

DISSERTATION

EXPLOITING DROUGHT TOLERANCE TRAITS AND GENETIC DIVERSITY OF
SYNTHETIC HEXAPLOID WHEAT IN WINTER WHEAT BREEDING

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ABSTRACT

EXPLOITING DROUGHT TOLERANCE TRAITS AND GENETIC DIVERSITY OF SYNTHETIC HEXAPLOID WHEAT IN WINTER WHEAT BREEDING

As the world's most widely dispersed food crop, wheat (*Triticum* spp.) is produced on approximately 22% of currently cultivated land. This wide distribution of environments and the large demand for consumption requires the adaptability of wheat to various environmental stresses. Therefore, this study was conducted to assess the benefits of introgressing synthetic hexaploid wheat (SHW) diversity into winter wheat in the U.S. Great Plains. The objectives of this study were to: 1) identify differences in drought tolerance-related physiological and morphological trait expression among SHW lines and adapted winter wheat cultivars; 2) evaluate and select SHW-derived backcross lines from multi-environment field studies for potential use as breeding germplasm; and 3) locate genomic regions of synthetic origin that show promise for yield improvements through targeted introgression into elite bread wheat backgrounds.

The instability of crop yields is predicted to increase due to climate change and environmental stresses, with drought considered the abiotic stress that is most associated with a loss of yield in cereal crops. The improvement of yield and drought stress tolerance in bread wheat (*Triticum aestivum* L.) through the introgression of novel alleles from ancestral species may be feasible. Synthetic hexaploid wheat, which incorporates the AABB genome from Durum wheat and the DD genome from the progenitor *Aegilops tauschii*, has shown promise for improving bread wheat yield and agronomic performance. In this study a group of six SHW lines were selected from a collection of 412 lines developed by the International Maize and Wheat Improvement Center (CIMMYT, El Batan, Mexico). A subset of SHW lines was selected

after two years of testing for visual agronomic traits and yield in Colorado. The six SHW lines were analyzed in greenhouse conditions for differences in drought tolerance traits relative to four adapted cultivars. This experiment focused on root morphology traits of these genotypes grown in one meter tall plastic tubes. Some synthetic lines ranked better than the adapted check varieties under greenhouse conditions for drought tolerance traits such as deep root biomass, longest root, and stomatal density. The plasticity of total root biomass and root biomass in the deepest third of the tubes was also found to be significantly correlated ($P < 0.05$, $r = 0.72$ and 0.70 , respectively) with a calculated drought index. Digital analysis of root length in five root diameter classes indicated a significant ($P < 0.05$) increase of small diameter root growth under drought stress for drought tolerant genotypes compared to drought sensitive genotypes.

The development and testing of 10 SHW-derived BC₁F_{1,2} populations (15 lines per population), with ‘Hatcher’ and ‘Goodstreak’ as the recurrent parents, identified six out of twelve SHW populations that showed the highest overall yield in eight Colorado and Nebraska environments in 2009-2010 and 2010-2011. Single plant selections were made from the highest yielding SHW-derived lines within each selected population and planted as headrows in 2011-2012. The top 10% of the headrows was selected based on visual assessment of yield potential and agronomic attributes such as early maturity date and lodging and disease resistance. Advanced yield testing of the selected fraction was then conducted in 2012-2013. Synthetic-derived lines with Hatcher as a recurrent parent showed a wide variety of yield under both irrigated and rainfed conditions in the summer of 2013. Two SHW-derived lines outranked Hatcher for grain yield in all three environments in 2013 with two additional lines ranking higher than all adapted check cultivars in both irrigated and rainfed environments. Of several agronomic traits evaluated, harvest index and canopy temperature showed the highest

correlations with grain yield under drought ($r = 0.55$ and -0.53 , respectively, $P < 0.05$). Canopy temperature was also assessed every other hour (0900 to 1730 h) for a single day during the grain fill period. This analysis identified high yielding SHW-derived lines showing contrasting responses (both continuous and variable period of low canopy temperature) relative to Hatcher under drought. Genotyping-by-sequencing data for the SHW-derived lines identified 13 single nucleotide polymorphisms which showed a preferential selection for the allele of synthetic origin after three selection events for improved grain yield. Our results demonstrate improvements in yield and agronomic performance in SHW-derived lines. Therefore we conclude that the use of SHW has good potential for introgression of diversity into elite winter bread wheat in the U.S. Great Plains.

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CHAPTER ONE:

LITERATURE REVIEW

Introduction

Wheat (*Triticum* spp.), is estimated to be part of a daily human diet for approximately 75% of the world's population and reaches an annual per person consumption of 300 kg in parts of northern Africa (Dixon et al., 2009). As the world's most dispersed food crop it is produced on 22% of currently cultivated land (Leff et al., 2004). Over 240 million hectares are dedicated to wheat crops yielding upwards of 0.6 billion tons of grain worldwide (Dixon et al., 2009). This wide distribution of environments and large demand for consumption requires the adaptability of wheat to various environmental stresses. Drought is the most yield limiting factor for cereals in many years and environments throughout the world (Araus et al., 2002). Its severity is determined by amount, intensity, and timing of precipitation. Increasing the tolerance of wheat to abiotic stresses such as drought is therefore essential for future global food security.

Origin of bread wheat (*Triticum aestivum* L.)

The origin of modern bread wheat (*Triticum aestivum* L.), an allohexaploid, has been traced to a hybridization between its ancestors some 10,000 years ago in the Fertile Crescent region of western Asia (Feldman, 2001). A natural cross between two wild grass species, *T. urartu* (genome AA, $2n=2x=14$) and 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, $2n=4x=28$) (Feldman, 2001). Selection of wild emmer for its larger heads resulted in its eventual domestication (*T. diccicum*) 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, 1944, 1946).

These wild grasses have contributed to the diversity of modern day durum and bread wheat through the presence of multiple allele combinations as seen in tetraploid and hexaploid plant species. However, the derivation of each of these wheat types began from a very rare, natural combination of genomes. The small number of progenitors that were involved in producing the hybrid plants represents a very narrow portion of the diversity that could have been present within each progenitor species (Feldman, 2001; Tanksley and McCouch, 1997). The founder effect associated with these events established a narrow genetic base limiting the amount of genetic variability found in the initial populations. This genetic bottleneck was further reduced as humans began to select for and against particular traits which contributed to the improvement of yield and adaptation to agricultural production. Initially this selection began through the indirect selection of free-threshing forms versus hulled varieties. A more active selection process eventually began to take place for uniformity, germination vigor, indehiscence, and of course yield and taste (Heiser, 1988; van Ginkel and Ogbonnaya, 2007). Notably, bread wheat has no wild forms in existence today (Kihara, 1944). However, those species involved in the original hybridization events still exist in either native or cultivated form.

Harnessing the diversity of bread wheat progenitors

The development of techniques for capturing wild diversity in wheat first began in 1944-1946 with independent researchers investigating the original progenitors of durum and bread

wheat (Kihara, 1944; McFadden and Sears, 1944, 1946). This began by crossing various grasses having the A, B and D genomes, which unintentionally introduced variation from a wide genetic basis into hexaploid progeny. Lines produced from these wild species opened the door for the introgression of traits that would prove useful in combating both biotic and abiotic stresses such as disease and insect pressure, drought, heat and salinity stress (van Ginkel and Ogonnaya, 2007). However, the potential use of these newly synthesized tetraploid and hexaploid lines for improving any lack of genetic diversity in cultivated wheat was not recognized until the 1980's (Gill et al., 1985).

Several avenues exist for the use of wild genetic resources for improving yield and agronomic performance of bread wheat. Direct-crossing involves making crosses between wheat and a progenitor or relative species of a different ploidy level for introgression of genetic diversity (Cox et al., 1990; Ogonnaya et al., 2013). This allows for capture of genetic diversity, on the D genome of wheat from *Ae. tauschii*, for example, while reducing the potential of linkage drag associated with the introduction of unadapted diversity from multiple genomes. Cox et al. (1995) reported a 96% recovery of the recurrent parent after two backcrosses using this technique. For parents that are both hexaploid this percentage of recovery would require four backcross generations. However, the ability of conducting the necessary procedures for embryo rescue of the aneuploid F₁ progeny and the instability of the aneuploid nature make the use of this procedure more challenging (Ogonnaya et al., 2013).

The development of synthetic hexaploid wheat (SHW) (*X Aegilotriticum* spp., genome AABBDD, 2n=6x=42) involves the use of crosses among species of different ploidy levels as well. It typically consists of crossing tetraploid durum varieties, with accessions of the diploid species *Ae. tauschii*, genome DD (Figure 1.1) (Ogonnaya et al., 2013).

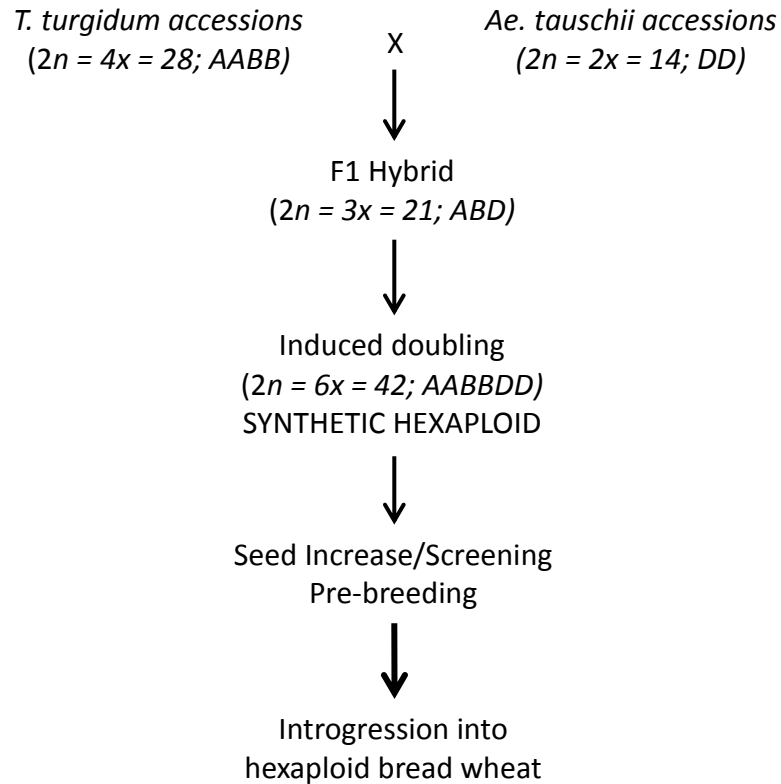


Figure 1.1 Development of synthetic hexaploid wheat by crossing durum wheat accessions with *Ae. tauschii* accessions.

After pollination, embryo rescue must take place to form plants of the genome composition ABD. Chromosome doubling with colchicine then occurs and the result of this cross is the synthetic version of hexaploid wheat (AABBDD) that can be directly crossed with hexaploid germplasm (Mujeeb-Kazi et al., 2008). Both parents in the production of SHW are sources of potentially novel genetic diversity that are combined into a single source. That single source acts as a bridge for crossing between the progenitor species of lower ploidy level and the hexaploid wheat that is targeted for improvement, producing genetically stable F₁ progeny without the need for further backcrossing (Mujeeb-Kazi et al., 2008). Also, the majority of the parental material used to develop SHW lines originates from areas prone to various levels of

biotic and abiotic stresses which promotes adaptive traits that can be targeted for introgression (Valkoun, 2001).

Both of these procedures (direct-crossing and SHW) have benefits and detriments to their use in breeding for improved bread wheat yield (Talbot, 2011). Cox (1997) explained his decision to use direct crossing in his research on winter wheat rather than SHW bridge crossing. Several reasons were given centering on the introgression of diversity from multiple genomes in SHW techniques. He stated that including the A and B genomes from spring-type durum wheat in SHW works well for crossing with spring-type bread wheat. However, the durum wheat accessions used to make SHW lines are not adapted to winter-type wheat production areas leading to the increased need for pre-breeding selection. On the other hand, the ability to select the most promising SHW lines from pre-established SHW collections and cross them into elite bread wheat lines allows for development of breeder-friendly germplasm stock (Ogbonnaya et al., 2013). The development of new germplasm via SHW-derived backcrossed lines with elite cultivars as the recurrent parent allows for the capture of novel SHW genes and SHW-by-elite gene interactions from multiple genomes simultaneously. Although the increased time associated with the backcross pre-breeding efforts may be considered a hindrance, the use of SHW derived from cultivated durum accessions may only require a single backcross event (Ogbonnaya et al., 2013; Wang et al., 2009).

The development of SHW collections on a large scale was established by the International Maize and Wheat Improvement Center (CIMMYT, El Batan, Mexico) (Mujeeb-Kazi et al., 1996) and the use of genetic variation from synthetic winter wheat in breeding programs was initiated by Cox et al. (1995). Crosses created from a wide variety of diploid and tetraploid native species began to re-introduce the genetic variability from each of the different

wild accessions that were not present in the natural creation of wheat (Reynolds et al., 2007; van Ginkel and Ogbonnaya, 2007). Cox et al. (1995) suggested that introgression of wild genetic diversity could be used to develop backcross derived lines that were agronomically equivalent or superior to their recurrent parent. Over 1000 spring habit SHW lines and 200 winter habit SHW lines have been produced by CIMMYT since the early 1990's. Also approximately one-third of the advanced wheat breeding lines that are produced by CIMMYT are developed with the use of SHW (van Ginkel and Ogbonnaya, 2007). Despite increases in genetic diversity and agronomic performance, breeders have been reluctant to use synthetic derived germplasm because of an expectation of linkage drag from deleterious alleles that may be present in the wild genomes (Mujeeb-Kazi et al., 2008).

Challenges of breeding for drought tolerance

Throughout North America, breeding goals for wheat and nearly all crops include maximizing yield potential in any given environment. Because the farming culture in the United States is that of achieving the highest monetary return, the end goal for breeders is increasing productivity under both good and poor growing conditions. Research suggests that selecting for maximum yield potential in an ideal environment will also increase the productivity of elite lines under drought scenarios (Trethowan et al., 2002). However, 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, p. 61). This negative relationship between yield and stress tolerance however, may only hold true for more extreme drought conditions. A general rule of thumb for most cereal crops was quoted by Blum (2011, p. 62) that "breeding for high yield potential will most likely help sustain yields under conditions where mean yield is not reduced more than about 50% of the potential." Alternatively, selection

for improved drought tolerance directly in water stressed environments shows a greater increase in yield gain when the varieties are targeted for consistently drought stressed environments (Trethowan et al., 2002).

A selection technique which may be beneficial for improving both maximum yield potential and drought tolerance is alternating the selection environment between stressed and ideal conditions (Trethowan et al., 2001). Therefore, selection for elite lines should involve the improvement of certain constitutive traits, depending on the population of target environments, 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, p. 128-129). Thus, utilizing certain constitutive traits as secondary selection criteria, or in the form of a selection index, may allow for maintenance of high yield potential while increasing the rate of gain for yield in drought prone areas.

Managing testing locations and protocols for imposing drought stress is often a limiting factor in measuring and quantifying drought stress. Phenotyping particular traits of interest often requires a high level of control over the testing conditions and the ability to relate the trait responses to differences seen in the growing environment. Blum (2011, p. 154) creates an image that represents the drought stress of a plant as being three-dimensional. He describes these dimensions as the growth stage at which the stress occurs; the rate of water deficit development; and the peak water deficit that is endured. Identifying how each of these relates to the population of target environments is critical for designing a drought stress study. Passioura (2007) stated “Research that aims to improve water-limited yields on farms must ultimately make sense on farms.” Often research may be carried out in growing conditions that are quite extreme compared to the typical conditions the crop would find in its adapted environment (Hargreaves et

al., 2009; Wojciechowski et al., 2009). Though testing in extreme conditions may be quite informative it may not relate well to the conditions typical for the three dimensions mentioned above. The challenge is to relate the work that is being done outside of the field setting to the challenges that are being faced on the farm.

Strategies for breeding with synthetic hexaploid wheat

The use of SHW wheat in crosses with elite cultivars that are well adapted to their growing environment can be very challenging due to the wild and unadapted genes that are being unintentionally introgressed into the new germplasm along with potentially favorable alleles (Huang et al., 2004). This linkage drag also disrupts many of the genetic interactions and favorable linkage blocks that were previously found in the elite haplotype of adapted cultivars. However, when breeding with SHW, backcross and selection techniques can be used to minimize the amount and size of unwanted SHW chromosomal regions through increased recombination between elite and synthetic genes to reduce linkage drag (Hospital, 2001; Valkoun, 2001).

Beginning the trait introgression process with pre-breeding techniques through early selection of the SHW lines *per se* may be beneficial to reducing the amount of initial crossing procedures and the number of lines that require testing in field plots. The choice of a subset of SHW lines or wild wheat relatives for use in crossing may be guided by information from genebanks such as the International Center for Agriculture Research in Dry Areas (ICARDA, Beirut, Lebanon) Germplasm Program (Valkoun, 2001). Information about the accessions held at these genebanks may be available for site ecology, biotic and abiotic stress tolerance, and to

some extent physiological and molecular data (Skovmand et al., 2001; Tanksley and McCouch, 1997; Valkoun, 2001).

Another option for identifying the most promising donors of beneficial diversity is the pre-breeding strategy of testing the SHW lines *per se* for certain phenotypic traits. This screening procedure should take place in a field trial which represents the population of target environments in order to assess the SHW adaptability (Reynolds and Trethowan, 2007). Though the overall productivity of these SHW lines will more than likely be low compared to that of the local check varieties, selection of donor lines for traits such as low canopy temperature or higher carbon isotope discrimination may be possible (Reynolds and Trethowan, 2007). This pre-breeding step will also allow for the selection of lines such as threshability, spike architecture, and general bread wheat-like appearance prior to production of the initial F₁ crosses. This selection should take place with the expectation that the predicted phenotypic outcome of quantitative traits in the F₁ or BC progeny may not resemble the phenotype of the parental lines (Tanksley and McCouch, 1997). However, due to the often limited resources available to plant breeders the visual exclusion of many SHW lines based on the presence of detrimental traits such as lodging, may be necessary to improve the potential for success from a limited number of crosses. Backcrosses from a reduced number of SHW lines can then be selected on a single plant basis for superior phenotypic attributes such as spike productivity and short plant stature as done by Valkoun et al. (2001).

Backcrossing procedures have been utilized for introgression of novel SHW genes into bread wheat with various numbers of backcross iterations. Trethowan and Mujeeb-Kazi (2008) suggested that at least one backcross event is required with an adapted recurrent parent to overcome the detrimental effects often found when crossing with unadapted germplasm. Spring-

type SHW lines have been shown to improve yield potential and adaptation to drought after backcrossing to the BC₂ generation (del Blanco et al., 2000; Kunert et al., 2007; Ogbonnaya et al., 2007; Trethowan and Mujeeb-Kazi, 2008), and BC₄ generation (Liu et al., 2006b). Three-way crosses of SHW with two different elite spring-type cultivars have also shown positive results (Mohammad et al., 2010; Trethowan and Mujeeb-Kazi, 2008). However, few studies have shown the beneficial use of crossing spring-type SHW with elite winter-type wheat cultivars in a single backcross (Cooper et al., 2012, 2013; Narasimhamoorthy et al., 2006).

The combination of backcrossing techniques with the use of marker-assisted selection may also contribute to improved efficiency in the capture of SHW germplasm. However, the use of markers usually involves the introgression of only a small, predetermined genomic target into the unaltered recurrent parent background. Capturing only this small genomic segment while eliminating the unwanted SHW regions may require over six generations of backcrossing (Talbot, 2011).

Contributions of synthetic wheat to increased genetic diversity

The use of SHW for introgression of diversity into elite germplasm backgrounds or breeding populations has proven to be a very successful in previous research (Trethowan and Mujeeb-Kazi, 2008; van Ginkel and Ogbonnaya, 2007). Molecular analysis of synthetic wheat lines and their synthetic-derived backcross lines has identified improvements in genetic diversity over modern cultivars (Das et al., 2007; Dreisigacker et al., 2008). However, whether or not there is a selective advantage in wild and alien genetic resources, such as in SHW, continues to be an issue impacting the utilization of this genetic resource.

In a research study by Zhang et al. (2005) the authors utilized simple sequence repeats to demonstrate that changes in SHW allele frequency occurred, following several generations of selection in the CIMMYT breeding program, in genomic regions from the D genome of *Ae. tauschii* accessions, as well as the A and B genome of durum wheat. Successful use of SHW can be seen in an analysis of varieties tested by CIMMYT in the Semi-Arid Wheat Yield Trial (SAWYT), a large, diverse study of approximately 50 bread wheat lines which are tested across environments in 30 countries (Lage and Trethowan, 2008). In this study the first appearance of SHW-derived lines took place in 1995 in the 5th SAWYT with only 8% of the lines having SHW in their pedigree. This number increased dramatically to 46% 10 years later (Lage and Trethowan, 2008). Also noteworthy in this analysis was the increase in average rank of SHW-derived lines across all locations from 30th (out of 50) in the 5th SAWYT to 25th by the 12th SAWYT. However, a reduction in the coefficient of parentage associated with these lines indicates that the percentage of SHW genome is small, suggesting that backcross development of lines is able to reduce deleterious SHW genes. Van Ginkel and Ogbannya (2007) reported on the changing genotypic diversity found in the lines produced by CIMMYT over the course of its history. Beginning with the level of genotypic diversity that is found in the landrace varieties a sharp reduction in diversity is seen between the 1950's to the 1980's. Following the introduction and use of synthetic wheat the diversity begins to climb beginning with the 1990's and into the present era, returning to nearly the diversity index level of the original landraces (van Ginkel and Ogbonnaya, 2007).

Efficient utilization of the genetic diversity found within SHW would be enhanced through the use of marker assisted backcrossing in order to target genes of interest that originate in the synthetic material. This would, however, require information relating to specific markers

that show a high contribution to improvements in yield or agronomic traits of interest. Mapping populations that have been developed through the use of SHW lines have identified QTL from synthetic wheat that have shown beneficial increases in traits such as kernel weight, kernels m^{-2} (Talbot, 2011), kernel number per spike, grain hardness, and tiller number (Narasimhamoorthy et al., 2006), thousand kernel weight (Huang et al., 2004), spike number, plant height, maturity date, spike length (Liu et al., 2006a), protein content and quality traits (Kunert et al., 2007). Of these traits, kernel weight is often cited as a key synthetic trait that contributes to increased yield in backcrosses with bread wheat (Cooper et al., 2012; Mohammad et al., 2010; Talbot, 2011). For this particular trait QTL of synthetic origin have been identified on chromosomes 1D, 2A, 3B, 4A, 5B, 6A, 7A, and 7D (Huang et al., 2004; Liu et al., 2006a). Also of interest when introgressing spring SHW into winter bread wheat is the vernalization requirement needed for crop production throughout the U.S. Great Plains. The major contributing genes to vernalization are the *Vrn-1* homoeologous genes that are located on chromosome 5L of each genome (Snape et al., 2001). The majority of SHW lines that have been produced have been spring-habit; thus, when crossing winter wheat with spring SHW retention of the recessive *Vrn-1* alleles is a necessity. Maintaining the vernalization genes necessary for winter-habit wheat may be a hindrance when crossing winter wheat with spring SHW due to the loss of potentially beneficial synthetic alleles in linkage disequilibrium with the alleles conferring spring habit (Hospital, 2001).

Contributions of synthetic wheat to increased agronomic productivity

Successful use of SHW for improved yield and resistance to abiotic stress has been shown in areas of spring wheat production (Cooper et al., 2012; Cooper et al., 2013; Dreccer et al., 2007; Inagaki et al., 2010; Lopes and Reynolds, 2011; Mohammad et al., 2010; Ogonnaya

et al., 2007). These improvements include many traits which contribute to yield such as large seed size, early maturity, number of tillers, high biomass, plasticity of root structure, and greater extraction of soil moisture. Average single grain weights for SHW lines have been up to 67 mg when tested by CIMMYT in field conditions in Mexico (Calderini and Reynolds, 2000). Field trials conducted in Mexico by Lopes and Reynolds (2011) indicated a 26% increase in average yield by the SHW-derived lines compared to their recurrent parents with no apparent reduction in yield under irrigated conditions. This study also showed a significant reduction in canopy temperature during the vegetative growth stage for the SHW-derived lines in contrast to the recurrent parents. The reduction in canopy temperature is often an indicator of increased soil moisture extraction capabilities due to increased soil exploration by roots (Lopes and Reynolds, 2010). Synthetic-derived wheat lines have been shown to produce less overall root biomass under drought yet demonstrated an increase in root mass at depth, enabling increased water extraction from deeper soil profiles (Reynolds et al., 2007). In similar studies, the yield of different populations of SHW-derived lines ranged from 32 to 130% and 73 to 118% of the highest yielding check variety according to Ogbannaya (2007) and Dreccer (2007), respectively. The low end of this range of yield results indicates the detrimental effects of linkage drag associated with the use of SHW; however, with proper experimental design and selection techniques beneficial transgressive segregants can also be found. Inagaki et al. (2010) showed an increased rank in grain yield over 10 rain-fed environments in Syria for a SHW-derived line relative to its recurrent spring wheat parent.

Baking quality and nutritional value are often important factors to take into consideration when breeding cereal crops such as wheat. Protein content is one of the quality traits in wheat that can have a significant impact on the end use quality of the product (Khan et al., 2000). It

has often been found that the protein content of wheat has an inverse relationship with grain yield as well as a low amount of genetic variation in breeding populations relative to environmental variation (Cox et al., 1995; Khan et al., 2000). However, diversity found within SHW lines has been shown to improve protein content along with several other important wheat quality traits such as falling number, sedimentation volume, and grain hardness (Kunert et al., 2007; van Ginkel and Ogbonnaya, 2007). For example, Lage et al. (2006) found a grain protein content of 15.5% in SHW-derived lines relative to 13.1% in bread wheat controls.

Research relating to the use of spring SHW for improvement of winter wheat germplasm, common in the U.S. Great Plains, is less prevalent. Cooper et al. (2012) reported variable success with SHW-derived crosses throughout portions of Texas. The authors attributed a reduction in yield of SHW-derived lines to the synthetic background which was unadapted to the cooler temperatures in the Plains region of Texas, similar to results seen in Australia (Dreccer et al., 2007). However, for one of the environments in the warmer South Texas region of their study the SHW-derived lines out-yielded the recurrent parents by 30.6% (Cooper et al., 2012).

Physiological traits beneficial for improving yield under drought

The identification of physiological traits which contribute improvements to drought stress tolerance has been the focus of many previous experiments (Praba et al., 2009; Rampino et al., 2006; Reynolds et al., 2007). Associating physiological traits with their contribution to yield for the target environment is key and has been shown to increase additive gene action and success of parental selection through trait-based strategic crosses (Mir et al., 2012; Reynolds and Trethowan, 2007). However, traits which are beneficial to improvements in yield depend on the population of target environments in which the crop will be grown, thus making it difficult to

generalize the benefit of a single trait in multiple regions of the world (Chenu et al., 2011). In order for a drought tolerance trait to be introgressed into a breeding population the potential negative impact of the trait in an ideal environment must be avoided or minimized.

Root morphology

To have a significant impact on improving drought tolerance traits must be plastic, adjusting to seasonal variation of specific environments without sacrificing yield. A primary trait of interest in drought tolerance research is root biomass, particularly deep roots, which may be beneficial to improving stability (Asseng et al., 1998; Lilley and Kirkegaard, 2011; Uga et al., 2013). The presence of reduced height (*Rht*) genes which are present in many varieties produced after the Green Revolution have been associated with a decrease in root growth in some studies. Historical wheat varieties and landraces have been shown to produce larger root systems relative to modern varieties by Waines and Ehdaie (2007). In that study, four landrace wheat lines produce a range of root biomass from 3.35 to 9.33 g, while the production of root biomass for 15 varieties produced after the Green Revolution ranged from 2.00 to 6.83 g. However, contradictory results have been seen for the effect of *Rht* genes on root growth depending on the experimental design and growth medium used (Wojciechowski et al., 2009). Due to the wild germplasm used in the creation of the SHW lines it could be hypothesized that SHW-derived varieties may produce an overall larger root system. However, Reynolds et al. (2007) demonstrated that an overall increase in root biomass does not indicate an increase in water uptake. These authors also indicated that SHW-derived lines, when grown in both well-watered and drought conditions, produced more root mass at depth yet reduced overall root biomass compared to check varieties. This response allowed for a reduced allocation of metabolites to

total root biomass production while increasing the efficacy of water uptake from greater soil depths.

The value of increasing roots at depth under drying soil can be seen also in the increased extraction of soil moisture by SHW-derived lines compared to their recurrent parents (Reynolds and Trethowan, 2007). As seen in research conducted by Ma et al. (2008), roots that had been trimmed to limit the total biomass of the root system showed improvements in shoot growth, water use efficiency, and a reduction in cost associated with greater root growth. Maximum root depth under drought has been shown to contribute to increased water extraction and often has a higher heritability (h^2) than most other root traits (Sayar et al., 2007). Maximum root depth is a constitutive trait which would be most beneficial in an environment where crops rely on moisture reserves in deeper soil profiles. Although the contribution of increased root dry weight at depths below 90 cm has been associated with lower canopy temperature and yield improvements under drought stress (Lopes and Reynolds, 2011), shallow root systems may also be beneficial for capturing rainfall that does not infiltrate to deeper soil profiles (Ehdaie et al., 2012). Crops grown under conditions of small, uniformly distributed rainfall events throughout the growing season would see a greater benefit from quicker water and nutrient capture from the upper soil profile (Ehdaie et al., 2012; Palta et al., 2011). Research in Australia showed that increasing the moisture extraction during the grain filling period by one mm may contribute to a 55 kg/ha increase in grain yield for rainfed wheat (Manschadi et al., 2006). Therefore, identifying a rooting structure that will lead to maximum extraction of soil moisture in the target environment will be beneficial to improvements in productivity under water stress.

Morphological root characteristics such as root length, root density, and root architecture are often difficult to assess. Technologies such as WinRhizo software (Regent Instruments Inc.,

Quebec, Canada), however, allow for detailed data that would otherwise be impossible to collect manually. The high-throughput nature of software programs such as WinRhizo and RootReader2D (Clark et al., 2013) make it possible to analyze the root morphology of more than 1000 plants per week (Clark et al., 2013).

Stomatal attributes

Stomatal conductance characteristics are responsible for regulating the amount of water lost via transpiration and maximizing the uptake of CO₂. The opening and closing of stomata in order to control the influx of CO₂ is regulated by environmental conditions such as light, CO₂ concentration, and atmospheric humidity (Blum, 2011, p. 21). Increasing stomate density was reported by Franks et al. (2009) to improve maximum potential conductance while Hetherington and Woodward (2003) stated that increasing aperture or size of the stomates will reduce conductance. Stomate density has been reported to be negatively correlated with aperture size, yet Wang and Clark (1993) showed that total stomatal pore area increased with increasing density in wheat genotypes. These authors also identified an increase in density under drought and no significant interaction between genotype and different environmental conditions. Substantial genotypic variation for stomatal attributes among bread wheat varieties was reported by Condon et al. (2007). They observed that leaf porosity, a surrogate for stomatal conductance, had a moderate h^2 , yet resulted in positive gains in yield when it was the target of retrospective selection. It has also been reported that smaller stomates are able to more rapidly adjust their turgor pressure to open and close more efficiently, thus minimizing water loss while maintaining adequate levels of CO₂ influx (Franks et al., 2009; Hetherington and Woodward, 2003).

Canopy temperature

Few practical, high-throughput measurements are able to indicate the water status of a large number of genotypes. Canopy temperature (CT) however, has been identified and used as a high-throughput screening technique in an applied breeding program (Blum, 2011, p. 188). Similar to crop yield, CT can be related to many aspects of plant water status. As leaf cooling is associated with the ability of the plant to transpire, increases in stress through lower water status will slow transpiration and increase CT. Many traits can contribute to the plant's water status such as soil moisture extraction efficiency, stomate attributes, leaf morphology, and others, therefore CT can act as an all encompassing measurement of the beneficial interactions of these traits. Olivares-Villegas et al. (2007) conducted trials on wheat over three years and various environmental conditions in Mexico to find that 'canopy temperature was the single-most drought-adaptive trait contributing to a higher performance, highly heritable and consistently associated with yield'. Throughout a variety of studies CT has been found to be strongly associated with several difficult to measure, water stress traits such as stomatal resistance (Jones et al., 2009), leaf water potential (Blum et al., 1982), leaf rolling (Garrity and O'Toole, 1995), soil moisture extraction (Olivares-Villegas et al., 2007), and carbon isotope discrimination (Zhu et al., 2008). Under drought Lopes and Reynolds (2011) identified a positive genetic correlation between vegetative CT and soil water content (SWC) in the 90 - 120 cm depth profile. These authors also reported phenotypic correlations of yield with vegetative CT and SWC at depth as -0.91 and 0.96, respectively. This indicates the importance of maintaining a low CT through more efficient soil moisture extraction and its relationship with higher yields under drought.

Drought avoidance

Earlier flowering and maturing genotypes may be able to increase yield in environments with a higher probability of terminal drought stress (Blum, 2011, p. 111). Genotypes containing traits which contribute to this situation promote drought escape rather than tolerance by completing their life cycle prior to the onset of drought stress. In terminal drought environments stress can be compounded by heat stress as well. There may also be drawbacks to selection for earliness in flowering and maturity. A shorter growth duration reduces the moisture demands of the plant through reduced evapotranspiration, however a loss in productivity may be seen due to the resulting reduction in carbon capture and growth duration in early maturing varieties (Lafitte and Courtois, 2002)

Of the traits associated with improvements in drought tolerance (root morphology, early maturity, stomatal conductance, canopy temperature, and others), very few have been incorporated into the selection procedures of a breeding programs with the possible exception of early maturity (Blum, 2011, p. 111; Palta et al., 2011; Richards and Passioura, 1989). The limited adoption of these traits by breeders in selection schemes is often due to the lack of validation in field conditions, difficulty of high-throughput screening, and difficulty in identifying patterns of h^2 .

Research objectives

Increasing genetic diversity may lead to long-term improvements in yield and stress tolerance. The exploitation of wild genetic resources for increased diversity has been accomplished in several different ways (Trethowan and Mujeeb-Kazi, 2008; van Ginkel and Ogbonnaya, 2007). This research focuses on the use of SHW lines previously derived by

crossing accessions of durum wheat with accessions of *Ae. tauschii* for the purpose of complementing the existing alleles and drought tolerance mechanisms found in local cultivars (Reynolds and Trethowan, 2007). The objectives of this study were to: 1) identify differences in drought tolerance-related physiological and morphological trait expression among SHW lines and adapted winter wheat cultivars; 2) evaluate and select SHW-derived backcross lines from multi-environment field studies for potential use as breeding germplasm; and 3) locate genomic regions of synthetic origin that show promise for yield improvements through targeted introgression into elite bread wheat backgrounds.

CHAPTER TWO:

PHYSIOLOGICAL AND MORPHOLOGICAL DROUGHT TOLERANCE TRAITS FROM SYNTHETIC HEXAPLOID WHEAT

Summary

Drought stress imposes major limits on bread wheat (*Triticum aestivum* L.) yield and is predicted to increase in frequency in many regions due to climate change. The aim of this study was to better understand the potential of synthetic hexaploid wheat (SHW) to improve productivity of adapted winter wheat cultivars under drought stress. Four winter wheat cultivars from the U.S. Great Plains and six SHW lines were evaluated in 1 m x 10 cm plastic tubes under drought and well-watered conditions in a greenhouse trial. We measured root morphology, stomatal attributes, biomass, plant water relations, and the response of these traits to drought stress. A strong correlation ($r = 0.72$, $P < 0.05$) was seen between root biomass plasticity at greater depths in response to moisture stress and the maintenance of plant water status. Root biomass in the bottom third of the tubes, length of longest root, stomatal conductance, and length of small diameter roots were all correlated ($P < 0.05$) with a calculated drought index. Synthetic line SYN-1 ranked highest for deep root biomass in both treatments, longest root under stress, and demonstrated plasticity of root biomass production from the upper third to the bottom third of the tubes when under water stress. Digital analysis indicated that SYN-1 and SYN-2 produced large amounts of small diameter roots at depth under both treatments. Synthetic SYN-5 showed high stomatal density and reduced stomatal aperture while maintaining leaf growth when stressed. Trait variability found within the SHW lines may contribute beneficial drought stress tolerance to Great Plains-adapted cultivars through introgression of novel allelic diversity.

Abbreviations: 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, length of longest seminal root; OP, osmotic potential; RWC, relative water content; LER, leaf elongation rate; g_s , adaxial (top) stomatal conductance; TSA, adaxial stomate aperture; BSA, abaxial (bottom) stomate aperture; TSD, adaxial stomate density; BSD, abaxial stomate density.

Introduction

Wheat (*Triticum* spp.) is consumed daily by more than 75% of the world's population. It is produced on over 240 million ha worldwide (Dixon et al., 2009), often under limited soil moisture conditions. Thus, increasing the tolerance of wheat to drought stress is essential for global food security.

The term drought represents a large diversity of conditions where soil moisture is limiting and reduces plant growth, reproduction, and yield (Blum, 2011, p. 2). Traits that are beneficial for drought tolerance are determined in large part by the population of target environments for production (Chenu et al., 2011). Ideally, the expression of drought tolerance traits will be plastic, allowing crop plants to adapt to seasonal variation of precipitation in moisture-limiting environments without sacrificing yield under more favorable moisture conditions. Drought tolerance can be conferred by reducing leaf water loss and/or increasing root water uptake.

A primary focus in drought tolerance research has been root morphology and an increase of root biomass, particularly of deep roots, which may be beneficial to improving yield stability. However, Reynolds et al. (2007) demonstrated that an overall increase in root biomass does not indicate an increase in water uptake. The authors reported that synthetic-derived wheat lines

produced less overall root biomass under drought, yet produced more root mass at depth, enabling more water extraction from deeper soil profiles. Maximum root depth has also been shown to contribute to improved water extraction and to have a higher heritability than most root traits (Ekanayake et al., 1985; Lopes and Reynolds, 2010; Sayar et al., 2007). Although the contribution of increased root dry weight at depths below 90 cm has been associated with cooler canopy temperatures and yield improvements under drought stress (Lopes and Reynolds, 2011), shallow root systems may also be beneficial for capturing rainfall that does not infiltrate to deeper soil profiles (Ehdaie et al., 2012). Research in Australia shows that for each mm of moisture absorbed throughout the grain filling period, a 55 kg/ha increase in grain yield occurred in rain fed environments (Manschadi et al., 2006). Therefore, identifying a rooting structure that will lead to maximum extraction of soil moisture at key growth stages in the target environment will be beneficial to improvements in productivity under water stress.

Leaf water loss through transpiration is driven by atmospheric demand and can be minimized by a reduction in stomatal conductance (g_s). Variation in stomatal conductance can be due to constitutive variation in stomatal size and/or density or to plastic responses in stomatal aperture. Franks et al. (2009) reported that an increase in stomatal density will increase maximum potential conductance (g_{wMax}), while an increase in stomatal size will reduce g_{wMax} (Hetherington and Woodward, 2003). Therefore, smaller stomates relates to greater stomatal control, as smaller guard cells are able to more rapidly adjust their turgor pressure to minimize water loss while maintaining adequate levels of CO_2 influx (Franks et al., 2009; Hetherington and Woodward, 2003).

A genetic bottleneck that has occurred due to the origin and subsequent domestication of bread wheat resulted in a limited pool of genetic and phenotypic diversity in modern bread

wheat. This reduction in diversity limits the potential for breeding for drought tolerance and other important traits such as root morphology, biomass and grain yield, and improved stomatal conductance (Gill et al., 1991). A possible solution to this reduction in variation is synthetic hexaploid wheat (SHW) (*X Aegilotriticum* spp.), which is produced by resynthesizing the cross between accessions of cultivated tetraploid wheat (*T. durum*) and accessions of *Aegilops tauschii* (Dreisigacker et al., 2008; Kihara, 1944; McFadden and Sears, 1944; Reynolds et al., 2007; van Ginkel and Ogonnaya, 2007). Subsequent crossing of SHW to domesticated hexaploid wheat has opened the door for the introduction of genotypic diversity and variation in traits needed to improve adaptation in elite breeding lines (Dreisigacker et al., 2008; Reynolds et al., 2007; Trethowan and Mujeeb-Kazi, 2008; van Ginkel and Ogonnaya, 2007). To date, the potential to increase the drought tolerance of winter wheat in the Great Plains region of North America through novel alleles present in SHW has not been fully explored.

The objectives of this research were to 1) compare selected SHW lines to locally adapted cultivars for traits that may improve drought tolerance in the U. S. Great Plains; and 2) to determine whether trait plasticity or constitutive expression of SHW drought tolerance traits is more important for improving productivity under ideal and drought stressed conditions.

Materials and methods

Germplasm

A population of 412 spring-habit SHW lines was grown under dryland conditions in Akron, CO in 2007 as a single replication of two-row plots, 75 cm long. These synthetic lines were developed by the International Maize and Wheat Improvement Center (CIMMYT, El Batan, Mexico) and kindly provided to us by Dr. Art Klatt, Oklahoma State University. Selection of a

subset of 45 lines was based on visual, agronomic traits including head type, tiller density, potential seed yield, well-filled seeds, and lodging resistance. This subset of 45 lines was again evaluated in 2008 for grain yield in a three-replication trial, resulting in the final selection of six high yielding synthetic lines for further testing. Entries (genotypes) in the following study included the subset of six spring-habit SHW lines and four locally adapted winter-habit cultivars (Table 2.1).

Experimental Design

Preliminary studies in 4 L pots were conducted on the entries listed in Table 2.1 in a greenhouse at Colorado State University in Dec. 2010. The experiment consisted of a well-watered treatment (saturated daily) and a drought stress treatment (imposed during the vegetative growth stage between approximate Zadoks stages 14 to 19 by reducing pot weights by three percent each day). These initial experiments did not indicate significant differences ($P < 0.05$) for photosynthetic capacity, transpiration, internal CO₂ concentration, or chlorophyll content (Appendix 1). However, significant genotypic differences ($P < 0.05$) for root biomass, above-ground biomass, stomate density, and stomatal conductance were found, and further research on these traits is presented here.

An initial root tube experiment was conducted in April, 2011 to develop experimental procedures and determine the effects of vernalization on the winter-habit cultivars versus the spring-habit synthetics. Due to the highly significant effect of vernalization on the cultivars the experiment was repeated without vernalization to remove this confounding effect.

Six replications of a split-plot design were grown with well-watered (WW) (fully saturated on a daily basis) and drought stressed (DS) (irrigation withheld one week after

emergence) conditions as the main plot treatments (T) and genotype (G) as the subplot factor. Three seeds of each genotype were planted in plastic tubes, 10 cm inside diameter and 99 cm tall in a greenhouse at Colorado State University (Fort Collins, CO) on 13 Oct. 2011. Each tube was lined with a 4 mil poly tube liner with two holes in the bottom for drainage (Uline, Pleasant Prairie, WI). Each tube was filled with 5.3 kg of dry GreensGrade fritted clay media (Profile Products LLC, Buffalo Grove, IL) and packed to a height of 98 cm, resulting in a bulk density of approximately 0.74 g/cm³ after irrigation. Greenhouse conditions consisted of 16h/8h of light/dark photoperiod at a temperature range from 18.3 to 25.5°C. Seven days after planting seedlings were thinned to a single plant per tube. Drip irrigation with Peters Professional 15-16-17 fertilizer (The Scotts Company, Marysville, OH) was applied at a concentration of 0.20 g/L with a 1:100 injector ratio. The water stress treatment was initiated 14 days after planting by terminating irrigation for a span of 18 days, corresponding to approximate Zadoks growth stages of 12 (two leaves unfolded) to 31 (first node detectable) (Zadoks et al., 1974).

Physiological and morphological measurements

Tubes in the drought stress treatment were weighed every three days for a total of six gravimetric water content measurements. Just prior to final collection of biomass samples, 2 cm leaf segments were collected from the middle of the fifth leaf for measuring relative water content (RWC) and osmotic potential. Relative water content measurements followed the procedure of Barrs and Weatherly (1962). Osmolality measurements were conducted by freezing the fresh leaf tissue at -20 °C and extracting the fluid by centrifuging at 14,000 RPM for 5 minutes. Samples were analyzed on a vapor pressure deficit osmometer (Wescor Model 5520, Wescor, Logan, UT). Leaf elongation rates of the fifth leaf were estimated 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). Above-ground biomass samples were collected at the termination of the experiment and dried at 70 °C for at least 24 hours prior to weighing. Measurement of g_s on the fourth fully developed leaf from the base of the plant began 11 days after treatment imposition. Measurements of g_s continued every other day, sampling complete replicates at midday in a randomized order each day, for a total of three repeated measurements. Both abaxial and adaxial surfaces of the leaf were measured with a Decagon SC-1 steady-state leaf porometer (Decagon, Inc., Pullman WA). However, abaxial conductance for the drought stressed treatment was too low to detect; thus, only adaxial conductance is reported. Leaf peels for measurement of stomatal density and aperture were collected through nail polish imprints adjacent to the site of porometer measurement on the fourth leaf. Stomatal counts were made under a light microscope at 16x magnification. Stomates were counted in five viewing areas of 2.18 mm² across the width of the leaf and were averaged for both adaxial and abaxial leaf surfaces. Stomatal length as an indication of aperture was estimated by measuring and averaging the total length of 10 closed stomates of both the adaxial and abaxial leaf surfaces (Malone et al., 1993).

Following collection of the above-ground biomass at Zadoks growth stage 30, the root systems were removed from the poly tube liners, washed free of growth medium, and measured for the longest seminal root length. The roots were divided into the upper (0-33 cm depth), middle (33-66 cm depth), and bottom (66-99 cm depth) sections of the tube. Individual root sections were floated on 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). Root morphology measurements recorded by WinRhizo included total root length, total root

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. A complete list of traits measured or calculated and their descriptions can be found in Appendix 2.

Statistical analysis

Unless otherwise stated, all statistical procedures were conducted in SAS software v. 9.2 (SAS Institute, 2008). Analysis of variance (ANOVA) for each trait was conducted in the PROC MIXED procedure where effects for G, T, and the G x T interaction were analyzed as fixed effects, while replicate was considered a random effect. Broad-sense plot mean heritability was calculated for all traits from mean squares estimates from a PROC GLM procedure according to Fehr (1987, p. 98, 228). In order to classify the water status of each entry under drought stress, least squares mean (LSmean) values of RWC were used to calculate a modified drought stress index (DI) similar to the formula of Ehdaie et al. (2012):

$$DI = 1 - \left(\frac{RWC_D}{RWC_W} \right) * 100$$

Where RWC_D is the RWC in the stressed treatment and RWC_W is the RWC of the well-watered treatment.

Traits associated with changes in plant water status were identified based on phenotypic correlations between least squares means of each trait and DI in JMP Pro 9.0.2 (JMP, 2010). Plasticity of each trait between the WW and DS treatments was calculated as the DS values subtracted from the WW values then divided by the grand mean across both treatments for that trait. Genotypes were classified into groups of drought tolerant and sensitive genotypes based on calculated DI values (smaller DI values are more tolerant). The three genotypes with the highest

DI value were considered drought sensitive and drought tolerant entries were the three lines with the lowest DI value. Only three entries were included in each group because DI values for entries within each group were found to be statistically different ($P < 0.05$) from entries in the opposite group. The PROC MIXED procedure was used to compare traits in tolerant versus sensitive groups. Entries within these groups were considered as random effects and groups as fixed effects.

Results

Analysis of variance

Significant genotypic differences ($P < 0.05$) were found for all physiological and morphological traits except root length for the 0.25 to 0.50 and 0.75 to 1.0 mm root diameter classes of the bottom tube section (Tables 2.2 and 2.3). Trait differences due to treatment effects were significant ($P < 0.01$) for all variables measured except for the root length of diameter class 0.75 to 1.00 mm in the middle tube section and longest root length for the diameter classes 0.00 to 0.25 and 0.75 to 1.00 mm in the bottom tube section. The effect of the moisture treatment began to be visually identifiable when three to four leaves were fully visible (Zadoks growth stage 14), as leaves in the DS treatment began to wilt at that stage. The G x T interaction effect was significant ($P < 0.05$) for above-ground biomass, upper tube section root biomass, relative water content, leaf elongation rate, adaxial stomate aperture, root length for all root diameter classes in the upper tube section, and root length for the 0.00 to 0.25, 0.50 to 0.75, and > 1.0 mm root diameter classes in the middle section. Under well-watered conditions, there were no differences among entries in production of bottom section root biomass; however, synthetic line SYN-2 showed significantly ($P < 0.05$) greater upper tube root biomass than all local cultivars.

Synthetic line SYN-1 had the longest seminal root as well as the largest root biomass in the bottom tube section under drought conditions. Adaxial g_s showed little difference among entries under drought stress, however synthetic line SYN-6 had the highest g_s of all entries.

Trait associations with drought stress index

To better understand the traits contributing to genetic variation in drought tolerance, we examined the correlations of DI with the traits in both WW and DS conditions and the plasticity of each trait (Tables 2.2 and 2.4, Appendix 3). Those traits significantly correlated ($r = -0.64$ to -0.86 , $P < 0.05$, $n = 10$) with DI in either treatment included bottom tube section root biomass, longest root, g_s , root length of the 0.00 to 0.25 mm root diameter class in the middle tube section, and root length of the 0.00 to 0.25, 0.25 to 0.50, and 0.50 to 0.75 mm diameter classes in the bottom tube section (Table 2.4). In both treatments root length of the 0.00 to 0.25 mm and 0.25 to 0.50 mm root diameter classes in the bottom tube section as well as the overall longest root were negatively correlated with DI. Bottom tube section root biomass, g_s , and root length of the 0.0 to 0.25 mm diameter class in the middle tube section were negatively correlated with DI in only the drought stressed treatment. Correlations among trait plasticity and DI showed only root biomass in the bottom tube section and total root biomass to be significant ($r = 0.72$ and 0.70 , respectively, $P < 0.05$, $n = 10$, Table 2.2). The traits that significantly ($P < 0.05$) correlated with DI also showed high correlations among themselves (Table 2.4). However, these large correlations were inconsistent across treatments; for example, no significant correlations with g_s were found in the WW treatment. Positive correlations were seen under drought conditions between plant water use and both root biomass and fine root growth in the bottom tube section.

Synthetic lines SYN-1 and SYN-2 as well as cultivar Byrd showed above average bottom tube section root biomass production under both treatments. When well-watered, synthetics SYN-1 and SYN-5 were the only two entries to rank above average for total root biomass and above average for percentage of roots produced in the upper section. However, SYN-1 was able to reallocate the production of root biomass to greater depths under drought, ranking third in overall root production with the largest percentage of root biomass produced in the bottom section (Table 2.3).

The classification of genotypes as drought tolerant versus drought sensitive identified Byrd, Ripper, and SYN-6 as drought tolerant whereas Goodstreak, Hatcher, and SYN-5 were considered drought sensitive. Least squares mean values for these groups and the interaction of groups with treatments identified significant ($P < 0.05$) differences between groups for above ground biomass, root biomass, longest root, g_s , water loss, and stomate aperture (Figure 2.1). Genotypes classified as drought tolerant had greater fine root lengths (diameter class 0.0 to 0.25 mm) ($P < 0.01$) throughout the root profile under both moisture treatments compared to the drought sensitive group (Figure 2.2). Synthetic lines SYN-1 and SYN-2 as well as the cultivar Byrd were consistently the largest producers of fine roots (diameter classes 0.00 to 0.50 mm) in the middle and bottom sections of the soil columns.

Correlations of stomatal density and aperture with DI were not significant ($P < 0.05$). However, ANOVA revealed differences between the drought tolerant and drought sensitive groups for stomatal aperture (Figure 2.1). Those genotypes classified as drought tolerant consistently exhibited a larger aperture under WW and DS conditions, however, lines SYN-5 and SYN-6 showed smaller aperture ($P < 0.05$) than nearly all other entries in both treatments.

Discussion

This set of SHW lines was found to vary significantly for various drought tolerance traits both among themselves and compared to cultivars adapted to the U.S. Great Plains. This study addressed our objectives by identifying beneficial SHW traits and demonstrating that both constitutive and plastic traits in SHW lines may improve plant water status under drought stress.

Physiological and morphological responses to water stress

Six of the seven traits correlated with DI were root traits such as bottom section root biomass and small diameter root length (Table 2.4, Appendix 3). The phenotypic correlations were all negative, indicating that an increase in those traits is related to a beneficial decrease in DI. This study confirms previous research on SHW and the importance of greater root biomass at depth and increased fine root production for enhancing water uptake at greater depths (Lopes and Reynolds, 2011; Reynolds et al., 2007). This study also demonstrated that trait variability, both constitutive and plastic, is present in diverse synthetic germplasm that may prove useful for the improvement of drought tolerance. Variation in root morphology and allocation of assimilates to deeper root growth facilitates greater water capture as seen by the correlations between DI, total water use, and bottom tube section root traits (Table 2.4). The plasticity values of only total root biomass and bottom tube section biomass were found to be significantly correlated with DI ($P < 0.05$) (Table 2.2). Positive correlations of these traits with DI indicate that increasing the amount of relative root biomass to deeper soil profiles under drought stress is associated with maintenance of leaf water status. An overall increase in root biomass may only be beneficial if the change occurs at deeper soil profiles. Plastic allocation of assimilates to deeper roots can also improve water capture with no reduction in grain yield (Blum, 2009;

Ehdaie et al., 2012; Lopes and Reynolds, 2011). Synthetic line SYN-6 and cultivar Byrd demonstrate this by showing small plasticity values (smaller is better) of above ground biomass and bottom tube section biomass as well as the smallest two DI values. Synthetic line SYN-1 produced the largest amount of bottom section root biomass under DS and ranked second in bottom root biomass production under WW conditions. Yet this synthetic line also produced more above-ground biomass than the cultivars when well-watered and was among the largest producers of above-ground biomass in the water stressed treatment.

Lengths of smaller diameter roots (0.00 to 0.5 mm) in the middle and bottom tube section were correlated with DI (Table 2.4). Negative correlations for these traits indicate improved drought tolerance from production of a greater length of fine roots, as also reported by Ehdaie et al. (2012). The importance of increased production of finer roots at depth can be seen in our results, as the length of fine roots is positively correlated with an increase in g_s and water use whereas bottom section biomass *per se* was positive but not significantly correlated with g_s in either treatment. In analysis of the drought tolerant versus sensitive genotypes, the tolerant genotypes produced a greater length of fine roots in both treatments at all tube depths (Figure 2.2). Synthetic line SYN-2 and cultivar Byrd consistently produced the largest amount of fine roots (0.00 to 0.25 mm diameter) in both treatments (Tables 2.5 and 2.6). Synthetic lines such as SYN-1 and SYN-2 that show greater root length in the 0.00 to 0.25 mm and 0.25 to 0.50 mm diameter classes in the middle and bottom tube section may contribute beneficial root traits useful in breeding for drought tolerance. Analysis of the upper root section indicated that the adapted cultivars had greater fine root length production than the synthetic lines in both treatments. This may be indicative of indirect selection for improved moisture and nutrient extraction from the upper soil section, as fine root tips near the soil surface are known to be a

primary location of moisture and nutrient uptake (Hopkins and Hüner, 2008). Selection for increased yield potential under more favorable conditions may have caused an indirect selection for increased shallow root biomass (Palta et al., 2011). However, as reported by Ehdaie et al. (2012), constitutive dispersion of fine, lateral roots throughout the soil profile, may contribute to improved moisture uptake due to greater soil surface area contact.

Stomatal aperture is proportional to g_w Max (Franks et al., 2009; Hetherington and Woodward, 2003). It would be expected that genotypes with larger apertures would lose more moisture and thus have a lower water status (lower RWC) (Hetherington and Woodward, 2003). However, we observed larger apertures for the drought tolerant group under both treatments (Figure 2.1). It was unclear if there was a causal relationship in this study between stomate aperture or density and an increase in g_s as no statistically significant ($P < 0.05$) correlations among these traits were found (data not shown). Water uptake traits may have overshadowed the effects of minor traits under drought stress, such as stomatal attributes and osmotic adjustment (Blum, 2011, p. 74, 91). Thus, the effect of longer fine diameter roots at depth, which was correlated with g_s (Table 2.4), may overshadow the effects of stomatal size and density. It could be argued that synthetic SYN-5 had an ideal combination of the densest stomates (12.3 adaxial and 9.1 abaxial stomata per mm^2) and the smallest aperture (46.4 μm adaxial and 46.3 μm abaxial) (Table 2.3). This would create a large leaf porosity with greater stomatal density and the potential for a quicker response time under drought stress due to smaller aperture (Franks et al., 2009). SYN-5 was the genotype with the lowest g_s , and second smallest bottom tube section root biomass under drought (Table 2.3). However, it was still able to maintain a relatively high leaf elongation rate, indicating that it may have minimized conductance and water loss while maintaining a sufficient amount of CO_2 uptake to continue photosynthetic activity and leaf

growth. However, the lack of any correlation between DI and both stomatal density and aperture supports the need for further study of the beneficial stomatal attributes of synthetic lines.

Conclusion

Synthetic hexaploid wheat had been shown previously to improve yield as well as resistance to biotic and abiotic stresses when crossed to adapted material. In this study, root morphology traits contributing to increases in water extraction from deeper depths in drying soil were found to contribute the most to the avoidance of plant water stress and thus the maintenance of productivity. These traits included increased deep root biomass, longer seminal roots, and increased production of small diameter roots at depth. Synthetic hexaploid wheat lines showed variation in constitutive and plastic root traits which may be beneficial in improving drought stress tolerance in elite cultivars. The genotypic diversity expected to contribute to these traits may be considered novel in many breeding programs and add to increases in productivity and stability in variable drought stressed regions of the U.S. Great Plains.

Table 2.1. Pedigrees of entries used in this study. Cultivar pedigrees are from Baenziger et al., (2004) and Haley et al. (2005, 2007, 2012a). Synthetic genotype nomenclature and pedigrees (SYN-1 through SYN-5) are from GRIN (<http://www.ars-grin.gov/>).

| Entry name | Pedigree | Plant Introduction Number |
|------------|---|---------------------------|
| Byrd | TAM 112/CO970547-7 | PI 664257 |
| Goodstreak | SD3055/KS88H164//NE89646 | PI 632434 |
| Hatcher | Yuma/PI372129//Tam-200/3/4*Yuma/4/KS91H184/Vista | PI 638512 |
| Ripper | CO940606/TAM107R-2 | PI 644222 |
| SYN-1 | 68112/Ward// <i>Ae. tauschii</i> (369) [†] | PI 648646 |
| SYN-2 | Garza/Boyerros// <i>Ae. tauschii</i> (241) | PI 648733 |
| SYN-3 | Laru/ <i>Ae. tauschii</i> (333) | PI 648758 |
| SYN-4 | Decoy 1/ <i>Ae. tauschii</i> (256) | PI 648810 |
| SYN-5 | Decoy 1/ <i>Ae. tauschii</i> (322) | PI 648823 |
| SYN-6 | Snipe/Yavaros 79//Dackiye/Teal/3/ <i>Ae. tauschii</i> (904) | BW27885 [‡] |

[†] Parentheses indicate *Ae. tauschii* accession number.

[‡] Inactive accession number as referenced on the GRIN database.

Table 2.2. Analysis of variance results for physiological and morphological traits under well-watered and drought stressed treatments and correlations (n = 10) of trait plasticity with drought index.

| Source | Traits [†] | | | | | | | | | | | | | |
|--------------------------------|---------------------|------|------|--------|--------|-------|-------|-----------------|------|----------------|-------|-------|------|-------|
| | ABM | TRBM | MRBM | BRBM | TotRBM | LR | OP | RWC | LER | g _s | TSA | BSA | TSD | BSD |
| Genotype (G) | *** | *** | *** | ** | *** | *** | *** | ** | ** | * | *** | *** | *** | *** |
| Treatment (T) | *** | *** | *** | *** | ** | * | *** | *** | *** | *** | *** | ** | ** | * |
| G x T | ** | ** | | | | | | * | ** | | * | | | |
| Trait <i>h</i> ² | 0.60 | 0.57 | 0.87 | 0.62 | 0.88 | 0.81 | 0.90 | 0.13 | 0.11 | 0.60 | 0.90 | 0.94 | 0.82 | 0.80 |
| <i>r</i> of plasticity with DI | 0.40 | 0.40 | 0.03 | 0.72 * | 0.70 * | -0.16 | -0.60 | NA [‡] | 0.45 | -0.01 | -0.25 | -0.43 | 0.09 | -0.24 |

*, **, *** Significant at the 0.05, 0.01, and 0.0001 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, length of longest seminal root; OP, osmotic potential; RWC, relative water content; LER, leaf elongation rate; g_s, adaxial stomatal conductance; TSA, adaxial (top) stomate aperture; BSA, abaxial (bottom) stomate aperture; TSD, adaxial (top) stomate density; BSD, abaxial (bottom) stomate density.

Table 2.3. Least squares means of physiological and morphological traits under well-watered and drought stress treatments.

| Genotype | Traits [†] | | | | | | | | | | | | | |
|-----------------|---------------------|------------|----------|------------|---------|----------|-----------|---------|---------|---------------------|----------|----------|---------------------|---------------------|
| | ABM | TRBM | MRBM | BRBM | TotRBM | LR | OP | RWC | LER | g _s | TSA | BSA | TSD | BSD |
| WW [‡] | g | g | g | g | g | mm | MPa | % | mm/day | μM/m ² s | μm | μm | cnt/mm ² | cnt/mm ² |
| Byrd | 1.20 b [¶] | 0.21 bcd | 0.05 abc | 0.0128 a | 0.26 b | 983 ab | -1.12 ab | 96.0 a | 55.9 ab | 350 bc | 58.0 a | 55.4 abc | 9.9 abc | 7.1 bc |
| GS | 1.14 bc | 0.18 ed | 0.04 bc | 0.0064 a | 0.23 bc | 899 ab | -1.17 bcd | 95.3 a | 57.7 a | 312 c | 55.1 bcd | 49.7 de | 10.7 ab | 7.1 bc |
| Hatcher | 1.16 bc | 0.22 bc | 0.04 abc | 0.0029 a | 0.27 b | 838 b | -1.13 abc | 96.3 a | 53.9 ab | 385 abc | 53.4 d | 52.4 cd | 11.1 ab | 7.9 ab |
| Ripper | 1.02 c | 0.17 e | 0.05 abc | 0.0084 a | 0.23 bc | 891 ab | -1.19 cd | 96.0 a | 50.3 b | 449 ab | 57.2 ab | 56.0 ab | 10.9 ab | 8.2 a |
| SYN-1 | 1.24 b | 0.19 cde | 0.04 bc | 0.0164 a | 0.24 bc | 941 ab | -1.10 a | 96.1 a | 51.8 ab | 374 bc | 50.6 e | 48.6 ef | 10.3 abc | 6.9 c |
| SYN-2 | 1.57 a | 0.26 a | 0.07 a | 0.0207 a | 0.35 a | 1009 a | -1.18 bcd | 95.9 a | 55.2 ab | 330 c | 58.1 a | 55.9 ab | 8.9 c | 7.1 bc |
| SYN-3 | 1.44 a | 0.17 e | 0.06 ab | 0.0102 a | 0.23 bc | 835 b | -1.22 de | 96.2 a | 56.2 ab | 361 bc | 56.3 abc | 53.1 bc | 9.6 bc | 7.7 abc |
| SYN-4 | 1.25 b | 0.18 ed | 0.05 abc | 0.0064 a | 0.24 bc | 865 ab | -1.25 e | 97.0 a | 52.7 ab | 521 a | 54.0 cd | 57.3 a | 10.6 ab | 7.5 abc |
| SYN-5 | 1.14 bc | 0.25 ab | 0.01 d | 0.0001 a | 0.26 b | 578 c | -1.13 abc | 95.5 a | 57.6 a | 370 bc | 45.0 f | 45.5 f | 11.2 a | 8.2 a |
| SYN-6 | 1.02 c | 0.17 e | 0.03 cd | 0.0033 a | 0.20 c | 860 ab | -1.17 bcd | 95.6 a | 52.0 ab | 404 abc | 49.8 e | 47.1 ef | 10.7 ab | 7.9 ab |
| DS | | | | | | | | | | | | | | |
| Byrd | 0.76 a | 0.15 a | 0.11 ab | 0.0378 ab | 0.29 a | 981 abc | -1.30 ab | 94.5 a | 37.5 b | 196 ab | 54.8 a | 50.1 bcd | 12.5 a | 8.4 abc |
| GS | 0.60 bcd | 0.12 abcd | 0.08 cd | 0.0029 e | 0.20 bc | 863 bcd | -1.41 cde | 89.8 c | 34.3 b | 125 d | 50.5 c | 47.2 de | 12.3 a | 8.9 ab |
| Hatcher | 0.56 d | 0.11 bcde | 0.08 bcd | 0.0155 cde | 0.20 bc | 937 abcd | -1.35 abc | 89.9 c | 32.0 c | 185 abc | 51.6 bc | 50.7 abc | 12.4 a | 8.6 ab |
| Ripper | 0.66 abcd | 0.14 ab | 0.07 d | 0.0280 abc | 0.25 ab | 1012 ab | -1.40 cde | 93.9 a | 39.8 ab | 183 abc | 55.7 a | 53.1 ab | 12.1 a | 8.2 bcd |
| SYN-1 | 0.75 ab | 0.12 abcde | 0.10 bc | 0.0449 a | 0.26 a | 1064 a | -1.30 a | 92.6 ab | 38.0 ab | 143 cd | 51.8 bc | 49.4 cde | 9.7 c | 6.9 e |
| SYN-2 | 0.73 abc | 0.13 abcd | 0.13 a | 0.0326 abc | 0.30 a | 942 abcd | -1.36 bc | 93.0 ab | 44.5 a | 158 abcd | 53.0 abc | 52.6 abc | 9.3 c | 7.3 de |
| SYN-3 | 0.77 a | 0.10 ed | 0.08 cd | 0.0236 bcd | 0.20 bc | 821 cd | -1.46 de | 90.6 bc | 41.6 ab | 150 bcd | 53.7 ab | 51.6 abc | 10.0 c | 8.1 bcd |
| SYN-4 | 0.69 abcd | 0.08 e | 0.07 d | 0.0270 abc | 0.19 c | 1016 ab | -1.46 e | 93.5 a | 40.4 ab | 150 bcd | 53.9 ab | 53.6 a | 10.1 bc | 7.6 cde |
| SYN-5 | 0.59 cd | 0.13 abc | 0.07 d | 0.0037 de | 0.21 bc | 794 d | -1.35 abc | 89.0 c | 44.3 ab | 144 cd | 46.4 d | 46.3 e | 12.3 a | 9.1 a |
| SYN-6 | 0.62 abcd | 0.09 de | 0.07 d | 0.0254 abc | 0.19 c | 941 abcd | -1.39 cd | 93.7 a | 38.8 ab | 211 a | 46.6 d | 46.9 e | 11.5 ab | 8.7 ab |

[†] 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, length of longest seminal root; OP, osmotic potential; RWC, relative water content; LER, leaf elongation rate; g_s, adaxial stomatal conductance; TSA, adaxial (top) stomate aperture; BSA, abaxial (bottom) stomate aperture; TSD, adaxial (top) stomate density; BSD, abaxial (bottom) stomate density.

[‡] WW, well-watered treatment; GS, Goodstreak; DS, drought stressed treatment.

[¶] Values with similar letters within a treatment are not statistically different based on LSD_{0.05} values.

Table 2.4. Phenotypic correlations ($n = 10$) among traits that were significantly correlated with the drought stress index. Correlations above and below the diagonal are associated with the drought stressed and well-watered treatments, respectively.

| Traits [†] | DI | BRBM | LR | gs | Mdia1 | Bdia1 | Bdia2 | Bdia3 | Water [‡] loss |
|---------------------|---------|---------|----------|---------|---------|----------|----------|----------|-------------------------|
| DI | | -0.73* | -0.68 * | -0.73 * | -0.70 * | -0.86 ** | -0.70 * | -0.67 * | -0.64 * |
| BRBM | -0.45 | | 0.78 ** | 0.55 | 0.63 | 0.87 ** | 0.79 ** | 0.84 ** | 0.86 ** |
| LR | -0.64 * | 0.78 ** | | 0.57 | 0.57 | 0.70 * | 0.73 * | 0.80 ** | 0.49 |
| gs | -0.44 | -0.03 | 0.00 | | 0.84 ** | 0.72 * | 0.64 * | 0.51 | 0.41 |
| Mdia1 | -0.56 | 0.68 * | 0.96 *** | 0.03 | | 0.69 * | 0.67 * | 0.65 * | 0.71 * |
| Bdia1 | -0.69 * | 0.90 ** | 0.82 ** | 0.08 | 0.70 * | | 0.93 ** | 0.86 ** | 0.74 * |
| Bdia2 | -0.64 * | 0.87 ** | 0.93 ** | -0.03 | 0.85 ** | 0.95 *** | | 0.95 *** | 0.66 * |
| Bdia3 | -0.61 | 0.78 ** | 0.97 *** | -0.15 | 0.91 ** | 0.83 ** | 0.94 *** | | 0.73 * |

*, **, *** Significant at the 0.05, 0.01, and 0.0001 probability levels, respectively.

[†] DI, drought stress index; BRBM, bottom tube section root biomass; LR, longest root; g_s , stomatal conductance; Mdia1, middle tube section root length for the 0.00 to 0.25 mm diameter class; Bdia1, bottom tube section root length for the 0.00 to 0.25 mm diameter class; Bdia2, bottom tube section root length for the 0.25 to 0.50 mm diameter class; Bdia3, bottom tube section root length for the 0.50 to 0.75 mm diameter class.

[‡] Measured only under drought stress.

Table 2.5. Analysis of variance for root traits measured with WinRhizo software under well-watered and drought stressed treatments.

| Source | Root Length per root diameter class (cm/mm) | | | | | | | | | | | | | | |
|--------------------------|---|-------------|-------------|-------------|-------|---------------------|-------------|-------------|-------------|-------|---------------------|-------------|-------------|-------------|-------|
| | 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 |
| Genotype (G) | *** | *** | ** | ** | *** | *** | *** | *** | ** | *** | * | | ** | | ** |
| Treatment (T) | *** | *** | *** | *** | *** | ** | *** | *** | | *** | | ** | ** | | ** |
| G x T | ** | ** | * | ** | ** | ** | | *** | | * | | | | | |

*, **, *** Significant at the 0.05, 0.01, and 0.0001 probability levels, respectively.

Table 2.6. Least squares means for root traits measured with WinRhizo software under well-watered and drought stressed treatments.

| Root diameter (mm) | Root Length per root diameter class (cm/mm) | | | | | | | | | | | | | | |
|--------------------|---|-------------|-------------|-------------|----------|---------------------|-------------|-------------|-------------|---------|---------------------|-------------|-------------|-------------|---------|
| | 0-33 cm tube depth | | | | | 33-66 cm tube depth | | | | | 66-99 cm tube depth | | | | |
| | 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 |
| Genotype | | | | | | | | | | | | | | | |
| WW [†] | | | | | | | | | | | | | | | |
| Byrd | 1828 a [‡] | 1103 b | 231 cd | 157 bc | 67.0 def | 395 a | 455 abc | 99 ab | 60 abc | 6.9 cde | 29.4 a | 16.5 abc | 47.7 ab | 28.8 a | 1.9 a |
| GS [†] | 1400 b | 970 b | 198 d | 123 d | 63.0 def | 215 b | 350 bc | 97 ab | 40 de | 2.2 f | 1.9 b | 8.6 abc | 21.3 ab | 12.5 a | 0.6 a |
| Hatcher | 1845 a | 1386 a | 251 bcd | 138 cd | 106.5 bc | 196 b | 464 abc | 80 b | 57 bc | 9.0 bcd | 0.9 b | 0.5 c | 7.9 b | 20.4 a | 1.8 a |
| Ripper | 1429 b | 1058 b | 237 cd | 144 bcd | 51.3 f | 241 ab | 428 abc | 103 ab | 52 bcd | 4.9 de | 8.0 ab | 5.9 abc | 21.7 ab | 16.0 a | 1.0 a |
| SYN-1 | 1145 cd | 1447 a | 243 cd | 170 ab | 78.6 cde | 294 ab | 380 bc | 83 b | 56 bcd | 5.5 de | 21.1 a | 52.3 ab | 39.4 ab | 28.8 a | 1.8 a |
| SYN-2 | 1248 bc | 1544 a | 324 ab | 196 a | 117.1 b | 397 a | 577 a | 137 a | 80 a | 13.4 bc | 22.6 a | 93.3 a | 58.7 a | 32.6 a | 1.8 a |
| SYN-3 | 982 de | 1076 b | 243 cd | 172 ab | 80.6 cde | 228 b | 492 ab | 103 ab | 67 ab | 33.4 a | 4.0 ab | 3.8 abc | 9.3 b | 28.3 a | 4.8 a |
| SYN-4 | 901 e | 1028 b | 266 abc | 159 bc | 88.5 bcd | 207 b | 428 abc | 73 b | 66 ab | 16.0 ab | 5.8 ab | 2.4 bc | 7.8 b | 22.2 a | 4.4 a |
| SYN-5 | 616 f | 1456 a | 340 a | 142 bcd | 186.3 a | 14 c | 38 d | 25 c | 34 e | 13.0 bc | 0.7 b | 0.1 c | 0.1 b | 8.1 a | 0.2 a |
| SYN-6 | 1221 bc | 1099 b | 257 bcd | 146 bcd | 59.1 ef | 179 b | 317 c | 84 b | 44 dce | 3.8 ef | 6.1 ab | 6.5 abc | 11.7 b | 22.7 a | 0.8 a |
| DS [†] | | | | | | | | | | | | | | | |
| Byrd | 1340 a | 765 a | 114 ab | 34 ab | 16.6 abc | 600 a | 924 a | 162 abc | ¶ | 13.6 ab | ¶ | 336.7 a | 71.3 ab | ¶ | 4.9 ab |
| GS | 1067 bc | 664 ab | 95 bc | 25 bcd | 13.0 bc | 226 b | 742 a | 120 cd | | 9.7 b | | 5.8 bc | 14.1 c | | 0.5 c |
| Hatcher | 1118 abc | 637 abc | 85 cd | 23 bcd | 12.0 bc | 273 b | 897 a | 125 bcd | | 10.7 b | | 29.7 abc | 23.3 bc | | 3.1 ab |
| Ripper | 1143 ab | 777 a | 120 ab | 28 abcd | 22.1 ab | 279 b | 741 a | 106 d | | 10.6 b | | 179.3 ab | 60.0 ab | | 3.8 ab |
| SYN-1 | 941 dc | 730 ab | 95 bc | 22 bcd | 11.4 bc | 238 b | 878 a | 136 bcd | | 19.9 ab | | 59.2 abc | 88.1 a | | 7.3 ab |
| SYN-2 | 778 d | 677 ab | 117 ab | 30 abc | 15.1 bc | 357 ab | 939 a | 202 a | | 26.1 a | | 61.2 abc | 62.1 ab | | 2.5 abc |
| SYN-3 | 774 d | 690 ab | 104 abc | 27 abcd | 19.0 abc | 209 b | 903 a | 152 abc | | 21.1 ab | | 37.2 abc | 43.3 abc | | 5.9 ab |
| SYN-4 | 493 e | 528 bc | 69 d | 16 d | 11.4 bc | 214 b | 742 a | 161 abc | | 25.3 a | | 154.0 ab | 61.5 ab | | 11.0 a |
| SYN-5 | 542 e | 707 ab | 129 a | 42 a | 31.1 a | 56 c | 480 b | 170 ab | | 16.9 ab | | 4.2 c | 6.8 c | | 1.9 bc |
| SYN-6 | 973 bc | 619 bc | 86 cd | 119 cd | 7.1 c | 331 b | 730 a | 133 bcd | | 11.0 b | | 16.3 abc | 23.4 bc | | 2.5 abc |

[†] WW, well-watered treatment; GS, Goodstreak; DS, drought stressed.

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

[¶] No significant treatment or genotype x treatment (G x Trt) effect was found. LS Mean values calculated across treatments.

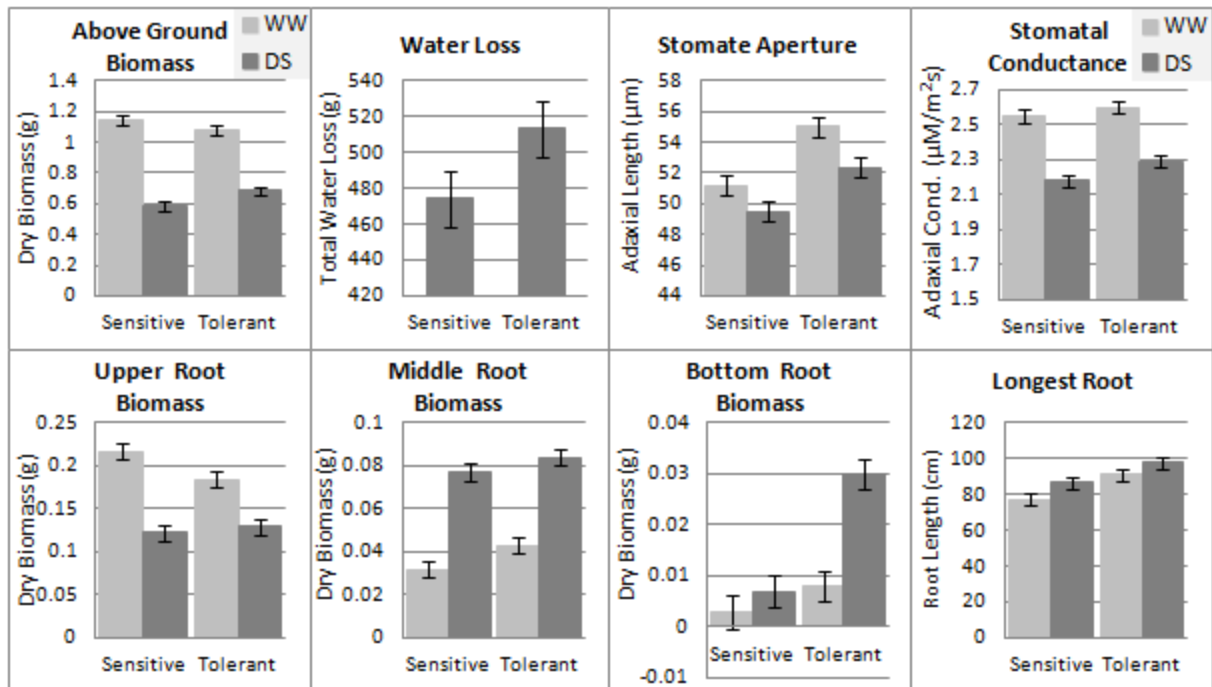


Figure 2.1. Traits with a significant difference ($P < 0.05$) between groups of genotypes classified as sensitive and tolerant according to the drought stress index. WW, well-watered treatment, DS, drought stressed treatment. Error bars indicate standard errors for each trait.

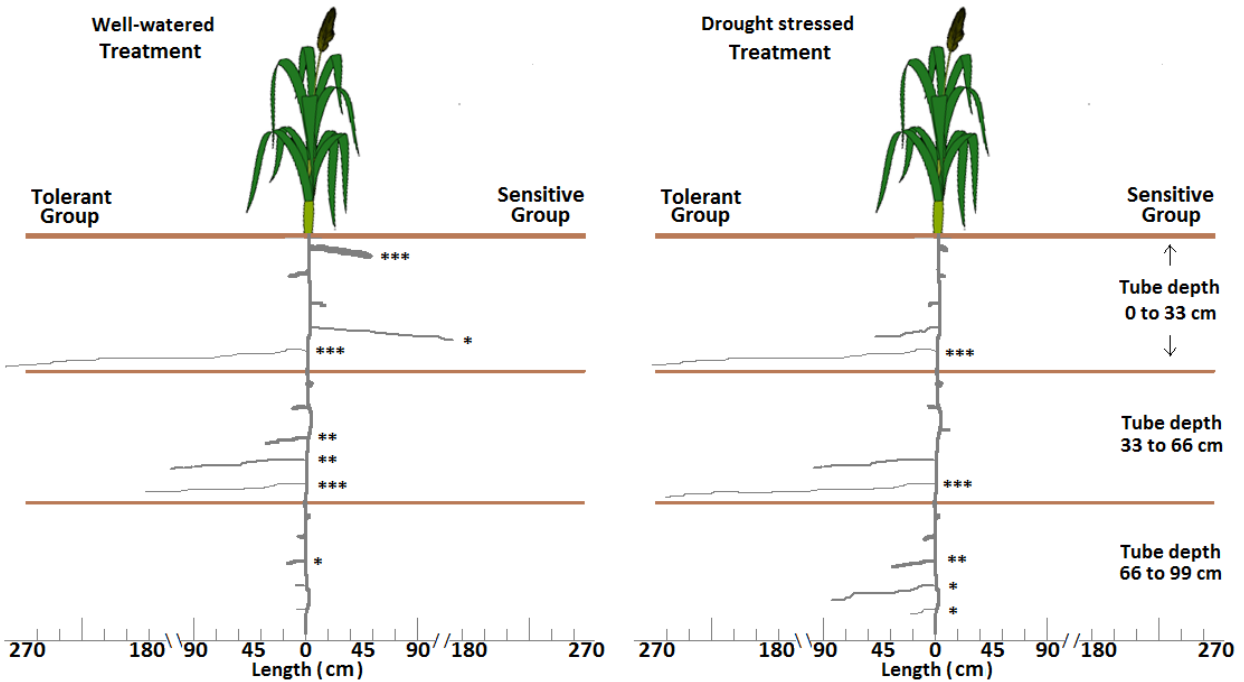


Figure 2.2. Root length per diameter class measured by WinRhizo software. The five diameter classes (0.00 to 0.25 mm, 0.25 to 0.50 mm, 0.50 to 0.75 mm, 0.75 to 1.00 mm, >1.0 mm) are portrayed by the thickness of the lateral roots in the figure. For example the thinnest lateral roots within each depth portray the 0.00 to 0.25 mm diameter class. Lateral root length under tolerant and sensitive headings represents the difference between groups in root length (cm) per diameter class. The group heading containing the root is the larger of the groups. For example, if the lateral root on the left side of the seminal root is 90 cm long, the tolerant group produced 90 cm more roots of that diameter class.

, * Significant at the 0.01 and 0.0001 probability level between groups within a treatment, respectively.

CHAPTER THREE:

INTROGRESSION OF SYNTHETIC HEXAPLOID WHEAT GERMPLASM INTO ADAPTED CULTIVARS IN THE U.S. GREAT PLAINS

Summary

Improving yield and yield stability of winter bread wheat (*Triticum aestivum* L.) is often a motivation for broadening the diversity of breeding populations. Introgression of novel diversity can be accomplished through the use of synthetic hexaploid wheat (SHW) lines, however, the detrimental effects of unadapted germplasm is a concern. The goal of this research was to evaluate the potential to introgress favorable SHW alleles into winter wheat with minimal pre-breeding requirements. Five pre-selected SHW lines were crossed and backcrossed to local cultivars ‘Hatcher’ and ‘Goodstreak’ to develop 10 backcross populations, consisting of 15 winter-habit SHW-derived BC₁F_{1,2} lines each. Yield trials conducted in eight environments in Colorado and Nebraska in 2009-10 and 2010-11 identified progeny with increased yield over the recurrent parent. The SHW-derived populations of 15 lines each created with the synthetics designated SYN-1 and SYN-2 consistently ranked among the top populations for average grain yield within an environment. Grain yield of SHW-derived lines varied significantly ($P < 0.05$) across all environments. Consistency of yield was also demonstrated, as the backcrossed line with the highest ranking grain yield overall also ranked higher than its recurrent parent Hatcher in all but one environment. Spike number m⁻², average kernel weight, and harvest index were consistently correlated ($r = 0.34$ to 0.62 , $P < 0.05$) with grain yield overall. However, correlation of grain yield with spike number m⁻² and average kernel weight varied among SHW-derived populations. Several SHW-derived lines showed promise for improvements in winter wheat yield and yield components across environments in the U.S. Great Plains.

Abbreviations: AKW, average kernel weight; BM, biomass; DF, date of 50% of heads reaching anthesis; DPM, date of 50% of peduncles senesced; GF, number of days in grain fill period from anthesis to senescence; GY, grain yield; HI, harvest index; KNA, kernel number m⁻²; KNS, kernel number per spike; PH, plant height; SDL, SHW-derived line; SHW, synthetic hexaploid wheat; SN, spike number m⁻²; TW, test weight.

Introduction

Abiotic stresses have a severe detrimental effect on crop yield throughout the world and achieving high yield along with yield stability despite these stresses is a primary concern of farmers and researchers (Passioura, 2007). Drought stress is the most limiting factor for cereal yield in many environments and years (Araus et al., 2002), so increasing crop tolerance to drought is essential for future global food security. The genetic variability found in modern crops including bread wheat (*Triticum aestivum* L.) has been reduced due to bottlenecks by both natural and artificial selection that has occurred since the first appearance of these species (Haudry et al., 2007; Tanksley and McCouch, 1997). In order to improve the productivity and stability of bread wheat under drought stress, breeders are seeking to increase the genetic variation for drought tolerance traits in their germplasm populations (Dreisigacker et al., 2008; Lopes and Reynolds, 2011; van Ginkel and Ogbonnaya, 2007). One approach that is being implemented to recover a portion of the lost variation is the use of synthetic hexaploid wheat (SHW). Synthetic wheat lines are typically developed through crosses between durum wheat (*T. turgidum* L.) accessions (AABB genome) and the wild wheat progenitor, *Aegilops tauschii* (DD Genome), to recreate the hexaploid genome (AABBDD) found in modern bread wheat (Mujeeb-Kazi et al., 2008). In this form the diversity found within both durum wheat accessions and *Ae.*

tauschii can easily be captured and crossed with elite bread wheat varieties (Trethowan and Mujeeb-Kazi, 2008).

Synthetic hexaploid wheat lines have been shown to contribute a large amount of genotypic diversity to crosses produced with modern cultivars (Dreisigacker et al., 2008; Talbot, 2011; van Ginkel and Ogbonnaya, 2007). However, it is also common that this diversity is quite detrimental rather than beneficial to the resulting progeny (Cox, 1997). Previous research suggests that multiple backcrosses of SHW lines to the adapted recurrent parent may be needed prior to selection (Lage and Trethowan, 2008). Spring SHW lines have been shown to improve yield potential and adaptation to drought when backcrossed or three-way crossed with elite spring wheat cultivars (del Blanco et al., 2000; Lage and Trethowan, 2008; Lopes and Reynolds, 2011; Mohammad et al., 2010; Trethowan and Mujeeb-Kazi, 2008). However, few studies have shown the beneficial use of crossing spring SHW with elite winter wheat cultivars in a single backcross (Cooper et al., 2012; Cooper et al., 2013; Narasimhamoorthy et al., 2006). Therefore, the objectives of this study were to determine the potential of SHW lines to contribute to high yielding, genetically diverse germplasm with a single backcross and to select the highest yielding and most stable lines for single plant selection and germplasm development adapted to the U.S. Great Plains.

Materials and methods

Population development

The steps involved in the development of germplasm used in this study are shown in Figure 3.1. Initial screening consisted of field evaluation of a collection of 412 spring SHW lines, developed by the International Maize and Wheat Improvement Center (CIMMYT, El

Batan, Mexico). The lines were grown under dryland conditions in Akron, CO (40.1548 °N, 103.142 °W, elevation, 1383 m) in 2007 as a single replication of two-row plots, 75 cm long. Forty-five SHW lines were selected and grown in a three replicate yield trial in Akron in 2008, using the same plot dimensions. A subset of five lines, listed in Table 3.1, was chosen based on visual, agronomic traits including head type, tiller density, lodging resistance, potential seed yield, and well-filled kernels. Each of these selected SHW lines was used as a donor parent and crossed and backcrossed to locally adapted winter wheat cultivars ‘Hatcher’ and ‘Goodstreak’ (Baenziger et al., 2004; Haley et al., 2005) as recurrent parents to create a total of 10 backcross-derived populations (e.g. Goodstreak x SYN-1 and Hatcher x SYN-1). Approximately 160 BC₁F₁ seeds from each of the 10 populations were space-planted in Yuma, AZ in December 2008 and single plant selection was conducted for wheat-like appearance, lodging resistance, and delayed heading indicative of winter habit. Twelve BC₁F_{1:2} seeds from each selected plant were planted without vernalization in a greenhouse at Colorado State University, in Fort Collins, CO in summer of 2009. Those BC₁F_{1:2} lines that entered the reproductive growth stage despite the lack of vernalization were considered spring habit and were not advanced for use in the current study. Lines in which all plants remained vegetative were considered winter habit and were used in the field evaluations.

Experimental design

Evaluation of the 10 backcross-derived populations (15 BC₁F_{1:2} SHW-derived lines in each) was conducted in four environments in 2009-2010: Greeley, CO fully irrigated (GreeleyF10), Greeley, CO limited irrigation (GreeleyL10), Lincoln, NE (Lincoln10), and Sidney, NE (Sidney10) (Table 3.2). Check cultivars included the recurrent parents Goodstreak and Hatcher along with ‘Ripper’, ‘Settler CL’, and ‘Snowmass’ (Baenziger et al., 2011; Haley et

al., 2007, 2011), for a total of 155 entries. For the Greeley location at the USDA Limited Irrigation Research Farm, genotypes were planted in 3.0 m long, two-row plots with 23 cm between rows and 28 cm between plots. Plots were randomized in a two-replication incomplete block Latinized row–column design created with CycDesign 3.0 (CycSoftware, 2006) with side-by-side full and limited irrigation treatments. Irrigation was supplied to both treatments by surface drip tube with a difference in weekly amounts, starting approximately at the booting stage, (Zadoks stage 40) (Zadoks et al., 1974) targeted at reducing the limited irrigation treatment yield by 50% of the full irrigation treatment (Table 3.2). The Lincoln10 and Sidney10 locations were planted under rainfed conditions with two replicates consisting of four-row plots, 2.4 m long with 30 cm between rows in an incomplete complete block design. At each location, measurements were made for grain yield (GY) and test weight (TW). Plots at Greeley were hand harvested and threshed with a Vogel thresher (Bill’s Welding Shop, Pullman, WA) for measurements of GY and TW. Test weight was measured by hand with a 151 filling hopper (Seedburo, Des Plains, IL). At Lincoln10 and Sidney10 plots were combine harvested and GY and TW were recorded by an on-combine HarvestMaster GrainGage (Juniper Systems, Logan, UT).

Selection to advance lines for further testing in 2011 was made based on a selection index that included GY and TW across all environments in 2010. In this index, GY and TW were standardized and GY was weighted at two to one over TW. An average index value for each population was calculated from the values of each line within each population. All populations derived from SYN-1, SYN-2, and SYN-4, whose lines averaged the highest index value across all locations, were selected for testing in 2011. However, due to space limitations the lowest yielding line of the 15 lines per population was excluded from the experiment in 2011. This

resulted in 14 BC₁F_{1:3} lines (from each of three SHW lines crossed to two cultivars) being advanced to testing in 2011.

Testing locations in 2011 consisted of side-by-side full and limited irrigation treatments at Greeley, CO (GreeleyF11 and GreeleyL11, respectively) and a rainfed location at Lincoln, NE (Lincoln11) as previously described, as well as an additional rainfed environment at Dailey, CO (Dailey11). Plots at Dailey consisted of six rows, 3.5 m long with the same experimental design as at the Greeley location. Additional location data is provided in Table 3.2. Entries in 2011 consisted of 14 BC₁F_{1:3} SHW-derived lines (SDL) within each of the six selected populations from 2010. Check cultivars included Hatcher, Goodstreak, Ripper, Settler CL, and Snowmass, for a total entry number of 89. Measurements in both treatments at Greeley in 2011 consisted of GY, TW, plant height (PH), above ground biomass (BM), kernel number per spike (KNS), average kernel weight (AKW), number of spikes m⁻² (SN), kernel number m⁻² (KNA), days to flowering (DF), days to physiological maturity (DPM), grain fill period (GF), and harvest index (HI). Above ground biomass was collected by harvesting all biomass above ground level from a 1 m long sub-sample of a single row in each plot, weighed and hand threshed as previously stated. A random sample of 10 spikes per plot was harvested and hand threshed in a Hege 16 thresher (Wintersteiger, Salt Lake City, UT). The 10-spike grain sample was counted and weighed and used for calculation of AKW, KNS, and SN as follows.

$$\text{AKW} = \text{Ten spike seed count} / \text{Ten spike seed weight}$$

$$\text{KNS} = \text{Ten spike seed count} / 10$$

$$\text{KNA} = \text{KNS} \times \text{SN}$$

$$\text{SN} = \text{Biomass kernel weight} / (\text{AKW} \times \text{KNS}) / 0.23 \text{ m}^2$$

Values for DF and DPM were calculated as the number of days from 1 Jan. until 50% of the plants in a plot showed anther exertion from the floret and peduncle senescence, respectively. Grain fill period was the number of days from DF to DPM. Harvest index was calculated as the ratio of biomass grain weight to total biomass. Measurements at all other locations in 2011 were GY and TW only. The SDLs within each population which ranked in the top three for GY across all environments in both 2010 and 2011 were selected for advanced testing. Selection between entries of similar rank was aided by an Eberhart-Russell stability index (Eberhart and Russell, 1966) across all 2010 and 2011 environments. If needed, those entries with a slope (b_i) closer to one and a deviation (s^2d_i) closer to zero were chosen.

Statistical analysis

All statistical procedures were conducted in SAS software version 9.2 (SAS Institute Inc., 2008) unless otherwise indicated. Analysis of variance for each trait was conducted using the PROC MIXED procedure. For each testing location in Nebraska, genotype was considered a fixed effect and incomplete blocks were considered random. For each trait within a treatment at Greeley and at Dailey three models were run in the PROC MIXED procedure to account for the expected spatial variation according to Butler et al. (2005). Genotype was considered a fixed effect and spatial variables were considered random. The best model was chosen for use based on the lowest Akaike information criterion value. Phenotypic correlations among all pairs of traits within each treatment at Greeley 2011 were calculated in JMP Pro 9.0.2 (JMP, 2010).

Results

Grain yield of synthetic-derived populations and lines

Least squares means for GY of the SHW-derived populations in 2010 varied significantly ($P<0.05$) within each environment (Table 3.3). The SHW-derived populations created with SYN-1 and SYN-2 consistently ranked in the top three for GY in each environment in 2010. Because all 15 segregating SDLs for a given cross were included in the GY values for the comparison of populations, it was not unexpected that population averages would rank lower than the recurrent parents. The overall GY of each population derived from Hatcher showed no difference ($P<0.05$) compared to the recurrent parent in 2010. However, the populations created from the crosses of Goodstreak with SYN-3, SYN-4, and SYN-5 yielded significantly less ($P<0.05$) than the recurrent parent in certain environments (Table 3.3). The populations derived from SYN-1 produced the highest overall GY (4611 and 4486 kg ha⁻¹) in both the Hatcher and Goodstreak genetic backgrounds, respectively (Table 3.3). Application of the selection index to the SDLs across all locations in 2010 identified that the populations derived from SYN-1, SYN-2, and SYN-4 produced the highest index of GY and TW. The 14 lines with the highest index values within the six populations derived from these three synthetics were advanced to yield testing in 2011.

Analysis of lines tested in 2011 found significant differences ($P<0.05$) for each trait in each environment. The population GxSYN-4 again showed a reduction of yield relative to the recurrent parent in 2011 (Table 3.4). This year also showed a significant reduction in the average GY for populations HxSYN-2 and HxSYN-4 compared to the recurrent parent Hatcher in the limited irrigation treatment at Greeley. In comparison of the full irrigation treatment at

Greeley, the limited irrigation treatment saw a reduction in grain yield of 21 and 28% in 2010 and 2011, respectively. Grain yield for individual lines within the selected populations was analyzed across all environments in 2010 and 2011. For each environment except GreeleyL11, at least one SDL ranked higher in GY than the adapted recurrent parent (Figure 3.2). A high yield potential was seen in line HxSYN-1-1 (designated as the number 1 in the HxSYN-1 population in each environment in Figure 3.2) because this entry ranked highest for GY among all SDLs when averaged across all environments. This line also outranked its recurrent parent Hatcher for GY in all environments except GreeleyL11 resulting in a 4% increase in yield overall. In general, the SDLs with Hatcher as the recurrent parent showed greater yield improvements over the recurrent parent than those produced from Goodstreak.

Selection of the three most productive SDLs within each of the six populations was conducted based on GY rank across all environments in 2010 and 2011. Overall Eberhart-Russell stability index scores resulted in slopes (b_i) ranging from 0.25 to 1.71 and variances (s^2d_i) ranging from 1.14 to 300.19. This high level of variability was reduced in the selected lines which had slopes ranging from 0.80 to 1.53 and variances ranging from 5.42 to 101.33 across all environments. From each of the 18 BC₁F_{1,3} lines selected for high yield, 104 single spike selections were made for advancement to further testing.

Correlation of traits with grain yield

The correlation of GY with the yield component traits, measured only in 2011 in the side-by-side treatments at Greeley, CO, identified seven and five traits to be significant ($P < 0.05$) under full and limited irrigation, respectively (Table 3.5). Traits significantly ($P < 0.05$) correlated with GY under fully irrigated conditions included PH ($r = -0.22$), BM ($r = -0.36$),

AKW ($r = 0.37$), SN ($r = 0.36$), KNA ($r = 0.40$), GF ($r = 0.21$), and HI ($r = 0.51$). Under limited irrigation conditions traits significantly ($P < 0.05$) correlated with GY included BM ($r = 0.24$), AKW ($r = 0.34$), SN ($r = 0.45$), KNA ($r = 0.47$), and HI ($r = 0.62$). When calculating phenotypic correlations with GY for each SHW population, each trait found significant in the overall correlations was also significant ($P < 0.05$) in at least one population, except for BM under drought (Table 3.6). Harvest index was, as expected, highly correlated with GY in all populations. Kernel number per area showed high correlation ($r = 0.40$ and 0.45 , $P < 0.05$) with GY under drought in the SYN-1 and SYN-4 populations. Average kernel weight was the only trait to be correlated ($r = 0.37$ and 0.43 , $P < 0.05$) with yield under both full and limited irrigation treatments, respectively, for the same population, SYN-4.

Discussion

Improvements in yield of SHW-derived lines in a single backcross

Efficient introgression of traits and reduced cycle time are critically important in crop breeding programs (Fehr, 1987, p. 220). Thus, the practical use of SHW lines in the U.S. Great Plains must incorporate an effective evaluation of SHW material in locally adapted backgrounds with only one generation of backcrossing. A two-step initial evaluation identified the most agronomically sound SHW lines out of a collection of 412 for yield testing in backcross populations. In this research, a single backcross led to SHW-derived $BC_1F_{1:2}$ and $BC_1F_{1:3}$ lines with improved yield rank over the recurrent parent in all but one environment despite the expected overall poor performance of the unadapted, synthetic germplasm (Fehr, 1987, p. 141) (Figure 3.2). When breeding for drought with diverse germplasm sources it is important that the resulting introgression of alleles does not have a detrimental effect on yield in ideal

environments (Lopes and Reynolds, 2011). A significant ($P<0.05$) reduction in GY compared to the recurrent parent was seen for the SHW-derived population GxSYN-3 at GreeleyF10 and Sidney10 and for GxSYN-4 and GxSYN-5 in Lincoln10 (Table 3.3). The GxSYN-4 population also showed a significant ($P<0.05$) reduction in GY compared to the recurrent parent under full and limited irrigation at Greeley in 2011 (Table 3.4). The populations with Hatcher as the recurrent parent showed no significant ($P<0.05$) reduction in yield in 2010, yet in 2011 HxSYN-2 and HxSYN-4 yielded significantly less than Hatcher in the limited irrigation treatment at Greeley.

In general, the variability of GY for SDLs was higher in the 2010 environments. The majority of this variability is at the low end of the yield range and indicates the detrimental effects that are expected when introgressing unimproved germplasm into elite lines (Blum, 2011, p. 221). There is, however, evidence of GY improvement in a number of individual SDLs compared to the adapted cultivars (Figure 3.2). In Figure 3.2, the line which ranked highest overall in GY for the HxSYN-1 population (HxSYN-1-1) also demonstrates stability across environments because it ranked higher than its recurrent parent in all environments except GreeleyL11 (Figure 3.2). It is common to find a crossover effect that occurs when high yielding lines perform poorly under stressed conditions and vice versa (Blum, 2011, p. 60). However, in comparing GY at the high yielding environment of Lincoln10 and the lower yielding Lincoln11, the lines HxSYN-1-1, HxSYN-1-2, and HxSYN-4-2 ranked higher than the recurrent parent in both environments.

The BC_1F_3 SDLs are expected to contain both genotypic and phenotypic variability among plants within each line (Fehr, 1987, p. 34). Therefore, selection and derivation of new lines from single plants within the BC_1F_3 plots may contribute to further improvement in yield

and stability beyond the average of the segregating lines from which they were derived. Under certain scenarios it may be beneficial to increase the number of backcrosses in the development of a population from wild germplasm sources (van Ginkel and Ogbonnaya, 2007; Wang et al., 2009). However, in this study it is shown that it is possible to derive lines which show high yield and yield stability with a single backcross event.

Traits associated with grain yield under full and limited irrigation environments

Evaluation of GY under full irrigation and limited irrigation treatments that are side-by-side within the same field allows for the direct comparison of the effect of available moisture on the productivity of the SDL in the same soil type and atmospheric conditions. Phenotypic correlations with GY identified variable associations for traits under fully irrigated versus drought stressed conditions as well as in different genetic backgrounds. When separating the correlations by the SDLs derived from each SHW line, AKW, SN, KNA, and HI were the only traits to show significance under drought stress (Table 3.6). Relative to adapted cultivars, SHW has been shown to produce larger kernels and kernel weight which contributes to improved GY (Talbot, 2011). Research also suggests that increasing kernel size may be beneficial for milling efficiency and has a commercial incentive as a breeding focus (Marshall et al., 1986). Average kernel weight was negatively correlated with SN and KNA (Table 3.5) under drought indicating the compensatory relationship between these traits previously noted by Slafer et al. (1996). For the SYN-4 populations however, AKW, SN, and KNA each showed a positive significant correlation ($P < 0.05$) with GY under drought (Table 3.6). This indicates that the SYN-4 line may be beneficial in increasing both AKW and KNA.

Production of biomass was significantly correlated ($P<0.05$) with GY, however this correlation was negative under fully irrigated conditions and positive under drought stressed conditions (Table 3.5). The change in sign for correlations of BM with GY indicates the importance of adaptable resource allocation, in terms of sink versus source, under variable conditions. Separated by recurrent parent however, biomass was positively correlated with GY only for those lines derived from the recurrent parent Goodstreak ($r = 0.57$ and 0.37 , $P<0.05$ under full and limited irrigation, respectively). A major phenotypic difference between the recurrent parents in this study is the semi-dwarf plant height status due to the reduced height gene, *Rht-B1b*, found only in Hatcher (Haley et al., 2005). Although the correlation between PH and BM was not significant ($P<0.05$) under fully irrigated conditions, a reduction in plant height associated with *Rht-B1b* is important under fully irrigated conditions to avoid excessive vegetative growth (Butler et al., 2005; Hedden, 2003).

Conclusions

Introgression of diverse genes and traits which are novel to winter wheat breeding populations can be accomplished through the use of spring SHW in a single backcross breeding program. In this study, SDLs selected from backcross populations created with SHW as the donor parent showed transgressive segregation relative to the elite cultivar recurrent parents. An overall reduction in GY was seen for the average of certain SHW populations, however, select SDLs produced with a single backcross showed improvements in yield as well as stability over eight environments across two years in Colorado and Nebraska. The individual SDL that ranked highest overall for GY also out-ranked its recurrent parent Hatcher in all but one environment. Traits positively correlated with yield under both full and limited irrigation included AKW, SN, KNA, and HI. Above ground biomass showed a negative and positive correlation with GY

under full and limited irrigation respectively, however, this varied depending on the recurrent parent background. With the variability found within the SDLs we conclude that further improvements in yield may be possible with continued selection and testing of synthetic-derived germplasm throughout the U.S. Great Plains. Future testing of single head selections and the association of trait variability with molecular markers of synthetic origin will aid in our ability to introgress favorable synthetic diversity into adapted bread wheat germplasm.

Table 3.1. Pedigrees of parents used to make crosses in this study. The synthetic hexaploid parents (SYN-1 through SYN-5) were developed at CIMMYT and pedigrees and plant introduction numbers were obtained from GRIN (<http://www.ars-grin.gov/>). Cultivar pedigrees are from Baenziger et al. (2004) and Haley et al. (2005).

| Parent | Pedigree | Plant Introduction Number |
|--------------------|---|---------------------------|
| SYN-1 [†] | 68112/Ward//Ae. tauschii (369) [‡] | PI 648646 |
| SYN-2 | Garza/Boyerros//Ae. tauschii (241) | PI 648733 |
| SYN-3 | Laru/Ae. tauschii (333) | PI 648758 |
| SYN-4 | Decoy 1/Ae. tauschii (256) | PI 648810 |
| SYN-5 | Decoy 1/Ae. tauschii (322) | PI 648823 |
| Goodstreak | SD3055/KS88H164//NE89646 | PI 632434 |
| Hatcher | Yuma/PI372129//Tam-200/3/4*8Yuma/r/KS91H184/Vista | PI 638512 |

[†] SYN-1 through SYN-5 are arbitrary designations assigned in this study.

[‡] Parentheses indicate *Ae. tauschii* accession number.

Table 3.2. Experimental locations in Colorado and Nebraska for 2010 and 2011 yield trials.

| Location | Lat. / Lon. | Elev. (m) | Prec. [†] (mm) | Irr. amount (mm) | Planting date | Major soil type |
|--------------------------------|----------------------|--------------|----------------------------|---------------------|------------------|--------------------------|
| Greeley F [‡] 2010 | 40.4484 / 104.636 | 1427 | 251 | 195 | 14-Oct 2009 | Olney fine sandy loam |
| Greeley L [‡] 2010 | 40.4484 / 104.636 | 1427 | 251 | 30 | 14-Oct 2009 | Olney fine sandy loam |
| Sidney 2010 | 41.3626 / 102.976 | 1246 | 515 | 0 | 10-Sep 2009 | Alliance loam |
| Lincoln 2010 | 40.8557 / 96.6083 | 358 | 652 | 0 | 16-Oct 2009 | Crete silt loam |
| Greeley F 2011 | 40.4492 / 104.639 | 1427 | 168 | 214 | 8-Oct 2010 | Otero sandy loam |
| Greeley L 2011 | 40.4492 / 104.639 | 1427 | 168 | 87 | 8-Oct 2010 | Otero sandy loam |
| Dailey 2011 | 40.5954 / 102.743 | 1231 | NA [§] | 0 | 28-Sep 2010 | Platner loam |
| Lincoln 2011 | 40.8557 / 96.6083 | 358 | 436 | 0 | 5-Sep 2010 | Crete silt loam |

[†] Precipitation amounts during growing season only.

[‡] F, Full irrigation treatment; L, Limited irrigation treatment.

[§] Error in weather station made precipitation records inaccurate.

Elev., Elevation; Irr., irrigation; Lat., latitude; Lon., longitude; Prec., precipitation.

Table 3.3. Least squares means for grain yield of populations (n = 15 lines/population) tested in four environments in Colorado and Nebraska in 2010.

| Populations | GreeleyF 10 | GreeleyL 10 | Lincoln 10 | Sidney 10 | Grand mean |
|-------------------------|------------------------------------|----------------|---------------|--------------|---------------|
| | Grain Yield (kg ha ⁻¹) | | | | |
| GxSYN-1 | 5618 ± 216 [†] | 4861 ± 272 | 4557 ± 128 | 3823 ± 157 | 4688 |
| GxSYN-2 | 5753 ± 215 | 4737 ± 271 | 4589 ± 127 | 3822 ± 157 | 4691 |
| GxSYN-3 | 5274 ± 215* | 4162 ± 272 | 4239 ± 127 | 3061 ± 156* | 4178 |
| GxSYN-4 | 5528 ± 216 | 4553 ± 272 | 4127 ± 127* | 3341 ± 157 | 4407 |
| GxSYN-5 | 5404 ± 215 | 4690 ± 272 | 4099 ± 128* | 3353 ± 157 | 4398 |
| HxSYN-1 | 6263 ± 212 | 4819 ± 268 | 4326 ± 128 | 3290 ± 157 | 4648 |
| HxSYN-2 | 5590 ± 206 | 4469 ± 272 | 3861 ± 128 | 3183 ± 156 | 4282 |
| HxSYN-3 | 5340 ± 214 | 4201 ± 273 | 3706 ± 127 | 2742 ± 157 | 3986 |
| HxSYN-4 | 5413 ± 226 | 4497 ± 275 | 3735 ± 128 | 3260 ± 157 | 4229 |
| HxSYN-5 | 5745 ± 232 | 4411 ± 275 | 3815 ± 131 | 2834 ± 161 | 4263 |
| Goodstreak [‡] | 6741 ± 694 | 5231 ± 670 | 5165 ± 496 | 4319 ± 600 | 5509 |
| Hatcher [‡] | 6651 ± 694 | 4362 ± 670 | 3867 ± 496 | 3877 ± 600 | 4678 |
| Mean | 5777 | 4583 | 4174 | 3409 | 4496 |
| CV (%) | 12.9 | 13.2 | 15.2 | 13.0 | |

* Significant difference between backcross populations and the respective recurrent parent at the 0.05 probability level.

[†] ± Standard error of the mean.

[‡] Indicates recurrent parent cultivar in backcross populations.

CV, coefficient of variation; GreeleyF, full irrigation treatment at Greeley, CO; GreeleyL, limited irrigation treatment at Greeley, CO.

Table 3.4. Least squares means for grain yield of selected populations (n = 14 lines/population) tested in four environments in Colorado and Nebraska in 2011.

| Populations | GreeleyF 11 | GreeleyL 11 | Lincoln 11 | Dailey 11 | Grand mean |
|------------------------------------|-------------------------|----------------|---------------|--------------|---------------|
| Grain Yield (kg ha ⁻¹) | | | | | |
| GxSYN-1 | 5590 ± 247 [†] | 4164 ± 192 | 3045 ± 110 | 4337 ± 220 | 4284 |
| GxSYN-2 | 5605 ± 247 | 4124 ± 192 | 2830 ± 110 | 4060 ± 221 | 4154 |
| GxSYN-4 | 5354 ± 246* | 3839 ± 192* | 3086 ± 110 | 4136 ± 220 | 4103 |
| HxSYN-1 | 6336 ± 249 | 4412 ± 193 | 3284 ± 110 | 4268 ± 222 | 4575 |
| HxSYN-2 | 5893 ± 247 | 4203 ± 192* | 3002 ± 110 | 4093 ± 220 | 4297 |
| HxSYN-4 | 5974 ± 247 | 4159 ± 193* | 3186 ± 110 | 4273 ± 221 | 4398 |
| Goodstreak [‡] | 6628 ± 572 | 4891 ± 415 | 3543 ± 110 | 4341 ± 416 | 4850 |
| Hatcher [‡] | 6204 ± 573 | 5129 ± 414 | 3442 ± 110 | 4843 ± 414 | 4904 |
| Mean | 5948 | 4265 | 3177 | 4293 | 4446 |
| CV (%) | 14.8 | 16.1 | 9.5 | 18.4 | |

* Significant difference between backcross populations and the respective recurrent parent at the 0.05 probability level.

[†] ± Standard error of the mean.

[‡] Indicates recurrent parent cultivar in backcross populations.

CV, coefficient of variation; GreeleyF, full irrigation treatment at Greeley, CO; GreeleyL, limited irrigation treatment at Greeley, CO.

Table 3.5. Phenotypic correlation coefficients (n = 86) among traits for grain yield and yield components for full and limited irrigation treatments in Greeley, CO 2011. Correlations above and below the diagonal relate to the full irrigation and limited irrigation treatments respectively.

| Traits [†] | GY | TW | PH | BM | KNS | AKW | SN | KNA | DF | DPM | GF | HI |
|---------------------|-------------|--------------|---------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|
| GY | | -0.04 | <u>-0.22</u> [‡] | <u>-0.36</u> | -0.00 | <u>0.37</u> | <u>0.36</u> | <u>0.40</u> | -0.20 | 0.09 | <u>0.21</u> | <u>0.51</u> |
| TW | -0.13 | | <u>0.57</u> | <u>0.36</u> | <u>-0.49</u> | -0.03 | <u>0.49</u> | 0.20 | <u>-0.35</u> | <u>-0.43</u> | -0.08 | -0.10 |
| PH | -0.09 | <u>0.47</u> | | 0.18 | -0.20 | -0.19 | <u>0.24</u> | 0.12 | -0.02 | <u>-0.27</u> | -0.16 | -0.09 |
| BM | <u>0.24</u> | <u>0.27</u> | <u>0.43</u> | | -0.20 | 0.06 | <u>0.58</u> | <u>0.56</u> | -0.14 | -0.13 | -0.02 | -0.01 |
| KNS | 0.06 | <u>-0.40</u> | -0.02 | -0.08 | | -0.18 | <u>-0.44</u> | 0.05 | <u>0.36</u> | <u>0.23</u> | -0.06 | -0.01 |
| AKW | <u>0.34</u> | 0.01 | -0.20 | -0.02 | <u>-0.23</u> | | -0.06 | <u>-0.22</u> | -0.11 | 0.09 | 0.11 | 0.16 |
| SN | <u>0.45</u> | <u>0.29</u> | 0.05 | <u>0.47</u> | <u>-0.43</u> | -0.05 | | <u>0.81</u> | <u>-0.26</u> | <u>-0.35</u> | -0.11 | <u>0.53</u> |
| KNA | <u>0.47</u> | 0.16 | <u>0.22</u> | <u>0.56</u> | -0.06 | <u>-0.21</u> | <u>0.83</u> | | -0.06 | -0.13 | <u>-0.06</u> | <u>0.65</u> |
| DF | -0.10 | <u>-0.28</u> | <u>0.31</u> | -0.03 | <u>0.27</u> | -0.08 | <u>-0.25</u> | -0.12 | | 0.20 | <u>-0.56</u> | -0.08 |
| DPM | -0.06 | -0.02 | 0.03 | -0.03 | 0.03 | 0.02 | -0.11 | -0.04 | 0.07 | | <u>0.63</u> | -0.04 |
| GF | 0.06 | 0.16 | <u>-0.32</u> | -0.01 | <u>-0.23</u> | 0.16 | 0.14 | 0.05 | <u>-0.73</u> | <u>0.55</u> | | 0.01 |
| HI | <u>0.62</u> | -0.06 | <u>-0.22</u> | -0.08 | -0.01 | <u>0.24</u> | <u>0.59</u> | <u>0.65</u> | <u>-0.19</u> | -0.09 | 0.12 | |

[†] GY, grain yield; TW, test weight; PH, plant height; BM, biomass; KNS, kernel number per spike; AKW, average seed weight; SN, spikes m⁻²; KNA, kernel number per area; DF, date of 50% of heads flowering; DPM, date of 50% of flag leaves senesced; GF, number of days in grain fill period from anthesis to senescence; HI, harvest index.

[‡] Underlined correlations are significant at the 0.05 probability level.

Table 3.6 Phenotypic correlation coefficients (n = 28) of traits with grain yield for each synthetic-derived population under full and limited irrigation treatments at Greeley, CO 2011.

| Traits [†] | GreeleyF 11 | | | GreeleyL 11 | | |
|---------------------|--------------------------|-------------|-------------|-------------|-------------|-------------|
| | SYN-1 | SYN-2 | SYN-4 | SYN-1 | SYN-2 | SYN-4 |
| TW | -0.14 | 0.03 | -0.09 | -0.17 | -0.02 | -0.35 |
| PH | <u>-0.49[‡]</u> | -0.15 | -0.14 | -0.19 | -0.06 | 0.00 |
| BM | 0.20 | <u>0.52</u> | 0.16 | 0.23 | -0.11 | 0.35 |
| KNS | 0.04 | 0.16 | 0.00 | -0.06 | 0.20 | 0.11 |
| AKW | 0.32 | 0.27 | <u>0.37</u> | 0.20 | <u>0.39</u> | <u>0.43</u> |
| SN | 0.22 | <u>0.47</u> | 0.15 | <u>0.46</u> | 0.00 | <u>0.38</u> |
| KNA | 0.20 | <u>0.59</u> | 0.27 | <u>0.40</u> | 0.32 | <u>0.45</u> |
| DF | -0.26 | -0.01 | -0.18 | 0.00 | -0.11 | -0.10 |
| DPM | 0.08 | -0.01 | 0.29 | -0.07 | 0.00 | 0.10 |
| GF | 0.29 | -0.05 | <u>0.41</u> | 0.02 | 0.07 | 0.24 |
| HI | <u>0.43</u> | <u>0.50</u> | <u>0.45</u> | <u>0.55</u> | <u>0.64</u> | <u>0.65</u> |

[†] TW, test weight; PH, plant height; BM, biomass; KNS, kernel number per spike; AKW, average seed weight; SN, spikes m⁻²; KNA, kernel number per area; DF, date of 50% of heads flowering; DPM, date of 50% of flag leaves senesced; GF, number of days in grain fill period from anthesis to senescence; HI, harvest index.

[‡] Underlined correlations are significant at the 0.05 probability level.

GreeleyF, full irrigation treatment at Greeley, CO; GreeleyL, limited irrigation treatment at Greeley, CO.

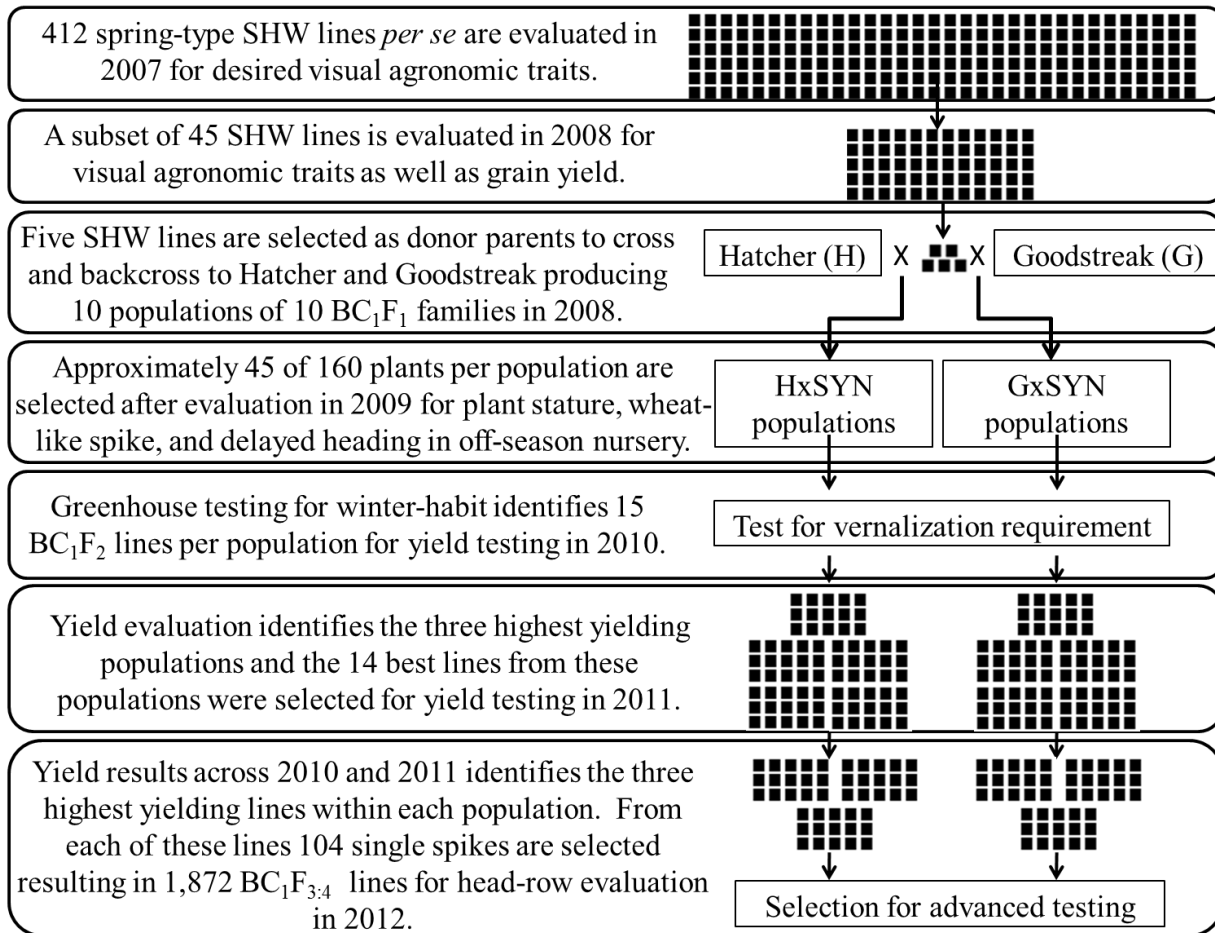


Figure 3.1. Schematic of pre-breeding procedures and selection events.

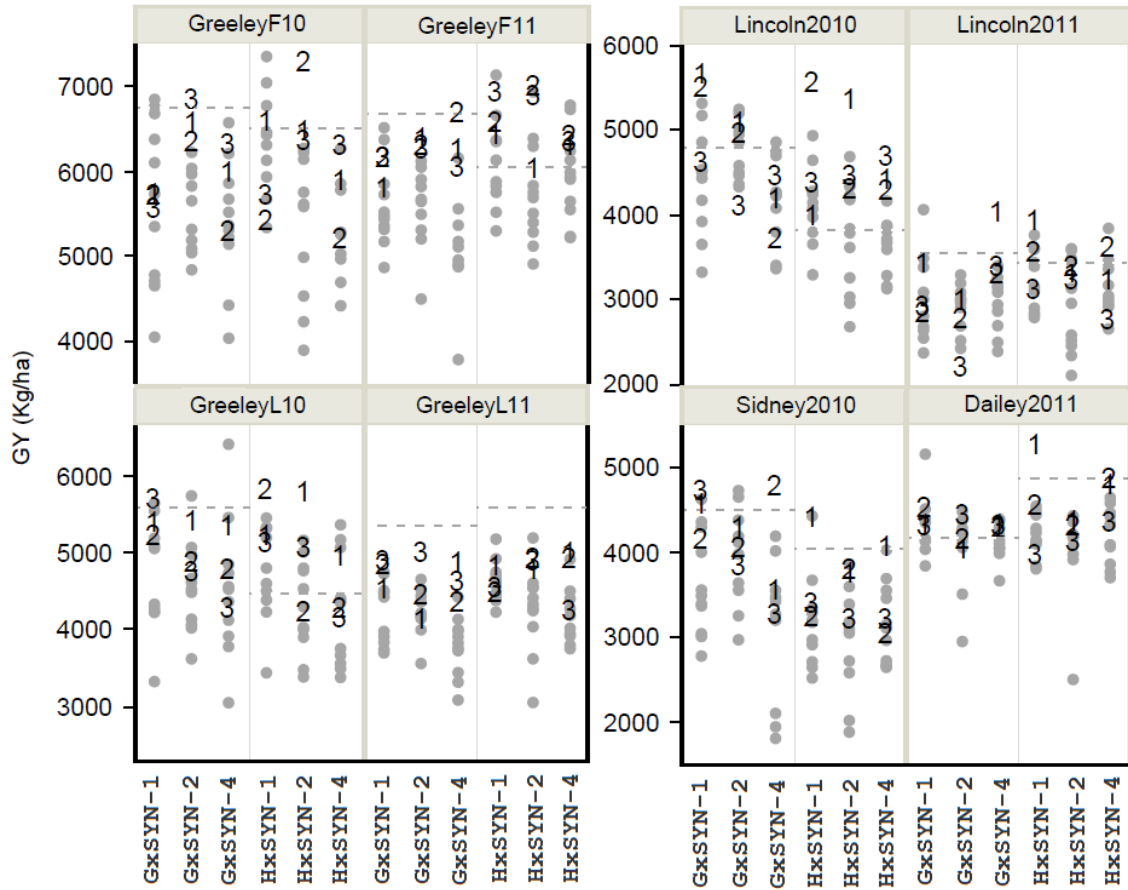


Figure 3.2. Distribution of grain yield for all lines within selected populations in each environment. Rankings across all environments within a population were determined. The numbers 1 to 3 indicate the top three SHW-derived lines across all environments within a population. Dashed lines indicate the average yield for each recurrent parent within each environment.

CHAPTER FOUR:

SYNTHETIC HEXAPLOID WHEAT IMPROVES WINTER WHEAT YIELD IN THE U.S.

GREAT PLAINS

Summary

Synthetic hexaploid wheat (SHW) has shown promise for improving bread wheat (*Triticum aestivum* L.) yield and agronomic performance. This study was conducted to assess the results of introgressing SHW into winter wheat. Ninety BC₁F_{3.5} synthetic-derived lines (SDLs) selected from crosses of three SHW accessions and the recurrent parent 'Hatcher' were grown in three irrigated and rainfed environments in Colorado. These SDLs showed a wide range of grain yield (GY) with the highest yielding line reaching 115% of the recurrent parent. Four SDLs outranked Hatcher for GY in all three environments with two lines ranking higher than all adapted check cultivars in both irrigated and rainfed conditions. Of several agronomic traits evaluated, harvest index and canopy temperature showed the highest correlations with GY under drought stress ($r = 0.55$ and -0.53 , respectively, $P < 0.05$). Average kernel weight was the only trait correlated ($r = 0.21$, $P < 0.05$) with GY under rainfed conditions only. When canopy temperature was measured every two hours for a single day during grain filling, measurements at 0900 and 1730 h had the highest correlations ($r = -0.44$ and -0.40 , respectively, $P < 0.0001$) with GY under drought. Genotyping-by-sequencing data for the SDLs identified 13 single nucleotide polymorphisms with an allele of synthetic origin showing preferential selection over the adapted allele after several generations of selecting for GY. Our results demonstrate improvements in yield and agronomic performance in SDLs relative to elite cultivars. Therefore we conclude that

SHW has good potential for the introgression of beneficial diversity into elite winter bread wheat in the U.S. Great Plains.

Abbreviations: AKW, average kernel weight; BM, above ground biomass; CTg, average canopy temperature during grain fill period; CTv, average canopy temperature during vegetative growth; DF, days to 50% of spikes with visible anther extrusion; DPM, days to 50% of peduncles senesced, GF, grain fill period; GY, grain yield; HI, harvest index; NDVIg, normalized difference vegetative index during GF; NDVIv, normalized difference vegetative index during vegetative growth; SN, spike number m⁻²; KNA, kernel number m⁻²; KNS, number of kernels per spike; SHW, synthetic hexaploid wheat; SDL, SHW-derived line TW, test weight.

Introduction

As the world's most widely dispersed food crop, wheat (*Triticum* spp.) is produced on approximately 22% of currently cultivated land (Leff et al., 2004). Over 240 million ha are dedicated to wheat crops yielding upwards of 600 million tons of grain worldwide (Dixon et al., 2009). This wide distribution of environments and large demand for consumption requires the adaptability of wheat to various environmental stresses. Drought is the most yield limiting factor for cereals in many years and environments throughout the world (Araus et al., 2002).

Increasing the tolerance of wheat to abiotic stresses such as drought is therefore essential for future global food security. One approach to accomplishing this is the introgression of genotypic diversity from synthetic hexaploid wheat (SHW) (*X Aegilotriticum* spp.), which has proven beneficial in increasing yield in various genetic backgrounds (Trethowan and Mujeeb-Kazi, 2008; van Ginkel and Ogbonnaya, 2007). The International Maize and Wheat Improvement Center (CIMMYT, El Batan, Mexico) produces SHW lines (genome AABBDD) through

interspecific hybridization between durum wheat (*T. turgidum* L.) varieties, donor of the genomes AABB, and accessions of wild goat grass (*Aegilops tauschii* L.), contributor of the DD genome (Lopes and Reynolds, 2011). These SHW lines are then crossed to hexaploid bread wheat (*T. aestivum* L.) in CIMMYT's breeding program as well as made available to breeders throughout the world.

The improvement of diversity in adapted bread wheat germplasm via crossing with SHW allows for the introgression of novel SHW genes and SHW-by-elite gene interactions from multiple genomes simultaneously (Ogbonnaya et al., 2013). Although the increased time associated with introgressing wild genes into adapted germplasm may be considered a hindrance, the use of SHW derived from cultivated durum accessions may only require a single backcross event (Ogbonnaya et al., 2013; Wang et al., 2009). Spring SHW lines have been shown to improve yield potential and adaptation to drought in spring wheat cultivars (del Blanco et al., 2000; Lopes and Reynolds, 2011; Ogbonnaya et al., 2007; Trethowan and Mujeeb-Kazi, 2008). These studies have shown not only improvements in the yield of SHW-derived lines (SDL), but also benefits from traits such as improved photosynthetic capacity, greater proportion of root dry weight in deep soil layers, early maturity, and stability of yield in many drought stressed environments. This research focuses on yield trials of SHW-derived winter wheat lines selected after a single backcross with the following objectives: to identify SDLs that show consistent high yield across multiple environments in Colorado; to assess SDLs for yield component traits which offer insight into their drought stress tolerance mechanisms; and to identify alleles, contributed by the SHW donor parents, that show a selective advantage over the adapted recurrent parent alleles.

Materials and Methods

Derivation of lines for advanced yield testing

Previous research identified six SHW-derived BC₁F₃ populations having the highest yield in four Colorado and Nebraska environments in 2010. Each population consisted of a SHW donor parent (designated SYN-1, SYN-2, and SYN-4) crossed and backcrossed to either adapted cultivar ‘Goodstreak’ or ‘Hatcher’ (Baenziger et al., 2004; Haley et al., 2005). Fourteen BC₁F_{1:3} lines within each of the six populations were tested in eight environments in 2010 and 2011, and from the three highest yielding lines individual spikes were harvested to develop families of 104 single plant-derived lines. These spikes were threshed individually and seed collected from each spike was planted at the Colorado State University Agricultural Research, Development, and Education Center (ARDEC) (40.6525 °N; 105 °W; Elevation, 1560 m) in Fort Collins, Colorado on 28 Sept. 2011. Plots consisted of two rows, 91 cm long with 23 cm between rows and 28 cm between plots. Each family of 104 spike-derived plots was planted in an unrandomized fashion to allow for visual selection within families. The recurrent parents were planted at the beginning and middle of each family as a visual check for selection. Plots were fully irrigated through all growth stages by a linear overhead sprinkler irrigation system. A selection of 10 lines from each family of 104 BC₁F_{3:4} lines was made, creating the nomenclature used to identify each line (e.g., HxSYN-1-1-01 to HxSYN-1-1-10 are the lines derived from the population HxSYN-1 (Hatcher as the recurrent parent backcrossed to SYN-1), family number 1 and lines 1 to 10 within this family). Selection of these lines was based on a visual score of yield component traits such as density of tillers and spike size, wheat-like spike morphology, similar maturity date relative to the recurrent parent, and disease and lodging resistance. At maturity, selected plots were hand harvested and threshed with a Vogel thresher (Bill’s Welding Shop, Pullman, WA).

Experimental design and environments

Yield trials for the selected SDLs involving the recurrent parent Hatcher were planted in three environments for the 2012-2013 growing season. Experimental design for the Hatcher-derived populations consisted of two replicates of 90 BC₁F_{3.5} SDLs (30 lines from each population) in an incomplete block, Latinized row-column design created with CycDesign 3.0 (CycSoftware, 2006). The first environment was rainfed at Dailey, CO (40.5954 °N; 102.743 °W; Elevation, 1230 m). At Dailey, a total of 104 entries were planted, including nine entries of bulked seed from the BC₁F₃ lines from which the single plant selections were derived, along with the cultivars ‘Byrd’, Hatcher, ‘McGill’, ‘Ripper’, and ‘Robidoux’ (Baenziger et al., 2011, 2012; Haley et al., 2007, 2012a). At the ARDEC location, side-by-side full irrigation (ARDEC IR) and rainfed only (ARDEC RF) treatments were grown. Bulk seed entries were not included but the additional cultivars of ‘Denali’, Goodstreak, ‘Prairie Red’, and ‘Settler CL’ were used for a total of 99 entries (Baenziger et al., 2011; Haley et al., 2012b; Quick et al., 2001). Plots in all environments consisted of six rows, 23 cm apart with 28 cm between plots. The ARDEC plots were 1.8 m long and the Dailey plots were 3.7 m long. Supplemental irrigation of 38 mm was applied to both the ARDEC IR and ARDEC RF treatments prior to planting. All environments were planted at a population density of approximately 280,000 seeds ha⁻¹. The trial at Dailey was planted on 25 Sept. 2012 and the trials at ARDEC were both planted on 2 Oct. 2012. Soil moisture at planting was assumed to be at field capacity for ARDEC following irrigation and rainfall events. Dailey soil moisture at planting is unknown but was considerably drier. Irrigation was supplied for ARDEC IR by a linear overhead sprinkler irrigation system. Early spring rainfall and cool temperatures did not allow for irrigation to begin until 21 May 2013 at approximately the booting stage, Zadoks scale 40 (Zadoks et al., 1974). Once initiated, irrigation

was applied to ARDEC IR on a weekly basis with the first three applications at 31 mm, the fourth and fifth application at 38 mm, and the sixth application at 12 mm (Figure 4.1).

Synthetic hexaploid wheat-derived lines developed with the recurrent parent Goodstreak were tested in a single location at Lincoln, NE with two replicates in an alpha-lattice incomplete block design. Planting occurred on 11 Sept. 2012. This location included 90 SHW-derived lines (30 lines from each population) along with the check cultivars used at the Dailey location.

Agronomic and physiological measurements

Grain yield (GY) was the only trait measured in all environments. Traits measured or calculated for the ARDEC environments included early vigor (NDVI_{ev}), canopy temperature (CT), normalized difference vegetative index (NDVI), days to flowering (DF), days to physiological maturity (DPM), plant height (PH), grain fill period (GF), above ground biomass (BM), average kernel weight (AKW), spike number m⁻² (SN), kernel number per spike (KNS), kernel number per area (KNA), test weight (TW), and harvest index (HI). Plots at ARDEC were harvested with a Hege combine (Wintersteiger, Salt Lake City, Utah) for measurements of GY and TW. Grain samples were weighed for calculation of GY, and TW was measured by hand with a 151 filling hopper (Seedburo, Des Plains, IL). At Dailey, plots were combine harvested and GY was recorded with an on-combine HarvestMaster GrainGage (Juniper Systems, Logan, UT). A GreenSeeker Handheld (Trimble, Ukiah, CA) was used for measuring NDVI, and NDVI values at the spring green-up growth stage (Zadoks scale 14 to 16) were used as a proxy for quantifying NDVI_{ev}. A CS-1000 datalogger (Cambell Scientific, Logan, Utah) was used to record data simultaneously from both an SI-131 infrared radiometer and an ST-100 thermistor air temperature sensor (Apogee Instruments, Logan, Utah) for use in measuring CT. Measurements

for both NDVI and CT were made in a serpentine walking pattern at repeated intervals through the late vegetative to early senescence period. Data presented for CT and NDVI were the means of 10 and 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 plot at a perpendicular angle to the rows of the plots. Similar to Prasad et al. (2007), average canopy temperature in the vegetative growth stage (CT_v) and the grain fill period (CT_g) was calculated as an average of the CT values made from 27 May to 3 June and 11 June to 21 June, respectively. On 21 June 2013, CT and NDVI measurements were made repeatedly every two hours starting at 0700 through 1730 h. Days to flowering was recorded as the number of days from 1 Jan. to the day when anther extrusion was visible on 50% of the spikes. Days to physiological maturity was recorded as the number of days from 1 Jan. to the day when 50% of the peduncles were senesced. Grain fill period was calculated as the number of days between DF and DPM. Above ground biomass was measured at maturity as the dry biomass of a 1 m-long section of a single row per plot. After drying and weighing the biomass sample the biomass grain was threshed with a Vogel thresher. A random sample of 10 spikes per plot was collected and hand threshed in a Hege 16 thresher (Wintersteiger, Salt Lake City, UT). The 10-spike grain and biomass grain were used for calculation of AKW, KNS, SN, KNA, and HI as follows.

$$\text{AKW} = 10\text{-spike seed count} / 10\text{-spike seed weight}$$

$$\text{KNS} = 10\text{-spike seed count} / 10$$

$$\text{SN} = \text{Biomass seed weight} / (\text{AKW} \times \text{KNS}) / 0.23 \text{ m}^2$$

$$\text{KNA} = \text{KNS} \times \text{SN}$$

$$\text{HI} = \text{Biomass seed weight} / \text{Total biomass weight}$$

Soil moisture samples during spring green-up were collected with a 3 cm diameter JMC Dry soil probe (Clements Associates, Inc. Newton, IA) at ARDEC on 5 Feb. 2013 from six random probe sites across both treatments and at Dailey on 7 Feb. 2013 at three random probe sites. Gravimetric water content was averaged for the upper soil profile (0 to 51 cm) and lower soil profile (52 to 101 cm) across all probe sites in each location: $20.3 \pm 1.0\%$ (upper profile) and $23.4 \pm 2.7\%$ (lower profile) for the ARDEC location; $22.0 \pm 1.8\%$ (upper profile) and $24.8 \pm 1.4\%$ (lower profile) for the Dailey location.

Genotyping-by-sequencing and molecular analysis

Genotyping-by-sequencing (GBS) library preparation was conducted according to Poland et al. (2012) on the 90 SDLs which had Hatcher as the recurrent parent, along with bulked BC₁F₂ lines from which they were derived and all of the parental lines. A bulk sample of leaf tissue from each genotype was collected from greenhouse flats of 10 random plants per entry at Zadoks stage 11. DNA was extracted with a DNeasy 96 Plant Kit (Qiagen, Inc., Valencia, CA) and barcoded for 96-plex multiplexing for sequencing (Poland et al., 2012). Parental and BC₁F₂ genotypes were included in two separate GBS libraries in order to achieve deeper coverage of markers. Sequencing was conducted on an Illumina HiSeq2000 (Illumina, San Diego, CA) by the DNA Core Facility at the University of Missouri - Columbia. The unfiltered, raw sequences were assigned to individual samples according to their barcodes and trimmed to 64 base pairs using the Tassel v. 4.2.1 Pipeline Java script for mapping GBS sequence reads (<http://www.maizegenetics.net/tassel/docs/TasselPipelineGBS.pdf>). Each tag was analyzed for single nucleotide polymorphisms with a GBS produced reference map developed with a biparental mapping population in which one parent was a SHW line (Poland et al., 2012).

Statistical analysis

Field data

Unless otherwise stated all statistical procedures were conducted in SAS software 9.2 (SAS Institute, 2008). A PROC MIXED procedure was used to generate best linear unbiased predictions (BLUPs) for each trait in each environment. Because of unknown spatial variation within each environment, six different spatially adjusted models were run to adjust trait values to account for this variability. The first model accounted for variation in rows and columns and the following five models adjusted for the following covariance structures: spherical, exponential, power, anisotropic power, and Matérn (<http://support.sas.com/documentation/>, Table 56.14, accessed 02 September 2013). Each model was compared and the model with the lowest Akaike information criterion value was chosen for use. In addition to the adjustments for spatial variation, an edge effect was present in the outside row of each treatment at ARDEC which was believed to be due to reduced irrigation amounts near the wheel tracks of the linear irrigation rig. This edge effect was accounted for by the use of a categorical covariate for those plots which were affected. The first row of the rainfed treatment and the last row of the full irrigation treatment were adjusted based on the average of all other plots within each treatment. Air temperature measured simultaneously with CT was used as an additional covariate in the analysis of CT. Trait values for each of the three populations of SDLs were calculated as the average of the BLUP values for lines within each population similar to Talbot (2011). Spearman rank correlation coefficients were calculated for GY among the different environments, excluding the bulked BC₁F₄ entries at Dailey prior to rank classification. For all traits, plot mean heritability (h^2) was calculated from variance components obtained by a PROC GLM procedure as:

$$h^2 = \frac{\sigma^2_G}{(\sigma^2_G + \sigma^2_{GE} + \frac{\sigma^2}{rn})}$$

where σ^2_G is the amount of genetic variance, σ^2_{GE} is the amount of genotype by environment variance, σ^2 is the error variance, r is the number of replicates and n is the number of environments.

As the genotype by environment effect for several of these traits made the combined h^2 values unexpectedly low, h^2 was also calculated on a per environment basis for comparison. Pearson phenotypic correlation coefficients were obtained between BLUPs for all traits from the ARDEC environments and the corresponding GY values for those environments in JMP Pro 9.0.2 (JMP, 2010). Scatterplots of GY as well as regression analysis graphs of GY on CT were produced in JMP Pro 9.0.2.

Genotyping-by-sequencing data

Data produced from the Tassel pipeline were assembled into HapMap format for analyzing single nucleotide polymorphism (SNP) markers. Lines which were sequenced in different libraries were merged into a single entry for each genotype. Synthetic hexaploid wheat-derived lines were parsed into separate files according to their SHW population. Analysis of a selective advantage of alleles, for only those markers where the BC₁F₂ bulk entry was called heterozygous, was done with a binomial distribution probability analysis (function BINOMDIST) in Microsoft Office Excel (Microsoft, 2007) (Zhang et al., 2005). Analysis of only those markers which were heterozygous in the bulk seed lot from which the advanced lines were derived was done to ensure that both alleles were present for potential selection. A selective advantage of an allele was determined at a probability level of 0.05. Though multiple

tests were done causing the probability of a Type I error to rise, no adjustment for multiple testing was done in order to ensure the identification of any potential allele of SHW origin for further testing.

Results

Environmental variation in yield

Grain yields were highest at ARDEC IR followed by Dailey and ARDEC RF (Table 4.1). Spearman rank correlation coefficients of GY among environments indicated that entry mean yields at Dailey were significantly ($P < 0.05$) correlated with those at both ARDEC IR and ARDEC RF ($r = 0.29$ and 0.39 , respectively, $n = 99$). However, there was not a significant correlation between GY rank at ARDEC IR and ARDEC RF. The effect of the drought stress treatment at ARDEC resulted in an average reduction in GY of 48.3% for ARDEC RF compared to ARDEC IR. The recurrent parent Hatcher showed a 49.3% reduction in GY, whereas the SDL HxSYN-1-2-06 had the smallest reduction in GY at 34.7%. However, this SDL also ranked as the 92nd lowest yield out of the 99 entries in the ARDEC IR treatment. Days to flowering had a mean of 157 days after 1 Jan. with a range of 155 to 160. The flowering date of the recurrent parent Hatcher equaled the mean of all entries. Reduction in the rainfed treatment for other yield component traits varied: BM (35.4%); DF (0.4%); DPM (4.2%); GF (37.7%); KNS (15.3%); SN (1.8%); KNA (14.6%); AKW (32.9%); and HI (14.4%).

Grain yield for synthetic hexaploid wheat-derived lines

The recurrent parent Hatcher yielded above average in each environment and a wide range of BLUP values for GY was seen for the SDLs (Table 4.1, Figure 4.2). The population derived from Hatcher and SYN-1 (HxSYN-1) averaged the highest GY of the SHW-derived

populations in the lower yielding environments of ARDEC RF and Dailey, however, this difference was slight and overall there was no significant difference in the population means. There was also no difference in GY between any SDL and Hatcher according to overlapping 95% confidence intervals of the BLUP values. However, for the SDLs within each population a significant ($P<0.05$) difference was seen. Individual lines yielded up to 109% of the recurrent parent Hatcher at ARDEC IR, 113% at ARDEC RF, and 115% at Dailey. In addition, nine, three and five SDLs out ranked all checks at ARDEC IR, ARDEC RF, and Dailey, respectively. No single SDL out yielded all check cultivars in all environments; however, two lines, HxSYN-1-1-08 and HxSYN-4-2-10 out ranked all checks in both ARDEC IR and Dailey, and HxSYN-1-1-06 and HxSYN-4-2-08 out ranked the recurrent parent Hatcher in all three environments (Figure 4.2).

Analysis of yield components at ARDEC

Phenotypic correlations between GY and all other traits at ARDEC IR and ARDEC RF revealed 21 and 20 significant ($P<0.05$) correlations, respectively (Tables 4.2 and 4.3, Appendix 5). All significant correlations with GY were positive except for DF at ARDEC RF and CT in both treatments. Average kernel weight was the only trait found to be significant ($r = 0.22$, $P<0.05$) under drought but not in irrigated conditions. Canopy temperature measurements throughout the day indicated contrasting results between the irrigated and rainfed treatment. In ARDEC IR, CT was not significantly ($P<0.05$) correlated with GY until 1100 h ($r = -0.30$) and this negative correlation increased to a max of -0.42 at 1300 h (Table 4.3). The opposite was true under rainfed conditions when correlations of CT and GY were strongest in the morning (0900 h) and decreased through midday (Table 4.3). Under rainfed conditions an increase in correlation between CT and GY occurred between 1300 and 1730 h as air temperatures and solar

irradiance began to drop. Trait heritability, measured across the treatments at ARDEC was highest for the traits associated with phenology (DF and DPM, $h^2 = 0.91$ and 0.69 , respectively).

The population derived from SYN-1 consistently averaged the lowest BM production in both ARDEC IR and ARDEC RF at 397 and 258 g respectively. However, this same population had the highest average HI score for ARDEC IR. The AKW among populations was found significantly ($P < 0.05$) different under rainfed conditions only. Population averages for AKW for HxSYN-1, HxSYN-2, and HxSYN-4 were 27.7, 26.2, and 29.0 mg, respectively. Populations HxSYN-1 and HxSYN-4 showed improvement in AKW over the recurrent parent Hatcher which averaged 26.3. Spike number was also significantly ($P < 0.05$) different among populations under the irrigated treatment. However, SN was reduced for all three SHW population averages relative to Hatcher. The population HxSYN-1 consistently showed the highest (worst) CTv and CTg in both environments despite having the highest average yield under drought. Correlations among all traits measured are available in Appendix 5.

Analysis of genotyping-by-sequencing molecular markers

Genotyping-by-sequencing reads from this study were mapped against sequences from a similar bi-parental GBS study (Poland et al., 2012), identifying 11,159 SNP markers between the SHW lines *per se* and the parental cultivar Hatcher. Marker counts for the A, B, and D genome were 2989, 5729, and 2441, respectively. Genetic distances among the SDLs in each population as well as the parental varieties can be seen in Appendices 6 through 8. Those markers not heterozygous in at least one of the BC₁F₂ entries from which the SDLs were selected were eliminated leaving 2900 SNPs for analysis. Counts of SNP markers per chromosome ranged from 54 to 462 with an average of 212 markers per chromosome. Out of the 2900 SNPs

identified for the lines with Hatcher as the recurrent parent, a total of 105 markers (3.6%) were identified as alleles which were preferentially selected in the backcross-derived lines (binomial probability of 0.05). Of these markers, 13 were found to have a selective advantage for the allele of SHW origin (Table 4.4). The HxSYN-4 population showed 11 loci with preferentially selected SHW alleles and the HxSYN-1 population showed only two. The HxSYN-2 population showed no selective advantage for SHW alleles. The percent of the markers per SDL that were called as synthetic in origin ranged from 10 to 51%. However, no association between GY and the percentage of synthetic alleles in the individual SDLs was found.

Discussion

Performance of synthetic derived populations

Variation in GY was apparent in all environments, however, the effect of the moisture treatment at ARDEC was the most prominent environmental difference as seen in the Spearman rank correlation coefficient results. The crossover effect of GY rank between the high yielding and low yielding environments indicates the importance of targeting different lines for testing and production under different environmental conditions (Chenu et al., 2011) (Table 4.1). For example, in the lower yielding environments of ARDEC RF and Dailey the HxSYN-4 population showed the largest range of yield and produced the highest yielding SDLs in these environments indicating that these conditions worked well to discriminate among lines for high yield in this population. In ARDEC IR, however, this same population showed the smallest range of yield as well as the lowest overall yield, again indicating this population is better suited for low yield environments.

Performance of individual synthetic hexaploid wheat-derived lines

The performance among all SDLs varied significantly ($P < 0.05$) in all environments. Though the population averages at each location were lower than the recurrent parent, two SDLs, HxSYN-1-1-06 and HxSYN-4-2-08, out ranked the recurrent parent Hatcher in all three environments (Figure 4.2). Synthetic-derived lines HxSYN-1-1-08 and HxSYN-4-2-10 out yielded not only the recurrent parent but also all checks in both ARDEC IR and Dailey (Figure 4.2). It may not be typical to see this type of performance from individual lines grown in environments with as much variability in average yield (Blum, 2011, p. 60), however, it is possible that the increased level of genetic diversity contributed by the SHW donor parents contributed to an increased level of trait plasticity between environments (Warburton et al., 2006). The four best ranking SDLs along with two other SDLs, HxSYN-4-2-05 and HxSYN-2-3-03, each yielded more grain across all environments than any check cultivar when GY was standardized against each environment mean. Figure 4.3 demonstrates the crossover effect that can be seen in multiple lines when compared across environments. However, when targeting non-irrigated locations in Colorado the SDL HxSYN-2-3-03 stood out as a leading GY producer (Figure 4.3). Though this line had a reduction in yield under full irrigation it may possess certain drought tolerant traits, such as low CT and recovery of low CT after stress (Figure 4.4) that allow for the increase in yield under stress.

Yield components and correlation of traits with grain yield

Correlation of yield components with GY indicated various traits which differed for importance under irrigated and rainfed conditions. Under rainfed conditions, which resulted in terminal drought stress (Figure 4.1) and a yield reduction of 48.3%, AKW showed a significant

correlation ($r = 0.22$, $P < 0.05$) with GY which was not the case in the irrigated treatment (Table 4.2). Kernel weight of SDLs has been previously shown to increase relative to a recurrent parent (Talbot, 2011). This was also the case in this study where under drought stressed conditions the overall AKW for populations HxSYN-1 and HxSYN-4 was larger than the recurrent parent Hatcher. The opposite was true for KNS where this trait was significant ($r = 0.33$, $P < 0.05$) only in irrigated conditions. The traits SN and KNS, components which comprised KNA, were minimally affected by the moisture treatments as they showed a 1.8 and 15.3% difference in treatment means, respectively, compared to a 32.9% reduction in AKW. Though the yield component traits of AKW and KNA typically have a negative relationship (Slafer et al., 1996), five SDLs, HxSYN-1-2-03, HxSYN-1-2-07, HxSYN-1-3-03, HxSYN-4-2-02, and HxSYN-4-3-03, showed a 2 to 14% increase in AKW as well as a 1 to 6% increase in KNA, relative to the recurrent parent.

The variation in traits related to spike development (AKW, SN, and KNS) indicates the importance of maintaining grain fill during late season drought stress (Blum, 2011, p. 134). The lack of treatment difference through booting (Zadoks stage 40) resulted in only a small difference in KNA between treatments relative to the change in AKW. Those entries with greater KNA under irrigation had a greater sink to capture more assimilates where under drought those with greater assimilate source capacity were able to maintain kernel weight. Because of this, the resulting yield reduction for the rainfed treatment was primarily associated with the lower assimilate source capacity not being able to completely fill the number of grains that were already set. On the other hand, under irrigated conditions sink capacity is often the limiting factor (Zhang et al., 2010). Because the heritability of AKW across treatments was relatively large ($h^2 = 0.66$), utilizing AKW as a secondary selection criterion may lead to an overall

increase in GY when breeding for drought stressed environments. This would also be beneficial in meeting commercial milling requirements (Marshall et al., 1986; Talbot, 2011).

An earlier flowering date also showed a significant correlation with GY under drought stress as expected (Lopes and Reynolds, 2011); however, an increase in GF period was not associated with an increase in GY. This may be due to the fact that under stress the majority of the entries entered senescence on roughly the same date.

Association of canopy temperature with grain yield

A reduction in canopy temperature was highly correlated with increasing GY under both irrigated and rainfed conditions similar to previous studies (Lopes and Reynolds, 2010, 2011; Olivares-Villegas et al., 2007; Reynolds and Trethowan, 2007) (Tables 4.2 and 4.3). It is presumed that the 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, CT_v was found to be more negatively correlated with GY values than was CT_g under both treatments at ARDEC (Table 4.2). It was expected that maintaining a cooler canopy during the greater stress levels seen during grain fill would relate to an increase in GY. It has been reported that variation in spike morphology after heading as well as the number of spikes per area can confound results for CT_g compared to CT_v (Hatfield et al., 1984). Heritability was also relatively low for both CT_v and CT_g under both irrigated and rainfed conditions (Table 4.2). This may be indicative of the range of various traits that contribute to maintenance of CT (Blum, 2011, p. 189) as well as variation in the environmental differences during CT collection on different days.

Measurements of CT taken at two hour intervals starting at 0700 h over the course of a day indicated a treatment effect as seen by the difference in time periods when cooler canopies were more highly associated with GY. Under irrigated conditions, CT had the strongest association with GY at 1300 h, indicating those entries which maintained greater transpiration at midday improved their GY (Izanloo et al., 2008) (Table 4.3, Figure 4.4). This association was reversed when entries were under drought stress where correlations were strongest in the morning at 0900 h and again at 1730 h. This suggests that the drought stress at mid-day was so extreme that differences among entries were obscured. These results do not agree with previous research that suggested CT under drought and irrigation was more strongly correlated with GY in the afternoon and morning, respectively (Olivares-Villegas et al., 2007). However, variation in the severity of drought stress and phenology during measurement may contribute to the differences seen between studies.

Associations of NDVI with GY in ARDEC IR increased slightly throughout the morning through 1300 h similar to CT. Andrade-Sanchez et al. (2013) suggests that this effect is due to leaf wilting that occurs as air temperatures rise and transpiration slows, even under full irrigation, exposing more of the soil. The increased associations of both CT and NDVI with GY under drought at 1730 h may be explained by a stronger recovery response resulting in improved GY as seen in the research by Vassileva et al. (2011). Figure 4.4 demonstrates the effects of recovery versus maintenance of transpiration as two of the highest yielding SHW-derived lines under drought conditions varied in their canopy temperature responses relative to the average. Lines HxSYN-4-2-02 and HxSYN-2-3-03 both showed cooler than average CT in the morning at 0900 h. At 1300 h however, it is assumed that line HxSYN-2-3-03 closed its stomates to preserve moisture until later recovery at 1730 h when it was again cooler than average. Yet, line HxSYN-

4-2-02 maintained better than average CT throughout the day, possibly contributing to its higher GY. Both of these characteristics can be said to be beneficial under some form of drought stress, however, it is a common conclusion that sustaining stomatal conductance and thus low CT is ideal for maximizing GY as long as available soil moisture is not exhausted (Blum et al., 1982; Fischer et al., 1998; Izanloo et al., 2008).

Selective advantage of synthetic genome regions

Following backcross introgression of synthetic diversity into the adapted cultivar Hatcher, GBS allowed for the identification of certain alleles of the synthetic genome that showed a potential selective advantage through several selection procedures (Table 4.4). In general, there was no association between the percentage of synthetic alleles retained in the SDLs and their GY under any environment (not shown). However, when analyzing the individual markers across each SHW-derived population several alleles from the SHW donor parent were present more often than the recurrent parent allele suggesting a selective advantage for the synthetic allele. Thirteen markers suggested a significant ($P < 0.05$) selective advantage for the synthetic allele (Poland et al., 2012). Eleven of these markers were found in the SYN-4 population and two markers in the SYN-2 population.

Eight of the 21 chromosomes of bread wheat were represented by selected synthetic alleles. Those chromosomes which do not contain synthetic alleles of selective advantage may be affected by a linkage drag associated with the selection of essential adaptation genes found in Hatcher. Prior to field testing winter x spring SHW crosses a test for vernalization is required. Those genotypes which successfully demonstrate the need for vernalization must also contain the major vernalization genes, the *Vrn-1* homoeologous genes, that are located on chromosome 5L

of each genome (Snape et al., 2001). A marker mapping to chromosome 5D on the reference map was identified as having a selective advantage for a SHW allele (Table 4.4). However, due to only a single backcross in the development of the lines used in this experiment it is expected that a major area associated with the loss of potentially beneficial SHW alleles is the area surrounding the vernalization genes of Hatcher.

A majority of the SNP markers (51%) identified in the GBS tags were found on the B genome. Therefore, it is not surprising that the majority (69%) of the selected synthetic alleles were also located on the B genome. It has been suggested that the introgression of the durum genomes (AABB) through SHW, often adapted to warmer climates, could be detrimental to winter varieties in the U.S. Great Plains (Cox, 1997). However, due to the increase in yield and agronomic performance identified in several SDLs, this research suggests otherwise.

The selective advantage of alleles was only considered to be significant ($P < 0.05$) if the BC₁F₂ genotype from which each BC₁F_{3.5} family was derived was called heterozygous for the marker in question. It may be argued that with an alpha level of 0.05 multiple testing across all markers allows too strong a possibility of a Type I statistical error. However, because this was exploratory research, the risk of this error was considered acceptable in order to identify regions of synthetic origin which should be explored further. Overall, due to the large amount of missing data that commonly occurs in GBS procedures, the number of SNP markers that were found to have a selective advantage for either allele was low despite the expected preferential selection of a majority of the adapted alleles from the recurrent parent.

Conclusion

As climates become more variable and unpredictable, improvements in yield and yield stability of bread wheat in the U. S. Great Plains will become more critical. The efficient introgression of novel genetic diversity found in relatives of bread wheat is simplified through the use of SHW. Spring-habit SHW accessions have been shown to improve yield in both irrigated and rainfed environments in Colorado with only a single backcross to the locally adapted cultivar Hatcher. Not only did certain SDLs out yield the recurrent parent but they were able to out-rank all elite check cultivars in certain environments. Canopy temperature shows potential for use in selecting for drought tolerance among SDLs under both rainfed and irrigated conditions. However, consideration of the phenological and diurnal differences that effect CT must be taken into account. Several SDLs showed consistently reduced CT through different time points in a day as well as a strong afternoon recovery response following a reduction in air temperature and radiation. A selective advantage was seen both for adapted and SHW alleles. Selected alleles of synthetic origin were primarily found on the B genome but were present on the A and D genomes as well. However, large amounts of missing data in the GBS procedures reduced our ability to more confidently identify a selective advantage of certain allelic positions. Overall, SHW has been demonstrated as beneficial in improving the yield and agronomic performance of winter wheat varieties after minimal backcrossing efforts. Synthetic hexaploid lines are a resource that should be exploited in wheat breeding programs throughout the U.S. Great Plains.

Table 4.1. BLUPs (\pm SE) for grain yield by environment. Range of grain yield is given for those populations which consisted of multiple entries.

| Grain yield (BLUPs) | ARDEC IR | | ARDEC RF | | Dailey | |
|------------------------|----------------|--------------|----------------|--------------|----------------|--------------|
| | Mean | Range | Mean | Range | Mean | Range |
| | kg/ha | | kg/ha | | kg/ha | |
| SYN-1 | 6220 | 5480 to 7180 | 3210 | 2920 to 3580 | 3940 | 3420 to 4500 |
| SYN-2 | 6290 | 4910 to 7020 | 3160 | 2850 to 3600 | 3870 | 3300 to 4460 |
| SYN-4 | 6030 | 5340 to 6930 | 3170 | 2790 to 3780 | 3930 | 3050 to 4590 |
| Hatcher | 6590 \pm 256 | | 3340 \pm 176 | | 3990 \pm 204 | |
| Ripper | 5980 \pm 253 | | 3570 \pm 176 | | 3750 \pm 204 | |
| Byrd | 6680 \pm 253 | | 3260 \pm 172 | | 4390 \pm 209 | |
| Settler CL | 5960 \pm 255 | | 3140 \pm 179 | | 3790 \pm 209 | |
| Prairie Red | 5400 \pm 256 | | 3360 \pm 173 | | † | |
| Goodstreak | 5790 \pm 256 | | 3390 \pm 172 | | | |
| Denali | 6400 \pm 252 | | 3180 \pm 173 | | | |
| McGill | 5910 \pm 260 | | 3310 \pm 173 | | 4150 \pm 205 | |
| Robidoux | 6000 \pm 257 | | 3050 \pm 173 | | 3950 \pm 205 | |
| HxSYN-1-1 Bulk | | | | | 4320 \pm 205 | |
| HxSYN-1-2 Bulk | | | | | 4030 \pm 205 | |
| HxSYN-1-3 Bulk | | | | | 3890 \pm 208 | |
| HxSYN-2-1 Bulk | | | | | 3650 \pm 206 | |
| HxSYN-2-2 Bulk | | | | | 4130 \pm 205 | |
| HxSYN-2-3 Bulk | | | | | 3620 \pm 208 | |
| HxSYN-4-1 Bulk | | | | | 3900 \pm 209 | |
| HxSYN-4-2 Bulk | | | | | 3970 \pm 205 | |
| HxSYN-4-3 Bulk | | | | | 3690 \pm 205 | |
| Environment mean | 6170 | | 3190 | | 3920 | |
| CV (%) | 12.5 | | 26.3 | | 11.9 | |

† Missing values indicate check entries that were not grown in some environments.
CV, coefficient of variation.

Table 4.2. Phenotypic correlations ($n = 99$) of traits that were significantly correlated with grain yield in either the full irrigation or rainfed treatment at ARDEC, and heritability estimates for each treatment and combined across treatments.

| Traits [†] | Irrigated | | Rainfed | | Combined |
|---------------------|-----------|-------|-----------|-------|----------|
| | r | h^2 | r | h^2 | h^2 |
| AKW | 0.17 | 0.80 | 0.22 * | 0.77 | 0.66 |
| BM | 0.42 *** | 0.00 | 0.44 *** | 0.05 | 0.24 |
| CTg | -0.31 ** | 0.29 | -0.31 ** | 0.37 | 0.08 |
| CTv | -0.49 *** | 0.23 | -0.53 *** | 0.34 | 0.00 |
| DF | 0.39 *** | 0.83 | -0.22 * | 0.86 | 0.91 |
| DPM | 0.59 *** | 0.88 | -0.09 | 0.52 | 0.69 |
| GF | 0.49 *** | 0.69 | 0.15 | 0.29 | 0.35 |
| HI | 0.59 *** | 0.70 | 0.55 *** | 0.33 | 0.30 |
| KNS | 0.33 ** | 0.56 | 0.19 | 0.61 | 0.55 |
| KNA | 0.44 *** | 0.26 | 0.27 * | 0.20 | 0.33 |
| NDVIg | 0.46 *** | 0.75 | 0.42 *** | 0.60 | 0.51 |
| NDVIv | 0.45 *** | 0.59 | 0.35 ** | 0.60 | 0.63 |
| SN | 0.29 ** | 0.20 | 0.34** | 0.02 | 0.30 |

[†] AKW, average kernel weight; BM, above ground biomass; CTv, average canopy temperature during vegetative growth; CTg, average canopy temperature during grain fill period; DF, days to 50% of spikes with visible anther extrusion; DM, days to 50% of peduncles senesced; GF, grain fill period; HI, harvest index; KNS, number of kernels per spike; KNA, kernel number m^{-2} ; NDVIv, normalized difference vegetative index during vegetative growth; NDVIg, normalized difference vegetative index during GF; SN, spike number m^{-2} .

*, **, *** Significant at the 0.05, 0.01, and 0.0001 probability levels, respectively.

Table 4.3. Correlations (n = 99) of grain yield with canopy temperature and normalized difference vegetative index (NDVI) measured throughout the day on 21 June 2013 at approximately the milk development stage (Zadoks stage 70) for the full irrigation treatment and the dough development stage (Zadoks stage 80) for the rainfed treatment at ARDEC.

| 21 June 2013 Hour (MST) | Atmospheric temperature °C | Solar Irradiance W / m ² | Canopy Temperature | | NDVI | |
|----------------------------------|----------------------------------|---|--------------------|-------------|-------------|-------------|
| | | | ARDEC IR | ARDEC RF | ARDEC IR | ARDEC RF |
| 0700 | 19.4 | 186 | -0.02 | -0.10 | 0.44 *** | 0.35 ** |
| 0900 | 25.1 | 616 | -0.12 | -0.44 *** | 0.43 *** | 0.31 ** |
| 1100 | 29.8 | 872 | -0.30 ** | -0.27 ** | 0.46 *** | 0.33 ** |
| 1300 | 34.3 | 965 | -0.42 *** | -0.22 * | 0.49 *** | 0.26 ** |
| 1500 | 32.3 | 430 | NA [†] | NA | 0.43 *** | 0.26 ** |
| 1730 | 33.9 | 465 | -0.30 ** | -0.40 *** | 0.54 *** | 0.30 ** |

[†] Canopy temperature not available due to error in measurement.

*, **, *** Significant at the 0.05, 0.01, and 0.0001 probability levels, respectively.

Table 4.4. Alleles of synthetic origin that were found to have a selective advantage over the Hatcher allele. Families whose BC₁F₂ lines were homozygous for either allele were not included in the allele counts. Lines with missing data were excluded from analysis.

| Chromosome | Population | Hatcher allele count | Synthetic allele count | Binomial <i>P</i> -value | Synthetic marker sequence |
|------------|------------|----------------------|------------------------|--------------------------|--|
| 1B | HxSYN-4 | 0 | 6 | 0.031 | CGCCTCCGCTGCACCCTTGGCGCCCCCTGCTCCACCGAGATCGGAAGAGCGGTTTCAGC |
| 1B | HxSYN-4 | 0 | 7 | 0.015 | ATAAGAGAGAGAGGGCTCGCCGCCGCGCAGAGACCTCAAAAGATCTCTCACAGGCAGGC |
| 2B | HxSYN-4 | 0 | 7 | 0.015 | CACTCGGCGTGTTCAGCCATAGCTCAAGTGGCCCCGTGGTGGCATCTCGTCGGCGAA |
| 2D | HxSYN-4 | 0 | 6 | 0.031 | TTGTTCCGGCTGACAAATCGACTTGGTTAAATTAATTGATTTCATAATAACTTCTTATACA |
| 3A | HxSYN-4 | 2 | 11 | 0.022 | GTTTGTGTGTGATACACACTATGTGGTGTATTCTTCATCAGTCCTTGTTTGAATAT |
| 3B | HxSYN-4 | 0 | 7 | 0.015 | TATAGTCCACCGATATAGTCCCCTTCCTTGATAGCGTTGTATTCTTAGCATAGTTCTAA |
| 3B | HxSYN-4 | 3 | 12 | 0.035 | CTTCTACTTATCCCATTTCGATTTCGATTCGATTCGCAGGTTGTTTACGGAAAGGGGCGACAGC |
| 3B | HxSYN-4 | 0 | 6 | 0.031 | TGCCCCATCGACCAAGTCGGAGCTGCTTCGTCCGGCGGACACCACGACGCTCACGCGAAA |
| 3B | HxSYN-4 | 1 | 8 | 0.039 | GCGACGACGGTGAGGATGTCGATGGGAAACGGGAACGAGGAGCATATCCTTGGAGAGTA |
| 5D | HxSYN-4 | 0 | 8 | 0.007 | CATCGCCGCGTCCCTCGGCGCCACGGGCTGCGCCGCGAGCTCCCCGAGATCGGAAGAAC |
| 6B | HxSYN-1 | 2 | 11 | 0.022 | ATGCTCATGACGAAGGACGAGGCGGAAACGGGGCTCCGTTCAGTTCCAGGCAGCGGCAGA |
| 6B | HxSYN-4 | 1 | 8 | 0.039 | ATTGTCCTAACTTTCCCATATGTGCCATCTTAGCGACTGTCGCGCCAACATTGATTTT |
| 6D | HxSYN-1 | 3 | 12 | 0.035 | TGCTCGACAACATCGGCCATCTCCTTCCCGAGGGGGTCCGTCTGTTGGTGCCATG |

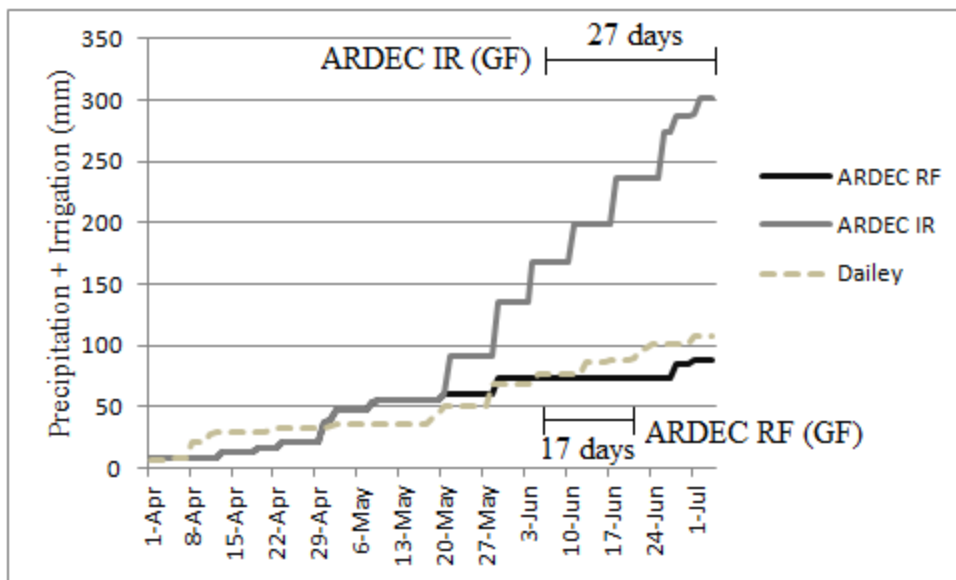


Figure 4.1. Total accumulated moisture through the spring and summer growth periods for all environments. Bars indicate the average grain fill (GF) period for the ARDEC location from the average date of anthesis to the average date of physiological maturity.

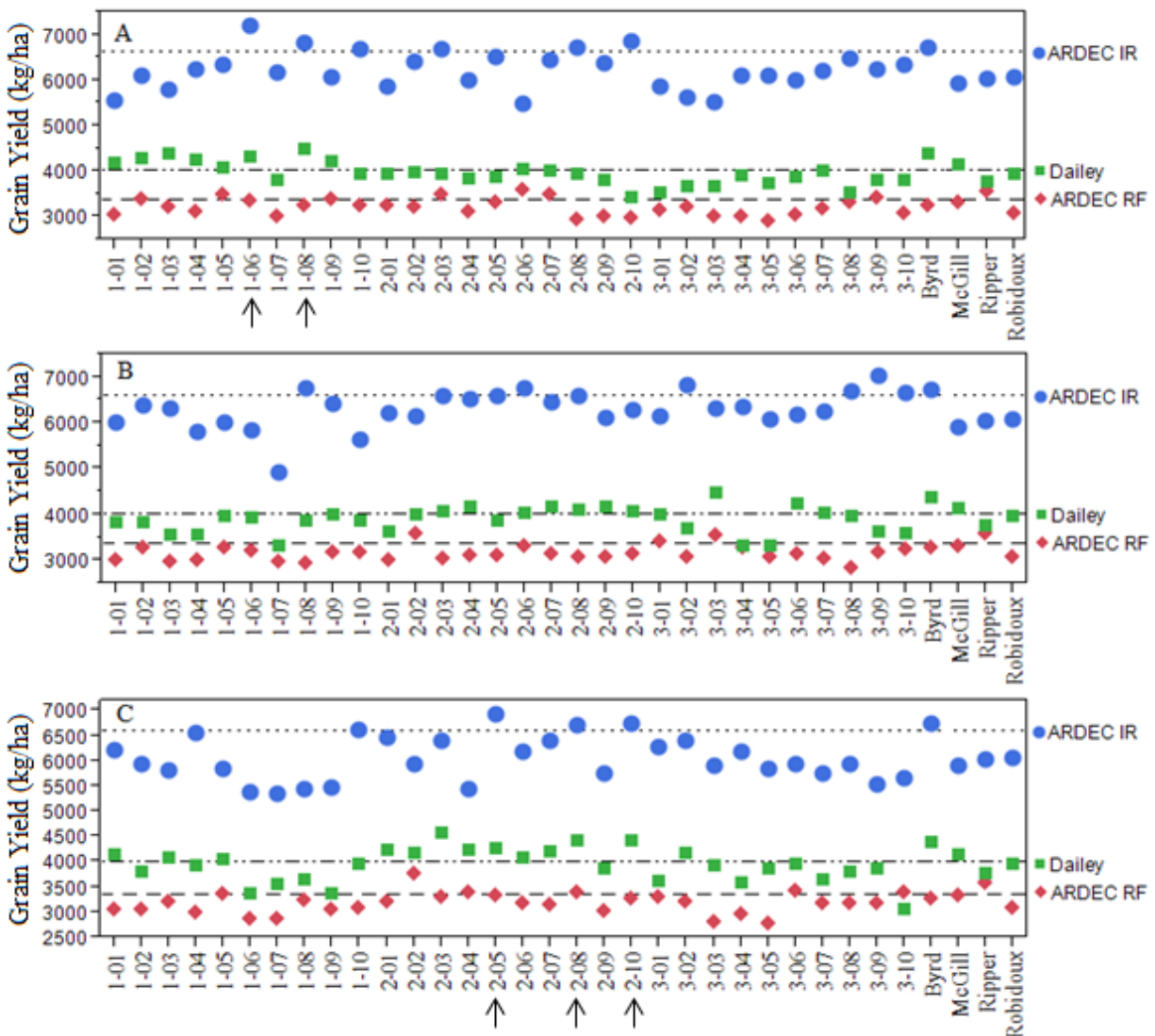


Figure 4.2. Grain yield for all SHW-derived lines comparing each population to the recurrent parent Hatcher. A) HxSYN-1 population. B) HxSYN-2 population. C) HxSYN-4 population. Lines within each graph indicate the grain yield of the recurrent parent Hatcher within each environment. Circles represent yield in the ARDEC IR environment, diamonds represent yield in the ARDEC RF environment, and squares represent yield at Dailey. Arrows indicate the top five yielding SHW-derived lines overall.

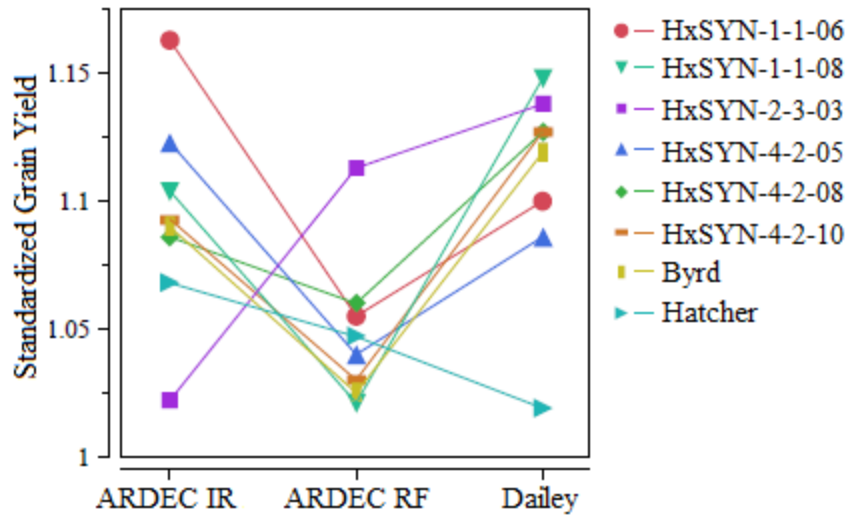


Figure 4.3. Standardized grain yield (entry mean/environment mean) for the best yielding synthetic-derived lines, the best yielding cultivar, Byrd, and the recurrent parent Hatcher.

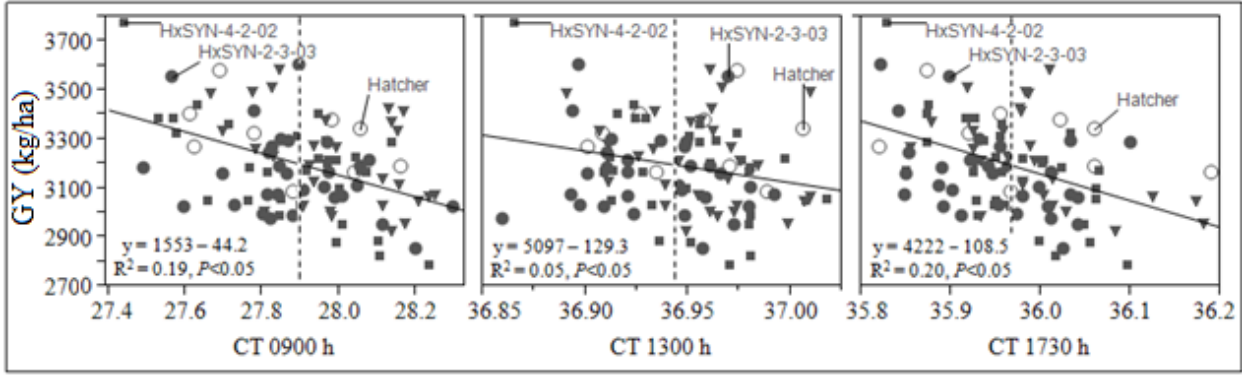


Figure 4.4. Regression analysis of grain yield (GY) on canopy temperature (CT) at ARDEC RF for time periods throughout a day during the grainfill stage of development. Dashed lines in each graph represent the average CT per time period.

LITERATURE CITED

- Andrade-Sanchez, P., M.A. Gore, J.T. Heun, K.R. Thorp, A.E. Carmo-Silva, A.N. French, M.E. Salvucci, and J.W. White. 2013. Development and evaluation of a field-based high-throughput phenotyping platform. *Funct. Plant Biol.*
- Araus, J.L., G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C3 cereals: What should we breed for? *Ann. Bot-London* 89:925-940.
- Asseng, S., J.T. Ritchie, A.J.M. Smucker, and M.J. Robertson. 1998. Root growth and water uptake during water deficit and recovering in wheat. *Plant Soil* 201:265-273.
- Baenziger, P.S., B. Beecher, R.A. Graybosch, D.D. Baltensperger, L. Nelson, J.M. Krall, D.V. Mcvey, J.E. Watkins, J.H. Hatchett, and M. Chen. 2004. Registration of 'Goodstreak' wheat. *Crop Sci.* 44:1473-1474.
- Baenziger, P.S., R.A. Graybosch, T. Regassa, L.A. Nelson, R.N. Klein, D.K. Santra, D.D. Baltensperger, J.M. Krall, L. Xu, S.N. Wegulo, Y. Jin, J. Kolmer, M.-S. Chen, and G. Bai. 2012. Registration of 'NI04421' hard red winter wheat. *J. Plant Regist.* 6:54-59.
- Baenziger, P.S., R.A. Graybosch, L.A. Nelson, T. Regassa, R.N. Klein, D.D. Baltensperger, D.K. Santra, A.M.H. Ibrahim, W. Berzonsky, J.M. Krall, L. Xu, S.N. Wegulo, M.L. Bernards, Y. Jin, J. Kolmer, J.H. Hatchett, M.-S. Chen, and G. Bai. 2011. Registration of 'NH03614 CL' wheat. *J. Plant Regist.* 5:75-80.
- Barrs, H.D., and P.E. Weatherley. 1962. A re-examination of relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.* 15:413-428.
- Blum, A. 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crop Res.* 112:119-123.
- Blum, A. 2011. *Plant breeding for water-limited environments.* Springer Science+Business Media, LLC, New York.
- Blum, A., J. Mayer, and G. Gozlan. 1982. Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. *Field Crop Res.* 5:137-146.
- Butler, J.D., P.F. Byrne, V. Mohammadi, P.L. Chapman, and S.D. Haley. 2005. Agronomic performance of *Rht* alleles in a spring wheat population across a range of moisture levels. *Crop Sci.* 45:939-947.
- Calderini, D.F., and M.P. Reynolds. 2000. Changes in grain weight as a consequence of de-graining treatments at pre- and post-anthesis in synthetic hexaploid lines of wheat (*Triticum durum* x *T. tauschii*). *Aust. J. Plant. Physiol.* 27:183-191.

- Cavanagh, C.R., S. Chao, S. Wang, B.E. Huang, S. Stephen, S. Kiani, K. Forrest, C. Saintenac, G.L. Brown-Guedira, A. Akhunova, D. See, G. Bai, M. Pumphrey, L. Tomar, D. Wong, S. Kong, M. Reynolds, M.L. Da Silva, H. Bockelman, L. Talbert, J.A. Anderson, S. Dreisigacker, S. Baenziger, A. Carter, V. Korzun, P.L. Morrell, J. Dubcovsky, M.K. Morell, M.E. Sorrells, M.J. Hayden, and E. Akhunov. 2013. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the National Academy of Sciences* 110:8057-8062.
- Chenu, K., M. Cooper, G.L. Hammer, K.L. Mathews, M.F. Dreccer, and S.C. Chapman. 2011. Environment characterization as an aid to wheat improvement: Interpreting genotype-environment interactions by modelling water-deficit patterns in North-Eastern Australia. *J. Exp. Bot.* 62:1743-1755.
- Clark, R.T., A.N. Famoso, K. Zhao, J.E. Shaff, E.J. Craft, C.D. Bustamante, S.R. Mccouch, D.J. Aneshansley, and L.V. Kochian. 2013. High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. *Plant, Cell & Environment* 36:454-466.
- Condon, A.G., M.P. Reynolds, G.J. Rebetzke, M.V. Ginkel, R.A. Richards, and G.D. Farquhar. 2007. Using stomatal aperture-related traits to select for high yield potential in bread wheat, In: H. T. Buck, et al. (Eds.), *Dev. Plant Breed*, Springer, Netherlands. pp. 617-624.
- Cooper, J.K., A.M.H. Ibrahim, J. Rudd, S. Malla, D.B. Hays, and J. Baker. 2012. Increasing hard winter wheat yield potential via synthetic wheat: I. Path-coefficient analysis of yield and its components. *Crop Sci.* 52:2014-2022.
- Cooper, J.K., A.M.H. Ibrahim, J. Rudd, D. Hays, S. Malla, and J. Baker. 2013. Increasing hard winter wheat yield potential via synthetic hexaploid wheat: II. Heritability and combining ability of yield and its components. *Crop Sci.* 53:67-73.
- Cox, T.S. 1997. Deepening the wheat gene pool. *J. Crop Prod.* 1:1-25.
- Cox, T.S., R.G. Sears, R.K. Bequette, and T.J. Martin. 1995. Germplasm enhancement in winter-wheat x *Triticum-Tauschii* backcross populations. *Crop Sci.* 35:913-919.
- Cox, T.S., J.H. Hatcher, B.S. Gill, W.J. Raupp, and R.G. Sears. 1990. Agronomic performance of hexaploid wheat lines derived from direct crosses between wheat and *Aegilops squarrosa*). *Plant Breeding* 105:271-277.
- CycSoftware. 2006. *CycDesign 3.0*. CycSoftware Ltd. Naseby, New Zealand.
- Das, M.K., G.H. Bai, and A. Mujeeb-Kazi. 2007. Genetic diversity in conventional and synthetic wheats with drought and salinity tolerance based on AFLP. *Can. J. Plant Sci.* 87:691-702.
- Del Blanco, I.A., S. Rajaram, W.E. Kronstad, and M.P. Reynolds. 2000. Physiological performance of synthetic hexaploid wheat-derived populations. *Crop Sci.* 40:1257-1263.

- Dixon, J., H.-J. Braun, P. Kosina, and J. Crouch. 2009. Wheat facts and figures. 2009, CIMMYT, D.F. Mexico.
- Dreccer, A.F., A.G. Borgognone, F.C. Ogonnaya, R.M. Trethowan, and B. Winter. 2007. CIMMYT-selected derived synthetic bread wheats for rainfed environments: Yield evaluation in Mexico and Australia. *Field Crop Res.* 100:218-228.
- Dreisigacker, S., M. Kishii, J. Lage, and M. Warburton. 2008. Use of synthetic hexaploid wheat to increase diversity for CIMMYT bread wheat improvement. *Aust. J. Agr. Res.* 59:413-420.
- Eberhart, S.A., and W.A. Russell. 1966. Stability parameters for comparing varieties. *Crop Sci.* 6:36-40.
- Ehdaie, B., A.P. Layne, and J.G. Waines. 2012. Root system plasticity to drought influences grain yield in bread wheat. *Euphytica* 186:219-232.
- Ekanayake, I.J., J.C. O'toole, D.P. Garrity, and T.M. Masajo. 1985. Inheritance of root characters and their relations to drought resistance in rice. *Crop Sci.* 25:927-933.
- Fehr, W.R. 1987 Principles of cultivar development. Macmillan Pub. Co, New York, NY.
- Feldman, M. 2001 Origin of cultivated wheat, In: Alain P. Bonjean and William J. Angus (Eds.), *The world wheat book: A history of wheat breeding*, Intercept, Andover, Hampshire. pp. 3-53.
- Fischer, R.A., D. Rees, K.D. Sayre, Z.-M. Lu, A.G. Condon, and A.L. Saavedra. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Sci.* 38:1467-1475.
- Franks, P.J., P.L. Drake, and D.J. Beerling. 2009. Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using *Eucalyptus globulus*. *Plant, Cell & Environment* 32:1737-1748.
- Garrity, D.P., and J.C. O'toole. 1995. Selection for reproductive stage drought avoidance in rice, using infrared thermometry. *Agron. J.* 87:773-779.
- Gill, B.S., H.C. Sharma, W.J. Raupp, L.E. Browder, J.H. Hatchett, T.L. Harvey, J.G. Moseman, and J.G. Waines. 1985. Evaluation of *Aegilops* species for resistance to wheat powdery mildew, wheat leaf rust, hessian fly, and greenbug. *Plant Dis.* 69:314-316.
- Gill, K.S., E.L. Lubbers, B.S. Gill, W.J. Raupp, and T.S. Cox. 1991. A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). *Genome* 34:362-374.
- Haley, S.D., J.S. Quick, J.J. Johnson, F.B. Peairs, J.A. Stromberger, S.R. Clayshulte, B.L. Clifford, J.B. Rudolf, B.W. Seabourn, O.K. Chung, Y. Jin, and J.A. Kolmer. 2005. Registration of 'Hatcher' wheat. *Crop Sci.* 45:2654-2655.

- Haley, S.D., J.J. Johnson, F.B. Peairs, J.S. Quick, J.A. Stromberger, S.R. Clayshulte, J.D. Butler, J.B. Rudolph, B.W. Seabourn, G. Bai, Y. Jin, and J. Kolmer. 2007. Registration of 'Ripper' wheat. *J. Plant Regist.* 1:1-6.
- Haley, S.D., J.J. Johnson, F.B. Peairs, J.A. Stromberger, E.E. Hudson, S.A. Seifert, R.A. Kottke, V.A. Valdez, J.B. Rudolph, G. Bai, X. Chen, R.L. Bowden, Y. Jin, J.A. Kolmer, M.-S. Chen, and B.W. Seabourn. 2012a. Registration of 'Byrd' wheat. *J. Plant Regist.* 6:302-305.
- Haley, S.D., J.J. Johnson, F.B. Peairs, J.A. Stromberger, E.E. Heaton, S.A. Seifert, R.A. Kottke, J.B. Rudolph, T.J. Martin, G. Bai, X. Chen, R.L. Bowden, Y. Jin, J.A. Kolmer, D.L. Seifers, M.-S. Chen, and B.W. Seabourn. 2011. Registration of 'Snowmass' wheat. *J. Plant Regist.* 5:87-90.
- Haley, S.D., J.J. Johnson, F.B. Peairs, J.A. Stromberger, E.E. Hudson, S.A. Seifert, R.A. Kottke, V.A. Valdez, J.B. Rudolph, T.J. Martin, G. Bai, X. Chen, R.L. Bowden, Y. Jin, J.A. Kolmer, M.-S. Chen, and B.W. Seabourn. 2012b. Registration of 'Denali' wheat. *J. Plant Regist.* 6:311-314.
- Hargreaves, C.E., P.J. Gregory, and A.G. Bengough. 2009. Measuring root traits in barley (*Hordeum vulgare* ssp. *vulgare* and ssp. *spontaneum*) seedlings using gel chambers, soil sacs and X-ray microtomography. *Plant Soil* 316:285-297.
- Hatfield, J.L., P.J. Pinter Jr, E. Chasseray, C.E. Ezra, R.J. Reginato, S.B. Idso, and R.D. Jackson. 1984. Effects of panicles on infrared thermometer measurements of canopy temperature in wheat. *Agricultural and Forest Meteorology* 32:97-105.
- Haudry, A., A. Cenci, C. Ravel, T. Bataillon, D. Brunel, C. Poncet, I. Hochu, S. Poirier, S. Santoni, S. Glemin, and J. David. 2007. Grinding up wheat: A massive loss of nucleotide diversity since domestication. *Mol. Biol. Evol.* 24:1506-1517.
- Hedden, P. 2003. The genes of the Green Revolution. *Trends Genet.* 19:5-9.
- Heiser, C. 1988. Aspects of unconscious selection and the evolution of domesticated plants. *Euphytica* 37:77-81.
- Hetherington, A.M., and F.I. Woodward. 2003. The role of stomata in sensing and driving environmental change. *Nature* 424:901.
- Hopkins, W.G., and N.P.A. Hüner. 2008. *Introduction to plant physiology*. 4th ed. John Wiley & Sons, Hoboken, NJ.
- Hospital, F. 2001. Size of donor chromosome segments around introgressed loci and reduction of linkage drag in marker-assisted backcross programs. *Genetics* 158:1363-1379.
- Huang, X.Q., H. Kempf, M.W. Ganai, and M.S. Röder. 2004. Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109:933-943.

- Inagaki, M.N., M. Mori, and M.M. Nachit. 2010. Yield comparison for synthetic-derived bread wheat genotypes with different water uptake abilities under increasing soil water deficits. *Cereal Res. Commun.* 38:497-505.
- SAS Institute Inc. 2008. *SAS/STAT 9.2 User's Guide*. SAS Institute. Cary, NC.
- Izanloo, A., A.G. Condon, P. Langridge, M. Tester, and T. Schnurbusch. 2008. Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. *J. Exp. Bot.* 59:3327-3346.
- JMP. 2010. Version 9.0.2. SAS Institute Inc. Cary, NC.
- Jones, H.G., R. Serraj, B.R. Loveys, L. Xiong, A. Wheaton, and A.H. Price. 2009. Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. *Funct. Plant Biol.* 36:978-989.
- Khan, I.A., J.D. Procnier, D.G. Humphreys, G. Tranquilli, A.R. Schlatter, S. Marcucci-Poltri, R. Froberg, and J. Dubcovsky. 2000. Development of PCR-based markers for a high grain protein content Gene from *Triticum turgidum ssp. dicoccoides* transferred to bread wheat. *Crop Sci.* 40:518-524.
- Kihara, H. 1944. Discovery of the DD analyser, one of the ancestors of *Triticum vulgare*. *Agric. Hort.* 19:889-890.
- Kunert, A., A. Naz, O. Dedeck, K. Pillen, and J. Léon. 2007. AB-QTL analysis in winter wheat: I. Synthetic hexaploid wheat (*T. turgidum ssp. dicoccoides* × *T. tauschii*) as a source of favourable alleles for milling and baking quality traits. *Theor. Appl. Genet.* 115:683-695.
- Lafitte, H.R., and B. Courtois. 2002. Interpreting cultivar × environment interactions for yield in upland rice. *Crop Sci.* 42:1409-1420.
- Lage, J., and R.M. Trethowan. 2008. CIMMYT's use of synthetic hexaploid wheat in breeding for adaptation to rainfed environments globally. *Aust. J. Agr. Res.* 59:461-469.
- Lage, J., B. Skovmand, R.J. Peña, and S.B. Andersen. 2006. Grain Quality of Emmer Wheat Derived Synthetic Hexaploid Wheats. *Genetic Resources and Crop Evolution* 53:955-962.
- Leff, B., N. Ramankutty, and J.A. Foley. 2004. Geographic distribution of major crops across the world. *Global Biogeochem. Cycles.* 18.
- Lilley, J.M., and J.A. Kirkegaard. 2011. Benefits of increased soil exploration by wheat roots. *Field Crop Res.* 122:118-130.
- Liu, S., R. Zhou, Y. Dong, P. Li, and J. Jia. 2006a. Development, utilization of introgression lines using a synthetic wheat as donor. *Theor. Appl. Genet.* 112:1360-1373.

- Liu, S.B., R.G. Zhou, Y.C. Dong, P. Li, and J.Z. Jia. 2006b. Development, utilization of introgression lines using a synthetic wheat as donor. *Theor. Appl. Genet.* 112:1360-1373.
- Lopes, M.S., and M.P. Reynolds. 2010. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. *Funct. Plant Biol.* 37:147-156.
- Lopes, M.S., and M.P. Reynolds. 2011. Drought adaptive traits and wide adaptation in elite lines derived from resynthesized hexaploid wheat. *Crop Sci.* 51:1617-1626.
- Ma, S.C., B.C. Xu, F.M. Li, W.Z. Liu, and Z.B. Huang. 2008. Effects of root pruning on competitive ability and water use efficiency in winter wheat. *Field Crop Res.* 105:56-63.
- Malone, S.R., H.S. Mayeux, H.B. Johnson, and H.W. Polley. 1993. Stomatal Density and Aperture Length in Four Plant Species Grown Across a Subambient CO₂ Gradient. *American Journal of Botany* 80:1413-1418.
- Manschadi, A.M., J. Christopher, P. Devoil, and G.L. Hammer. 2006. The role of root architectural traits in adaptation of wheat to water-limited environments. *Funct. Plant Biol.* 33:823-837.
- Marshall, D., D. Mares, H. Moss, and F. Ellison. 1986. Effects of grain shape and size on milling yields in wheat. II. Experimental studies. *Aust. J. Agr. Res.* 37:331-342.
- Mcfadden, E.S., and E.R. Sears. 1944. The artificial synthesis of *Triticum spelta*. *Records of the Genetics Society of America* 13:26-27.
- Mcfadden, E.S., and E.R. Sears. 1946. The origin of *Triticum spelta* and its free threshing hexaploid relatives. *Heredity* 37:81-89.
- Microsoft. 2007. Microsoft Excel 2007. Redmond, Washington.
- Mir, R.R., M. Zaman-Allah, N. Sreenivasulu, R. Trethowan, and R.K. Varshney. 2012. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theor. Appl. Genet.* 125:625-645.
- Mohammad, F., O.S. Abdalla, S. Rajaram, A. Yaljarouka, S.K. Khalil, N.U. Khan, I.H. Khalil, and I. Ahmad. 2010. Yield of synthetic-derived bread wheat under varying moisture regimes. *Pak. J. Bot.* 42:4103-4112.
- Mujeeb-Kazi, A., V. Rosas, and S. Roldan. 1996. Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. non L.) in synthetic hexaploid wheats (*T. turgidum* L. s.lat. x *T. tauschii*; 2n=6x=42, AABBDD) and its potential utilization for wheat improvement. *Genetic Resources and Crop Evolution* 43:129-134.

- Mujeeb-Kazi, A., A. Gul, M. Farooq, S. Rizwan, and I. Ahmad. 2008. Rebirth of synthetic hexaploids with global implications for wheat improvement. *Aust. J. Agr. Res.* 59:391-398.
- Narasimhamoorthy, B., B.S. Gill, A.K. Fritz, J.C. Nelson, and G.L. Brown-Guedira. 2006. Advanced backcross QTL analysis of a hard winter wheat x synthetic wheat population. *Theor. Appl. Genet.* 112:787-796.
- Ogbonnaya, F.C., G.Y. Ye, R. Trethowan, F. Dreccer, D. Lush, J. Shepperd, and M. Van Ginkel. 2007. Yield of synthetic backcross-derived lines in rainfed environments of Australia. *Euphytica* 157:321-336.
- Ogbonnaya, F.C., O. Abdalla, A. Mujeeb-Kazi, A.G. Kazi, S.S. Xu, N. Gosman, E.S. Lagudah, D. Bonnett, M.E. Sorrells, and H. Tsujimoto. 2013. Synthetic hexaploids: Harnessing species of the primary gene pool for wheat improvement. *Plant Breeding Reviews* 37:35-122.
- Olivares-Villegas, J.J., M.P. Reynolds, and G.K. McDonald. 2007. Drought-adaptive attributes in the Seri/Babax hexaploid wheat population. *Funct. Plant Biol.* 34:189-203.
- Palta, J.A., X. Chen, S.P. Milroy, G.J. Rebetzke, M.F. Dreccer, and M. Watt. 2011. Large root systems: are they useful in adapting wheat to dry environments? *Funct. Plant Biol.* 38:347-354.
- Passioura, J. 2007. The drought environment: physical, biological and agricultural perspectives. *J. Exp. Bot.* 58:113-117.
- Poland, J.A., P.J. Brown, M.E. Sorrells, and J.-L. Jannink. 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS ONE* 7:e32253.
- Praba, M.L., J.E. Cairns, R.C. Babu, and H.R. Lafitte. 2009. Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. *J. Agron. Crop Sci.* 195:30-46.
- Prasad, B., B.F. Carver, M.L. Stone, M.A. Babar, W.R. Raun, and A.R. Klatt. 2007. Genetic analysis of indirect selection for winter wheat grain yield using spectral reflectance indices. *Crop Sci.* 47:1416-1425.
- Quick, J.S., J.A. Stromberger, S. Clayshulte, B. Clifford, J.J. Johnson, F.B. Peairs, J.B. Rudolph, and K. Lorenz. 2001. Registration of 'Prairie Red' wheat. *Crop Sci.* 41:1362-1363.
- Rampino, P., S. Pataleo, C. Gerardi, G. Mita, and C. Perrotta. 2006. Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotypes. *Plant Cell Environ.* 29:2143-2152.
- Reynolds, M., F. Dreccer, and R. Trethowan. 2007. Drought-adaptive traits derived from wheat wild relatives and landraces. *J. Exp. Bot.* 58:177-186.

- Reynolds, M.P., and R.M. Trethowan. 2007. Physiological interventions in breeding for adaptation to abiotic stress. *Wag. Ur. Fron.* 21:129-146.
- Richards, R.A., and J.B. Passioura. 1989. A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain-yield in rain-fed environments. *Aust. J. Agr. Res.* 40:943-950.
- Sayar, R., H. Khemira, and M. Kharrat. 2007. Inheritance of deeper root length and grain yield in half-diallel durum wheat (*Triticum durum*) crosses. *Ann. Appl. Biol.* 151:213-220.
- Skovmand, B., M.P. Reynolds, and I.H. Delacy. 2001. Mining wheat germplasm collections for yield enhancing traits. *Wheat in a Global Environment* 9:761-771.
- Slafer, G., D. Calderini, and D. Miralles. 1996. Yield components and compensation in wheat: opportunities for further increasing yield potential. *Increasing yield potential in wheat: Breaking the Barriers.* 101-133.
- Snape, J.W., K. Butterworth, E. Whitechurch, and A.J. Worland. 2001. Waiting for fine times: genetics of flowering time in wheat. *Euphytica* 119:185-190.
- Talbot, S.J. 2011. Introgression of genetic material from primary synthetic hexaploids into Australian bread wheat (*Triticum aestivum* L.). Masters thesis. The University of Adelaide. Adelaide.
- Tanksley, S.D., and S.R. Mccouch. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277:1063-1066.
- Trethowan, R., M. Van Ginkel, and S. Rajaram. 2002. Progress in breeding wheat for yield and adaptation in global drought affected environments. *Crop Sci.* 42:1441-1446.
- Trethowan, R.M., and A. Mujeeb-Kazi. 2008. Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. *Crop Sci.* 48:1255-1265.
- Trethowan, R.M., J. Crossa, M. Van Ginkel, and S. Rajaram. 2001. Relationships among bread wheat international yield testing locations in dry areas. *Crop Sci.* 41:1461-1469.
- Uga, Y., K. Sugimoto, S. Ogawa, J. Rane, M. Ishitani, N. Hara, Y. Kitomi, Y. Inukai, K. Ono, N. Kanno, H. Inoue, H. Takehisa, R. Motoyama, Y. Nagamura, J. Wu, T. Matsumoto, T. Takai, K. Okuno, and M. Yano. 2013. Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under drought conditions. *Nature Genetics* 45:1097-1102.
- Valkoun, J. 2001. Wheat pre-breeding using wild progenitors. *Wheat in a Global Environment* 9:699-707.
- Van Ginkel, M., and F. Ogonnaya. 2007. Novel genetic diversity from synthetic wheats in breeding cultivars for changing production conditions. *Field Crop Res.* 104:86-94.

- Vassileva, V., C. Signarbieux, I. Anders, and U. Feller. 2011. Genotypic variation in drought stress response and subsequent recovery of wheat (*Triticum aestivum* L.). *J. Plant Res.* 124:147-154.
- Waines, J.G., and B. Ehdaie. 2007. Domestication and crop physiology: Roots of Green-Revolution wheat. *Ann. Bot-London* 100:991-998.
- Wang, H., and J.M. Clarke. 1993. Genotypic, intraplant, and environmental variation in stomatal frequency and size in wheat. *Can. J. Plant Sci.* 73:671-678.
- Wang, J., R. Singh, H.-J. Braun, and W. Pfeiffer. 2009. Investigating the efficiency of the single backcrossing breeding strategy through computer simulation. *Theor. Appl. Genet.* 118:683-694.
- Warburton, M.L., J. Crossa, J. Franco, M. Kazi, R. Trethowan, S. Rajaram, W. Pfeiffer, P. Zhang, S. Dreisigacker, and M. Ginkel. 2006. Bringing wild relatives back into the family: Recovering genetic diversity in CIMMYT improved wheat germplasm. *Euphytica* 149:289-301.
- Wojciechowski, T., M.J. Gooding, L. Ramsay, and P.J. Gregory. 2009. The effects of dwarfing genes on seedling root growth of wheat. *J. Exp. Bot.* 60:2565-2573.
- Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. *Weed Research* 14:415-421.
- Zhang, H., N.C. Turner, and M.L. Poole. 2010. Source–sink balance and manipulating sink–source relations of wheat indicate that the yield potential of wheat is sink-limited in high-rainfall zones. *Crop and Pasture Science* 61:852-861.
- Zhang, P., S. Dreisigacker, A.E. Melchinger, J.C. Reif, A.M. Kazi, M. Van Ginkel, D. Hoisington, and M.L. Warburton. 2005. Quantifying novel sequence variation and selective advantage in synthetic hexaploid wheats and their backcross-derived lines using SSR markers. *Mol. Breeding* 15:1-10.
- Zhu, L., Z.S. Liang, X. Xu, S.H. Li, J.H. Jing, and P. Monneveux. 2008. Relationships between carbon isotope discrimination and leaf morphophysiological traits in spring-planted spring wheat under drought and salinity stress in Northern China. *Aust. J. Agr. Res.* 59:941-949.

APPENDIX

Appendix 1. Analysis of variance results from initial greenhouse pot study of pre-anthesis stress of six selected synthetic hexaploid wheat lines and recurrent parents Hatcher and Goodstreak.

| Trait | Entry | Treatment | Entry * Treatment |
|-------------------------------------|-------|-----------|-------------------|
| CIRAS measurements [†] | | | |
| Transpiration | ns | *** | ns |
| Leaf temperature | ns | *** | ns |
| Photosynthesis | ns | *** | ns |
| Internal CO ₂ | ns | ns | ns |
| Volumetric water content | *** | *** | *** |
| Stomatal conductance | ns | *** | ns |
| Porometer measurements [‡] | | | |
| Adaxial stomatal conductance | ** | ** | ns |
| Abaxial stomatal conductance | ns | * | ns |
| Adaxial stomatal density | *** | Irr. Only | |
| Abaxial stomatal density | *** | Irr. Only | |
| Above ground biomass | *** | *** | ** |
| Root biomass | *** | *** | *** |
| SPAD meter | *** | ns | ns |

*, **, *** Significant at the 0.05, 0.01, and <0.0001 probability levels, respectively.

[†] CIRAS-2 Portable Photosynthesis System, PP Systems, Amesbury, MA, USA.

[‡] SC-1 Leaf Porometer, Decagon Devices, Pullman, WA, USA.

Appendix 2. Description of physiological and morphological traits measured in root tube study.

| Trait | Units | Description |
|-----------------------------------|----------------------------|---|
| Above ground biomass | g | Above ground biomass including root crown |
| 0-33 cm section root biomass | g | Root biomass for upper third of the root column tube |
| 33-66 cm section root biomass | g | Root biomass for middle third of the root column tube |
| 66-99 cm section root biomass | g | Root biomass for bottom third of the root column tube |
| Total root biomass | g | Total root biomass from combined tube sections |
| Osmotic potential | MPa | Leaf # 5 osmotic potential |
| Leaf 5 elongation rate per day | mm | Leaf # 5 average growth rate per day |
| 0-33 cm section root length | log10(mm) | Total root length for upper tube section across diameter classes |
| 33-66 cm section root length | log10(mm) | Total root length for middle tube section across diameter classes |
| 66-99 cm section root length | log10(mm) | Total root length for bottom tube section across diameter classes |
| Longest root length | cm | Length of the longest root |
| Relative water content | % water | Relative water content according to Barrs and Weatherly (1962) |
| Drought stress index | | % reduction in RWC due to drought stress |
| Adaxial (upper) stomate density | Count/area | Count of adaxial stomates averaged across ten viewing areas |
| Abaxial (bottom) stomate density | Count/area | Count of abaxial stomates averaged across ten viewing areas |
| Adaxial (upper) stomate aperture | µm | Estimate of stomate aperture as a measure of closed stomate length |
| Abaxial (bottom) stomate aperture | µm | Estimate of stomate aperture as a measure of closed stomate length |
| Water use | g | Gravimetric water content lost to evapotranspiration |
| Water use efficiency | w/w | Calculation of water use efficiency as ABM / total water use in GWC |
| Leaf elongation rate | mm | Average leaf growth per day of leaf number five |
| Stomatal conductance | log10(µM/m ² s) | Stomatal conductance on leaf #4 with a steady state porometer |
| Upper root length / diameter 1 | Log10(cm) | Root length for diameter class 1 for the upper third of the tube |
| Upper root length / diameter 2 | Log10(cm) | Root length for diameter class 2 for the upper third of the tube |
| Upper root length / diameter 3 | Log10(cm) | Root length for diameter class 3 for the upper third of the tube |
| Upper root length / diameter 4 | SQRT(cm) | Root length for diameter class 4 for the upper third of the tube |
| Upper root length / diameter 5 | SQRT(cm) | Root length for diameter class 5 for the upper third of the tube |
| Middle root length / diameter 1 | Log10(cm) | Root length for diameter class 1 for the middle third of the tube |
| Middle root length / diameter 2 | SQRT(cm) | Root length for diameter class 2 for the middle third of the tube |
| Middle root length / diameter 3 | SQRT(cm) | Root length for diameter class 3 for the middle third of the tube |
| Middle root length / diameter 4 | Log10(cm) | Root length for diameter class 4 for the middle third of the tube |
| Middle root length / diameter 5 | Log10(cm) | Root length for diameter class 5 for the middle third of the tube |
| Bottom root length / diameter 1 | Log10(cm) | Root length for diameter class 1 for the bottom third of the tube |
| Bottom root length / diameter 2 | Log10(cm) | Root length for diameter class 2 for the bottom third of the tube |
| Bottom root length / diameter 3 | SQRT(cm) | Root length for diameter class 3 for the bottom third of the tube |
| Bottom root length / diameter 4 | Log10(cm) | Root length for diameter class 4 for the bottom third of the tube |
| Bottom root length / diameter 5 | Log10(cm) | Root length for diameter class 5 for the bottom third of the tube |

Appendix 3. Phenotypic correlations among selected traits and the drought stress index within well-watered and drought stressed treatments in the root tube study. (A) Physiological and morphological traits, (B) root length per root diameter class as measured by WinRhizo within the upper, middle and bottom tube sections.

| (A) | Well-watered | Drought Stressed | (B) | Well-watered | Drought Stressed | |
|-------------------------------------|----------------------|------------------|---------------------|----------------|----------------------|----------|
| Trait | ----- <i>r</i> ----- | | Root diameter class | Tube section | ----- <i>r</i> ----- | |
| Above ground biomass | 0.11 | -0.47 | 0-0.25 mm dia. | Upper section | -0.36 | -0.34 |
| Upper root biomass | 0.22 | -0.17 | 0.25-0.50 mm dia. | | 0.23 | -0.17 |
| Middle root biomass | -0.37 | -0.35 | 0.50-0.75 mm dia. | | 0.12 | -0.02 |
| Bottom root biomass | -0.45 | -0.73 * | 0.75-1.0 mm dia. | | -0.35 | 0.2 |
| Total root biomass | 0.05 | -0.53 | >1.0 mm dia. | | 0.61 | 0.41 |
| Longest root | -0.64 * | -0.68 * | 0-0.25 mm dia. | Middle section | -0.56 | -0.70 * |
| Stomatal conductance | -0.44 | -0.73 * | 0.25-0.50 mm dia. | | -0.44 | -0.34 |
| Upper stomate density | 0.33 | 0.13 | 0.50-0.75 mm dia. | | -0.52 | 0.02 |
| Bottom stomate density | 0.26 | 0.37 | 0.75-1.0 mm dia. | | -0.24 | -0.4 |
| Upper stomate aperture | -0.40 | -0.36 | >1.0 mm dia. | | 0.28 | -0.01 |
| Bottom stomate aperture | -0.40 | -0.31 | 0-0.25 mm dia. | Bottom section | -0.69 * | -0.86 ** |
| Osmotic potential | 0.05 | -0.17 | 0.25-0.50 mm dia. | | -0.64 * | -0.70 * |
| % Leaf elongation rate [†] | NA [‡] | 0.4 | 0.50-0.75 mm dia. | | -0.61 | -0.67 * |
| Water use efficiency | NA | -0.43 | 0.75-1.0 mm dia. | | -0.45 | -0.38 |
| Total water loss | NA | -0.64 * | >1.0 mm dia. | | -0.22 | -0.35 |

[†] Percent reduction in leaf elongation rate under drought stress relative to well-watered treatment.

[‡] Not applicable to different values for each treatment as these measurements were based on the relationship between treatments. These measurements are also not causal traits but possible indicators of drought tolerance.

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

Appendix 4. Grain yield of synthetic populations for crosses with recurrent parent Goodstreak at Lincoln, NE in 2012-2013. A set of 30 lines in each population were used, along with nine adapted check varieties including Robidoux.

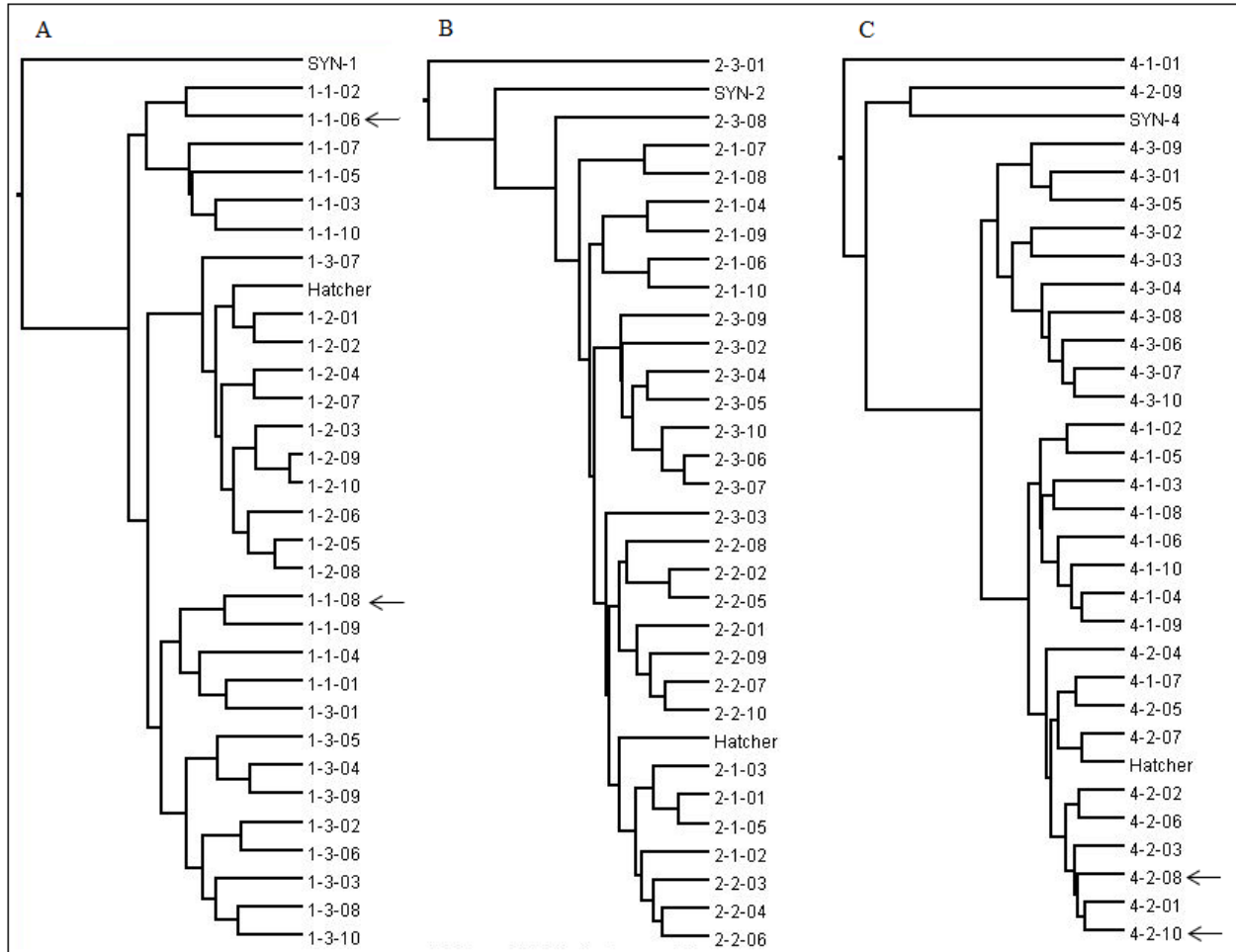
| | | Lincoln NE |
|---------------------------------|-------|--------------|
| Grain Yield | | kg/ha |
| Grand mean | | 3830 |
| Robidoux mean | | 5100 |
| -----Synthetic populations----- | | |
| SYN-1 | Mean | 3750 |
| | Range | 2630 to 4510 |
| SYN-2 | Mean | 3540 |
| | Range | 2930 to 4010 |
| SYN-4 | Mean | 3740 |
| | Range | 3280 to 4480 |

Appendix 5. Phenotypic correlations (n = 99) for all traits measured at the ARDEC location in 2013. Correlations above and below the diagonal are associated with the irrigated and rainfed treatments, respectively.

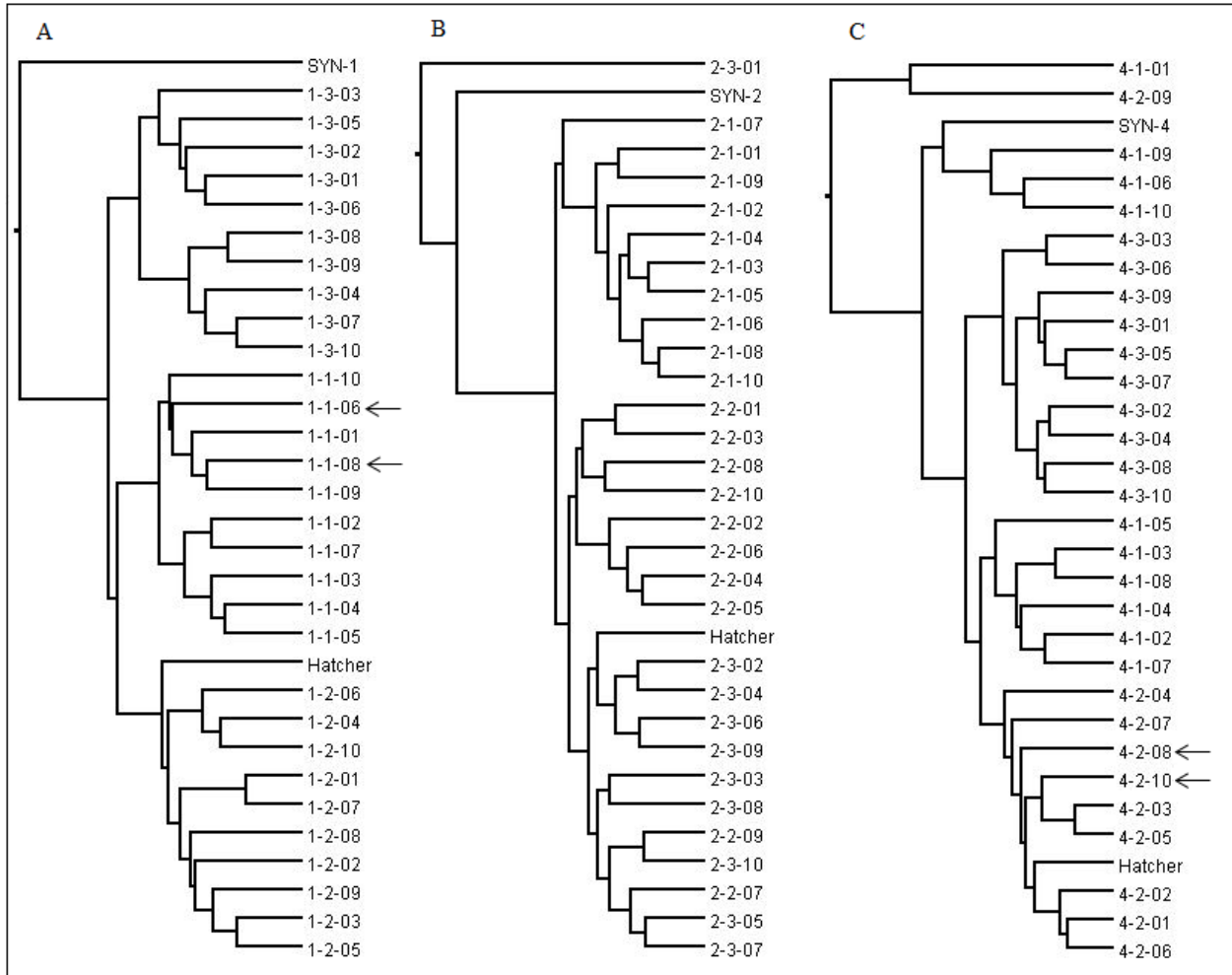
| Trait [†] | GY | TW | AKW | BM | CTg | CTv | DF | DPM | GF | HI | KNS | KNA | NDVIg | NDVIv | SN | NDVIev |
|--------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|
| GY | | 0.08 | 0.17 | <u>0.42</u> | <u>-0.31</u> | <u>-0.49</u> | <u>0.39</u> | <u>0.59</u> | <u>0.49</u> | <u>0.59</u> | <u>0.33</u> | <u>0.44</u> | <u>0.46</u> | <u>0.45</u> | <u>0.29</u> | 0.12 |
| TW | 0.12 | | <u>-0.23</u> | <u>0.23</u> | -0.10 | -0.05 | 0.16 | 0.02 | -0.06 | -0.20 | -0.16 | 0.10 | 0.07 | -0.20 | <u>0.21</u> | -0.12 |
| AKW | <u>0.22</u> | 0.18 | | -0.12 | -0.16 | 0.01 | -0.05 | 0.00 | 0.03 | 0.17 | <u>-0.21</u> | <u>-0.30</u> | 0.08 | 0.20 | <u>-0.26</u> | 0.18 |
| BM | <u>0.44</u> | 0.08 | 0.07 | | <u>-0.44</u> | <u>-0.37</u> | <u>0.36</u> | <u>0.25</u> | 0.06 | <u>0.25</u> | <u>0.24</u> | <u>0.52</u> | <u>0.27</u> | <u>0.22</u> | <u>0.73</u> | 0.05 |
| CTg | <u>-0.31</u> | <u>-0.24</u> | 0.06 | -0.17 | | <u>0.69</u> | -0.20 | -0.19 | -0.11 | -0.07 | -0.14 | -0.19 | -0.37 | <u>-0.26</u> | <u>-0.21</u> | -0.19 |
| CTv | <u>-0.53</u> | -0.14 | -0.10 | <u>-0.28</u> | <u>0.61</u> | | <u>-0.29</u> | <u>-0.37</u> | <u>-0.26</u> | -0.12 | <u>-0.24</u> | <u>-0.29</u> | <u>-0.40</u> | <u>-0.38</u> | -0.18 | -0.16 |
| DF | <u>-0.22</u> | -0.06 | <u>-0.27</u> | -0.12 | -0.07 | 0.01 | | <u>0.63</u> | 0.14 | 0.07 | <u>0.38</u> | <u>0.31</u> | <u>0.38</u> | 0.15 | 0.03 | -0.05 |
| DPM | -0.09 | -0.10 | -0.12 | 0.10 | -0.20 | -0.11 | <u>0.63</u> | | <u>0.85</u> | <u>0.39</u> | <u>0.39</u> | <u>0.38</u> | <u>0.63</u> | <u>0.36</u> | 0.06 | -0.01 |
| GF | 0.15 | -0.02 | 0.15 | <u>0.23</u> | -0.17 | -0.14 | <u>-0.47</u> | <u>0.37</u> | | <u>0.45</u> | <u>0.24</u> | <u>0.28</u> | <u>0.54</u> | <u>0.36</u> | 0.05 | 0.04 |
| HI | <u>0.55</u> | 0.08 | 0.18 | 0.09 | -0.12 | <u>-0.24</u> | <u>-0.24</u> | -0.15 | 0.12 | | <u>0.34</u> | <u>0.38</u> | 0.16 | <u>0.30</u> | 0.35 | 0.00 |
| KNS | 0.19 | -0.03 | -0.06 | 0.00 | -0.16 | <u>-0.26</u> | 0.01 | -0.03 | -0.04 | <u>0.27</u> | | <u>0.38</u> | <u>0.22</u> | 0.19 | <u>-0.21</u> | <u>-0.21</u> |
| KNA | <u>0.27</u> | -0.02 | <u>-0.22</u> | <u>0.51</u> | -0.11 | -0.02 | -0.17 | -0.06 | 0.13 | <u>0.31</u> | <u>0.30</u> | | 0.19 | <u>0.30</u> | <u>0.46</u> | 0.00 |
| NDVIg | <u>0.42</u> | -0.03 | -0.02 | <u>0.27</u> | <u>-0.47</u> | <u>-0.46</u> | <u>0.33</u> | <u>0.51</u> | 0.16 | 0.16 | 0.12 | 0.11 | | <u>0.46</u> | 0.07 | 0.01 |
| NDVIv | <u>0.35</u> | -0.19 | -0.19 | <u>0.20</u> | <u>-0.23</u> | <u>-0.22</u> | 0.01 | <u>0.21</u> | <u>0.24</u> | 0.17 | -0.05 | 0.09 | <u>0.61</u> | | 0.16 | <u>0.33</u> |
| SN | <u>0.34</u> | -0.02 | <u>-0.29</u> | <u>0.61</u> | -0.18 | -0.12 | -0.07 | 0.04 | 0.15 | <u>0.25</u> | <u>-0.43</u> | <u>0.40</u> | 0.19 | 0.33 | | 0.14 |
| NDVIev | 0.12 | -0.07 | 0.08 | 0.20 | -0.03 | -0.03 | -0.15 | -0.07 | 0.13 | -0.12 | <u>-0.28</u> | -0.04 | 0.13 | <u>0.32</u> | 0.16 | |

[†] GY, grain yield; TW, test weight; AKW, average kernel weight; BM, above ground biomass; CTg, average canopy temperature during grain fill period; CTv, average canopy temperature during vegetative growth; DF, days to 50% of spikes with visible anther extrusion; DPM, days to 50% of peduncles senesced, GF, grain fill period; HI, harvest index; KNS, number of kernels per spike; KNA, kernel number m⁻²; NDVIv, normalized difference vegetative index during vegetative growth; NDVIg, normalized difference vegetative index during GF; SN, spike number m⁻²; NDVIev, early vigor as measured by NDVI.

Appendix 6. Genetic mean distance dendrogram for grain yield calculated by TASSEL software for genome A by SHW populations and parental lines. Arrows indicate the four best yielding SHW-derived lines overall. A) HxSYN-1 population, B) HxSYN-2 population, and C) HxSYN-4 population.



Appendix 7. Genetic mean distance dendrogram for grain yield calculated by TASSEL software for genome B by SHW populations and parental lines. Arrows indicate the four best yielding SHW-derived lines overall. A) HxSYN-1 population, B) HxSYN-2 population, and C) HxSYN-4 population.



Appendix 8. Genetic mean distance dendrogram for grain yield calculated by TASSEL software for genome D by SHW populations and parental lines. Arrows indicate the four best yielding SHW-derived lines overall. A) HxSYN-1 population, B) HxSYN-2 population, and C) HxSYN-4 population.

