0.55	0.56	0.53	0.57
SD01237	10993	3032	28	3407	31	4556	41	0.55	0.52	0.57	0.56

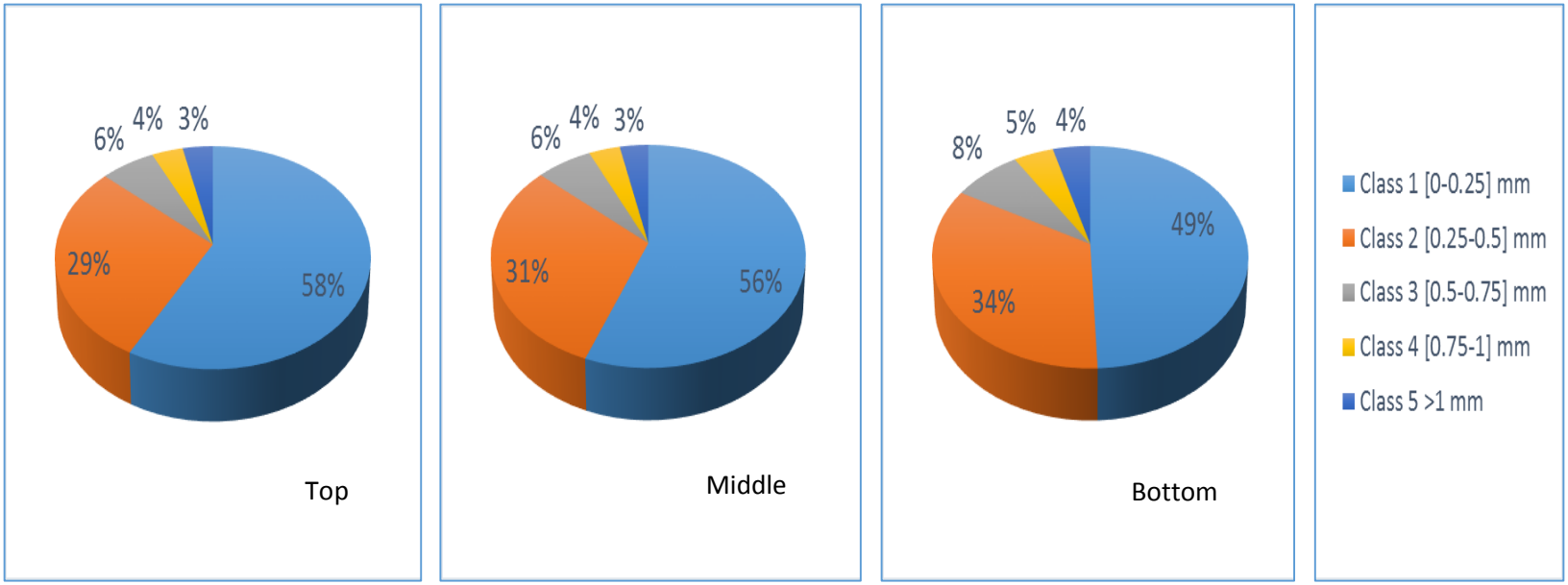
TAM 111	10045	2690	27	3303	33	4008	40	0.58	0.58	0.63	0.54
TX04V075	9703	2729	28	3217	33	3785	39	0.57	0.64	0.54	0.53
TX05A001	10312	2753	27	3474	34	4064	39	0.55	0.57	0.50	0.59
TX07A001	11119	3372	30	3522	32	4269	38	0.52	0.49	0.55	0.52
Vona	9343	2710	29	3485	37	3161	34	0.64	0.63	0.64	0.67
Wendy	8654	2357	27	3267	38	3535	41	0.58	0.53	0.59	0.60
Kharkof	8602	2529	29	2834	33	3224	37	0.54	0.46	0.57	0.56
Wichita	9246	2436	26	2998	32	3824	41	0.55	0.51	0.53	0.61
Mean	9573	2694	28	3211	34	3747	39	0.57	0.56	0.56	0.57

† TL, total length; TLTS, total length for top section; RAT, root allocation in the top section; TLMS, total length for middle section; RAM, root allocation in the middle section; TLBS, total length for bottom section; RAB, roto allocation in the bottom section; AD, average diameter; ADTS, average diameter for top section; ADMS, average diameter for middle section; ADBS, average diameter for bottom section.

Appendix 7. Total root length percentage per diameter class for the three root sections: Top section, Middle section and Bottom section in Chapter 3, Study I (Colorado entries) under drought stress.



Appendix 8. Total root length percentage per diameter class for the three root sections: Top section, Middle section and Bottom section in Chapter 3, Study II (Great Plains entries) under drought stress.



LIST OF ABBREVIATIONS

Abbreviation	Description
ABM	Above Ground Biomass
AD	Average root diameter
ADBS	Average diameter for bottom root section
ADMS	Average diameter for middle root section
ADTS	Average diameter for top root section
AM	Association mapping
BLUPs	Best Linear Unbiased Prediction
BRBM	Bottom root biomass
T_c	Canopy temperature
CID	Carbon isotope discrimination
DSI	Drought susceptibility index calculated based on grain yield
ETr	Estimated transpiration
GAPIT	Genomic Association and Prediction Integrated Tool
GBW	Grain biomass weight in the 1 m sampled section
g_s	Stomatal conductance
GY	Grain yield per six-rows plot
QTL	Quantitative trait loci
GWAS	Genome-wide association study
HI	Harvest index
LER	Leaf elongation rate
LD	Linkage disequilibrium
LR	Longest root depth
MRB	Middle root biomass

PCA	Principle components analysis
Ht	Plant height
RWC	Relative water content % of leaves
SNP	Single nucleotide polymorphism
TBM	Total biomass weight
TL	Total root length
TLBS	Total length for bottom root section
TLMS	Total length for middle root section
TLTS	Total length for top root section
TotRBM	Total root biomass
TRBM	Top root biomass
WS	Water-stressed
WW	Well-watered