GENETIC DIVERSITY AMONG HARD RED WINTER WHEAT CULTIVARS AND EXPERIMENTAL LINES AND THEIR ASSOCIATION WITH WATER LIMITATION

By
MESHAL MUNAWIR R. ALMUTAIRI

Bachelor of Agriculture and Science
Qassim University
Kingdom of Saudi Arabia
2004

Master of Food and Agricultural Science
King Saud University
Kingdom of Saudi Arabia
2009

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY

December, 2017

GENETIC DIVERSITY AMONG HARD RED WINTER WHEAT CULTIVARS AND EXPERIMENTAL LINES AND THEIR ASSOCIATION WITH WATER LIMITATION

Dissertation Approved:

Dr. Michael P. Anderson

Dissertation Advisor

Dr. Yanqi Wu

Dr. Vijaya G. Kakani

Dr. Justin Q. Moss

ACKNOWLEDGEMENTS

First and foremost, I would like to thank GOD whose many blessing have made me who I am today. In addition, this research could not be done and accomplished without help from some people who contributed so much value and knowledge. I fell to express my grateful of acknowledgment for them. I'm indebted for their contribution in my research and study.

I would like to express the deepest appreciation to my academic advisor, Dr. Michael Anderson, who always encourages me to do my best work and directs me into good methodical working. In addition, his counseling helped me during my Ph.D. study and courses at Oklahoma State University. I could not have imagined having a better advisor and mentor for my Ph.D. study and thus I am grateful to him.

I also thank my wife who has not only supported me throughout my academic trajectory despite our alienation but also to stood with me through all my travails, and my absences.

I'm also a favor to express my thanks to committee members, Dr. Yanqi Wu, Dr. Vijaya Kakani, and Dr. Justin Moss, and other faculty, Drs. Brett Carver, Joshua Habiger, and Carla Goad. They helped me by discussion a great idea for this research for the better processes.

Names: MESHAL MUNAWIR R. ALMUTAIRI

Date of Degree: December, 2017

Title of Study: GENETIC DIVERSITY AMONG HARD RED WINTER WHEAT CULTIVARS AND EXPERIMENTAL LINES AND THEIR ASSOCIATION WITH WATER LIMITATION
Major Field: CROP SCIENCE

Abstract The effect of water limitation on Hard Red Winter Wheat (HRWW) production is a major concern for the wheat industry throughout the Southern Great Plains. Average HRWW yield was compared between irrigated and rainfed conditions at four locations within the High Plains region of Texas and New Mexico, and then screened among 19 HRWW cultivars and experimental lines were selected and screened to identify best performance under three levels of water limitations under greenhouse condition. From the 19 HRWW cultivars, nine were selected to undergo further screening under the same treatment conditions for yield characteristics. Finally, genetic diversity for these 19 HRWW cultivars and experimental lines were analyzed using genotyping by sequencing (GBS) for genetic relatedness. Results from the panhandle of Texas showed that CJ, TX02A0252, and Ruby Lee, had the highest rainfed/irrigation yield ratio. On the other hand, Iba, Billings, and Mace had the lowest rainfed/irrigation yield ratio. Under greenhouse condition, results under severe water limitation in preliminary evaluation of the 19 cultivars and experimental lines showed that Byrd, Ruby Lee, TAM 113, and Duster showed higher average seed weight whereas Endurance, Chisholm, and Gallagher showed lower average in the same trait across two cycles of growth and development. In the final screening, results showed that Gallagher, Ruby Lee, and Endurance showed higher average seed weight whereas Jagger, Byrd, and Cedar showed lower average seed weight. The result of discriminant analysis showed that seed number and spike number were the most important traits that contributed to discrimination among 19 HRWW cultivars and experimental lines across all cycles of growth and development. The Neighbor joining tree divided 19 HRWW cultivars and experimental lines into five groups consisting of group one Iba, Duster, OK12621, OK10126, Cedar, Garrison, and Hatcher, group two Byrd, TAM 112, TAM 113, and TAM 111), group three Endurance and Ruby Lee, group four Chisholm and Gallagher, and group five Jagger, OK Bullet, Bentley, and OK11D25056. The higher genetic similarity was found between Duster and Iba whereas the least similar was found between Jagger and Hatcher.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

CHAPTER III:

CHAPTER IV:

LIST OF FIGURES

CHAPTER I

GENERAL INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is one of the two most important cereal crops for human consumption along with rice (Gary and James, 2008). Two-thirds of wheat production is used for human consumption while one third is used as animal feed (Araus et al., 2007; Krishna, 2015). Wheat is one of the healthiest grains for human nutrition with many complex carbohydrates, vitamins, and minerals providing substantial daily caloric intake as a principal source of energy to humans (Bushuk and Rasper, 2012). Furthermore, wheat gluten, a commercial product of the wheat industry, is used in bread making. The added wheat protein, gliadin and glutenin, improves the bread-making properties of wheat flour significantly (Goutam et al., 2013) and is used extensively in the baking industry. In fact, hard red winter wheat (HRWW) flour is very desirable because of its high protein content (14%), mellow gluten content, and chewy texture, which contributes to improved bread making and nutritional value (Pomeranz et al., 1985).

Wheat is classified as winter and spring wheat (*Triticum aestivum* L.) depending on the season when the seed is sown. Farmers in the northern part of United States typically grow spring wheat planted in late winter and harvested in early summer while farmers in the south central parts of the United States typically grow winter wheat planted in late September and harvested in May. Winter wheat is ranked second behind spring wheat for its contribution to international of wheat production (United States Department of Agriculture (USDA-ARS, 2010). The large wheat producing nations are the European Union, China, India, Russia, and the United States (USDA-ARS, 2016). China uses most of its wheat production for internal consumption while the United States exports more than 40% of its wheat internationally (Asseng et al., 2015). The United States provides more wheat for the international market than any other country [United States Department of Agriculture Economic Research Service (USDA-ERS), 2016].

Production of wheat in the United States has decreased considerably from 1990 to 2016. The trend of production in the past 26 years decreased -0.602%/per year. This reflects the decrease in both planted (-1.14%) and harvested (-1.53%) acreage. However, the yield average in these 26 years was 11.36 metric tons per acre and the trend for yield has increased by 0.94%/year (USDA-ARS, 2016). In 2016, USDA-ARS data showed that among all wheat classes, production was 62.82 million metric tons where 25.85 million metric tons were exports, 26.62 million metric tons for food, 1.72 million metric tons as seed, and 8.8 million metric tons for feed and residual uses (USDA-ARS, 2016). Approximately 40% of all wheat planted in the United States is HRWW, which is principally grown in Oklahoma, Kansas, Colorado, and Texas. In 2015, the production of HRWW in Oklahoma was 2.88 million metric tons (USDA-ARS, 2016; Plains Grains Inc., 2015).

From the earliest time, most breeding programs were focused on creating new varieties with high yield potential. An understanding of genetic diversity and relatedness of wheat selections is an important goal in most breeding programs. The genetic diversity refers to the numbers of alleles within a particular cultivar of wheat. Genetic diversity within a particular species plays a fundamental role allowing wheat to survive and adapt to changing environments. Creating a new wheat cultivar involves the shuffling and selection of adaptive genes through genetic selection. Improvement of modern wheat cultivars response to drought will rests primarily on the breeding and selection of genetic elements that permit adaptation to water limitations (Budak et al., 2013).

Modern molecular technologies have been utilized to improve and develop modern wheat cultivars. These technologies are used in genetic investigation that introduce novel genetic material through genetic transformations (Nelson et al., 1995). Many technologies for improving tolerance to drought stress have been utilized at numerous universities, non-governmental organizations, and private companies including: Oklahoma State University, International Maize and Wheat Improvement Center (CIMMYT), and Monsanto, Inc. Some of these technologies include restriction fragment length polymorphisms (RFLP) (Neuhausen, 1992), random amplified polymorphic DNA (RAPD) (Garcia, 1998), amplified fragment length polymorphism (AFLP), simple sequences repeats (SSR) (Katzir et al. 1996) and most recently GBS (Elshire et al., 2011). These technologies are readily utilized by many breeding programs for marker selection and genotyping to provide information to breeders concerning desirable lines for cultivars improvement.

The wheat genome is one of the largest of any agricultural crop with an overall genome size of approximately 17 giga-base pairs (Hart and Ruvolo, 2012). Hexaploid wheat genome consists of three separate and functionally distinct genomes called A, B and D. Characterizing this complex multi-genome and its many variants continues to be a major challenge for wheat scientists. Advanced genotyping technologies may offer a better and more precise and powerful genome comparison than any other methodology (Salem et al., 2008; Hart and Ruvolo, 2012). Jordan and Humphries (1994) developed a method that provides a high degree of resolution by identifying nucleotide differences known as single nucleotide polymorphisms (SNPs). These polymorphisms can be analyzed in multiple cultivars simultaneously, comparing the sequences and identifying variants sequences using a protocol known as GBS. The many nucleotide variants are then analyzed to characterize genetic relatedness and to associate with specific traits.

Wheat production needs adequate water and nutrients in order to obtain optimal yield. Adequate water can be supplied through irrigation or by rainfall. Typically, in rainfed areas, soil moisture in the winter and the spring in Oklahoma averages 69% of field capacity and in the summer declines to below 29% of field capacity when water is most needed. Typically, in the summer months, wheat fields are left fallow to conserve valuable soil moisture for fall plantings. Soil moisture is impacted by current precipitation, air temperature (Deliberty and Legates, 2003) and soil texture. Soils with a relatively high clay content hold moisture more than sandy soils, whereas sandy clay loam and silt loam are somewhat in between. In addition, the lack of good soil moisture overtime will greatly impact wheat yield. Thus, soil moisture management is critical for maximizing wheat yield for sustained production (Nolz et al., 2016).

Yield parameters are the most useful of all factors to assess cultivar response to drought. To do so, it would be useful to locate the experiment in an area where water deficits are common. One such area is in the High Plains region of Texas and New Mexico where rainfed wheat receives on average less than 22 inches of rainfall per year, well below its optimal. Comparing rainfed wheat with irrigated wheat provides a measure of a particular cultivars tolerance to drought. Fortunately, data by the Texas Agricultural Experiment Station is available for multiple years for this comparison (Deliberty and Legates, 2003).

The hypothesis of the first study was that there were differences among cultivars and experimental lines for their field performances under water limitation. Thus, the first objective of this study was to use the irrigated and rainfed cultivar trials from the High Plains region of Texas and New Mexico over multiple years to identify HRWW cultivars that are best drought response. While this approach has its advantages there are significant interactions that one must consider when interpreting the results. Yield data from the field is not solely influenced by soil water availability. Other factors such as soil types, biotic stressors, management and the degree of rainfed contribution to productivity influences significantly the interpretation of any field-based analysis. Thus, additional screenings are necessary under more controlled and monitored conditions to assess performance under a range of water deficits in order to assess the true genetic potential of a given cultivar (Nolz et al., 2016).

The hypothesis of the second study was that there are differences among cultivars and experimental lines for their drought performance under greenhouse conditions. The second objective of this study is to assess the level of best drought response of HRWW under greenhouse conditions using cultivars from the High Plains region data of Texas and New Mexico, selected cultivars and experimental lines under development by the Oklahoma State Wheat Breeding program. These selections will be grown under controlled conditions over multiple cycles of growth and development under non-stressed, moderate and severe stress conditions. Here we anticipate the true level of best drought response will show forth.

The hypothesis of the third study was that there was genetic relatedness among cultivars and experimental lines. The third objective was to assess the genetic relatedness among 19 tested HRWW cultivars and experimental lines using GBS. The data from our sequencing efforts will provide clues as to the overall genetic background of a given wheat genotype and their association with yield performance under moderate and severe water limitation.

REFERENCE

Araus, J., J. Ferrio, R. Buxo, and J. Voltas. 2007. The historical perspective of dryland agriculture: lessons learn from 10,000 years of wheat cultivation. J. Expt Bot, 85:131-145. DOI: 10.1093/jxb/erl133.

Asseng, S., F. Ewert, P. Martre, R. Rötter, B. Lobell, D. Cammarano, and P. Reynolds. 2015. Rising temperatures reduce global wheat production. N. C. C. 5(2), 143-147. doi:10.1038/nclimate2470.

Budak, H., M. Kantar, and K. Kurtoglu. 2013. Drought tolerance in modern and wild wheat. H. P. C. 2013:16

Bushuk, W., and V. Rasper. 2012. Wheat production, properties and quality. S. S. & B. M. 239 pages.

Deliberty, T., and D. legates. 2003. Internal and seasonal variability of modelled soil moisture in Oklahoma. Int. J. of C. 23:157-1086.

Elshire, R., J. Glaubitz, Q. Jesse, J. Poland, K. Kawamoto, E. Bukler, S. Mitichell. 2011. A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. PLoS ONE 6(5): e19379. doi:10.1371/J. P. 0019379.

Garcia, E., M. Jamilena, J. Alvarez, T. Arnedo, J. Oliver, and R. Lozano. 1998. Genetic relationships among melon breeding lines revealed by RAPD markers and agronomic traits.Theor. A. G. 96:878–885.

Gary, M., and S. James. 2008. The early history of wheat improvement in the Great Plains. A. J. 100:70–78. doi:10.2134/agronj2006.0355c.

Goutam, U., S. Kukerja, R. Tiwari, A. Chaudhury, R. Gupta, B. Dholakia, and R. Yadav. 2013. Biotechnological approach for grain quality improvement in wheat: Present status and future possibilities. A. J. of C. S. 7(4):469-483.

Hart, D. and M. Ruvolo. 2012. Genetic: Analysis of Genes and Genomes. 8th ed. Printed in the United States of America.

Jordan, S., and P. Humphries. 1994. Single nucleotide polymorphism in exon 2 of the BCP gene on 7q31-q35. Human Mol Genet 3:1909-1915.

Katzir, N., Y. Danin-Poleg, G. Tzuri, Z. Karchi, U. Lavi, and P. Cregan. 1996. Application of RAPD and SSR analysis to the identification and mapping of melon (*Cucumis melo* L.) varieties. In:Lester GE, Dunlap JR (eds). Proc. Cucurbitaceae. 94: evaluation enhancement cucurbit germplasm. Gateway Printing and Office Supply, Edinburg, Tex., p 19.

Krishna, K. R. 2015. Agricultural Prairies: Natural Resources and Crop Productivity. Florida: CRC Press. A. A. P. 445.

Nelson, J., E. Sorrells, E. Van Deynze, H. Lu, M. Atkinson, M. Bernard, P. Leroy, D. Faris, and A. Anderson. 1995. Molecular mapping of wheat: major genes and rearrangements in homologous groups 4, 5, and 7. G, 141: 721-731.

Neuhausen, S. 1992. Evaluation of restriction fragment length polymorphism in Cucumis melo. T. A. G. 83:379-384.

Nolz, R., P. Cepuder, J. Balas, and W. Loiskandl. 2016. Soil water monitoring in vineyard and assessment of unsaturated hydraulic parameters as threshold for irrigation management. A. W. M. 164:235-242. http://dx.doi.org/10.1016/j.agwat.2015.10.030

Plains Grains Inc. 20015. Production. Retrieved September 5, 2016 fro https://plainsgrains.org/our-product/.

Pomeranz, Y., L. Bolte, K. Finnet, and M. Shogren. 1985. Effect of variations in tempering on micromilling of hard winter wheat. Cereal Chem. 62(1):47-50.

Salem, K. F., M. El-Zanaty, and M. Esmail. 2008. Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatellite markers. W. J. A. S. 4(5): 538–544. DOI:10.1111/j.1744-7348.2007.00201.x.

United States Department of Agriculture (USDA). 2010. United States Department of Agriculture. Agricultural Research Service. Wheat Data. (n.d.). Retrieved December 18, 2015, from https://www.nrcs.USDA-ARS.gov/wps/portal/nrcs/main/soils/survey/office/ssr9/tr/ United States Department of Agriculture.

United States Department of Agriculture (USDA). 2016. United States Department of Agriculture. Economic Research Service. Wheat Data. (n.d.). Retrieved August 10, 2017 from http://www.ers.USDA-ARS.gov/data-products/wheat-data.aspx. United States Department of Agriculture.

CHAPTER II

REVIEW OF THE LITERATURE

Impact of drought stress upon winter wheat cultivars

The Southern Great Plains is a semiarid region effected by severe to extreme droughts, which are projected to persist and intensify due to a changing climate, leading to an intensification of wind, air temperature, and low water availability (Patrignani et al. 2014). The wheat growing area of this region receives on average less than 22 inches of rainfall per year but accounts for 29% of the United States wheat production. In the High Plains region there were more than 30 million acres under irrigation and 4 million acres under of rainfed management. The Ogallala aquifer is the primary water source for crop irrigation in the region (Krueger et al., 2015). This aquifer is being continually depleted, which ultimately will lead to significantly lower water availability in the near future (Krueger et al., 2015). Therefore, it is essential to study the effect of drought stress in anticipation of projected trends (Patrignani et al. 2014).

Previous work has shown significant differences in average winter wheat yield under drought stress in many parts of the world. Investigation of winter wheat performance under drought stress helps researchers to identify traits in an effort to create drought tolerant cultivars. Chen et al. (2012) evaluated several morphological traits for 90 winter wheat cultivars under water stress conditions during one growing season. Winter wheat cultivars were planted in the field under two irrigated regimes at 2000 m^3 irrigation/hectare and 1200 m^3 irrigation/hectare. They found significant variation under the different water levels for most yield traits, including: thousand-kernel weight, grain yield/plant, plant height, spike length, and grain number of spikes; however, there was no significant difference in spikelet number under the two water levels. In addition, they found that spikelet number, grain number per spike were significantly and positively correlated with yield. These two traits explain 92% of the total variation in wheat grain yield and are therefore useful indicators to evaluate the impact of drought on wheat yield. They concluded that the drought tolerant cultivars revealed under this study were good candidates for inclusion in a breeding program. The authors showed that single morphological traits cannot reveal the complex variation associated with drought tolerance in wheat performance. Moreover, according to Blum et al. (1988), selection for best drought performance under field conditions is complicated by large in-field variations and a low degree of heritability necessitating multiple replication and year to year sampling.

Others have found additional whole plant morphological traits across developmental stages associated with grain yield. Ranjbar et al. (2015) evaluated 30 wheat cultivars under rainfed conditions and found that the tiller number/plant had the highest variation and 1000-grain weight showed the lowest under drought stress. In addition, the simple correlation analysis showed a high positive relationship between grain yield and number of spikes/plant ($R^2 = 0.88$). These results indicated the importance of number of spikes for improving grain yield. In the same study, many variables including soil components were included in the analysis to determine the effect of drought on grain yield parameters. According to the authors, cluster analysis indicated that the number of spikes/plant, soil acidity, tiller number s, and sodium soil concentration were grouped apart in a multivariate analysis from spike length, number of grain $spike^{-1}$, soil calcium, and magnesium levels. Thus, the effect of soil and yield parameters differed in their effects on grain yield under drought conditions. Correlation and cluster analysis of yield components and soil factors provide a benefit to select traits for breeding and valuable information for wheat production managers.

It is suggested that imposition of drought stress during the vegetative stages of wheat development will be beneficial in order to select criteria for wheat improvement. El Hafid et al. (1998) evaluated the effect of early drought stress on six spring wheat cultivars at the vegetative stage. The authors screened six cultivars of winter wheat for phenotypic variation across three levels of drought stress. All these cultivars significantly differed number of tillers. One experimental line, LA V18, achieved the earliest physiological maturity than others under severe drought stress. Three of the cultivars showed the highest yield: LA V17, Karim and Marzak with the highest number of spikes and highest number of kernel per spike. These tillered earlier than others (ACSAD 65). Three of the cultivars (LA V17, Karim and Marzak) were shown to speed up development in response to drought stress. In addition, there was a strong positive correlation between the grain yield components and the tiller number.

Fischer and Maurer (1978) suggested that evaluating wheat under drought conditions at every wheat stage, but especially at flowering, is critical to the maximization grain yield under drought stress (Flohra et al., 2017). Drought at pre-anthesis delayed flowering in bread wheats. Khakwani et al. (2012) evaluated the impact of two levels of drought stress on the booting and the flowering stages of six wheat cultivars (Damani, Hashim-8, Gomal-8, DN-73, Zam-04 and Dera-98). Days of 50% heading was measured, which is the time it takes for 50% of the wheat in the field to produce a spike. The authors found varietal differences where DN-73 took 78 days to produce 50% of spikes whereas Hashim-8 reached 50% of spikes in 74 days. Flowering was advanced more rapidly in one cultivar compared to the other under drought conditions under drought stress compared to well water plants. Fewer tillers (35%) were observed under drought stress in all wheat cultivars.

Grain filling is also very sensitive to drought stress. Primarily, grain yield is partially dependent on the grain weight and grain weight depends on the ability of the wheat plant to translocate carbohydrate into the growing kernel. In the field, Abdoli et al. (2013) examined eight wheat cultivars effected by drought during grain filling. They reported that drought did not influence the number of spikes/m^2 and the number of grains/spike but caused 26% reduction in grain weight. The average grain yield was 701g/m^2 under well water condition, declining significantly to 463g/m^2 under drought stress conditions. The reason for this reduction is that physiologically, wheat exhibited a decreased power for absorbing the photo-assimilates from the rest of the plant during drought, thus reducing overall yield. In other words, wheat is assimilation limited in terms of grain filling.

Wheat can be induced to be more resistant to water limitation if a priming stress was imposed early in wheat development. In the greenhouse, Abid et al. (2016) evaluated two winter wheat cultivars Luhan-7 and Yangmai-16 under drought stress during anthesis and grain filling when earlier primed in comparison to no priming event. The priming event increased resistance to yield loss more so in Luhan-7 than in Yangmai-16 indicating a varietal difference in the priming effect. Therefore, previous drought events in development can affect overall response to drought in later developmental stages, and this aspect is likely to be genetically determined.

Developing a drought index that captures the differences between rainfed and irrigated condition is an important goal for the identification of drought tolerant cultivars. Drought indices depend on duration of drought (time) and magnitude (drought threshold) to measure the reduction of yield. One of these indices is the susceptibility index, which is described by Fischer and Maurer (1978). The drought susceptibility index refers to the relationship between yield under drought stress (x axis) and yield under well watered conditions (y axis). This relationship is plotted using regression analysis and is expressed as a slope. The lower the value of this index the more drought tolerant is the cultivar; the higher the value of the index the more drought susceptibility is the cultivar Fischer and Maurer (1978). This susceptibility index has been used as a selection method for identification optimal yield for wheat cultivars under drought stress. This index also reflects the assessment of drought stress at targeted locations (Hayes et al. 2000).

At Texas A&M University, Lazar et al. (1995) evaluated the effect of drought stress on nine winter wheat cultivars, in the Bushland region within the High Plains region of Texas under irrigation condition, and Washburn, Etter and Stinnett under rainfed condition. They found that TAM-107 produced more yield under rain-fed condition when compared to other cultivars in the study. They found that TAM-107 exhibited a low relative susceptibility index and therefore was considered drought tolerant whereas TAM-105 showed a relatively high susceptibility index indicating a susceptibility to drought stress.

Screening among winter wheat cultivars for drought tolerance

Choice of screening methods is important for evaluating the effect of drought stress on wheat productivity among wheat cultivars. Screening methods for best drought performance among cultivars should utilize a uniformly applied water limitation across all experimental units (Khakwani et al., 2011) and should use multiple growing seasons across multiple locations to better represent field conditions (Farooq et al., 2015). In addition, the timing of drought stress in wheat development is an important factor to consider in any screening program. Khakwani et al. (2011) screened six wheat cultivars 'Damani, Hashim-8, Gomal-8, DN-73, Zam-04, and Dera-98' for drought tolerance under three levels of water stress (100%, 35% and 25% of field capacity) measuring seed germination, tiller number/plant, number of spikes/plant and using the susceptibility indices as described by Fischer and Maurer (1978). There was no difference among cultivars in percentage of seed germination under all three levels of water stress. A significant reduction in tiller number/plants among cultivars was observed under severe stress with one experimental line, Dera-98 showed more tiller (4 tillers) than any other cultivars. Severe stress significantly affected wheat performance among all cultivars by decreasing number of grain/spike compared to well water plants at 100% of field capacity. The authors showed that cultivar, Hashim-8, had a low value of the susceptibility indices indicating drought tolerance based on number of grain/ spikes. This Hashim-8 showed its ability tolerate water stress by producing minimum vegetative structures (3 tillers) with high weight grains per plant compared with other cultivars under drought stress.

The susceptibility index has not proven to be an infallible tool in screening wheat cultivars for drought stress. Mortazavian et al. (2015) screened 39 wheat cultivars for drought tolerance under two years of drought stress using the susceptibility indices method during 2010 to 2012 of growing seasons by applying stress during flowering. The most tolerant cultivars were Hirmand, Star, and Toos in the first year and Zarrin, Akbari, and Sardari in the second year. The difference between the two years indicated a significant environmental interaction with this index which must be taken into account by performing multiple screening events by location and year.

The yield components are the best measurements used under any cultivar screening program for best drought performance, representing the end product of all farming operations. Jatoi et al. (2011) studied the effect of withholding water for 20 days during the early grain filling on 12 selected wheat cultivars in an effort to identify high yielding cultivars. Measured traits included the number of grains/spike, 1000 kernel weight and relative water content of leaf tissues as a main sources of genetic variation among wheat cultivars. Among these 12 wheat cultivars, Inqilab, Anmol, and Imdad-05 produced less number of grains/spikes (35 grains) under water stress illustrating drought susceptibility, whereas TD-1 and SKD-1 produced more grains/spike (more than 50 number of grains) exemplifying of drought tolerance.

Xue et al. (2014) investigated the genetic screening of ten HRWW cultivars under water limitation condition conducted in the fields of Bushland, TX at Texas A&M AgriLife Research station. These ten cultivars were TAMs cultivars (105, 110, 111, and 112), Dumas, Jagalene, TX99A0153-1, TX86A5606, TX88A6880, and TX86A8072. They found that TAM 112, TAM 111, and TAM 110 had higher yield and higher stem dry weight than TAM 105 under dryland condition. In addition, the authors showed that TAM 112 and TAM 111 had higher yield than the older related cultivar TAM 105, which showed significant genetic improvement under dryland conditions in Texas. Moreover, germplasm of these two cultivars, TAM 112 and TAM 111, have been used in may breeding program to improve production of Southern Great Plains.

Another study in the Southern Great Plains in Bushland, TX was performed by Reddy et al. (2014) who evaluated the performance of TAM 111 and TAM 112 under water deficit in two gallon pots under greenhouse condition. The plants were watered to 50% (stressed) and 100% (well water) of gravimetric water content. This measurement is based on the mass of water per mass of soil. They found that cultivar TAM 112 produced more grain yield and showed reduced stomatal conductance leading to reduce photosynthesis compared with cultivar TAM 111. These results are in accordance with studies by Reddy et al. (2014) and Xue et al. (2014) which showed TAM 112 to be more drought tolerance than other cultivars.

Genetic diversity among winter wheat cultivars

The genetic diversity of breeding lines is the main resources used by a plant breeding program for the purpose of improving wheat response to drought stress. A high level of genetic diversity provides breeders with a wide source of variation for productive traits. There are several marker systems that can be used to measure genetic diversity, such as: RFLP (Neuhausen, 1992), RAPD (Garcia, 1998), AFLP, and SSR (Katzir et al. 1996) and more recently GBS (Elshire et al., 2011). In Japan, Kobayashi et al. (2016) examined the genetic diversity among Japanese wheat cultivars using the GBS method through Neighbor-Joining (NJ) methods as describe by Saitou and Nei (1987) to infer a phylogenetic tree. From the phylogenetic tree, the authors were able to classify these cultivars into three groups (Hokkaido area, Southwest Japan, and landraces). The first group and the second group had comparative low genetic diversity and the last group (landraces) had a much higher genetic diversity. Thus, the landrace group was recommended for use as germplasm for breeding research. Thus, GBS methods are good for the estimation of genetic diversity among breeding lines and cultivars.

Allelic variation is important as well and can be determined by genotype by sequencing. Bajgain et al. (2016) estimated the genetic diversity using GBS method among 141 $F_{6:7}$ recombinant inbred lines of wheat. These lines were created by crossing the spring wheat lines RB07 with MN06113-8. This genetic diversity or the frequency of number of difference alleles was calculated based on the Kosambi mapping distance (Kosambi, 1943) as a measure of the average distance of linked genes on the same chromosome. The results revealed the number of polymorphism that were found was 932 single nucleotide polymorphisms (SNP) among these lines. The authors found that 46% of SNP markers came from MN06113, whereas 49% derived from RB07, and the remaining 5% were heterozygotes. These heterozygotes, which were the main sources of genetic variation were used to enrich the alleles for targeted traits.

Genetic diversity is also important for the adaption of wheat to environmental changes to different locations, and breeders need to make selections based on local environments. The genetic diversity of 242 accessions of wheats from around the world, developed from 1940 to 1990, was estimated using AFLP markers in order to track breeding progress over time (Tian et al., 2005). The authors calculated the genetic diversity as total gene frequency divided by total loci detected using five AFLP primers. They found that highest genetic diversity was found in the 1950 population, which came predominantly from landraces whereas the lowest genetic diversity index was found in the 1990s population. An average of 245 polymorphic bands were detected among these accessions of wheat. This result demonstrated that genetic diversity declined from 1940 to 1990 resulting in a narrowing of the genetic pool from which to form new cultivars. The decline in genetic diversity may be due to a bias in the exchange the germplasm among different breeding programs world-wide over time. The lower genetic diversity has significant effects on cultivar creation in that this decrease will limit the creation new genotypes of wheat adapted to a broad range of environment conditions. Other markers were used by Couviour et al. (2011) who screened 195 winter wheat cultivars for genetic diversity using SSRs markers and diversity array technology (DArT). These DArTs are dominant markers for sequences the genotyping based on SNPs (White et al. 2007). These cultivars were obtained from 18 companies from France, the United Kingdom, and Germany. The results of their study showed an average of 7.49 alleles per gene across all cultivars from a total of 1191 alleles. Both chromosome 5B and 7B were similar with a number of common translocations detected. Likewise, the results of DArT showed that average distance of the 634 markers was low. The analysis separated all the cultivars into two clusters. The first cluster constituted United Kingdom wheat cultivars whereas the second cluster included both French and German cultivars, indicating differences in breeding stocks.

Hexaploid winter wheat genomes are complex in structure due the combination of three separate and distinct genomes. For example, Hanif et al. (2014) discussed a crucial role for genetic diversity in plant breeding especially in terms of genome evolution. They evaluated Ds genome for genetic diversity using fifty-eight synthetic hexaploid cultivars of winter wheat and 71 SSR primers. These synthetic hexaploid wheats were created by crossing 9 wheats with 31 of *Ae. tauschii* followed by colchicine treatment to create double haploids in the F1 hybrids. These results showed that chromosome 4D and 6D demonstrated higher diversity of alleles than the other chromosomes and that alleles from chromosome D could be favorably used in targeted breeding program and wheat improvement.

REFERENCE

Abdoli, M., M. Saeidi, S. Jalali-Honarmand, S. Mansourifar, and S. Ghobadi. 2013. Effect of post-anthesis water deficiency on storage capacity and contribution of stem reserves to the growing grains of wheat cultivars. P. K. J. *2*(3):99-107.

Abid, M., Z. Tian, S. Ata-Ul-Karim, Y. Liu, Y. Cui, R. Zahoor, D. Jiango, and T. Dai. 2016. Improved tolerance to post-anthesis drought stress by pre-drought priming at vegetative stages in drought-tolerant and -sensitive wheat cultivars. P. P. and B. 106: 218-227. Doi: 10.1016/j.plaphy.2016.05.003.

Bajgain, P., M. Rouse, and J. Anderson. 2016. Comparing Genotyping-by-Sequencing and Single Nucleotide Polymorphism Chip Genotyping for Quantitative Trait Loci Mapping in Wheat. C. S. 56:232–248. Doi: 10.2135/cropsci2015.06.0389.

Chen, X., D. Min, T. Yasir, and Y. Hu. 2012. Evaluation of 14 morphological, yield-related and physiological traits as indicators of drought tolerance in Chinese winter bread wheat revealed by analysis of the membership function value of drought tolerance (MFVD). F. C. R. 137:195–201. Doi: org/10.1016/j.fcr.2012.09.008.

Couviour, F., S. Faure, B. Poupard, Y. Flodrops, P. Dubreuil, and S. Praud. 2001. Analysis of genetic structure in a panel of elite wheat varieties and relevance for association mapping. T. A. G. 1233: 715-727. Doi: 10.1007/s00122-011-1621-9.

El Hafid, R., D. Smith, M. Karrou, and K. Samir. 1998. Morphological attributes associated with early season drought tolerance in spring durum wheat in a Mediterranean environment. E. 101: 273–282.

Farooq, S., M. Shahid, M. Khan, M. Hussain, and M. Farooq. 2015. Improving the Productivity of bread wheat by good management practices under terminal drought. J. A. C. S. 201:173-188.

Fischer, R., and R. Maurer. 1978. Drought resistance in spring wheat cultivars. I. Grain yield response. A. J. A. R. 29:897-912.

Flohra, B., J. Huntb, J. Kirkegaarda, and R. Evans. 2017. Water and temperature stress define the optimal flowering period for wheat in south-eastern Australia. F. C. R. 209: 108–119. http://dx.doi.org/10.1016/j.fcr.2017.04.012.

Garcia, E., M. Jamilena, J. Alvarez, T. Arnedo, J. Oliver, and R. Lozano. 1998. Genetic relationships among melon breeding lines revealed by RAPD markers and agronomic traits. T. A. G. 96:878–885.

Hanif, U., A. Rasheed, A. Kazi, F. Afzal, M. Khalid, M. Munir, A. Mujeeb-Kazi, A. 2014. Analysis of genetic diversity in synthetic wheat assemblage (*T. turgidum*×*Aegilops tauschii*; 2n=6x=42;AABBDD) for winter wheat breeding. C. 79(4):485-500.

Hayes, M.J., D. Svoboda, and A. Wilhite. 2000. Monitoring drought using the standardized precipitation index. In Drought: A Global Assessment. Edited by D.A. W. R. London, UK. pp. 168–180.

Jatoi, A., Baloch, M., Kumbhar, M., Khan, N., & Kerio, M. 2011. Effect of water stress on physiological and yield parameters at anthesis stage in elite spring wheat cultures. S. J. A. 27(1): 59-65.

Katzir, N., Y. Danin-Poleg, G. Tzuri, Z. Karchi, U. Lavi, and P. Cregan. 1996. Application of RAPD and SSR analysis to the identification and mapping of melon (*Cucumis melo* L.) varieties. In:Lester GE, Dunlap JR (eds). Proc. Cucurbitaceae. 94: evaluation enhancement cucurbit germplasm. Gateway Printing and Office Supply, Edinburg, Tex., p 19.

Khakwani, A., M. Dennett, M. Munir, and M. Abid. 2012. Growth and yield response of wheat varieties to water stress at booting and anthesis stages of development. ak. J. Bot. 44(3): 879-886.

Khakwani, A., M. Dennett, and M. Munir. 2011. Drought tolerance screening of wheat varieties by inducing water stress conditions. Songhlanakarin J. S. T. 33(2), 135-142.

Kobayashi, F., T. Tanaka, H. Kanamori, J. Wu, Y. Katayose, and H. Handa. 2016. Characterization of a mini core collection of Japanese wheat varieties using single nucleotide polymorphisms generated by genotyping-by-sequencing. B. S. 66: 213–225. Doi:10.1270/jsbbs.66.213.

Krueger, E. S., E. Ochsner, M. Engle, D. Carlson, D. Twidwell, and D. Fuhlendorf. 2015. Soil moisture affects growing-season wildfire size in the Southern Great Plains. S. S. S. of A. J. 79(6), 1567. Doi: 10.2136/sssaj2015.01.0041.

Lazar, M. C. Salisbury, and W. Worrall. 1995. Variation in drought susceptibility among closely related wheat lines. F. C. R. 41(3): 147-153.

Mortazavian, S., H. Ramshini, M. Mohseni, T. Nabavi. 2015. Assessment of wheat yield response to water shortage using various tolerance indices. P. A. S. 98(3):262-269.

Neuhausen, S. 1992. Evaluation of restriction fragment length polymorphism in *Cucumis melo*. T. A. G. 83:379-384.

Patrignani, A., R. Lollato, T. Ochsner, C. Godsey, J. Edwards. 2014. Yield gap and production gap of rainfed winter wheat. Agronomy, soil & environmental quality. 106:1329-1339. Doi:10.2134/agronj14.0011.

Ranjbar, A., A. Sepaskhah, and S. Emadi. 2015. Relationships between wheat yield, yield compoents and physico-chemical properties of soil under rain-fed condition. I. J. of P. P. 9(3) 434-465.

Reddy, S., S. Liu, J. Rudd, Q. Xue, P. Payton, S. Finlayson, J. Mahan, A. Akhunova, S. Holalu, and N. Lu. 2014. Physiology and transcriptomic of water-deficit stress responses in wheat cultivars TAM 111 and TAM 112. J. of P. P. 171: 1289-1298. http://dx.doi.org/1Xue0.1016/j.jplph.2014.05.005.

Tian, Q., R. Zhou, and J. Jia. 2005. Genetic diversity trend of common wheat (Triticum aestivum L.) in china revealed with AFLP markers. G. R. and C. E. 52: 352-331.

Xue, Q., J. Rudd, S. Liu, K. Jessup, R. Devkota, and J. Mahan. 2014. Yield determination and water use efficiency of wheat under water-limited conditions in the U.S. Southern High Plains. C. S. 54: 34–47. Doi:10.2135/cropsci2013.02.0108.

CHAPTER III

IMPACT OF IRRIGATED AND RAINFED WATER CONDITIONS ON YIELD OF HARD RED WINTER WHEAT CULTIVARS AND EXPERIMENTAL LINES IN THE HIGH PLAIN REGION OF TEXAS AND NEW MEXICO

ABSTRACT

The main factor that causes yield loss in wheat is drought stress, which is especially evident in the Southern Great Plains. Therefore, increasing our knowledge about the performance of Hard Red Winter Wheat (HRWW) (*Triticum aestivum* L.) cultivars under drought stress is an essential step to evaluate these cultivars at their targeted location. The objective of this research was to compare HRWW yield data under irrigated and rainfed conditions from publicly available data in the Panhandle of Texas and New Mexico from 2009 to 2012. The results showed that cultivars CJ, TX02A0252, and Ruby Lee, had the highest ratio of yield from rainfed over irrigation of all 36 cultivars considered and are therefore considered the best drought responsive cultivars. The cultivars Iba, Billings, and Mace had the lowest ratio of rainfed/irrigation and, therefore, these cultivars are considered the most drought susceptible. Overall, CJ showed the highest rainfed yield and the highest ratio of rainfed/irrigated yield of all 36 cultivars. Of the cultivars released by Oklahoma State University, Duster, Garrison, Billings, and Ruby Lee are the

best drought responsive cultivars. The best cultivars across environments were Winterhawk, TX02A0252, and Hatcher, primarily due to their high yield under irrigated and above average yield under rainfed conditions across this highly variable environment.

INTRODUCTION

It is projected that there will be increased occurrence of drought in the Southwestern part of the United States where HRWW is grown. In 2016, Texas experienced a severe drought resulting in a HRWW yield loss of 65 million metric tons. Yield has varied substantially due environmental interactions in the region under field conditions. This yield variability is projected to become worse with a changing climate. Similar yield instability is projected to occur throughout the winter wheat growing areas that in the United Sates including Colorado, Kansas, Oklahoma, and Texas. To limit the effects of drought on wheat productivity, breeders throughout the region have been actively seeking stable germplasm that they can use in developing new cultivars with improved drought resistance. These efforts are directed at screening programs among current and potential wheat accessions that are currently used or are under development. Once promising germplasm are identified, these can be readily incorporated into current breeding pipelines for improving drought resistance in future cultivars (Xue et al., 2014).

Breeders are currently screening promising breeding lines and new cultivars to identify traits that are significantly impacted by water limitations. Ranjbar et al. (2015) evaluated 30 wheat cultivars for yield characteristics under rainfed conditions at physiological maturity and found that the tiller number/plant had the highest variation whereas 1000-grain weight showed the lowest under drought stress. In addition, simple correlation analysis showed a high positive relationship between grain yield and number of spikes/plant ($R^2 = 0.88$). Consequently, drought stress at the grain filling stage can dramatically reduce overall wheat yield. Jatoi et al. (2011) studied the effect of withholding irrigation water at the anthesis stage for 20 days during the early grain filling on 12 selected wheat cultivars. Among these 12 wheat cultivars, Inqilab, Anmol, and Imdad-05 produced less number of grains/spikes (35 grains) under water stress illustrating drought susceptibility, whereas TD-1 and SKD-1 produced more number of grains/spike (more than 50 number of grains) exemplifying of drought tolerance. Primarily, grain yield is dependent on the grain weight and grain weight depends on the ability of the wheat plant to translocate carbohydrate into the growing kernel. In the field, Abdoli et al. (2013) examined eight wheat cultivars effected by drought during grain filling. They reported that drought did not influence number of spikes/m^2 and the number of grains/spikes but caused 26% reduction in grain weight. The reason for this reduction is that physiologically, wheat exhibited a decreased power for absorbing the photo-assimilates during drought reducing overall yield. Furthermore, priming events during early vegetative stages can make wheat more resistant at later stages of development (Abdoli et al., 2013).

Selection of measurement parameters is also important in estimating drought tolerance in the field and greenhouse conditions. A cultivar of drought indices has been used such as susceptibility indices as described by Fischer and Maurer (1978), and tolerance index (Fermandez, 1992). Drought tolerance of winter wheats was evaluated (Hirmand, Star, and Toos) under drought stress among 39 winter wheat cultivars as noted by Mortazavian et al. (2015) who used the susceptibility index and tolerance index in their evaluation. Both of these indices were able to discriminant drought tolerant cultivars under drought stress among wheat cultivars. One cultivar, Hashim-8, had a low value of the susceptibility indices indicating drought tolerance based on number of grain/ spikes compared with other wheat cultivars (Damani, Gomal-8, DN-73, Zam-04, and Dera-98) (Khakwani et al., 2011). Overall the susceptibility indices have been shown to yield the most stable and reliable approach for screening wheat for yield characteristics under water limitation.

The High Plains region of Texas and New Mexico has shown some of most severe impact of drought stress on wheat yield due to lower average precipitation during late winter and early spring. Wheat producers compensate for limited water availability by irrigating. In the High Plains region, there were more than 30 million acres under irrigation and 4 million acres under of rainfed management. Irrigation depend on the availability of water from the Ogallala Aquifer, but that aquifer due to depletion is expected to decline under the current rate of water demand (Johnson et al., 2009). A decline in water availability will increase the reliance of wheat farmers on rainfed conditions. Under these conditions, the development of a drought resistant cultivar would be very advantageous. The initial stages of such a project must include a preliminary screen of wheat germplasm. Fortunately, the data for such a screen is immediately available online in the form of the Texas Wheat Variety Trials in the High Plains region (http://varietytesting.tamu.edu/wheat/). A distinctive feature of this data is that it includes yield data from the same location under both rainfed and irrigated conditions.

The objective of this study was to identify the best cultivars or experimental lines under irrigated and rainfed conditions from this publicly available data from 2009 to 2012 at four locations where side by side comparisons of rainfed and irrigated conditions are possible including: Bushland, Clovis, NM, Etter, and Perryton. The difference between irrigated and rainfed provides an opportunity to measure the degree of varietal best drought response. The locations are scattered throughout the High Plains region of Texas and New Mexico representing a wide range of environmental variation. By using rainfed and irrigated datasets from this large area over four years we will be able to better identify winter wheat cultivars best adapted to drought prone areas.

MATERIALS AND METHODS

Plant materials

Yield data for HRWW cultivars and experimental lines across multiple locations and from 2004 onward are present online at the Texas Wheat Variety Trials website (http://varietytesting.tamu.edu/wheat/). Some of these trials include irrigated and rainfed yield data from the same locations. A total of 36 cultivars and experimental lines were selected from locations that included irrigation and rainfed treatments (Table 1). Sources for the wheat cultivars include university programs and commercial entities, such as: Oklahoma State University, Texas A&M AgriLife Extension, which is a unit of the Texas A&M University, Monsanto Company, CIMMYT, Syngenta, Colorado Wheat Research Foundation and other sources. Most cultivars and experimental lines were tested across four years and four locations with the exceptions being: Byrd, Doans, Garrison, Gallagher, Mace, and Iba. Locations included Bushland, TX, Etter, Perryton, and Clovis. These four locations run southwest to the northwest starting at Clovis, NM located at 34°24′45″N 103°12′17″W, Bushland, TX at 35°11′31″N 102°03′53″W, then Etter, TX at 36°2′46″N 102°0′8″W and finally Perryton, TX at 36°23′30″N 100°48′22″W, respectively. The environmental factors under which the wheat cultivars and experimental lines were tested, included planting deviations, insect infestations, heat and drought stress, and disease across four years and locations (Table 2). Bushland in particular showed drought stress throughout the four-year periods. Other locations typically showed two out of four years with drought, the exception being Perryton, which exhibited drought stress only in 2009. Heat stress was common in Clovis for three out of four years, Clovis being the westerly location. Etter showed greenbug infection in three out of four years. Drought stress was present at all locations in 2009. In 2010 and 2011, also head stress showed in three out of four locations. In 2012, all locations had insect pests and viral diseases. Air temperatures for Texas High Plains region for each year by location across growing months are presented from 2009 to 2012 (Table 3). The temperature dropped to -7 °C on January and February in 2011, which caused a late frost and loss of yield (Xue et al,. 2014). At the end of the season, the temperatures (25± 4 °C) were high during April and May in 2010 and 2012. The minimum temperatures were noted in February across four years.

Statistical analysis

Average yield data across year and location for each of 36 cultivars and experimental lines was determined for rainfed and irrigated plots along with their standard deviations. The ratio of rainfed to irrigated yield was calculated and the data was sorted based on this ratio (Table 4). From this data, a scatter plot was created with the x axis consisting of the irrigated yield and the y axis representing the rainfed yield. The scatter plot was divided up into four quadrants based on the average overall cultivar yield for both rainfed and irrigated. Quadrant 1 included cultivars and experimental lines that were above average in both irrigated and rainfed situations. Quadrant 2 included cultivars and experimental lines that were above average in rainfed and below average in irrigated. Quadrant 3 included cultivars and experimental lines that were above average in irrigated and below average in dryland. Quadrant 4 included cultivars and experimental lines below average in both irrigated and rainfed. A linear regression line and equation (slope and intercept) was determined along with the R^2 value to indicate goodness of fit. From this data, the most representative cultivars and experimental lines across all quadrants were selected for further greenhouse evaluation (Chapter IV).

RESULTS AND DISCUSSION

The average yield for 36 cultivars and experimental lines grown in the High Plains region of Texas during 2009 and 2012 at two to four locations under irrigated and rainfed conditions at the same location ranged from 4455.6 to 3156.6 kg/ha under irrigation and ranged from 2614.4 to 1509.6 (kg/ha) under rainfed (Table 4). The data was sorted from high to low based on the rainfed yield values. The inclusion of irrigated and rainfed data at the same location was a valuable and provided us an opportunity to evaluate the yield response under well water vs rainfed conditions. This option was taken to give us a more reliable estimate of best drought response in a field setting. When interpreting the results one must take into account the number of years by location and the variance associated with each cultivar. The data consisted of six to 14 location x year data points. The entries CJ, TX02A0252, Dumas, and Ruby Lee, showed the highest average rainfed yield. The overall coefficient of variation across all 36 cultivars and experimental lines was 56%. Among the five best CJ also had the lowest variance and coefficient of variation (31%) than all other cultivars and experimental lines indicating a high level of yield across variable environments. Cultivars: Iba, Fannin, Shocker, TAM W-101, and TAM 401 showed the lowest average rainfed yield. The ratio of rainfed/irrigated reflects the proportion of rainfed yield over the well water yield, which is a better indicator of the level of best drought response than just rainfed yield alone. This ratio takes into account the inherent yield ability of a given cultivar to withstand drought. The cultivars CJ, TX02A0252, and Ruby Lee, had the highest ratio and were therefore considered the best drought response cultivars. Cultivars: Iba, Billings, and Mace had the lowest ratio of rainfed/irrigation and therefore these cultivars were considered as drought susceptible. Overall, CJ showed the highest rainfed yield and the highest ratio of rainfed/irrigated yield of all 36 cultivars and experimental lines. The total average of rainfed was 1948.2 kg/ha whereas the total average of irrigated was 3919.8 kg/ha. Thus, rainfed wheat production in the region on average yield 50% of compared to irrigated wheat production.

The average yields expressed by kilogram per hectare (kg/ha) for all winter wheat cultivars across locations and years are presented in Figure 1. This option was provided to give us a different view of the relationship between rainfed yield and irrigated yield. Whereas Table 4 provided data that reflects the effect of rainfed conditions compared to rainfed on yield this figure visualizes the relationship between rainfed vs irrigated. This is best understood by examining the experimental line CJ which showed the highest average rainfed yield and the highest ratio of rainfed to irrigated yield (Table 4). From that data, it would appear that CJ was the best one overall in terms of drought response. However, in this alternative visualization we find that CJ is found in quadrant 2 indicative of its excellent response to rainfed condition but had suboptimal response to irrigated conditions. Breeders and farmers are more interested in varietal response across variable environments. The High Plains region data illustrates this very well with across years and locations that exhibited a wide range of variation. The irrigated average yield is plotted on the x-axis with the average rainfed yield plotted on the y-axis. Lines dissecting the chart into four quadrants represent the average overall rainfed (1948.2 kg/ha) and irrigated yields (3919.8 kg/ha). Quadrant 1 reveals the above average yield of some cultivars and experimental lines under rainfed and irrigated conditions such as: TX2A0252, Dumas, Winterhawk, and Hatcher. Quadrant 2 reflects the best cultivars and experimental lines under only rainfed condition such as CJ, Ruby Lee, Doans, and Endurance. Quadrant 3 displays best cultivars and experimental lines under irrigated condition only, such as: Iba. Quadrant 4 reflects the poorest cultivars and experimental lines under two conditions such as: Fannin, Shocker, Jagger, TAM W-101, TAM401, and OK Bullet. Those cultivars and experimental lines in Quadrant 1 show above average yield across this highly variable environment. Accordingly, the best cultivars and experimental lines across environments would be Winterhawk, TX02A0252 and Hatcher: Winterhawk primarily due to their very high yield under irrigated and above average yield under rainfed; TX02A0252 due to its high yield under rain-fed and above average yield under irrigated and Hatcher with its above average yield under rainfed and irrigated conditions. Other cultivars and experimental lines of interest would include: TAM 111, Duster, Garrison and Dumas. These results are similar with Xue et al. (2014), which showed both TAM 112 and TAM 111 cultivars differed significantly in tiller number in field of Bushland at Texas A&M AgriLife research station and dissimilar with Reddy et al. (2014) who found that cultivar TAM 112 produced more biomass and yield under water deficit compared with cultivar TAM 111. Of the cultivars and experimental lines release by Oklahoma State University Duster, Garrison, Billings, and Ruby Lee showed the best response in terms of yield and yield variation under water limited conditions across these variable environmental conditions.

The field results presented here are not only associated with water limitations but with all environmental variables including heat, cold, disease. In 2011 and 2010, the High Plains region areas had severe drought at Bushland and Clovis. In addition, heat stress was reported in Perryton and Etter in 2010 and 2011, which worsened conditions. In 2012, wheat cultivars were in poor conditions because drought stress was continuing and pests and wheat streak mosaic virus was present. In Bushland, Barley yellow dwarf virus was present in 2010, pests and wheat streak mosaic virus was found in 2012. The air temperature was reported as well, and the average high (30± 1^0C) was found during September and May in 2010, 2011, and 2012. This high temperature is considered above the temperature where wheat growth is inhibited. With projected future change in climate these drought conditions are expected to increase so that the conditions resident in the High Plains region will likely be more common to future conditions in the primary wheat growing areas of Oklahoma.

CONCLUSION

The results of this study showed the effect of the rainfed conditions on wheat yield in the High Plains regions of Texas and New Mexico. Superior cultivars with respect to rainfed conditions include: CJ, TX02A0252, Dumas, and Ruby Lee. Superior cultivars with respect to drought response based on the rainfed to irrigated ratio included: CJ, TX02A0252, and Ruby Lee. Finally, the best cultivars based on yield across the variable environments include: TX2A0252, Dumas, Winterhawk, and Hatcher. The best cultivars for a given location will depend whether we are targeting a rainfed and irrigated production system. Rainfed yields would favor CJ, TX02A0252, Dumas, and Ruby Lee while irrigated yields would favor under drought conditions, but under variable or irrigated conditions may favor TX2A0252, Dumas, Winterhawk, and Hatcher. Breeding for best drought response is most likely best using genetic backgrounds containing CJ, TX02A0252, Winterhawk, Hatcher and Ruby Lee.

Table 1. Cultivars and experimental lines used in the field research at Bushland (B) ‡, Etter (E) ‡, Clovis (C) ‡, and Perryton (P) ‡ in the High Plains region during 2009 to 2012 from the Texas Wheat Variety Trials.

Cultivars and/or Experimental lines	**Sources†**	**Locations‡**	**Years**
Armour	WestBred	BECP	2009, 2010, 2011, 2012
Art	AgriPro	BECP	2009, 2010, 2011, 2012
Bill Brown	CSU	BECP	2009, 2010, 2011
Billings	OSU	BECP	2009, 2010, 2011, 2012
Cedar	WestBred	BECP	2009, 2010, 2011
CJ	AgriPro	BECP	2010, 2011
Doans	Syngenta	BECP	2009, 2010, 2011, 2012
Dumas	Virginia Grain	BECP	2009, 2010, 2011, 2012
Duster	OSU	BECP	2009, 2010, 2011, 2012
Endurance	OSU	BECP	2010, 2011, 2012
Fannin	Syngenta	BECP	2009, 2010
Fuller	KSU	BECP	2011, 2012
Gallagher	OSU	BECP	2010, 2011
Garrison	OSU	BEC	2011, 2012
Greer	Syngenta	BEP	2010, 2011, 2012
Hatcher	CSU	BECP	2010, 2011, 2012
Iba	OSU	BECP	2009, 2010, 2011
Jackpot	Syngenta	BECP	2009, 2010, 2011, 2012
Jagalene	AgriPro	BECP	2009, 2010, 2011, 2012
Jagger	Syngenta	BECP	2009, 2010, 2011, 2012
Mace	UNL	BE	2011, 2012
OK Bullet	OSU	BECP	2009, 2010, 2011
Pete	OSU	BECP	2009, 2010, 2011, 2012
Ruby Lee	OSU	BECP	2009, 2010, 2011, 2012
Santa Fe	Westbred	BECP	2009, 2010, 2011, 2012
Shocker	Westbred	BECP	2009, 2010, 2011, 2012
T136	ARDS Turda	BECP	2009, 2010, 2011, 12
T197	Limagrain	BECP	2009, 2010, 2011, 2012
T81L	Limagrain	BECP	2010, 2011
TAM 111	TAMU	BEC	2010, 2011, 2012
TAM 112	TAMU	BECP	2009, 2010, 2012
TAM W-101	TAMU	BECP	2009, 2010, 2011
TAM 113	TAMU	BECP	2009, 2010, 2011, 2012
TAM 203	TAMU	BECP	2009, 2010, 2011, 2012
TAM 304	TAMU	BECP	2009, 2010, 2011, 2012
TAM 401	TAMU	BEP	2010, 2011, 2012

TX06A001263	TAMU	BECP	2009, 2010
Winterhawk	WestBred	BECP	2009, 2010, 2011, 2012

† CSU: Colorado State University, OSU: Oklahoma State University, KSU: Kansas State University, TAMU: Texas A&M University. UNL: University of Nebraska, Lincoln.

‡ Locations where the study was performed: B: Bushland, E: Etter, C: Clovis New Mexico, P: Perryton

Table 2. The descriptions of biotic and abiotic growing conditions at the High Plains region from 2009 to 2012

Location	2009	2010	2011	2012
Perryton, TX	Drought, Heat	Heat, Greenbug	Some lodgings	Pests, Wheat streak mosaic virus
Etter, TX	Drought, Greenbug	Heat, Greenbug	Heat, Greenbug	Drought
Bushland, TX	Drought	Drought, Barley dwarf virus	Drought	Drought, Pests, Wheat streak mosaic virus
Clovis, NM	Drought, Heat, Greenbug	Drought, Heat	Heat, Greenbug	Pests, Wheat streak mosaic virus

Table 3. The high, average, and low temperatures in the High Plains region during the growing seasons between 2009 to 2012

Months	**2009-10**			**2010-11**			**2011-12**			**2012-13**		
	High	**Ave.**	**Low**	**High**	**Avg.**	**Low**	**High**	**Avg.**	**Low**	**High**	**Avg.**	**Low**
September	26	19	12	31	23	15	29	22	14	30	22	14
October	19	12	4	24	17	8	23	16	7	23	14	6
November	18	11	2	17	8	-1	17	9	1	21	12	3
December	7	1	-7	13	5	-3	6	1	-4	14	6	-3
January	10	2	-6	11	2	-7	14	6	-3	11	4	-4
February	6	1	-4	11	2	-7	12	5	-2	12	4	-3
March	17	9	1	19	11	2	22	14	5	19	11	2
April	22	14	7	26	16	6	26	17	9	22	13	3
May	25	18	11	29	19	9	29	21	13	29	21	11

Table 4. The average yield for each of the 36 cultivars and experimental lines grown under irrigated and rainfed conditions in the High Plains region between 2009 and 2012 sorted by rainfed yield

Cultivars and Experimental lines	Sites x Years	Average Grain Yield kg/ha Rainfed ± st.dev	Irrigated ± st.dev	Ratio Rainfed/Irrigated
CJ	14	2614.4±813.1	3729.6±906.5	0.701
TX02A0252	8	2495.9±1276.6	4132.6±1258.2	0.604
Dumas	14	2302.9±1070.4	4059.6±1324.9	0.567
Ruby Lee	12	2294.2±1033.3	3814.5±1002.4	0.601
T81	6	2293.5±1142.6	3916.1±1352.5	0.586
Winterhawk	6	2247.8±1228.4	4531.4±977.2	0.496
Hatcher	10	2160.6±1277.9	4350.0±997.2	0.497
TAM 112	8	2126.1±1108.0	4083.6±1152.7	0.521
TX06A001263	14	2113.0±1249.7	4083.2±992.5	0.517
T197	14	2102.8±1222.0	3978.8±992.1	0.528
TAM304	14	2095.9±1267.3	4248.3±993.8	0.493
Art	14	2075.3±1126.0	3959.2±1502.5	0.524
Doans	6	2059.5±1126.5	3787.5±1156.3	0.544
Garrison	10	2056.3±1205.7	4127.8±1060.7	0.498
TAM 111	10	2046.3±1237.5	4332.9±1270.3	0.472
Duster	14	2036.0±1208.9	4184.5±1155.3	0.487
Endurance	6	1994.3±1194.5	3747.8±1003.3	0.532
TAM203	14	1989.8±1180.6	4181.1±1092.7	0.476
Armour	12	1975.0±1195.4	3961.7±1137.3	0.499
Greer	14	1957.8±1211.6	4050.5±1031.5	0.483
Bill Brown	10	1949.4±1238.9	4124.9±1155.6	0.473
Jackpot	10	1918.5±1028.2	3581.9±946.6	0.536
Billings	14	1906.9±1155.3	4106.1±1291.5	0.464
Mace	12	1903.2±1193.2	4049.9±908.1	0.470
Santa Fe	8	1898.3±1050.4	3642.5±1104.1	0.521

T136	8	1861.1±1097.7	3763.6±1064.6	0.495
Fuller	14	1857.5±1152.6	3733.9±1105.1	0.497
Jagger	14	1766.5±1062.5	3423.2±954.3	0.516
Pete	10	1761.3±1060.3	3463.6±1060.6	0.509
Bullet	10	1752.3±1087.8	3577.9±1080.4	0.490
TAM401	10	1729.3±1009.1	3579.1±850.0	0.483
TAM W-101	8	1717.7±970.3	3580.6±1050.7	0.480
Shocker	8	1699.5±1051.6	3388.0±1100.3	0.502
Fannin	10	1679.1±932.6	3156.0±880.9	0.532
Jagalene	12	1632.9±1247.1	3797.6±1314.1	0.430
Iba	10	1509.6±718.9	4455.6±647.1	0.339

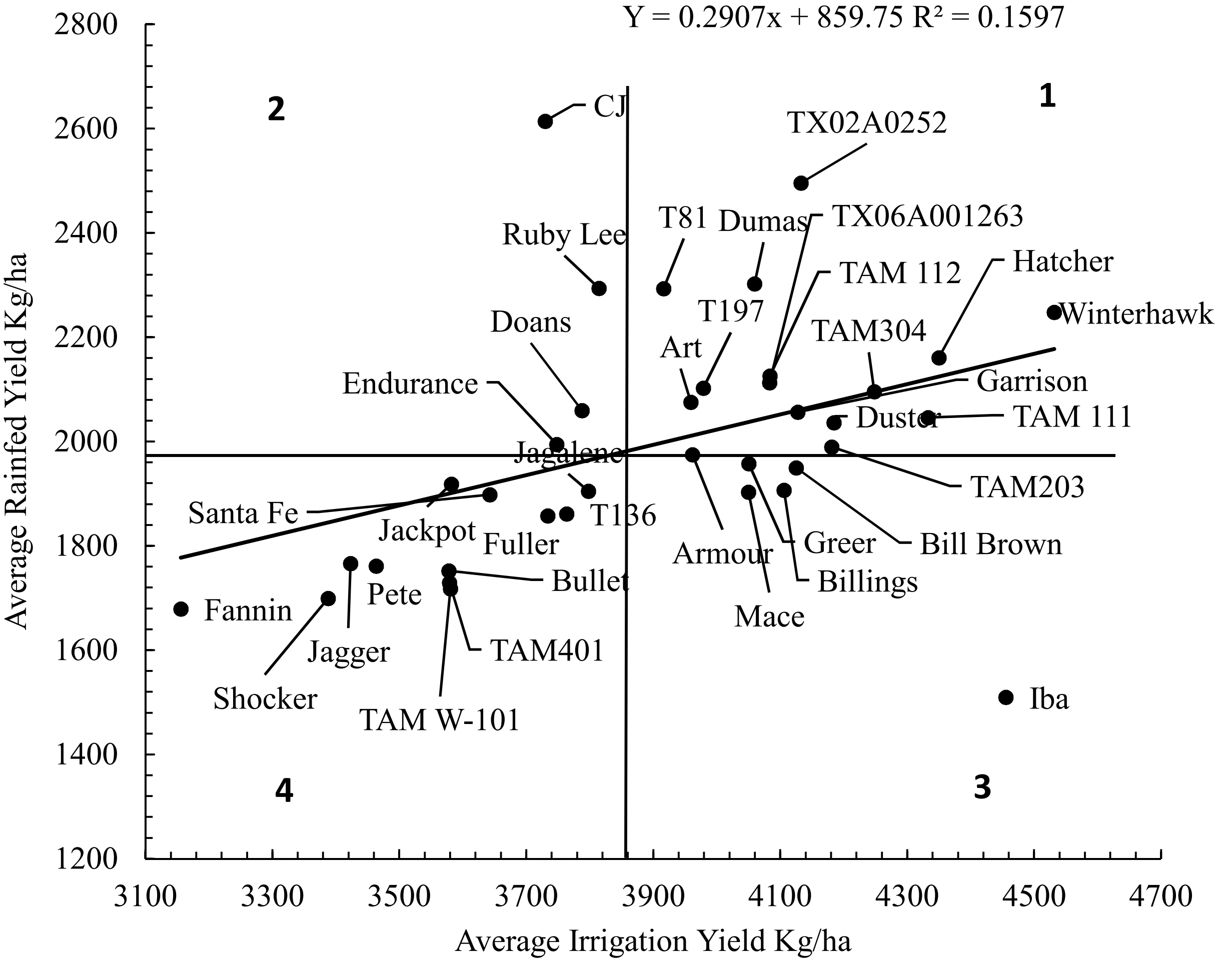

Figure 1. Relationship between average irrigated (x axis) and rainfed (y axis) yield of winter wheat cultivars and experimental lines in the High Plain region between 2009 to 2012. Vertical and horizontal lines represent the average yield across the data set for irrigated and rainfed conditions. The dissecting lines divide the chart into 4 numbered quadrants. Quadrant 1 represents above average cultivars and experimental lines under both conditions, Quadrant 2 above average in rainfed, Quadrant 3 above average under irrigation, Quadrant 4 below average under both conditions. All data points were taken from locations with both irrigated and rainfed plots.

REFERENCE

Abdoli, M., M. Saeidi, S. Jalali-Honarmand, S. Mansourifar, and S. Ghobadi. 2013. Effect of post-anthesis water deficiency on storage capacity and contribution of stem reserves to the growing grains of wheat cultivars. P. K. J. 2(3):99-107.

Fischer, R., and R. Maurer. 1978. Drought resistance in spring wheat cultivars. I. Grain yield response. Aust. J. A. R. 29:897-912.

Jatoi, A., Baloch, M., Kumbhar, M., Khan, N., and Kerio, M. 2011. Effect of water stress on physiological and yield parameters at anthesis stage in elite spring wheat cultures. S. J. A. 27(1): 59-65.

Johnson, J., P.N. Johnson, E. Segarra, and D. Willis. 2009. Water Conservation Policy Alternatives for the Ogallala Aquifer in Texas. W. P. 11: 537-552

Khakwani, A., M. Dennett, and M. Munir. 2011. Drought tolerance screening of wheat varieties by inducing water stress conditions. S. J. S. T. 33(2), 135-142.

Ranjbar, A., A. Sepaskhah, and S. Emadi. 2015. Relationships between wheat yield, yield components and physico-chemical properties of soil under rain-fed condition. I. J. of P. P. 9(3) 434-465.

Reddy, S., S. Liu, J. Rudd, Q. Xue, P. Payton, S. Finlayson, J. Mahan, A. Akhunova, S. Holalu, and N. Lu. 2014. Physiology and transcriptomic of water-deficit stress responses in wheat cultivars TAM 111 and TAM 112. J. of P. P. 171: 1289-1298. http://dx.doi.org/1Xue0.1016/j.jplph.2014.05.005.

Xue, Q., J. Rudd, S. Liu, K. Jessup, R. Devkota, and J. Mahan. 2014. Yield determination and water use efficiency of wheat under water-limited conditions in the U.S. Southern High Plains. C. S. 54: 34–47. Doi:10.2135/cropsci2013.02.0108.

CHAPTER IV

GENETIC DIVERSITY AMONG HARD RED WINTER WHEAT CULTIVARS AND EXPERIMENTAL LINES AND THEIR ASSOCIATION WITH WATER LIMITATION

ABSTRACT

Increasing knowledge about winter wheat yield response under limited water availability is critical to the development of drought adapted cultivars. The objective of this study was to assess the level of yield response under water limiting greenhouse conditions using cultivars and experimental lines from the Texas study and selected cultivars under development by the Oklahoma State Breeding Program. Nineteen cultivars and experimental lines were used in a preliminary screen using two cycles of growth and development followed by an additional focused screen with replication an additional two cycles of growth and development. These cultivars and experimental lines were grown under three levels of water availability (well water, moderate drought stress, severe drought stress). In the initial preliminary screen, seed weight under severe stress showed the highest average in cultivars: Byrd, Ruby Lee, TAM 113, and Duster whereas cultivars: Endurance, Chisholm, and Gallagher showed the lowest average seed weight. In the later replicated study, average seed weights were significantly higher in Gallagher, Ruby Lee, and Endurance and significantly lower in Jagger, Byrd, and Cedar. Result of discriminant analysis showed that spike weight and number of spikes were the two traits that discriminated among cultivars across all cycles of

growth and development. The 19 cultivars and experimental lines clustered based on SNPs based genotype by sequencing methods and the Neighbor Joining algorithm into five groups: First, Iba, Duster, OK12621, OK10126, Cedar, Garrison, and Hatcher; Second, Byrd, TAM 112, TAM 113, and TAM 111; Third, Endurance, Ruby Lee; Forth, Chisholm, and Gallagher; Fifth, Jagger, OK Bullet, Bentley, and OK11D25056. Cultivars that revealed best yield response under moderate and severe water limiting conditions can be used to generate improved drought adapted lines in breeding programs.

INTRODUCTION

It is projected that there will be increased occurrence of drought in the Southwestern part of the United States where HRWW is grown. In 2016, Texas experienced a severe drought resulting in a HRWW yield loss of 65 million metric tons. Yield has varied substantially due the substantial environmental interactions in the region under field conditions. This yield variability is projected to become worse with a changing climate. Similar yield instability is projected to occur throughout the winter wheat growing areas that include Colorado, Kansas, Oklahoma, and Texas. To limit the effects of drought on wheat productivity breeders throughout the region have been actively seeking stable sources for drought resistance that they can use in developing new drought tolerant cultivars. These efforts are directed at screening programs among current and potential wheat accessions that are currently used or are under development. Once promising germplasms are identified, these can be readily incorporated into current breeding pipelines for improving drought tolerance in future cultivars (Xue et al., 2014).

Breeders are currently screening promising breeding lines and new cultivars to identify traits that are significantly impacted by water limitation. Ranjbar et al. (2015) evaluated 30 wheat cultivars for yield characteristics under rain-fed condition at physiological maturity and found that the tiller number /plant had the highest variation whereas 1000-grain weight showed the lowest under drought stress. In addition, the simple correlation analysis showed a high positive relationship between grain yield and number of spike/plant ($R^2 = 0.88$). Consequently, drought stress at the grain filling stage can dramatically reduce overall wheat yield. Jatoi et al. (2011) studied the effect of withholding irrigation water at anthesis stage for 20 days during the early grain filling on 12 selected wheat cultivars. Among these 12 wheat cultivars, Inqilab, Anmol and Imdad-05 produced less number of grain/spike (35 grains) under water stress illustrating drought susceptible, whereas TD-1 and SKD-1 produced more number of grain/spike (more than 50 number of grains) exemplifying of drought tolerance. Primarily, grain yield is partially dependent on the grain weight and grain weight depends on the ability of the wheat plant to translocate carbohydrate into the growing kernel. In the field, Abdoli et al. (2013) examined eight wheat cultivars effected by drought during grain filling. They reported that drought did not influence number of spike/m^2 and the number of grain/spike but caused 26% reduction in grain weight. The reason for this reduction is that physiologically, wheat exhibited a decreased power for absorbing the photo-assimilates during drought reducing overall yield. Furthermore, priming events during early vegetative stages can make wheat more resistant at later stages of development (Abdoli et al., 2013).

According to Blum et al. (1988) selection for drought tolerance under field conditions is complicated by large in-field variations and high interaction between wheat cultivars and environment. The large environmental variations can be normalized by selecting for drought responses across multiple years and across multiple locations representative of a given local. However, to access and isolate the genetic potential of a given cultivar it is necessary to analyze the yield response under greenhouse controlled conditions. Greenhouses studies allow for the control of water availability, temperature and the use of a homogenized and nutritionally defined soil substrate compared to most field studies. Reduction in variation in these parameters permits the genetic potential to water limitation to be revealed and existed. In this study we use controlled conditions to isolate the true genetic potential of wheat cultivars.

Selection of measurement parameters is also important in estimating drought tolerance in the field and greenhouse conditions. A cultivar of drought indices has been used such as susceptibility indices as described by Fischer and Maurer (1978), and tolerance index (Fermandez, 1992). Drought tolerance of winter wheats was evaluated (Hirmand, Star, and Toos) under drought stress among 39 winter wheat cultivars as noted by Mortazavian et al. (2015) who used the susceptibility index and tolerance index in their evaluation. Both of these indices were able to discriminate drought tolerance cultivars under drought stress among wheat cultivars. One cultivar, Hashim-8, had a low value of the susceptibility indices indicating drought tolerance based on number of grain/ spikes compared with other wheat cultivars (Damani, Gomal-8, DN-73, Zam-04, and Dera-98) (Khakwani et al., 2011). Overall the susceptibility indices have been shown to yield the most stable and reliable approach for screening wheat for yield characteristics under water limitation.

Current cultivars can be assessed concerning their genetic relatedness using a number of modern technologies. An understanding of the genetic relatedness among cultivars under water limiting conditions can serve to identify common genetic elements that correlate with drought adaptation. Jordan and Humphries (1994) developed a method that provides a high degree of resolution of focusing on the identification of single nucleotide polymorphisms (SNPs) for genetic diversity analysis. SNPs refer to nucleotide differences that are found among cultivars. These SNPs are highly abundant and widely distributed throughout the wheat genome, much more so than in any other marker based system. Their abundance and wide distribution throughout the wheat genome make SNPs good tools for breeding program seeking to perform association mapping (Semagn et al, 2006). In addition, high resolution sequencing approaches to identify SNPs known as Genotyping by Sequencing (GBS) can be used to analyze genetically relatedness among wheat cultivars (Varshney et al., 2014). With the advent of massively parallel sequencing procedures, decrease in sequencing costs and increase in technical accessibility, GBS is becoming the method of choice for plant breeders to characterize the genetic diversity and relatedness (Deschamps et al., 2012).

The objectives of this study were to first screen among winter wheat cultivars and experimental lines for best drought response under controlled environmental conditions. The procedure was accomplished in two steps by using a preliminary screen with 19 cultivars and experimental lines followed by a more focused screen with nine selected cultivars and experimental lines including selections from the preliminary screen and promising selections from the Oklahoma State University breeding program. The second objective was to assess the genetic diversity among 19 tested winter wheat cultivars and experimental lines using GBS.

MATERIALS AND METHODS

Plant Materials

The total of 19 HRWW cultivars and experimental lines used in this study are presented in Table 1. These cultivars and experimental lines are among advanced breeding lines from the Texas field evaluation and New Mexico, from the Oklahoma State Breeding Program and from other sources such as WestBred, Colorado Wheat Research, and Kansas State University. Some of these cultivars and experimental lines are genetically related to one another, such as Bentley and Jagger, OK10126 and OK Bullet, OK12621 and Duster, and OK11D25056 and Gallagher.

Prior to planting under greenhouse conditions three wheat seeds were planted in plastic trays containing 4.5 width x 4.1 length x 7.0 height cm wells filled with a pine bark and perlite Miracle Grow premium potting mix (Scotts Marysville, OH), 200 wells per tray. Wheat was thinned to one plant per cell after two weeks, and was moved to a cold room for vernalization for six weeks at 5 ± 2 ºC temperature under a fluorescent 14-hour photoperiod. During six weeks, all cultivars were faithfully watered to avoid any water stress and treated with Neem oil to avoid insect and disease problems such as aphids and powdery mildew.

After vernalization seedlings were gently transferred to 29 liter Tray10 boxes (Stuwe and Sons Inc, OR) filled with homogenized Eastspur silt loam soil from the Oklahoma State University Stillwater Experiment Station with a wheat growing history. The soil was fertilized with ammonium nitrate and super phosphate to 100 kg/ha and 60 kg/ha of P_2O_4, respectively. Plants were grown under a 14-hour photoperiod, watered with care, sprayed for insect pests and disease, and weeds removed manually. A total of 12 plants were grown per Tray 10 box consisting of a single cultivar. Each of 19 cultivars and experimental lines was exposed to three different watering treatments (well water WW, moderate drought stress stress MS, and severe drought stress SS). Watering was performed before the WW plants were exposed to water limiting conditions as judged by a calibrated soil tensiometer. Well water wheats were provided with 2000 ml of water, MS wheat with 1000 ml, and SS wheat with 500 ml. Boxes were moved throughout the greenhouse space at least 25 times so that each box sampled multiple regions within the greenhouse space throughout development. This system of watering was initiated at Feekes stage 5 and continued to physiological maturity. This system of planting continued for two cycles: cycle one from September 2015 to January 2016, and cycle two from Mid-January 2016 to May 2016.

An additional more focused screening was implemented using the best drought responding cultivars and experimental lines from the preliminary screen and a few that were less adapted for two additional cycles (cycle three and cycle four). These were vernalized and planted in Tray 10 boxes as in the preliminary screen with three replications and nine plants per Tray 10 for a total of 27 wheat plants overall for each cultivar/treatment combination. Cycle three began from the end of September 2016 to February 2017 and cycle four from Mid- February to end of April 2017.

At the end of each growth cycle and after two weeks of drydown, all winter wheat cultivars and experimental lines were harvested and evaluated individually on a plant by plant basis. Biomass was collected, bagged, and air dried in the greenhouse for one week. Seed heads were hand harvested and threshed manually. The measurements taken on a per plant basis included: seed weight, shoot weight, spike weight, spike number, tiller number, and seed number were determined. All the data was entered into an Excel spreadsheet for analysis.

Statistical Analysis

The control greenhouse experiment was analyzed based on two factorial analysis of variance design with the first factor being cultivar or experimental lines and the second water treatment. The dependent variables were (spike weight, shoot weight, seed weight, spike number, tiller number, and seed number were determined). Cycle one and cycle two were analyzed based on a single Tray 10 containing 12 plants per cultivar or experimental lines without replication. Cycle three and cycle four were analyzed based on the average value per plant per replicate Tray 10 (3 replications) for each cultivar or experimental lines treatment combination. The two factorial analysis was computed using PROC GLM procedure at significant level of $p \leq 0.05$ in SAS computer packages version 9.2 for Windows (SAS Institute Inc.). Overall there were 684 observations in cycle one and cycle two and were 730 observations in cycle three and cycle four. Discriminant analysis was used to test which variables were most important under treatment conditions. Lastly, the correlation analysis was computed to test the degree of relationship among the six dependent variables.

Genetic Relatedness of 19 HRWW Cultivars and Experimental Lines

The genetic relatedness of 19 HRWW cultivars and experimental lines was determined by GBS methodology using SNPs and Neighbor Joining algorithms. The young leaves of the same 19 winter wheat cultivars and experimental lines in cycle one and cycle two were harvested, frozen, and stored in liquid nitrogen for later DNA extraction. Fresh Leaf (0.167 grams) was ground in liquid nitrogen in a mortar and pestle to a fine powder, transferred to a 2 ml micro-centrifuge tube and placed on ice. A total of 1.5 ml of hexadecyltrimethyl ammonium bromide (CTAB) with 5µl of β-mercaptoethanol (BME) was added to the micro-centrifuge tube containing the ground young leaf tissue (Murray and Thompson 1980). The ingredients of the CTAB buffer are: 27 Millimolar (mM) of CTAB, 690 mM of NaCl, 49 mM of TRIS buffer, and 10 mM of NaEDTA adjusted to pH 8. Each tube was incubated in a water bath at 70°C for 30 min, then three to four metal beads were added to the tube. The tubes were shaken at 4000 oscillations/minute using a BioSpec BeadBeater (Biospec, OK) to break up the tissue. Then all the supernatant was transferred to a new 1.5 mL tube and an equal volume of chloroform: isomyl-alcohol (24:1 v/v) (CI) was added, and the tubes were centrifuged for 30 min at 10000 rpm. The CI extraction was repeated twice. Then all supernatant layers were combined into a new 1.5 ml tube and a double volume of Isopropyl alcohol (IPA) was added to precipitate the DNA, on ice for one hour. The tubes were centrifuged at 10,000 rpm for 5 min and the IPA decanted and the pellet air-dried. Then the DNA pellet was dissolved in 200µl TE (100 Mm TRIS and 10 Mm EDTA pH 8.0). Then, the DNA concentrations were determined by using NanoDrop Spectrophotometer based on absorbance at 260 nm. The DNA qualities were assessed by the ratio of A260/280 nm (wavelength) absorbance reading (Table 2).

Acceptable purity was between 1.8 and 2.0 as an A260:280 nm spectrophotometric ratio. In addition, DNA concentrations were verified again by using a PicoGreen fluorescent assay as recorded by the NanDrop Spectrophotometer according to the Thermo Fisher instructions.

DNA sequencing and polygenetic analysis

The optimal DNA concentration was adjusted to between 20 ng/µl and 150ng/µl. The dsDNA of all wheat cultivars and experimental lines were submitted to Kansa State University Wheat Genetics and Germplasm Improvement laboratory (http://wheatgenetics.org/) for barcoding, Illumina library development and sequencing on their Illumina HiSeq 2000 sequencer. Following sequencing single nucleotide polymorphisms (SNPs) were identified for all cultivars and experimental lines using Trait Analysis by Association, Evolution and Linkage (TASSEL) software (Bradbury et al., 2007). All reads were filtered in order to match perfectly one of the barcodes and the expected four-base pair indicator for each cultivar or experimental lines. Then all reads were sorted into files according to their barcodes. Next, all reads were aligned to the Chinese Spring Wheat reference genome seq v1.0 based Basic Local Alignment Search (BLAST) alignment algorithm. All reads were constructed as contigs which ended with the collapsing all identical reads down to single unique sequence. All SNPs were then identified using TASSEL. Sequences were filtered at the 50% level. TASSEL software was used to generate the dendrogram using the Neighbor-joining algorithm and a distance matrix. The clustering dendrogram visualizes the genetic distances between two winter wheat cultivars and experimental lines (Saitou and Nei, 1987).

RESULTS AND DISCUSSION

Our first greenhouse experiments involved screening 19 cultivars and experimental lines in order to rank the cultivars and experimental lines in order of grain yield. The average of six traits across cycle one and cycle two under WW conditions for 19 winter wheats cultivars and experimental lines grown under greenhouse conditions are presented in Table 2. These cultivars and experimental lines were sorted based on seed weight. Cultivars and experimental lines with the highest average of seed weight were Ruby Lee, OK12621, Duster, and TAM 112. Cultivars and experimental lines with the lowest average seed weight included Endurance, OK Bullet, Iba, Gallagher, and Chisholm. In terms of vegetative yield which showed very little correlation with seed weight (R^2=0.05) Cedar, Ruby Lee, and TAM 111 had the highest yield overall and Hatcher, Chisolm and Bentley the lowest vegetative yield. Spike weight showed the highest correlation with seed weight (R^2=0.97) that was highest in Ruby Lee, Jagger, TAM 112 and Duster and lowest in OK Bullet, Iba and Gallagher. Spike number was highly correlated with seed weight (R^2= 0.74) and was greatest in Duster, Hatcher and TAM 112, and lowest in Endurance, OK Bullet and Chisholm. The tiller number were negatively correlated overall with seed weight (R^2=0.59) and was greatest in Endurance, Iba and TAM 111 and lowest in Ruby Lee, OK11D25056, and TAM 112. The seed number was highly correlated with seed weight (R^2=0.93) highest in Duster, Jagger, and Ruby Lee and lowest in OK Bullet Chisholm and Gallagher. Overall, the highest seed yield resulted from a larger number of spikes and seed number per plant. Tillering appears to be negatively correlated with seed yield.

The average of the yield traits across cycle one and cycle two under MS for 19 winter wheats is presented in Table 3. These cultivars and experimental lines were sorted based on seed weight/plant. Some cultivars and experimental lines showed highest average of seed weight such as Ruby Lee, OKD1125056, Byrd, and TAM112 whereas other cultivars and experimental lines showed lowest of average in the same trait including Jagger, Endurance, Iba, and OK Bullet. Seed weight was very little correlated with shoot weight (R^2=0.02) and Byrd, Cedar, and TAM 111 had highest average of shoot weight whereas Endurance, Bentley, and Duster had the lowest average of shoot weight. Seed weight was highly correlated with spike weight (R^2=0.88) and Ruby Lee, Jagger, Byrd, and OK11D25056 had the highest spikes number whereas Endurance, Iba, and Chisholm had the lowest average of spikes number. Seed weight correlated with number of spikes (R^2=0.77) and Ruby Lee, Duster, Jagger, and Hatcher had the highest average of spikes number whereas Endurance, Iba, OK Bullet, and Gallagher were lowest average of spikes number. Seed weight had moderate correlation (R^2=0.55) with tiller number and Cedar, Iba, OK Bullet, and Garrison had the highest average of tiller number whereas Ruby Lee, TAM 112, OK10126, and OKD1125056 had lowest average of tiller number. Seed weight was weakly correlated with seed number (R^2=0.01) and showed that Iba, Bentley, OK10126 had the highest average of seed number whereas Endurance, OK11D25056, Cedar had the lowest average of seed number. Overall, the highest seed yield resulted from a larger spikes number and partially with the tiller number. This is a reaction to moderate stress to increase the spikes number and tillers number. These initial screens when compared to the field data from the High Plains region of Texas showed no correlation between seed weight and seed yield under irrigated conditions (R^2=0.00) but higher correlation under rainfed condition (R^2=0.28).

The average of yield traits across cycle one and cycle two under SS for 19 winter wheats is shown in Table 4. These cultivars and experimental lines were sorted based on seed weight/plant. Some cultivars and experimental lines showed the highest average of seed weight including Byrd, Ruby Lee, TAM113, and Duster whereas others showed lowest average in the same trait such as Endurance, Chisholm, and Gallagher. Seed weight had no correlation with shoot weight (R^2=0.00). TAM 112, Garrison, OK11D25056, and TAM 111 had the highest average shoot weight whereas OK10126, Chisholm, Duster, and Bentley had the lowest average shoot weight. Seed weight had the high correlation with spike weight (R^2=0.95) and Byrd, Duster, and TAM 112 had the highest average spike weights whereas Chisholm, Endurance, and Gallagher had the lowest average spike weight. Seed weight was correlated with spike number (R^2=0.71) and Duster, Ruby Lee, and Jagger had the highest of spikes number whereas Chisholm, Endurance, Gallagher, and Cedar had the lowest average of spikes number. Seed weight had a weak correlation with tiller number (R^2=0.22) and Garrison, TAM 111, and Cedar had the highest average of tiller number whereas OK101256, Ruby Lee, Duster, and Byrd had the lowest average of tiller number. Seed weight was highly correlated with seed number (R^2=0.89) and Byrd, Jagger, Duster, and TAM 113 had the highest average of seed number whereas Endurance, Chisholm, Gallagher, and Cedar had the lowest average of seed number. Overall, the highest seed yield resulted from a larger spikes number and seed number. These initial screens when compared to the field data from the High Plains region showed limited correlation between seed weight and seed yield under rainfed conditions (R^2=0.30).

These initial screens when compared to the field data from the High Plains region of Texas showed very little correlation between seed weight under WW and seed yield under irrigated conditions (R^2 =0.02). There were slightly higher correlations found from field data under rainfed conditions those under MS and SS (R^2= 0.28, 0.30). These results indicate a different response in seed yield under greenhouse for our preliminary screen. Part of the confounding problems with the greenhouse data are at least partially related to incomplete vernalization and slow germination for OK Bullet, and Endurance resulting in very low seed yield during cycle one. Also, the cycle one and cycle two screening procedure used a single replication which is insufficient in sampling the environmental variation in the greenhouse. A more statistically rigorous examination of the relationship between drought stress and yield response in the greenhouse is in order. On the other hand, field screening under rainfed and irrigated conditions reflects more the response to a localized variable environment consisting of multiple yield effectors, not solely a response to water limitation.

To better visualize the data presented in Tables 2-4. The average seed weight for all winter wheat cultivars and experimental lines across cycle one and cycle two under MS and WW is plotted in Figure 1. The WW response is plotted at the x-axis with total average seed yield at 1.22 g/plant represented as a vertical line whereas the MS is plotted at y-axis with a total average seed yield at 0.54 g/plant presented as a horizontal line. The intersection of the plot represents the overall average across all treatments. This plot was added for better visualization of the result and was divided into four quadrants. Quadrant 1 reveals above average seed weight under WW and MS in cultivars: Ruby Lee, OK11D25056, Bentley, Byrd, Duster, Hatcher, OK10126, OK12621, TAM 113, and TAM 112. Quadrant 2 reflects the best cultivar and experimental line under only the MS condition and below average under WW condition in which no cultivar or experimental lines is shown. Quadrant 3 displays above average cultivar and experimental line under WW and below average under MS Garrison. Quadrant 4 reflects below average response under the two conditions including cultivars: OK Bullet, Endurance, Gallagher, Iba, Chisholm, and Cedar. Wheat cultivars and experimental lines in quadrant 1 are considered the best drought response cultivars and experimental lines because of above average response to both conditions whereas wheat cultivar and experimental line in quadrant 4 are considered as drought susceptibility.

Of the 19 cultivars and experimental lines in the preliminary greenhouse study nine of these are common with the field study: Ruby Lee, Jagger, TAM 111, TAM 112, TAM 113, Duster, OK Bullet, Endurance, and Iba. Not surprisingly, the results of the preliminary greenhouse study under MS shows some similarity with the field research. When examining the quadrant location for each of the nine common cultivars and experimental lines between the field study and the preliminary greenhouse study, respectively: Quadrant 1 contains TAM 111, TAM 112, TAM 113, and Quadrant 4 contains Duster in both studies. The differences between the two studies were found with 4 cultivars: Ruby Lee changed from Quadrants 2 to 1, Jagger from 4 to 1, Endurance from 2 to 4 and Iba from 3 to 4, respectively, comparing field study with the preliminary screenings. Thus five out of the nine retain their same relative placements under MS conditions. Those that did change quadrants the shifts were substantial reflecting a large environmental impact not associated with drought response with those cultivars.

The average seed weight for all winter wheat cultivars across cycle one and cycle two under SS and WW conditions is plotted in Figure 2. The WW response is plotted at x-axis with overall average seed yield at 1.22 g/plant represented as a vertical line whereas the SS is plotted at y-axis with total average seed yield at 0.12 g/plant represented as an horizontal line. Quadrant 1 reveals the above average seed weight under WW and SS, including Ruby Lee, Duster, OK11D25056, TAM 113, Hatcher, OK10126, OK12621, TAM 112, and Bentley. Quadrant 2 reflects above average cultivars and experimental lines under SS conditions but below average under WW: no cultivar. Quadrant 3 displays above average cultivar and experimental line under WW and below average under SS: TAM 111 and Garrison. Quadrant 4 reflects the below average for cultivars and experimental line under WW and SS: OK Bullet, Endurance, Gallagher, Chisholm, Cedar, and Iba. The positions of these wheat cultivars are relatively similar in both Figures 2 and 3 indicating that there is little difference in cultivar response between MS and SS. It can be concluded that entries in quadrant 1 should be considered the best drought response cultivars and experimental lines. These most likely include Ruby Lee, Duster, Byrd, Jagger, and TAM 113 whereas wheat cultivars in quadrant 4 are considered as the most drought susceptibility such as Gallagher, OK Bullet, and Endurance. When comparing the field response with the preliminary screen under SS the results matched very closely those under MS with the exception that TAM 111 changed from quadrant 1 to quadrant 3 under SS compared to quadrant 1 under MS. These initial screens when compared to the field data from the High Plains region of Texas and New Mexico showed high correlation with seed weight under rainfed conditions (R^2 =0.82) for the five cultivars.

In further screenings nine cultivars from cycle one and cycle two were selected for further screening based upon the best drought responsive strains (Byrd, Ruby Lee, TAM 112, and Duster) as well as one moderately responsive strain (Bentley) and three susceptible strains (Endurance, Gallagher, and Cedar). This more focused screening used the same WW, MS, and SS conditions, but this time with three replications and with nine plants per Tray 10 for 27 plants per cultivar/treatment combination over two cycles of growth and development. Of the nine cultivars, five were common to the field study permitting limited correlation with field data. This final screening is referred to as cycle three and four. The average for yield traits across cycle three and four under WW conditions is shown in Table 5. There was a high level of consistency between cycle three and four as far as seed weight is concerned. Ruby Lee, TAM 112, Duster, and Gallagher showed highest average seed weight whereas Jagger, Bentley, Endurance, and Cedar showed lowest average in the same trait. Seed weight was correlated with shoot weight (R^2=0.65).

TAM 112, Ruby Lee, Gallagher, and Duster had the highest average of shoot weight whereas Jagger, Cedar, and Endurance had the lowest average. Seed weight was correlated with spike weight (*R^2*=0.75). TAM 112, Duster, and Ruby Lee had the highest average of spike weight whereas Bentley, Endurance, and Jagger had the lowest average of spike weight. Seed weight was correlated with spike number (*R^2*=0.35). Duster, Ruby Lee, and Gallagher had the highest average of spike number whereas Jagger, Cedar, and Endurance had the lowest average. Seed weight had no correlation with tiller number (*R^2*=0.00). Endurance, Duster, and Cedar having the highest average of tiller number whereas Jagger and TAM 112 had the lowest average of tiller number. Seed weight was highly correlated with seed number (*R^2*=0.89). Duster, Ruby Lee, and Gallagher had the highest average of seed number whereas Jagger, Bentley, and Endurance had the lowest average of seed number. Compared with the field research, these cultivars responded similarity under irrigated conditions: Ruby Lee, Hatcher, and TAM 112. Other cultivars such as Endurance and Jagger were similar with the field study results in that they exhibited the lowest average seed yield. Overall, the highest seed yield resulted from a larger spikes number and seed number.

The average yield traits across cycle three and cycle four under MS for nine winter wheats is illustrated in Table 6. Bentley, Byrd, and Ruby Lee showed high average seed weight whereas Jagger, Cedar, and Duster showed lowest average in seed weight. Seed weight was correlated with shoot weight (*R^2*=0.67) with Bentley, Byrd, and Gallagher having the highest average shoot weight whereas Endurance, Jagger, and Duster had the lowest average shoot weight. Seed weight was correlated with spike weight (*R^2*=0.74) Bentley, TAM 112, and Byrd had the highest average of spike weight whereas Cedar, Jagger, and Duster had the lowest average of spike weight. Seed weight showed little correlation with spike number (*R^2*=0.19) with Jagger, Bentley, and Endurance having the highest average of spike number whereas Cedar, Gallagher, and Duster had the lowest average spike number. Seed weight was not correlated with tiller number (*R^2*=0.02) with Endurance, Jagger, Cedar had the highest average of tiller number whereas Duster, TAM 112, and Gallagher had the lowest average of tiller number. Seed weight was moderately correlated with seed number (R^2=0.49) with Bentley, Endurance, and TAM 112 had the highest average of seed number whereas Duster, Cedar, and Jagger had the lowest average of seed number. Overall, the highest seed yield resulted from larger spikes number and seed number under MS. These screens when compared to the field data from the High Plains region of Texas showed weak correlations with seed yield under rainfed conditions (R^2 =0.25).

The average of yield traits across cycle three and cycle four under SS for nine winter wheats are demonstrated in Table 7. Gallagher, Ruby Lee, and Endurance showed highest average seed weight whereas Jagger, Byrd, and Cedar showed lowest average seed weight. Seed weight was moderately correlated with shoot weight (R^2=0.36) with Endurance, Gallagher, and Ruby Lee had the highest average of shoot weight whereas Cedar, Byrd, and Bentley had the lowest average of shoot weight. Seed weight was moderately correlated with spike weight (R^2=0.35) with Bentley, Endurance, and Gallagher having the highest average of spike weight whereas TAM 112, Cedar, and Byrd had the lowest average spike weight. Seed weight was not correlated with spike number (R^2=0.00) with Jagger, Endurance, and Gallagher having the highest average of spike number whereas Ruby Lee, TAM 112, and Cedar had the lowest average of spike number. Seed weight showed very little correlation with tiller number (R^2= 0.03) with TAM 112, Endurance, Byrd, and Jagger having the highest average of tiller number whereas Ruby Lee, Duster, and Cedar had the lowest average of tiller number. Seed weight was moderately correlated with seed number (R^2=0.54) with Gallagher, Bentley, Ruby Lee, and Byrd having the highest average of seed number whereas Jagger, TAM 112, Cedar, and Endurance had the lowest average seed number. Overall, the highest seed yield resulted from a larger seed number. These initial screens when compared to the field data from the High Plains region of Texas showed moderate correlation between seed weight and seed yield under rainfed conditions (R^2 =0.35).

The average seed weight for all winter wheat cultivars across cycle three and cycle four under MS and WW is plotted in Figure 3. The WW is plotted on the x-axis with total average seed weight at 4.71 g/plant represented as a vertical line whereas the seed weights under MS is plotted at y-axis with total average seed weight at 2.16 g/plant presented as a horizontal line. Quadrant 1 revealed the highest average seed weights under WW and MS including Duster, Gallagher, Bentley, and Ruby Lee. Quadrant 2 reflects the above average seed weight cultivars under only MS condition, including Byrd. Quadrant 3 displays above average seed weight and below average under WW, including TAM 112. Quadrant 4 reflects below average seed weights in cultivars under WW and MS, including Cedar, Jagger, and Endurance. Some of wheat cultivars in Figure 3 showed the same relatively position compared with Figure 1 in cycle one and two. For example, Duster, Bentley, Ruby Lee, and TAM 112 are situated in quadrant 1 and Endurance and Cedar are found in in quadrant 4 in both studies.

The average seed weights for all winter wheat cultivars across cycles three and cycle four under SS and WW is plotted in Figure 4. The WW seed weights are plotted on the x-axis with total average seed weight at 4.71 g/plant presented as a vertical line whereas under SS seed weights are plotted on the y-axis with total average seed weight at 1.19 g/plant represented as a horizontal line. Quadrant 1 includes cultivars with a high average of seed weight under WW and SS including Duster, Ruby Lee, Gallagher, and Bentley. Quadrant 2 reflecting above average cultivars seed weight under SS but below average under WW contains no cultivars. Quadrant 3 displays above average seed yield under WW and below average under SS includes TAM 112. Quadrant 4 reflects the below average cultivars under WW and SS includes Cedar, Jagger, Endurance, and Byrd. Since, the positions of some cultivars are relatively similar between Figure 3 and Figure 4, it can be concluded that Duster, Gallagher, and Ruby Lee are considered as the best drought responsive cultivars overall.

Specific yield traits that differentiates among cultivars are important to identify in that they provide information concerning which traits are important in distinguishing cultivars in a breeding program. Discriminant analysis is often used for this purpose. The discriminant analysis is shown in Figure 5. The traits that most differentiates among cultivars are those which are found farthest from the center. Seed number and spike number are the two traits that discriminated most among the 19 cultivars selected for cycle one and two. The green arrow in traits shoot weight and tiller number indicated the lowest contribution to the discrimination among winter wheat cultivars. This discriminant analysis includes two factors: factor 1 is correlated with spikes number, seed weight, spike weight, and seed number explaining 80.47% of total variation, factor 2 is correlated with shoot weight and tiller number explaining only 9.06% of total variation. The correlation and the discriminant analysis among some traits allowed us to choose the most effective traits for directing selection methods and improving wheat breeding program.

A better understanding of the genetic relationships among HRWW cultivars is essential for selecting genetic backgrounds for new cultivar creation. Genetic relatedness among cultivars are best determined using GBS methodology coupled with SNP analysis. The genetic distance of 19 HRWW cultivars and experimental lines are illustrated (Table 8). The highes genetic dissimilarity was found between Jagger and Hatcher representing the two cultivars that are the least related to each other, genetically. The lowest genetic dissimilarity was found between Duster and Iba representing the closest cultivars in terms of genetic relatedness. Low genetic diversity in a breeding program will affect negatively the improvement of wheat yield and adaptions to harmful condition such as drought stress. On other hand, high genetic diversity will help breeders to select breeding materials that can promote a higher wheat yield for cultivar development. The GBS data coupled to with the SNPs analysis can also be used to associate specific traits with tolerance to water limiting conditions, which will be conducted at a future date.

Genetic relatedness can be best visualized using a number of dendrogram variants. These dendrograms are created from the GBS SNP analysis using he Neighbor joining algorithm (Figure 1). The analysis divides the 19 cultivar entrants into five groups, namely: first group, Iba, Duster, OK12621, OK10126, Cedar, Garrison, and Hatcher; second group, Byrd, TAM 112, TAM 113, and TAM 111; third group, Endurance, Ruby Lee; forth group, Chisholm, and Gallagher; fifth group, Jagger, OK Bullet, Bentley, and OK11D25056. It is noted that wheat cultivars and experimental lines from Oklahoma State University are clustered in two groups (groups one, three, four and five) indicating the divergence of the genetic materials withing the breeding program. Interesting, wheat cultivars from Texas A&M University are clustered in the second group, only. Hatcher and Byrd from Colorado State University Breeding programs was found in groups one and two. The only entry from the Kansas State Breeding program was Jagger and it was found in group 5. Kobayashi et al. (2016) who were able to demonstrate the ability of GBS method to classify Japanese wheat cultivars developed in between 1940 and 1990 into three groups.

When performing an analysis on genetic relatedness in wheat using the GBS approach coupled to SNP analysis researchers often use the wheat reference genome from the cultivar Chinese Spring, the wheat genome that was partially sequenced. The SNP analysis permits the identification and quantification to the total number of SNPs for each cultivar, which represents the heterozygosity between a given cultivar and the reference genome. The higher the number the most differentiated from the reference genome. The highest heterozygosity was found in Gallagher (18,235 SNPs) and OK11D25056 (17,078 SNPs) whereas lowest was found in TAM 111 (2,871 SNPs) and OK Bullet (7,459 SNPs) (Table 3). Wheat cultivars and experimental lines that originate from the Oklahoma State University breeding program showed the least heterozygosity in OK12621, OK Bullet, and OK10126, whereas Duster, Iba, Bentley, Ruby Lee, Endurance, OK11D25056, and Gallagher showed highest heterozygosity.

CONCLUSION

The cultivars that gave the best response to water limitations across all cycles of growth and development under greenhouse conditions are Byrd, Ruby Lee, TAM 112, and Duster. Under field, rainfed and irrigated conditions presented in the High Plains region of Texas and New Mexico Winterhawk, TX02A0252, and Hatcher provided the best response. The results from the field differs substantially from those from the controlled greenhouse study which is most likely due to factors other than just water limitations. That being said, the field study indicates the best cultivars and experimental lines that are adapted to the High Plains region of Texas and New Mexico, while the Greenhouse study indicates cultivars that are best adapted to MS and SS water limitations. The best Oklahoma State

Cultivars and experimental lines under field conditions are Duster and Garrison and under controlled water limitations conditions are Duster, Gallagher, Bentley, and Ruby Lee.

Table 1. Names and origins of 19 winter wheat cultivars and experimental lines used in greenhouse research

Name	Seed Sources
Cedar	WestBred
TAM 111	AgriLife research, Texas A&M
OK Bullet	Oklahoma Foundation Seed Services, OSU
Ruby Lee	Oklahoma Foundation Seed Services, OSU
Iba	Oklahoma Foundation Seed Services, OSU
Duster	Oklahoma Foundation Seed Services, OSU
Bentley	Oklahoma Foundation Seed Services, OSU
OK10126	Breeding Program, OSU
Gallagher	Oklahoma Foundation Seed Services, OSU
Hatcher	Colorado Wheat Research Foundation
OK12621	Breeding Program, OSU
TAM 113	AgriLife Research, Texas A&M
TAM 112	AgriLife Research, Texas A&M
Chisholm	Breeding Program, OSU
Endurance	Oklahoma Foundation Seed Services, OSU
OK11D25056	Breeding Program, OSU
Byrd	Colorado Wheat Research Foundation
Garrison	Oklahoma Foundation Seed Services, OSU
Jagger	Kansas Agricultural Experiment Station

Table 2. The average value for yield traits across cycle one and cycle two for 19 wheat cultivars and experimental lines under well water and greenhouse conditions

cultivar and experimental lines	Seed weight	Shoot weight	Spike weight	Spike Number	Tiller Number	Seed Number
	g/plt	*g/plt*	*g/plt*	#	#	#
Ruby Lee	2.04	5.75	2.85	10.92	3.04	53.38
OK12621	1.75	5.00	2.56	10.71	3.13	48.38
Duster	1.70	4.40	2.63	16.29	3.29	57.83
TAM 112	1.69	4.88	2.71	11.17	2.38	49.38
Jagger	1.69	5.48	2.74	10.83	3.88	53.50
TAM 113	1.62	5.19	2.63	10.46	3.83	49.08
OK11D25056	1.48	4.63	2.28	10.21	3.04	40.58
Bentley	1.40	4.27	2.03	10.17	3.13	42.84
OK10126	1.38	3.66	2.12	8.75	3.58	48.79
Byrd	1.37	4.54	2.24	4.79	3.04	40.75
Hatcher	1.32	4.03	2.03	13.38	3.54	38.67
Garrison	1.31	5.18	2.14	8.83	3.92	45.25
TAM 111	1.29	5.61	2.08	4.79	4.88	35.75
Cedar	1.10	5.77	1.79	5.54	4.29	29.25
Chisholm	0.70	4.22	1.41	2.29	4.17	19.34
Gallagher†	0.65	4.33	1.04	3.38	4.00	22.25
Iba	0.63	4.55	1.04	3.17	5.38	25.67
OK Bullet†	0.06	5.01	0.24	0.88	4.54	10.71
Endurance†	0.03	4.67	0.10	0.21	5.63	1.00
LSD	1.05	3.24	1.90	9.66	2.74	31.20

† Issues vernalization.

Table 3. The average value for yield traits across cycle one and cycle two for 19 wheat cultivars and experimental lines under moderate stress and greenhouse conditions

cultivar and experimental lines	Seed weight	Shoot weight	Spike weight	Spike Number	Tiller Number	Seed Number
	g/plt	*g/plt*	*g/plt*	#	#	#
Ruby Lee	0.90	2.06	1.17	9.25	1.25	14.38
OK11D25056	0.78	2.14	1.05	7.04	1.67	7.50
Byrd	0.75	2.57	1.08	6.46	2.08	18.67
TAM 112	0.70	1.92	1.00	7.08	1.29	21.00
OK10126	0.69	1.89	0.99	5.50	1.63	21.13
Bentley	0.68	1.80	0.94	5.88	1.79	23.67
TAM 111	0.68	2.37	0.94	2.50	2.13	16.00
Duster	0.67	1.81	1.01	9.08	1.92	15.04
OK12621	0.65	2.06	0.92	5.33	1.75	16.29
Hatcher	0.59	1.91	0.83	7.29	1.71	16.17
TAM 113	0.55	1.97	0.77	4.67	2.17	14.38
Cedar	0.34	2.56	0.52	1.96	3.25	10.63
Garrison	0.30	2.03	0.74	4.42	2.38	19.25
Chisholm	0.27	2.06	0.41	2.75	2.25	14.29
Gallagher†	0.27	2.00	0.45	1.88	2.17	16.08
OK Bullet†	0.27	2.12	0.43	1.71	2.67	17.46
Iba	0.25	2.07	0.38	1.42	3.13	25.38
Endurance†	0.22	1.72	0.00	0.25	2.17	6.83
Jagger	0.79	2.35	1.09	7.38	2.08	20.96
LSD	0.5	1.41	0.69	5.1	1.91	21.07

† Issues vernalization.

Table 4. The average value for yield traits across cycle one and cycle two for 19 wheat cultivars and experimental lines under severe stress and greenhouse conditions

cultivar and experimental lines	Seed weight	Shoot weight	Spike weight	Spike Number	Tiller Number	Seed Number
	g/plt	*g/plt*	*g/plt*	#	#	#
Byrd	0.26	0.72	0.36	3.00	0.96	9.92
Ruby Lee	0.24	0.78	0.29	5.33	0.92	5.71
TAM 113	0.22	0.86	0.34	3.00	1.29	7.46
Duster	0.20	0.68	0.34	5.54	0.92	7.96
Jagger	0.20	0.82	0.28	5.08	1.00	9.83
OK12621	0.17	0.69	0.23	2.67	1.17	4.58
OK11D25056	0.16	1.03	0.23	2.83	1.21	5.08
Bentley	0.15	0.69	0.22	3.71	1.08	4.92
TAM 112	0.14	1.09	0.23	3.17	1.13	4.54
OK10126	0.14	0.63	0.19	1.08	0.88	4.54
Hatcher	0.13	0.72	0.21	4.25	1.08	4.17
TAM 111	0.09	0.93	0.15	0.00	1.50	3.08
Garrison	0.09	1.04	0.10	0.92	1.54	3.50
OK Bullet†	0.01	0.77	0.09	0.50	1.08	1.21
Iba	0.01	0.89	0.02	0.17	1.29	0.54
Cedar	0.00	0.80	0.01	0.00	1.46	0.25
Gallagher†	0.00	0.71	0.01	0.00	1.42	0.17
Chisholm	0.00	0.66	0.00	0.00	1.13	0.00
Endurance†	0.00	0.88	0.00	0.00	1.04	0.00
LSD	0.23	0.73	0.3	2.99	0.82	7.26

† Issues vernalization.

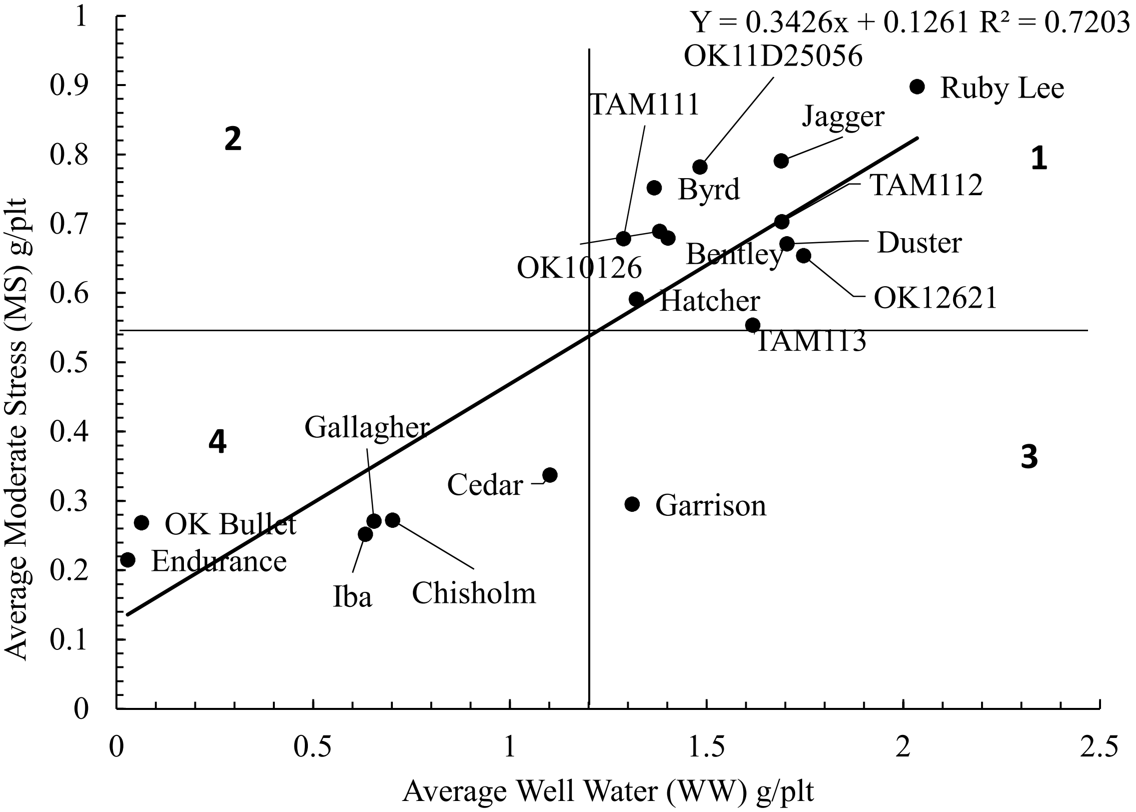

Figure 1. The average total seed weight/plant across cycle one and cycle two under well water (WW) and moderate stress (MS) under greenhouse conditions

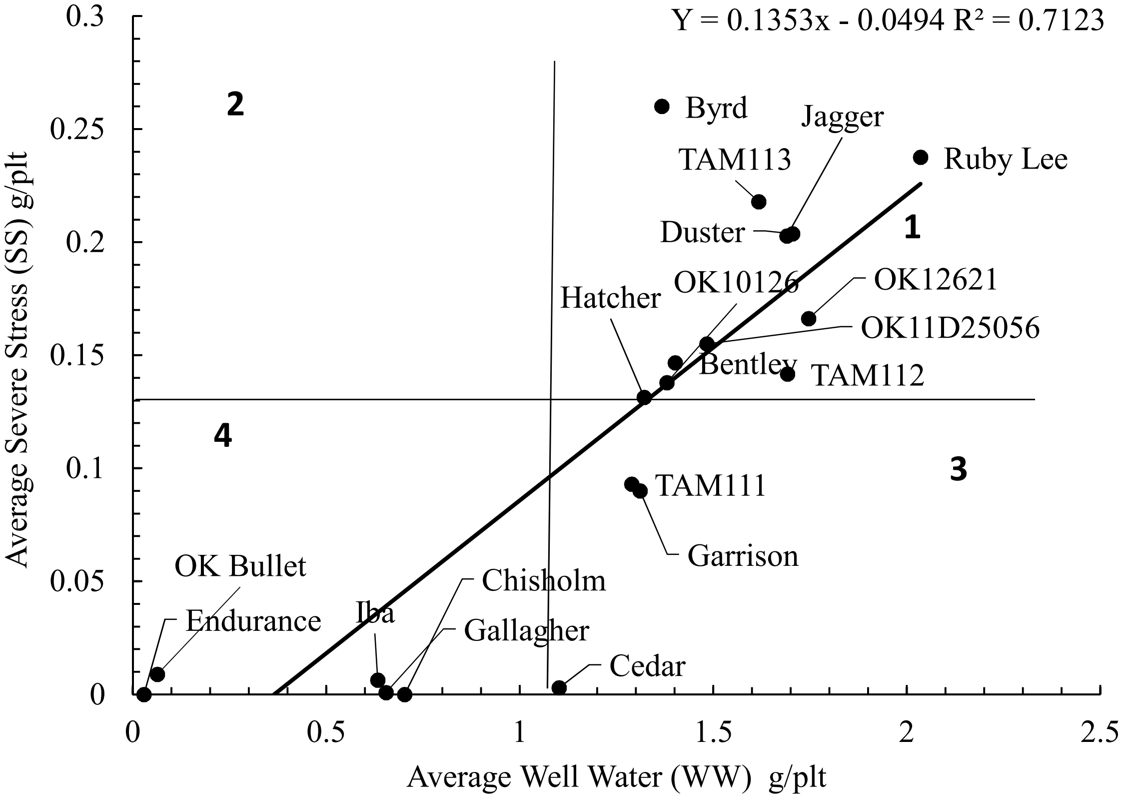

Figure 2. The average total seed weight/plant across cycle one and cycle two under well water (WW) and severe stress (SS) under greenhouse conditions

Table 5. The average value for yield traits across cycle three and cycle four for nine wheat cultivars under well water and greenhouse conditions

cultivar and experimental lines	Seed weight	Shoot weight	Spike weight	Spike Number	Tiller Number	Seed Number
	g/plt	*g/plt*	*g/plt*	#	#	#
Ruby Lee	6.33	15.62	8.42	3.74	3.89	107.24
TAM 112	6.28	15.92	10.65	3.17	3.30	97.91
Duster	5.75	15.49	8.94	4.31	4.91	110.72
Gallagher	5.18	15.57	7.76	3.65	3.83	98.89
Byrd	4.95	14.38	7.60	3.48	3.59	85.83
Cedar	4.32	10.25	8.06	2.98	3.94	81.11
Endurance	3.74	11.14	5.70	3.15	5.57	68.94
Bentley	3.06	13.66	4.77	3.48	3.61	67.19
Jagger	2.76	8.62	6.37	2.57	3.07	61.15
LSD	1.44	4.03	2.11	0.88	0.93	18.84

Table 6. The average value for yield traits across cycle three and cycle four for nine wheat cultivars under moderate stress and greenhouse conditions

cultivar and experimental lines	Seed weight	Shoot weight	Spike weight	Spike Number	Tiller Number	Seed Number
	g/plt	*g/plt*	*g/plt*	#	#	#
Bentley	3.10	8.37	4.74	2.24	2.31	56.80
Byrd	2.75	6.90	3.34	2.15	2.28	52.56
Ruby Lee	2.51	6.19	3.23	2.17	2.28	52.06
Gallagher	2.31	6.35	3.12	1.63	1.63	48.28
Endurance	2.21	4.25	3.28	2.22	2.57	55.59
TAM 112	2.11	5.97	3.76	1.83	1.87	53.56
Duster	1.60	4.99	2.25	1.69	1.63	29.19
Cedar	1.52	5.23	2.48	1.54	2.31	44.74
Jagger	1.38	4.75	2.48	2.26	2.37	47.07
LSD	0.65	1.85	1.04	0.59	0.81	13.64

Table 7. The average value for yield traits across cycle three and cycle four for nine wheat cultivars under severe stress and greenhouse conditions

cultivar and experimental lines	Seed weight	Shoot weight	Spike weight	Spike Number	Tiller Number	Seed Number
	g/plt	*g/plt*	*g/plt*	#	#	#
Gallagher	1.66	4.05	2.37	1.93	1.93	39.50
Ruby Lee	1.44	3.62	1.97	1.39	1.39	34.76
Endurance	1.42	5.60	2.43	2.09	2.30	31.41
Bentley	1.24	3.06	3.27	1.65	1.69	35.57
TAM 112	1.11	3.52	1.46	1.44	2.83	28.46
Duster	1.09	3.29	1.59	1.50	1.52	32.30
Cedar	1.02	2.72	1.51	1.54	1.57	30.39
Byrd	0.96	2.76	1.50	1.91	2.15	34.35
Jagger	0.79	3.27	1.52	2.11	2.15	27.11
LSD	0.43	1.16	1.15	0.57	0.64	10.41

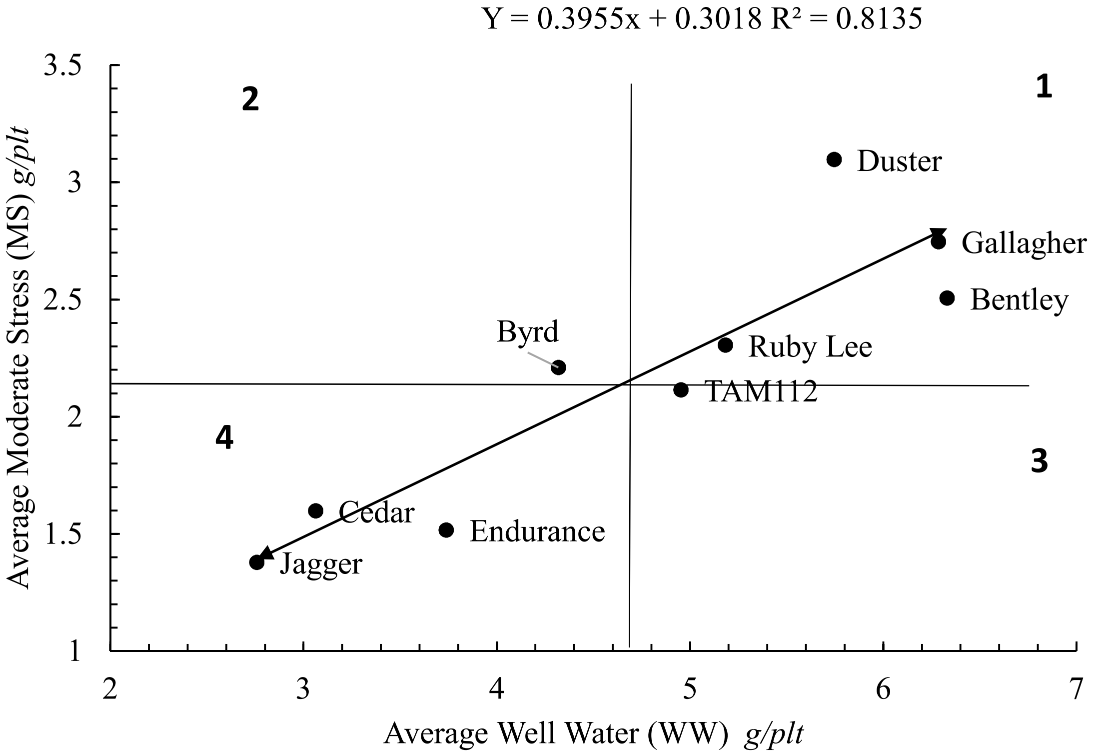

Figure 3. The average total seed weight/plant across cycle three and cycle four under well water (WW) and moderate stress (MS) under greenhouse conditions

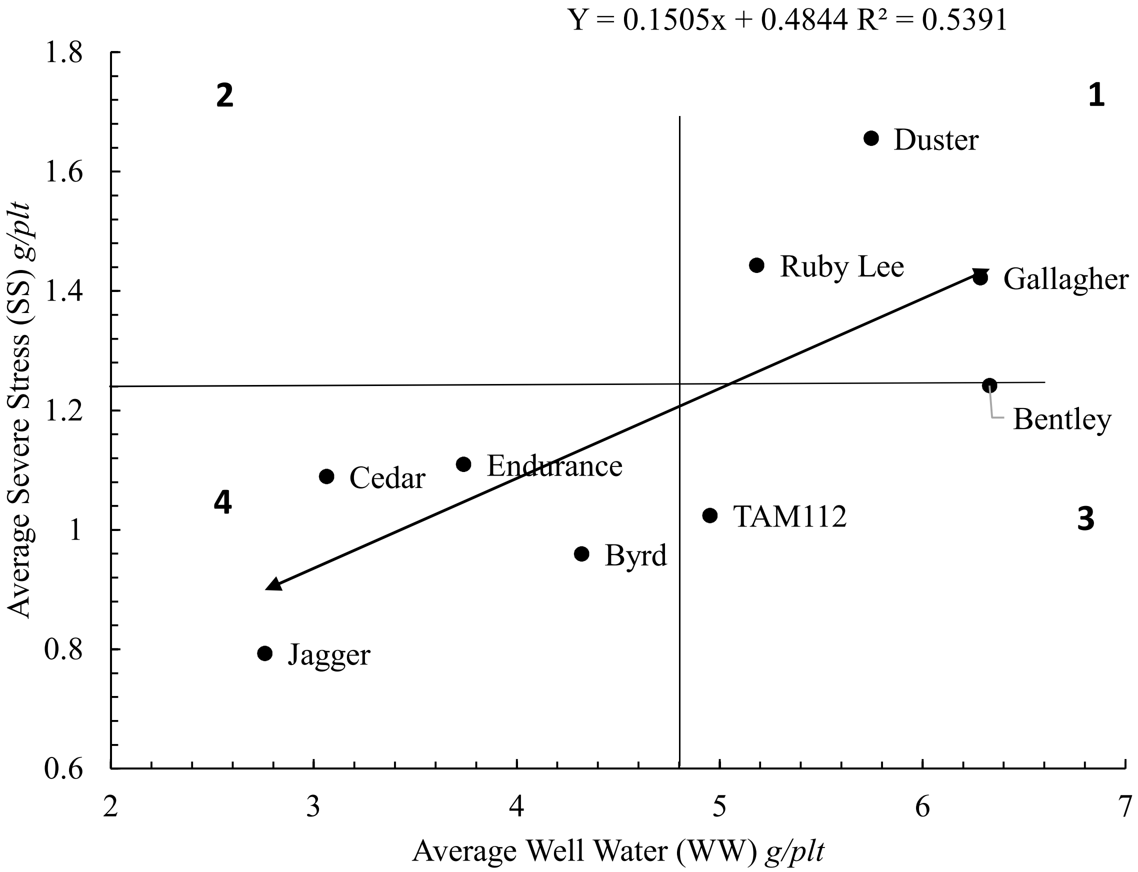

Figure 4. The average total seed weight/plant across cycle three and cycle four under well water (WW) and severe stress (SS) under greenhouse conditions

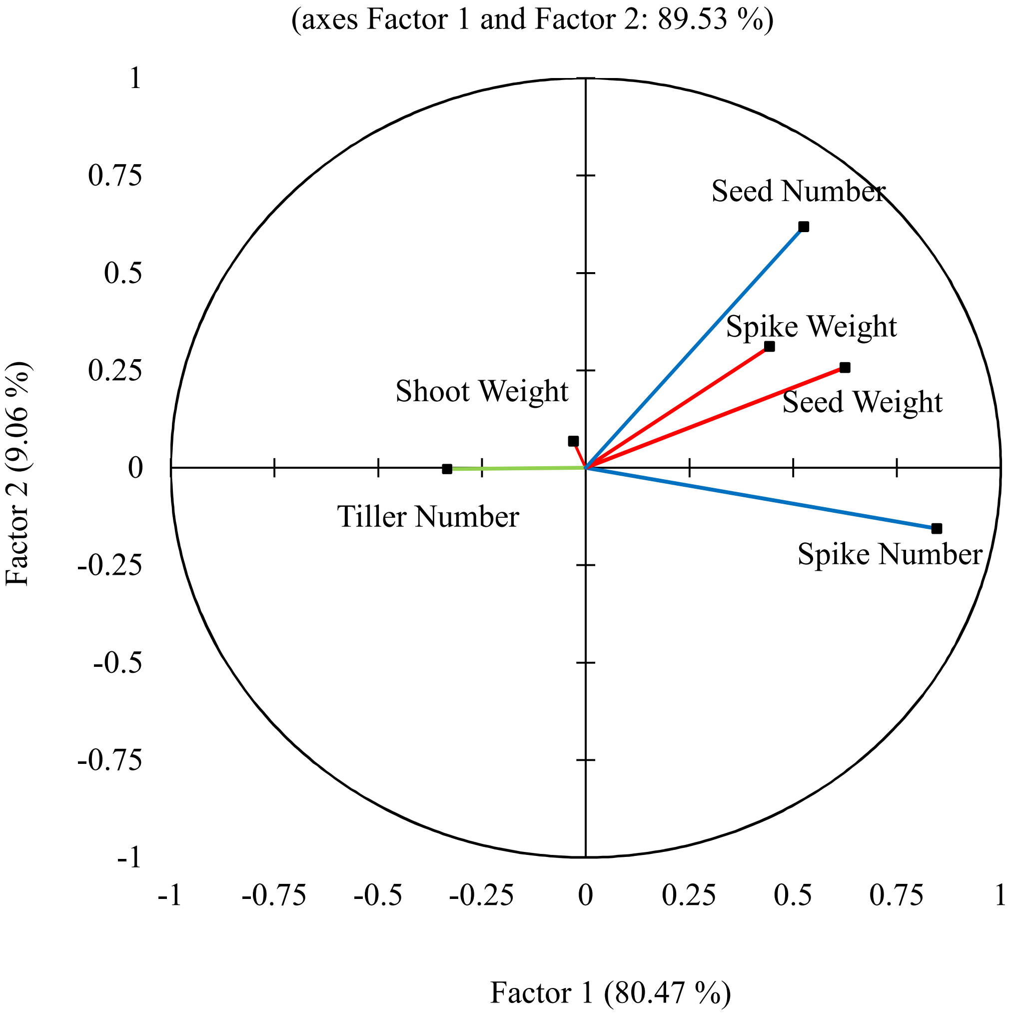

Figure 5. The discriminant analysis across four cycles of greenhouse studies; arrow length represents importance of a particular trait in discriminating among cultivars and experimental lines. Blue arrows indicate highly discriminating traits whereas green arrows indicate less discriminating traits.

Table 8. Distance Matrix based on similarity index among 19 hard red winter wheat cultivars and experimental lines using the Neighbor Joining (NJ) algorithm

cultivar and experimental lines	Cedar	OK Bullet	TAM 111	Iba	Ruby Lee	Bentley	Duster	Gallagher	OK10126	Hatcher	Chisholm	TAM 112	TAM 113	OK12621	Garrison	Byrd	OK11D25056	Endurance	Jagger
Cedar	0	0.274	0.232	0.24	0.269	0.279	0.232	0.258	0.262	0.257	0.237	0.256	0.26	0.245	0.26	0.27	0.278	0.249	0.289
OK Bullet		0	0.227	0.249	0.235	0.194	0.248	0.258	0.271	0.257	0.237	0.228	0.243	0.255	0.26	0.222	0.209	0.248	0.18
TAM111			0	0.227	0.224	0.224	0.229	0.237	0.259	0.229	0.21	0.211	0.211	0.25	0.24	0.212	0.22	0.237	0.254
Iba				0	0.244	0.26	0.148	0.247	0.225	0.241	0.222	0.239	0.23	0.206	0.25	0.243	0.248	0.232	0.28
Ruby Lee					0	0.236	0.239	0.256	0.264	0.257	0.233	0.224	0.241	0.272	0.27	0.228	0.236	0.214	0.279
Bentley						0	0.257	0.252	0.279	0.25	0.227	0.224	0.234	0.279	0.27	0.226	0.215	0.25	0.217
Duster							0	0.243	0.194	0.244	0.215	0.238	0.224	0.164	0.25	0.24	0.236	0.228	0.274
Gallagher								0	0.279	0.26	0.207	0.259	0.257	0.233	0.26	0.253	0.226	0.231	0.286
OK10126									0	0.273	0.264	0.268	0.255	0.239	0.27	0.27	0.229	0.234	0.285
Hatcher										0	0.23	0.241	0.231	0.261	0.26	0.241	0.247	0.24	0.291
Chisholm											0	0.237	0.222	0.243	0.26	0.235	0.225	0.237	0.27
TAM112												0	0.177	0.263	0.25	0.172	0.225	0.24	0.277
TAM113													0	0.251	0.23	0.2	0.24	0.238	0.28
OK12621														0	0.25	0.266	0.259	0.24	0.281
Garrison															0	0.253	0.264	0.247	0.277
Byrd																0	0.219	0.234	0.276
OK11D025056																	0	0.235	0.248
Endurance																		0	0.271
Jagger																			0

Table 9. The number of SNPs for 19 hard red winter wheat cultivars and experimental lines

Cultivar and experimental lines	Number of SNPs
Gallagher	18,235
OK11D25056	17,078
Hatcher	15,774
Jagger	14,535
Endurance	13,947
Cedar	13,815
Bentley	11,939
Ruby Lee	11,474
Chisholm	11,350
Duster	11,035
TAM 112	10,931
Iba	10,638
OK10126	10,124
Byrd	9,900
TAM 113	9,355
Garrison	7,890
OK12621	7,790
OK Bullet	7,459
TAM 111	2,871

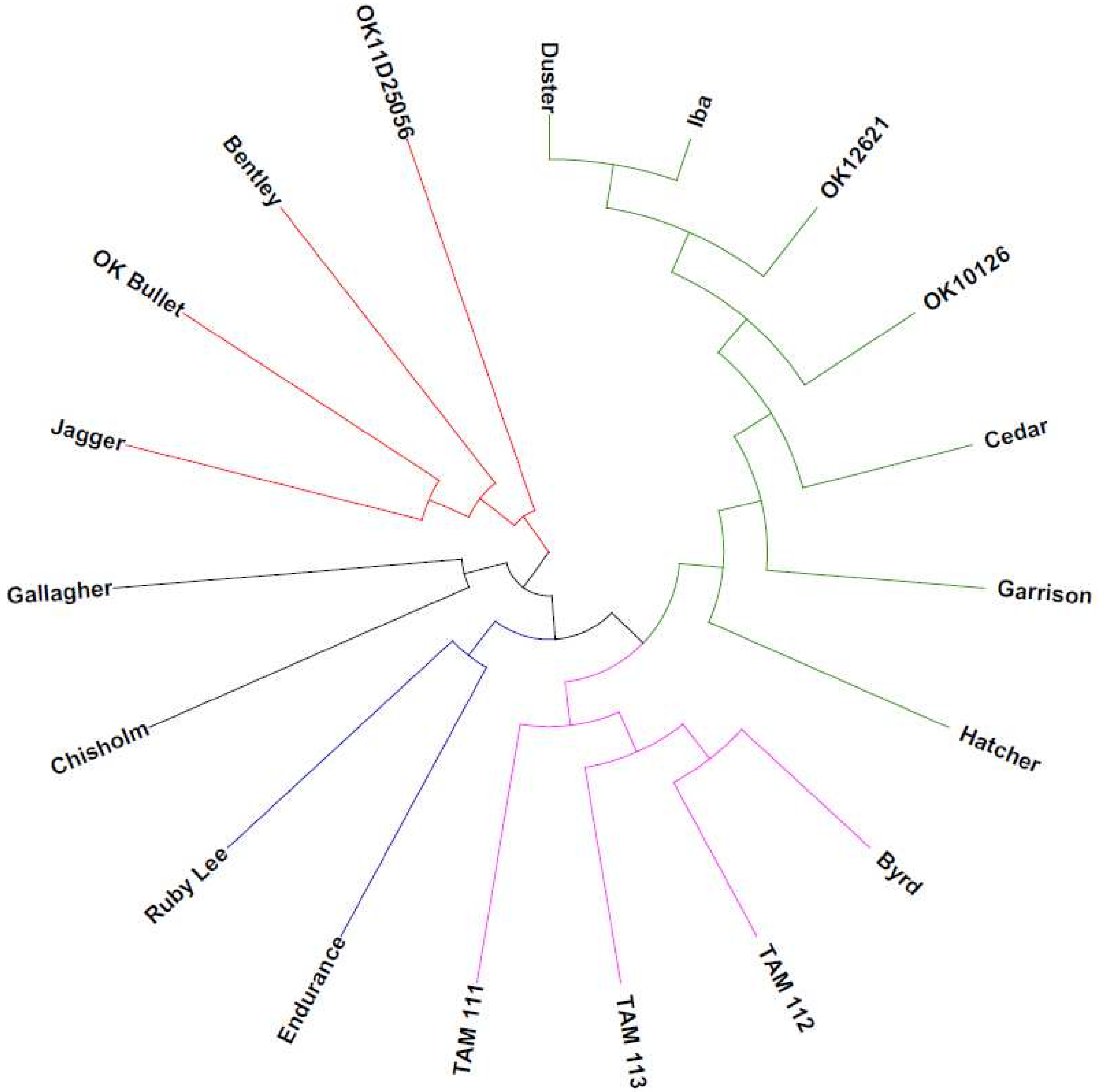

Figure 6. Dendrogram illustrating the genetic relatedness among 19 hard red winter wheat cultivars and experimental lines based on SNPs analysis data using Neighbor joining (NJ) algorithm as processed through TASSEL software

REFERENCES

Abdoli, M., M. Saeidi, S. Jalali-Honarmand, S. Mansourifar, and S. Ghobadi. 2013. Effect of post-anthesis water deficiency on storage capacity and contribution of stem reserves to the growing grains of wheat cultivars. Plant Knowledge Journal, *2*(3):99-107.

Bajgain, P., M. Rouse, and J. Anderson. 2016. Comparing Genotyping-by-Sequencing and Single Nucleotide Polymorphism Chip Genotyping for Quantitative Trait Loci Mapping in Wheat. Crop Sci. 56:232–248. doi: 10.2135/cropsci2015.06.0389.

Deschamps, S., V. Llaca, and D. Gregory. 2012. Genotyping-by-Sequencing in Plants. Biology, 1, 460-483. doi:10.3390/biology1030460.

Fischer, R., and R. Maurer. 1978. Drought resistance in spring wheat cultivars. I. Grain yield response. Aust. J. Agric. Res. 29:897-912.

Hart, D. and M. Ruvolo. 2012. Genetic analysis of genes and genomes. 8th ed. https://books.google.com/books?id=jxhH8sWRn_oC&printsec=frontcover&dq=genetic&hl=en&sa=X&ved=0ahUKEwjk6sXm_Y_TAhVhxYMKHQeWCIEQ6AEIGjAA#v=onepage&q=genetic&f=false (accessed 1 August. 2017).

Jordan, S., and P. Humphries. 1994. Single nucleotide polymorphism in exon 2 of the BCP gene on 7q31-q35. Human Mol Genet 3:1909-1915.

Khakwani, A., M. Dennett, M. Munir, and M. Abid. 2012. Growth and yield response of wheat varieties to water stress at booting and anthesis stages of development. ak. J. Bot. 44(3): 879-886.

Kobayashi, F., T. Tanaka, H. Kanamori, J. Wu, Y. Katayose, and H. Handa. 2016. Characterization of a mini core collection of Japanese wheat varieties using single nucleotide polymorphisms generated by genotyping-by-sequencing. Breeding Science, 66: 213–225. doi:10.1270/jsbbs.66.213.

Mortazavian, S., H. Ramshini, M. Mohseni, T. Nabavi. 2015. Assessment of wheat yield response to water shortage using various tolerance indices. Phillipp Agric. Science. 98(3):262-269.

Ranjbar, A., A. Sepaskhah, and S. Emadi. 2015. Relationships between wheat yield, yield compoents and physico-chemical properties of soil under rain-fed condition. International journal of plant production, 9(3) 434-465.

Reddy, S., S. Liu, J. Rudd, Q. Xue, P. Payton, S. Finlayson, J. Mahan, A. Akhunova, S. Holalu, and N. Lu. 2014. Physiology and transcriptomic of water-deficit stress responses in wheat cultivars TAM 111 and TAM 112. Journal of Plant Physiology, 171: 1289-1298. http://dx.doi.org/1Xue0.1016/j.jplph.2014.05.005.

Riaz, R. and M. Chowdhry. 2003. Genetic analysis of some economic traits of wheat under drought condition. Asian Journal of Plant Sciences 2(10): 790-796.

Salem, K. F., M. El-Zanaty, and M. Esmail. 2008. Assessing Wheat (Triticum aestivum L.) Genetic diversity using morphological characters and microsatellite markers. World J Agric Sci. 4(5): 538–544. DOI:10.1111/j.1744-7348.2007.00201.x.

Semagn, K., A. Bjornstad, and M. Ndjiondjop. 2006. An overview of molecular marker methods for plants. African Journal of Biotechnology. 5(25): 2540-2568.

Tadesse, W., F. Ogbonnaya, A. Jighly, M. Sanchez-Garcia, Q. Sohail, S. Rajaram, and M. Baum. 2015. Genome-Wide Association Mapping of Yield and Grain Quality Traits in Winter Wheat Genotypes. Plos One 10(10) 1-18 doi:10.1371/journal.

Varshney, R.K., R. Terauchi, and R. McCouch. 2014. Harvesting the promising fruits of genomics: Applying genome sequencing technologies to crop breeding. PLoS Biol. 12(6): 8 pages. doi:10.1371/journal.pbio. 1001883.

Xue, Q., J. Rudd, S. Liu, K. Jessup, R. Devkota, and J. Mahan. 2014. Yield determination and water use efficiency of wheat under water-limited conditions in the U.S. South-ern High Plains. Crop Sci. 54: 34–47. doi:10.2135/cropsci2013.02.0108.

VITA

MESHAL MUNAWIR R. ALMUTAIRI

Candidate for the Degree of

Doctor of Philosophy

Dissertation: GENETIC DIVERSITY AMONG HARD RED WINTER WHEAT CULTIVARS AND EXPERIMENTAL LINES AND THEIR ASSOCIATION WITH WATER LIMITATION

Major Field: Crop Science

Biographical:

Education:

I have completed the requirement for the Bachelor of Agriculture and Science Specialty of Horticulture and Forestry from Qassim University, Kingdom of Saudi Arabia, in 15/02/2004.

And completed the requirement for the Master of Food and agricultural Science Specialty of Horticulture, King Saud University, Kingdom of Saudi Arabia in 16/06/ 2009.

Experience:

I was a director of the Alkafi computer for one years in the Albukairah agency. Worked as Agricultural engineer for four years in the Special Security Forces. Also, Worked as Member in quality and Development in King Saud University for six months. Lastly, I have been working as academic researcher at King Abdulaziz City for Science and Technology since 2009.