Phenotypic, Spectral Reflectance and Genetic Analysis of Spring Wheat Accessions from the NSGC

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Authorization to Submit Dissertation

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Abstract

This study was designed to assess spring wheat accessions found in the USDA-ARS National Small Grains Collection using a combination of genetic markers, agronomic characteristic, and a novel spectrometer based high-throughput phenotyping platform. The panel of spring wheat accessions selected for this study originated from six continents and 81 countries. This panel included wheat accessions classified by the USDA as 'Cultivars', 'Breeding lines', 'Landraces' and 'Uncertain'. Cultivars and breeding lines were developed through modern breeding techniques, while landraces developed through farmer selections. In the present study, the following measurements were made in irrigated and water stressed environments: yield, grain protein, grain volume by weight (test weight), plant heights, and days to heading. Spectrometer readings were made throughout the growing season and markers were used to map genes influencing traits of interest. Selection of accessions using genetic markers and spectral reflectance found that each of these methods was able to identify a significant proportion of the highest yielding accessions each year. Several of the landrace accessions selected by markers, spectral reflectance and yield, were found to have relatively high yields that were consistent across both years and irrigations regimes. The accessions identified here may contain novel genes not currently found in modern cultivars and could be used to introduce genetic variation into current breeding programs.

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Dedication

I would like to thank my uncle George for being a ……, I wish you were still around, but know you are in a better place; Bird for putting up with George's antics; my grandparents Paul and Sarah; my parents Bruce and Ann for putting up with me over the years; my brother Travis for covering for me many times; cheese and peanut butter sandwiches; that Brazilian guy who spent more time going to Las Vegas than working (congrats on your first child); that dude from England that hates American food; braided fishing line for being superior in every way; Deepak, Santosh and Shawn for making curry that mere mortals are unable to eat; Junli for wearing Tickle-me-Elmo shirts into clubs while rolling with a Mexican crew and being the sole member of the 0.005% Asian demographic in Aberdeen Idaho; Patrón for moments of inspiration followed by hours of fuzzy memory gaps; Yeti and Founders for making the best breakfast on this planet; extra chunky peanut butter; Microtech for hours of masochistic fun; BRS for making the best flipper known to man; BT and all the masters there for pushing the limits of balisong flipping; and finally thanks to the JDBA for being a constant source of distractions, from dogpiling "what should I buy" and "vs" threads to teaching me new and unique vulgar expressions, as well as methods of "marking" knives so no one will ever try to steal my T2, let alone want touch it. Oh yeah, to my sexy beast Celina for keeping me sane.

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    - μ = treatment mean, *i* = single accession mean

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- Water Index (WI) = 970nm / 900nm
- Normalized Water Index 1 (NWI1) = (970nm - 900nm) ÷ (970nm + 900nm)
- Normalized Water Index 1 (NWI2) = (970nm - 850nm) ÷ (970nm + 850nm)
- Normalized Water Index 1 (NWI3) = (970nm - 920nm) ÷ (970nm + 920nm)
- Normalized Water Index 1 (NWI4) = (970nm - 880nm) ÷ (970nm + 880nm)
- Simple Ratio (SR) = 900nm ÷ 680nm
- Normalized Difference Vegetation Index (NDVI) = (900nm - 680nm) ÷ (900nm + 680nm)
- Red NDVI (RNDVI) = (780nm - 670nm) ÷ (780nm + 670nm)
- Photochemical Reflectance Index (PRI) = (531nm - 570nm) ÷ (531nm + 570nm)
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Literature Review

WHEAT PRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most widely grown crop plants world-wide. It has been cultivated for the past 8,000 to 12,000 years since its origin in the fertile crescent (Abbo et al., 2006; Cavanagh et al., 2013). Wheat accounts for nearly 20% of human daily caloric intake and occupies 17% of the world crop production acreage (Gupta et al., 2008). In the United States, six classes of wheat account for most of the wheat grown. In general, specific regions of the US will grow certain classes of wheat. Hard red winter and spring wheat cultivars are grown in the Great Plains region and used for leavened bread. Soft red winter wheat is grown east of the Mississippi and soft white wheat is grown in the Pacific Northwest, both are used for cereals, flat breads, pastries and crackers. Durum wheat (*Triticum durum*) is grown in the same regions as hard red spring wheat and is used for pasta. The most recent class of wheat to be grown in the USA is hard white. Hard white wheat has similar quality characteristics to hard red, but without the colored outer seed coat. While hard white wheat has been grown in Asia and Australia, it has not been widely grown in the United States until the past 10 to 15 years (Clay et al., 2012, Ransom et al., 2006).

Wheat yields have increased significantly since the introduction of modern breeding techniques in the mid-20th century. The Green Revolution began in the mid-1960s, and since then the human population has more than doubled, thanks in part to the increased availability of food. Small grain production allowed for populations to increase without a decrease in

available food supplies by tripling production with only a 30% increase in crop land, amounting to a 200% increase in yield per hectare (Pingali, 2012). International organizations have been created around the world to support continued small grains improvement. The success of the International Center for Wheat and Maize Improvement in Mexico (CIMMYT) and the International Rice Research Institute (IRRI) in coordinating research in their respective crops spurred the creation of new international agricultural research centers that now fall under the Consultative Group for International Agricultural Research (CGIAR). These international institutes allowed for access and exchange of germplasm and breeding methods between researchers from around the world (Evenson and Gollin, 2003). The dissemination of knowledge and technologies from developed nations and the adoption of modern cultivars reduced poverty in developing countries. It has been estimated that for every 1% increase in crop yields the number of impoverished people was reduced by 0.5% in eastern Asia and 1.9% in India (Pingali, 2012).

ISSUES IN MODERN WHEAT BREEDING

The largest genetic gains in wheat yield have been attributed to the introduction of dwarfing genes, deployment of disease resistant cultivars, and the development of regionally adapted varieties (Fischer and Edmeades, 2010). In addition to genetic gains, past yield increases have also been attributed to management practices such as weed control, precise fertilizer applications and minimal tillage (Fischer and Edmeades, 2010). The USDA monitored genetic gains in wheat through the implementation of regional trials starting in 1930. New cultivars were compared to a historic wheat cultivar Kharkof. Comparisons to

Kharkof allowed for increases in yield that are due to genetic gain to be distinguished from yield increases due to management practices (Graybosch and Peterson, 2010). Genetic improvement in grain yield from 1932 to 1959 are estimated to be 30% and between 1959 and 1984 to be 49% (Graybosch and Peterson, 2010). These yield gains have, until recently, allowed wheat production to keep pace with increasing world demand. Since the mid-1980s, annual yield increases have fallen off from 3-4% before 1984 to the current estimate of less than 1% (NASS, 2010).

The decline in the rate of annual yield increase has become a concern as the current rate is insufficient to meet the projected 1.4% to 2.4% annual production increase needed to keep pace with the growing world population (NASS, 2010; Ray et al., 2013). It is estimated that global crop production must increase by 60% to 110% by the year 2050 to ensure adequate food for human populations. Corn, rice, soybean and wheat are the most widely grown crops worldwide, but each are failing to achieve annual yield gains sufficient to double production in the next 40 years. Current yield increases in wheat will only meet 38% of the expected global demand by the year 2050, and the United States has the lowest projected annual yield gains at 0.8% when compared to other top wheat producing countries (Ray et al., 2013).

Achieving the yield gains, comparable to those seen during the Green Revolution is a challenging task. The problems facing modern breeders are formidable. Global warming and reduced availability of water for irrigation likely will cause the amount of arable land to decrease significantly over the next few decades. Wheat is potentially the most critical crop for world food security as it is the most widely grown (Reynolds et al., 2012). In addition to

climate changes, breeders are restricted by the low genetic diversity in modern wheat cultivars. Combating these problems will require an understanding of the physiological and genetic factors underlying yield potential and their responses to water limited environments, increasing the genetic resources available to breeders, efficient screening of physiological traits and increased use of molecular breeding practices.

CLIMATE AND YIELD POTENTIAL

Climate change is possibly the biggest threat to food security worldwide. Many of the issues facing wheat production are linked to the projected climate change. Access to clean water is a concern for around 800 million people worldwide. Drinking water and water used for industrial manufacturers are beginning to compete with the water available for crop irrigation (Stamp and Visser, 2012). Drought conditions due to a changing climate would put further stain on the amount of available water. Crop development over the past 100 years has been able to keep pace with climate changes due to the small steady increases in temperature and carbon dioxide. Adapting wheat to more extreme changes in the environment would be difficult for breeders, even with access to the most up-to-date technologies (Stamp and Visser, 2012). In addition, the amount of arable land in the world is finite. Each year, 70 to 140 thousand square kilometers of arable land is lost due to erosion and urban expansion. Extreme examples of the loss of arable land can be found in eastern Asia where Japan and South Korea have lost an estimated 50% of their crop land over the past 100 years (Stamp and Visser, 2012). Currently the loss of crop land has been offset by increased yields. Climate change is projected to increase the rate of farm land loss. An estimated 10% of

current crop land may become unusable in the future due to climate change (Zhang and Cai, 2013).

A heat-wave in 2003 gave a glimpse of what wheat growers could face in the future if the predicted climatic changes occur. An increased in temperatures 3.5^0C above average in much of Europe caused a 20% to 36% decrease in grain and fruit yields. Climate experts predict that global temperatures will continue to rise in the future (Fedoroff et al., 2010). Climate change in the United States is predicted to have the largest effect on annual precipitation patterns. Southern regions of the US are likely going to see the most drastic changes. Northern and Corn Belt regions will be less affected, but will see the most substantial economic loss of up to $3 billion per year due to climate change and shifting pest populations (Malcolm et al., 2012).

The susceptibility of wheat production areas worldwide to drought is because most wheat is grown in water-limited environments (Fleury et al., 2010). The United States has 125.3 million hectares of arable land under cultivation, with over 80 percent of the area reliant on rainfall to provide water for the crops being grown (Schaible and Aillery, 2012; Karl et al., 2012; Johnson, 2013). Several forecasting models project that wheat crop area will decrease by up to 8% by the year 2030 (Malcolm et al., 2012). In addition, extreme heat exposure during the reproductive stage of wheat will likely increase in most growing areas worldwide, increasing yield loss potential in drought-prone environments (Gourdji et al., 2013). Currently, 75 to 95 percent of wheat in the Western United States is grown in rainfed environments and vulnerable to drought induced yield loss (Malcolm et al., 2012; Schaible and Aillery, 2012; Al-Kaisi et al., 2013). Identifying new sources of germplasm and alleles

that can improve the drought tolerance of current wheat cultivars is essential for ensuring adequate yields in water-limited environments. Increasing yield potential while taking climate change in consideration will be complicated, but researchers have identified several areas of focus that would improve both yields and tolerance in hotter, dryer climates.

DROUGHT TOLERANCE

Developing drought tolerant wheat cultivars could increase both yields and yield stability in much of the world's wheat producing area (Dodig et al., 2012). There are specific plant traits that researchers are focusing on to increase yield in wheat. Yield potential in wheat can be thought of as a function of light interception, radiation use efficiency and partitioning of assimilates (Reynolds et al., 2012). The traits that affect yield potential are dynamic and interconnected. Each has its own set of constituents that can positively or negatively affect yield directly or indirectly through its effects on other yield related traits (Witcombe et al., 2008).

Light interception is influenced by the amount of above ground biomass, the amount of phytochemicals, mainly chlorophyll, and canopy architecture. Canopy architecture is probably the most straight forward trait to manipulate. The photosynthetic machinery saturates at about 50% of the normal intensity of sunlight. Therefore wheat plants that have vertical leaves decrease the intensity of the sunlight on these leaves while allowing more light to permeate the canopy to lower leaves, raising the overall area of light interception. In contrast, horizontal leaves absorb too much light, saturating the photosynthetic machinery of

leaves near the canopy top and blocking sunlight from lower leaves (Araus et al., 2002; Reynolds et al., 2012).

Radiation use efficiency is a measure of how effectively the plant as a whole and on a cellular level can utilize the light intercepted by the canopy. Plants, in general, lose a large percentage of the potential energy from light due to the inability of photochemicals to absorb certain wavelengths (~50% of the light spectrum), reflection of useful wavelengths, saturation of the photosynthetic machinery, and photoinhibition (Condon et al., 2004; Parry et al., 2011; Reynolds et al., 2012).

The partitioning of assimilates is the end result of light energy converted into a grain yield. Harvest index, the ratio of grain yield to above ground biomass, is a measurement used by breeders to determine the efficiency of grain production without that direct measurements of traits that affect grain yield. Genes for dwarfing (*Rht*), photoperiodism (*Ppd*), and vernalization (*Vrn*) have been deployed in modern cultivars that directly affect the harvest index (Gupta et al., 2008; Kiss et al., 2014). While the wheat plant grows, flowers, and matures, various parts of the plant are competing for finite resources. This competition is the main cause of complexity when breeding for yield potential. Ideally all available assimilates would be converted into grain, but this is not possible. Roots need nutrients to grown and access water in the ground, the canopy needs to have enough biomass to capture optimal amounts of light, and flowering needs to occur at the correct time to make efficient use of resources. Each of these processes removes assimilates available for grain production. The dwarfing genes, *Rht*, decrease plant height allowing assimilates to be used elsewhere without reducing photosynthetic biomass, and a large part of the success of *Rht* gene deployment is

the reduction of lodging which can reduce yields up to 80% (Reynolds et al., 2012). The *Ppd* and *Vrn* genes allow for adaption of cultivars to specific regions based on the length of daylight and winter temperatures. This allows for adapted lines to have optimal flowering time and efficient use of the growing season (Reynolds et al., 2012).

GENETIC RESOURCES

Molecular marker and genomic sequence data all indicate that during the domestication of wheat some of the genetic diversity found in *Triticum aestivum* was lost. Domestication puts significant selective pressure on the adaption of crops (Doebley, et al., 2006; Gross and Olsen, 2010). Usually, a small number of founder genotypes form a base for the majority of the genetic variation found within a crop. The reduction in genetic diversity during the development of crops has been termed the domestication bottleneck (Doebley et al., 2006). In wheat it is estimated that the shift from progenitor genomes to landrace genomes caused a 19% to 69% reduction in genetic diversity, based on sequence analysis of 21 loci and genome wide molecular markers (Reif et al., 2005; Haudry et al., 2007). Reif et al. (2005) also reported a 5% reduction in genetic diversity between landrace and modern cultivars, but an increase in genetic diversity since the advent of international agricultural research centers. Lower levels of linkage disequilibrium in landrace accessions, when compared to modern cultivars, is also indicative of a decrease in genetic diversity (Cavanagh et al., 2013).

The genetic resources available to breeders can be divided into improvement categories. Modern cultivars are the easiest source of genes to incorporate into a breeding

program as there is a reduced chance of linkage drag compared to others genetic sources. Obsolete or historic cultivars have similar advantages as modern cultivars but are slightly less desirable. Landraces are wheat genotypes that were developed through natural selection or farmer selection and usually adapted to localized regions and often carry deleterious alleles (Jaradat, 2011). Closely related *Triticum* species, more distantly related *Aegilops spp.* and rye substitution lines are all potential donors of novel alleles, albeit not as easily integrated into modern breeding programs (Feuillet et al., 2008). Worldwide, there are more than 640,000 accessions available that could potentially serve as sources of genetic material for wheat breeders (Reynolds et al., 2001).

The United States Department of Agriculture's (USDA) National Small Grains Collection (NSGC) is a source of genetic variation that breeders and researchers could exploit to develop new and improved wheat cultivars. The NSGC contains over 54,000 wheat accessions developed by farmers and plant scientists from around the world. Landrace accessions make up around 36% of the collection. Primary cultivars or breeding lines account for the remainder and these accessions were derived by plant breeders through systematic hybridization of specific wheat genotypes to combine or improve traits of interest (Bonman et al., 2005, 2006). Landrace accessions were not developed by modern breeding practices (Newcomb et al., 2013). Characterization of wheat genotypes found in the NSGC for agronomic and phenotypic traits has been ongoing for the past 30 years. Information on individual accessions and data collected is available through the USDA-ARS Germplasm Resources Information Network (GRIN) at www.ars-grin.gov/npgs.

The NSGC is a particularly promising source of new alleles or novel genes. Disease resistance has been found among accessions in the NSGC, including resistance to *Stagonospora nodorum* blotch (Adhikari et al., 2011), spot blotch (Gurung, et al., 2012) common and dwarf bunt (Bonman et al., 2006), tan spot (Gurung et al., 2009) and stem rust (Bonman et al., 2007; Newcomb et al., 2013). Landrace accessions, by definition, have not benefitted from modern breeding techniques such as marker-assisted-selection (MAS) but may likely possess new sources of genetic diversity. In addition, the multitude of regional environments from which landrace accessions originated increase the likelihood of finding novel gene alleles.

A recent example of large scale screening of germplasm collections, including the NSGC, is the search for new sources of resistance to the stem rust strain UG99, first identified in Uganda in 1998 (Pretorius et al., 2000). UG99 and related new races of the pathogen overcome most of the stem rust resistance deployed in modern wheat cultivars. The prospects of a global stem rust epidemic spurred the formation of the Borlaug Global Rust Initiative (http://www.globalrust.org), and the concerted efforts of wheat breeders, plant pathologists, and research organizations, such as CIMMYT or the USDA, to identify additional sources of resistance to UG99. Over 200,000 wheat genotypes (*Triticum sp.*) as well as related species, such as barley, *Aegilops sp.*, *Thinopyrum sp.* and *Secale sp.*, have been evaluated for resistance to UG99 since 2005 (Singh et al., 2011; Periyannan et al., 2013; Saintenac et al., 2013b). For example, 2500 landrace accessions and 700 cultivars or breeding lines from the NSGC were screened for resistance to UG99 (Newcomb et al., 2013, Rouse et al., 2011). Newcomb et al. (2013) found 278 (11%) of the landrace accessions tested showed resistance

to UG99 and Rouse et al. (2011) found 88 (13% of those tested) resistance cultivar or breeding line accessions. The results of these two studies show the value of the NSGC. Rouse et al. (2011) identified advanced lines that could be used immediately as sources of stem rust resistance with minimal linkage drag, and Newcomb et al. (2013) identified resistance that likely has not been deployed in modern cultivars.

HIGH-THROUGHPUT PHENOTYPING

Canopy spectral reflectance (CSR) is a high-throughput phenotyping platform for field assessment of crops (Aparicio et al., 1999). CSR measures light reflectance from the wheat canopy at photosynthetically active wavelengths (400-700 nm) and infrared wavelengths (700 - 1000 nm). The intensity of light reflections at specific wave lengths has been shown to be related to important plant characteristics that affect yields or other agronomic traits. Directly measuring plant biomass (van Ginkel et al., 1997), chlorophyll content, water status (McCaig and Romagosa, 1989) and leaf nitrogen (Feng et al., 2011) is time consuming, expensive and potentially destructive. CSR is a non-destructive method and is able to assess these characteristics quickly and in a field environment (Babar et al., 2006; Penuelas et al., 1997; Wright et al., 2001; Zhu et al., 2008b). More importantly, CSR indices have been found to be predictors of yield in several crop plants including wheat (Aparicio et al., 1999; Babar et al., 2006; Gutierrez, et al., 2010; Hansen, et al., 2002; Prasad et al., 2007) barley (Hansen, 2002), rice (Inoue et al., 1998), and corn (Teal et al., 2006). CSR has the potential to be useful to breeders because it could simultaneously measures multiple traits in diverse genotypes before grain harvest.

MARKER ASSISTED BREEDING

Current wheat breeding techniques are insufficient for keeping pace with projected global population increases. The cyclic breeding practiced by most breeders relies on small yield increases achieved by traditional breeding techniques within the gene pools of modern cultivars. The recently sequenced wheat genome will aid researchers in determining combination of genes that complement one another to increase yields and optimize adaption to targeted environments (Reynolds et al., 2012; Cavanagh et al., 2013). Currently there are hundreds of thousands of molecular markers in wheat. Efficient use of molecular marker technologies in breeding will be essential to the success of increasing wheat yields. Association mapping and genomic selection are two methods that can be applied to wheat genetic analysis and wheat improvement, respectively. The use of these techniques could aid in broadening the genetic base of wheat and in identifying new alleles and allelic combinations.

Molecular markers function as a means to artificially select for traits of interest based on a molecular fingerprint or signpost. In some instances, the use of molecular markers is less expensive, faster, and more reliable than phenotypic assays. There have been several types of molecular markers used for artificial selection in wheat, each with their own set of strengths and weaknesses. Restriction fragment length polymorphism (RFLP) were the first class of markers used for MAS in wheat (Anderson et al.,1992). RFLP markers were replaced with polymerase chain reaction (PCR) markers, due to the large amounts of DNA needed and the time required. PCR based random fragment length polymorphism markers (RAPDs), amplified fragment length polymorphism markers (AFLP), and sequence tag sites (STS) have

been used for MAS but each have their own set of advantages and disadvantages. RAPDs had low levels of polymorphisms and were inconsistent, AFLPs are time consuming, and STS are developed from existing RFLPs, RAPDs, or AFLPs. Simple sequence repeat (SSR) have, until recently been the marker of choice for MAS. SSRs are easy to manipulate, automate and relatively frequent within the wheat genome.

Currently SSR, diversity arrays technology (DArT) and genotype by sequencing (GBS) derived single nucleotide polymorphism (SNP)markers are the markers of choice. These groups of markers show a shift in focus from single genes to genome wide analysis. DArTs, a propriety marker, were the first genome wide platform for large scale, high throughput genotyping in wheat. DArTs have been used for identifying wheat resistance genes (Adhikari et al., 2011; Bhavani et al., 2011), quality traits (Emebiri et al., 2010; Kulwal, et al., 2012), and genome wide diversity analyses (Rong et al., 2005; Saintenac et al., 2013a). SNPs and GBS markers are the most recent advancement in MAS technologies and are an improvement on DArT because they are significantly more numerous within the wheat genome and derived from genes or sequenced regions. The ability to automate GBS-SNP assays with next-generation sequencing and convenient chip arrays has expedited the collection of genetic marker data (Deschamps et al., 2012).

The final goal of any MAS study is the identification of markers that can be used to select traits of interest without tedious phenotypic, biochemical, or physiological assays. MAS Wheat (http://maswheat.ucdavis.edu/Education/) is a source of information on genetic markers identified in wheat, as well as markers specifically described Functional Markers (FM). FM are able to distinguish specific allelic variations found in the wheat genome. There

are 97 FMs identified for 30 loci in wheat. FMs are different from the majority of markers because they are considered to be gene-specific or 'perfect' makers instead of being linked to loci of interest. While the usefulness of other marker types depends on their genetic distance from loci of interest, FMs are developed from the targeted genes themselves. As a result, FMs are more efficient at MAS than are linked markers (Liu et al., 2012).

MARKER ASSISTED SELECTION

Since their advent, molecular markers have been touted as a means to increase the rate of cultivar development. Unfortunately molecular markers are underutilized by modern breeders and phenotypic traits are used most often for selection (Paux, et al., 2012). In most cases, markers are used to select for traits that are fairly easy to select for phenotypically, reducing the potential impact of the markers used. A gap between breeders and genomic research scientists is found in the rationale behind developing new markers for MAS. In a majority of published genetic mapping publications, the researchers validate their study by stating the utility of new markers for MAS. This rationale is accepted by both breeders and research scientists, even when it is widely acknowledged that MAS has the potential to expedite breeding efforts, but has had minimal impact on cultivar development in wheat (Xu and Crouch, 2008; Gupta et al., 2008; Collard and Mackill, 2008; Paux et al., 2012; Stamp and Visser, 2012). Theoretical MAS models show the time needed to develop a new cultivar can be reduced by several generations when using 'background' markers that are linked or unlinked to traits of interest (Xu and Crouch, 2008). Therefore, even in the absence of tightly linked markers to genes of interest, background markers could facilitate reducing

heterogeneity and selecting for the genetic background of the more desirable parent. In addition, the focus of research scientists is on developing new markers, not validating the utility of markers in validation studies. Fortunately, there are large scale efforts in the United States (Wheat MAS Consortium), Mexico (CIMMYT) and Australia to increase the usefulness of molecular markers and integrate markers in public breeding programs (Gupta et al., 2008).

While artificial selection for single gene traits such as insect (*Gb3, H23* and *Dnx*) or disease resistance (*LR34, Yr18, Pm38* and *Pm3a*), dwarfing (*Rht-B1, Rht-D1*), and photoperiodism (*Ppd-D1*) is relatively simple, selecting for complex traits such as yield is difficult. Identification of complex loci has been limited to main effect QTLs through single marker regression and interval mapping. Much of the problem with MAS of complex traits is that there are many small effect loci that contribute to the trait of interest but most are highly affected by environmental interactions (Gupta et al., 2008; Jannink et al., 2010). Recent advancements in marker technologies have allowed researchers to saturate genetic linkage maps and undertake genome wide analysis of complex traits that was not possible in the past.

ASSOCIATION MAPPING

Genome wide association studies (GWAS) are a recent advancement in the analysis of complex traits in many plant species (Murray et al., 2009; Jin et al., 2010; Roy et al., 2010; Kloth et al., 2012; Mandel et al., 2013; Zhang et al., 2014). The first association studies attempted to find the limits of this type of analysis. It was found that GWAS is applicable for identifying large effect QTLs that control complex traits such as yield (Breseghello and

Sorrells, 2006) and quality traits such as kernel weight, grain protein content, flour sedimentation, falling numbers and starch content (Reif et al., 2011; Zhang et al., 2014). In addition, meta-analyses found historic trait data collected over ten years could be used to identifying important loci associated with their traits of interest (Neumann et al., 2010). These studies showed that GWAS has a broad range of applications for analysis of many traits.

GWAS relies on linkage disequilibrium for identifying significant marker-trait associations. Linkage disequilibrium is based on the assumption that in a population that is not affected by selection pressure, genes will be randomly distributed across individual genotypes. This means that in randomly mating populations, gene frequencies and combinations are at equilibrium. Gene combinations or molecular markers that are found together at a rate higher than expected are said to be in linkage disequilibrium. While linkage disequilibrium can occur because of population stratification or admixture, GWAS accounts for these through the use of mixed linear models. When identifying marker-trait associations population structure or groups of related genotypes and the relationship between all individuals are accounted for in the statistical analysis (Yu et al., 2006; Zhu et al., 2008a). The genetic Q (structure) + K (kinship) model was designed to prevent spurious associations (Yu et al., 2006). In addition to population structure, the success of association studies is often limited by the density of molecular markers available to researchers. But, with the recent increase in availability of genome-wide markers, adequate marker coverage has become less of a concern.

The largest problem with GWAS is that the identification of novel alleles is limited by their occurrence within the mapping panel. Important, highly effective loci may be missed by GWAS due to their low frequency within the mapping panel, or the relationship of superior alleles to the population structure. For example, highly effective yield loci that are found only in lines from a specific region or in groups of related genotypes may be missed by GWAS. The identification of new loci may still require biparental population development and QTL mapping (Yu et al., 2006; Zhu et al., 2008a).

GENOME SEQUENCE

Wheat has one of the most complex genomes to be sequenced to date, with over 124,000 genes distributed across 17 gigabases (Marcussen et al., 2014). The recent sequencing of wheat has opened new avenues by which researchers can characterize important agronomic traits. Wheat has a hexaploid genome with three progenitors, *T. urartu* (AA), *Aegilops speltoides* (BB) and *Ae. tauschii* (DD) (Ling et al., 2013; Marcussen et al., 2014). The wheat genome has a complex lineage that started 5.5 million years ago. The first hybridization event occurred between ancient *Triticum* and *Aegilops* species, which eventually gave rise to the DD genome of *Ae. tauschii*. *T. urartu* (AA) and *Aegilops speltoides* (BB) hybridized to give the allotetraploid Emmer wheat (*T. turgidum*; AABB), which then hybridized with *Ae. tauschii* (DD) to give modern bread wheat (*T. aestivum*; AABBDD) (Marcussen et al., 2014).

The complexity of the wheat genome has made the identification of important genes difficult. In the past, cytogenetic stocks that have either missing, duplicated, or altered

chromosomes were used to identifying loci (Gupta et al., 2008). A problem with cytogenetic stocks is that in most cases the resolution is extremely low. Molecular markers helped increase the resolution of characterized loci, but large regions of the wheat genome remained inaccessible to wheat breeders. Sequencing the wheat genome opens a myriad of possibilities for breeders and researchers. A good example of using sequenced genomes for investigating traits is found with soybean (Schmutz et al., 2010). When the unassembled soybean genome was published in 2008, several studies utilized the sequence data to identify genes of interest for various traits (Maroof et al., 2010; Schmutz et al., 2010; Suh et al., 2011). In addition, the wheat genome will allow additional gene-specific functional markers to be developed. Wheat breeders and researchers will now be able to look at the sequence underlying QTLs of interest, and developed better markers or identify candidate genes.

GENOMIC SELECTION

Genomic selection (GS), like GWAS, relies on linkage disequilibrium between markers and traits but takes genome wide analysis of genetic markers to a higher level. GS assigns a breeding value to a genotype based on the additive genetic value of markers across the entire genome regardless of how large or small the effect (Meuwissen et al., 2001). GS models are developed through the use of training panels that contain both phenotypic and genotypic data. The developed models are then applied to the test populations. Until recently, the markers available to breeders made GS difficult to practice on a large scale. GS addresses some of the disadvantages of GWAS and QTL mapping. GWAS identifies significant marker trait associations but is limited to identifying large effect QTLs that are

highly significant. The problem with GWAS is the markers that are nearly significant often get ignored by the analysis (Meuwissen et al., 2001). Linkage mapping of QTLs often find that important loci can vary in significance from year to year and environment to environment. Researchers have circumvented this problem by combining data sets or raising the significance level used for identifying QTLs. While the marker significance levels can be changed in GWAS, it is probable that many small effect QTLs are still missed.

One of the first studies in simulating genomic selection for crop breeding was in corn and barley (Lorenzana and Bernardo, 2009). Biparental populations of corn and barley were evaluated to generate genome wide breeding values based on different levels of genome coverage and population sizes. Accuracy increased with higher numbers of molecular markers and progeny, and identifying the most beneficial progeny based on genome wide markers was possible. The methods of analyzing thousands of markers in GS studies posed additional problems for researchers. In traditional linkage mapping, QTL identified through interval mapping often over or under estimates the effects of loci (Jannink et al., 2010; Reynolds et al., 2011). Much work has gone into developing models for GS that accurately estimate the effects of loci on a genome wide level and not limited to within single biparental populations (Crossa et al., 2010; De los Campos et al., 2009). CIMMYT has put significant efforts into generating GS models, using historical maize and wheat data sets, for breeding efforts worldwide (Crossa et al., 2014). The advantages of GS are, like GWAS, important loci will likely be applicable across a wide range of genotypes, but significance levels of maker trait associations are not as important as in GWAS (Cabrera-Bosquet et al., 2012). In

addition, GS identifies the most beneficial genotypes for inclusion in breeding programs and reduces costs (Bernardo and Yu, 2007).

Recently, the accuracy of GS was empirically tested in elite hybrid rye populations across multiple years and locations. Wang et al. (2014) found that GS significantly out preformed MAS in accuracy of predictions of yield, plant height, and quality traits. In addition, combining test populations increased both the genetic diversity and accuracy of prediction (Wang et al., 2014). The results in rye show that GS has the potential to increase the genetic gains realized by breeders. For genomic selection to become a feasible method for breeders to use to make selections, comprehensive analysis of the entire wheat genome and available germplasm will be needed to develop genome-wide selection models. Studies such as the one presented here could provide data for the development of genomic selections models.

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Chapter II:

Evaluation of Grain Yield and Agronomic Characteristics of NSGC Spring Wheat Accessions

ABSTRACT

Tolerance to water stress is an important trait of wheat (*Triticum aestivum* L.) in water limiting environments of the world. A total of 540 spring wheat accessions from the NSGC core subset were evaluated for grain yield, grain test weight, grain protein content, days to heading and plant height under irrigated (IR) and terminal drought (DR) environments in 2012 and 2013. The objectives of this study were: 1) to evaluate grain yield and agronomic characteristics of spring wheat accessions in the National Small Grain Collection (NSGC); 2) to compare wheat quality classes and accession improvement status groups, and 3) identify high yielding drought tolerant materials in the NSGC. The panel of accessions comprised three improvement status groups, cultivars or breeding lines, landraces, and accessions of uncertain status, and four market classes, hard red (HR), hard white (HW), soft red (SR) and soft white (SW) spring wheat. Drought effects on yield were quantified using the drought susceptibility index (DSI) and the effect of drought on grain protein, test weight, height and days to heading was quantified using the DSI equation. Agronomic traits explained from 15 to 25% of the yield variation and indexed traits explained 44% of DSI in 2011 and 34% in 2012. Drought responses were compared among the three improvement groups and the four market classes. We found, in general, few statistical differences in yield, grain protein or test weight between wheat improvement status groups or market classes. But, we were able to

identify 39 accessions with low drought susceptibility and yielding greater than treatment means in DR and IR treatments in 2011 and 2012.

INTRODUCTION

Worldwide, most wheat (*Triticum aestivum* L.) is grown in water-limited environments where drought is the leading environmental stress affecting yield (Fleury et al., 2010). The United States has 125.3 million hectares of arable land under cultivation, with over 80 percent of the area reliant on rainfall to provide water for the crops being grown (Schaible and Aillery, 2012; Karl, et al., 2012; Johnson, 2013). It is projected that extreme heat exposure during the reproductive stage of wheat will likely increase in most growing areas worldwide, increasing yield loss in drought-prone environments (Gourdji et al., 2013). Currently, 75 to 95 percent of wheat in the Western United States is grown in rainfed environments and vulnerable to drought induced yield loss (Malcolm et al., 2012; Schaible and Aillery, 2012; Al-Kaisi et al., 2013). Developing drought tolerant wheat cultivars could increase both yields and yield stability in much of the world's wheat producing areas (Dodig et al., 2012). Identifying new sources of alleles that can improve the drought tolerance of current wheat cultivars is essential for ensuring adequate yields in water-limited environments.

Drought tolerance is a complex trait and phenotypic responses to drought can differ by year, genotype, and environment (Saint Pierre et al., 2010a,b) . Drought resistance, drought tolerance, drought susceptibility, yield potential, transpiration efficiency, and water use efficiency have been used independently or in combination to describe drought response in

wheat (Condon et al., 2004,; Blum et al., 2005; Lopes and Reynolds, 2010; Richards et al., 2010; Saint Pierre et al., 2010a,b). The drought susceptibility index (DSI) is a commonly accepted method to evaluate response to drought, and is an estimate of relative yield stability in water limited environments (Ahmad et al., 2003; Bahar and Yildirim, 2010; Li et al., 2011, 2012; Maccaferri et al., 2011; Li et al., 2011, 2012). DSI quantifies the effects of drought by comparing the percentage of yield loss due to drought by a single line grown in irrigated and drought treatments to the percent yield loss of all lines grown in irrigated and drought treatments. In addition to yield based DSI values, the change in grain protein (GP), test weight, (TW), days to heading (DH) and plant height (Ht) can also be indicators of drought tolerance (Gebeyehou et al., 1980; Nezhadahmadi et al., 2013; Sinoh et al., 1973).

Germplasm repositories such as the National Small Grains Collection (NSGC) are a source of new alleles for plant breeders, and have been exploited by small grains researchers to identify new forms of disease resistance and tolerance to some abiotic stresses (Bonman et al., 2006; Bonman, et al., 2005; Gurung, et al.,2009, 2012; Gutierrez et al., 2010; Maccaferri et al., 2011). Previous research indicates that wheat landraces can have higher, more stable yields over a wider range of environmental conditions than many elite cultivars (Dencic et al., 2000; Blum et al., 2005). The genetic diversity found within the NSGC makes this collection a valuable resource to breeders and researchers who seek to increase drought tolerance in crop plants.

The purpose of this study was to assess the drought tolerance of accessions from the NSGC and the response of grain yield and agronomic traits to water stress applied at reproductive growth stages. The DSI was used to quantify drought tolerance and the DSI

equation was applied to quantify the effects of drought on GP, TW, DH, and Ht. Comparisons were made between accessions of different improvement status and accessions of different quality classes to determine if any category of accessions exhibited significantly higher levels of drought tolerance than others.

MATERIALS AND METHODS

Plant Material

Spring wheat accessions were selected from the USDA-ARS National Small Grains Collection wheat core subset, which represents 10% of the *T. aestivum* accessions in the collection. Accessions from a 2010 trial with uniform appearance, little to no lodging and heading between 58 and 78 days after planting were selected for further evaluations. In total, 540 accessions from 81 countries of origin were selected. This panel contained 254 cultivars or breeding lines, 183 landraces, and 103 accessions of uncertain improvement status. In this study cultivars and breeding lines are considered to be 'Advanced' (Adv) accessions, having been developed through breeding programs, 'Landrace' (LR) accessions are considered to be the least improved, having been developed in traditional farming systems without modern plant breeding practices (Jaradat, 2011), and some accessions are designated as 'Uncertain' (Unc) as their improvement status is unknown (Table 2.1). All accessions, except for seven, were placed in wheat quality classes by the Idaho Wheat Quality Laboratory with measurements of seed hardness and visual classification of seed color. In total, 194 hard red, 133 hard white, 100 soft red and 106 soft white accessions were identified (Table 2.1).

Three locally developed lines IDO599, IDO686, IDO702, and two cultivars, Agawam and Alpowa were used as checks in 2011. These check lines have been used in previous drought studies at the same field location and have described responses to drought environments (Li et al., 2011, 2012). In 2012, IDO702 and PI648027 were replaced by accessions PI428506 and PI520108 from Netherlands and Mexico, respectively. Neither of the replacement check lines used in 2012 had previously been evaluated at the experimental site, except in the t 2011 trialb. IDO702 and PI648027 were removed from subsequent trials in order to include checks from the NSGC, and so as not to have an over-representation of checks that originated in Idaho or surrounding states.

Field Design and Experimental Conditions

Experiments were conducted in the 2011 and 2012 growing seasons at the University of Idaho Aberdeen Research and Extension Center in Aberdeen, Idaho (42°57'36'' N, 112°49'12'' W, and elevation 1342 m). The field soils are Declo-loam (coarse-loamy, mixed, superactive, mesic Xeric Haplocalcids) with 0 to 2% slopes and pH of 8.1. Historical information on climate conditions for Aberdeen Idaho is available through AgriMet (http://www.usbr.gov/). The 540 accessions and 60 check plots were planted side by side in two irrigation regimes (treatments), irrigated (IR) and terminal drought (DR). Treatments of 600 plots were arranged in an un-replicated augmented complete block design, 20 plots wide by 30 plots deep, with replicated checks in each treatment (Federer and Ragavarao, 1975). Within each treatment, accessions were classified into early, medium, and late maturity groups. The maturity groups were determined by days to heading: Early (E, 58-66 days),

Medium (M, 66-70 days), and Late (L, 70-75 days) within 180 lines and five checks replicated 4 times in each group. Maturity groups were further divided into four sub-blocks of 50 plots which included each of the 5 checks to ensure uniform distribution through the field. Across an entire treatment, checks were arranged with two unique checks in each of the 30 rows and three unique checks in each of the 20 columns. All plots had seven-rows of 1.83 m by 1.5 m and a seeding density of 225 seeds m^{-2}. Check plots were used to detect and correct field variation within each treatment as described in data analysis.

Precipitation between planting and physiological maturity was 66.3 mm in 2011 and 46.2 mm in 2012. Precipitation, high temperature averages, and irrigation regime are shown in Table 2.2. Plots were planted in April and harvested in August in both 2011 and 2012. Average monthly temperatures ranged from 5.2 to 23.0 ˚C in 2011 and 6.5 to 25.4 ˚C in 2012. Detailed weather data for Aberdeen Idaho are available through the National Oceanic and Atmospheric Administration or Agrimet (http://www.usbr.gov/pn/agrimet/webarcread.html).

A drip tape irrigation system was used for precise water application to each treatment. Irrigation was provided from canopy closure to physiological maturity for irrigated treatments. Terminal drought conditions were simulated by shutting off drip irrigation when 95% of the plots in a maturity group were at heading or anthesis. In each year, the early, medium, and late maturing plots in the terminal drought treatments received respectively 354, 236, and 117 mm less irrigation than the irrigated plots. Irrigated trials received an average of 1,063 mm of irrigation in addition to natural precipitation (Table 2.2). The irrigation rate used exceeded the evapotranspiration each year. Nitrogen fertilizer was applied (N = 280 kg/ha) before planting at suggested rates for target yields of 6.7 tons/hectare.

Phenotypic Measurements

In both years, individual plots were harvested using a Wintersteiger Classic Plot Combine (1998 Wintersteiger Elite, Wintersteiger Seedmech, Salt Lake City, UT). Plot harvest weights were recorded using a HarvestMaster HM400 Plot Harvest Data System Classic Graingage (Juniper Systems; Logan, UT) and converted to kg/ha for further analysis. Whole grain protein was measured by a Foss 6500 NIR Spectrometer and test weights were measured using certified dry pint container and Boehmer funnel. Heading dates were recorded daily from the onset of booting until all plots had headed. Heading dates were determined once 50% of individual plants within a plot had complete head emergence from the flag leaf sheath. Plant height was measured at physiological maturity from the ground to the top of the spike, not including awns.

Drought Susceptibility Index

Accessions were evaluated for drought tolerance based on yield stability using the drought susceptibility index (DSI) described by Fischer and Maurer (1978). The DSI is defined as $(1-Yd/Yi)/(1-\mu Yd/\mu Yi)$, where Yd = plot yield under drought, Yi = plot yield under irrigation, μYd = mean treatment yields under drought and μYi = mean treatment yields under irrigation, excluding checks. This calculation allows the yield stability across irrigated and water stressed environments of individual accessions to be compared to the average change in yield of all genotypes evaluated (Ahmad, 2003; Li et al., 2011). To quantify drought effects on other characteristics, the DSI equation was applied to GP, TW, DH, and Ht. Here, the response of agronomic traits to drought will be shown as a drought index (DI)

values, GPDI, TWDI, DHDI and HtDI. Values closer to 1 indicate smaller effects of drought. The percent change in yield, GP, TW, HT and DH was also calculated to show the difference between treatments for individual quality classes and improvement status groups. DSI values for individual lines were compared in 2011 and 2012. The distribution of low DSI accessions was evaluated across market classes and improvement status groups.

Statistical Analysis

Best linear unbiased predictors (BLUPs) were calculated for each trait in each treatment using lines as random effects in JMP Version 9.0 (JMP, Version 9.0 SAS Institute Inc., Cary, NC, 1989-2012) statistical software from SAS. The spatial adjustment model included maturity blocking effects and days to heading by restricted maximum likelihood (REML). A generalized heritability was computed using the predicted error variance from the mixed model or the mean variance of a difference between BLUPs (Table 2.3).

Analysis of variance (ANOVA), Student's t-test, single or multiple regression, and correlation coefficients were calculated using JMP statistical software. Comparison of treatments within a single year and between years was performed using ANOVA or t-tests ($p < 0.05$) where appropriate. Single and multiple regressions were performed using JMP within individual treatments and combined treatments within years. BLUPs were used for analyses within treatments and uncorrected data used for comparisons between treatments.

RESULTS

Analysis of Variance

Significant differences between irrigated and drought environments were found each year. Irrigated treatments had significantly higher yield and TW, lower GP, and shorter Ht. Irrigated treatments yielded 5795.22 kg/ha in 2011 and 4503.95 kg/ha in 2012. Drought treatments yielded 5376.78 kg/ha in 2011 and 3697.30 kg/ha in 2012 (Table 2.3). GP was 0.97 lower in 2011 IR than 2011 DR and 0.32 lower in 2012 IR than 2012 DR. There was a 14.91 (kg/m3) difference between treatment test weights in 2011 and 18.22 g difference in 2012. Plant heights were 2.4 cm and 3.6 cm taller in DR treatments than IR in 2011 and 2012, respectively. Between treatments, there were 0.33 days to heading difference in 2011 and a 0.93 difference in 2012 (Table 2.3).

Treatment Correlations and Heritability

Yield heritabilities were always the highest in the IR treatments both years at 0.64 in 2011 and 0.30 in 2012. The highest heritabilities for TW were for 2011 IR at 0.90 and 2012 DR at 0.58. Irrigated treatments had the highest heritabilities for GP at 0.77 and 0.65 and for DH at 0.88 and 0.32 in 2011 and 2012, respectively (Table 2.3).

Correlations between treatments each year and between years were significant for all traits at $p < 0.001$. Correlation of yields between IR and DR was 0.46 in 2011 and 0.36 in 2012. GP was correlated at 0.67 in 2011 and 0.29 in 2012. For TW, correlations between treatments were 0.49 and 0.52 in 2011 and 2012, respectively. Plant heights were correlated at 0.85 in 2011 and 0.64 in 2012. DH had the highest correlation coefficients for all

comparisons with values between treatments at 0.85 and 0.86 in 2011 and 2012, respectively (Table 2.4). Correlations between years were highest in IR treatments for all traits. Yield had correlation coefficients at 0.28 in IR and 0.27 in DR. GP had correlations of 0.51 and 0.21, TW had correlations at 0.41 and 0.25, and HT was correlated at 0.73 and 0.70 in IR and DR treatments, respectively. DH correlations between years were 0.80 for both treatments (Table 2.4).

Yield Response to Drought

Across treatments there was a 7.8% decrease in yield in 2011 and a 21.8% yield decrease in 2012 attributed to accessions being grown in drought conditions. Significant effects of drought were found in each improvement status group and market class, in both years. Cultivars showed an 8.28% decrease in yield in 2011 and a 22.2% decrease in 2012 (Table 2.3 and Table 2.5). Landrace accessions had a 6.79% yield decrease due to drought in 2011 and a 24.7% decrease in 2012. Yield loss due to drought was 5.39%, 8.61%, 9.45 % and 7.5% for SWS, SRS, HWS and HRS, respectively in 2011 and 27.51%, 18.58%, 21.77% and 20.65% in 2012 (Table 2.5 and Table 2.6).

No significant differences in DSI were found for accessions of different improvement status groups or market classes. The drought susceptibility index (DSI) value for Lr accessions was 0.66 and for Adv accession DSI was 0.77 in 2011. In 2012, LR had DSI values of 0.7 and Adv accessions had a DSI value of 0.92. In 2011, DSI values for HRS, HWS, SRS and SWS were 0.63, 1.02, 0.85 and 0.46, respectively. In 2012 DSI values for the same market classes were 0.72, 0.65, 0.57 and 0.95, respectively (Table 2.7).

Grain Protein Response to Drought

Significant effects of drought were found in both 2011 and 2012. Improvement status groups and market classes showed significant increases in GP due to drought each year. GP was 7.4% lower in 2011 IR than 2011 DR, and 2.1% lower in 2012 IR than 2012 DR (Table 2.3). Cultivars had a 7.31% increase in GP and LR had a 6.95% increase in GP due to drought in 2011. In 2012, cultivars experienced a 2.32% increase in GP and LR had a 3.16% increase in GP. SWS, SRS, HWS and HRS experienced 5.3%, 5.9%, 8.2% and 9.1% increase in GP due to drought in 2011 and in 2012 these market classes had a 2.6%, 4.6%, 4.8% and 4.0% increase, respectively (Tables 2.5 and 2.6).

Significant differences in drought response between market classes were found in 2011 but not 2012. HR and HW accessions had, on average, significantly larger increases in protein (GPDI) than SR or SW in 2011, when compared to the average loss of all accessions. Landrace and cultivar or breeding line accessions showed similar levels of drought response in terms of the change in GP (Table 2.7).

Test Weight Response to Drought

Significant differences in TW between treatments were found each year. Improvement status groups and market classes all showed significant effects of drought on TW (TWDI). In 2011 drought caused a 1.6% decrease in TW and in 2012 drought was responsible for a 2.1% decrease in TW (Table 2.3). Adv accessions showed a 1.88% decrease in TW in 2011 and a 2.13% decrease in 2012. LR accessions had a 1.1% decrease in TW in 2011 and 2.61% decrease in 2012. Market classes had a 0.9% to 1.9% decrease in TW in

2011 and a 1.4% to 3% decrease in 2012 (Tables 2.5 and 2.6). Market classes showed similar levels of TW drought index. Adv had significantly higher loss in TW than LR in 2011 but not 2012, when compared to the mean reduction of TW each year (Table 2.7).

Hd and Ht Response to Drought

Between treatments, plots experienced less than 1% change in DH in 2011 and a 1.3% increase in DH for 2012. A 2.2% and 4.2% increase in height from IR to DR was found between treatments in 2011 and 2012, respectively. No significant difference in DH was found for LR or Adv accessions in 2011, but a 1.6% and 1.4% increase in DH was found in 2012, respectively. Landrace accessions showed a 2.4% increase in height from IR to DR in 2011 and a 5% increase in 2012. Adv accessions had a 2.3% increase in height in 2011 from IR to DR and 2.7% increase in 2012 (Table 2.5). No significant changes in HT or DH were found for any of the four market classes in irrigated treatments either year. Soft white accessions were the only quality class to show significant differences in DH, 1.8%, due to drought conditions in 2012. HRS, HWS and HWS showed significant differences in HT between treatments in 2012 DR, ranging from a 4% increase to 4.76% increase (Table 2.6).

Both years, advanced accessions showed significantly lower increases in height due to drought, quantified by height drought index (HtDI) than LR accessions, but no significant differences between LR and Adv accessions were found days to heading drought index (DHDI) either year. No significant difference in HtDI response was found between quality classes in 2011 or 2012, and the only significant differences in DH between quality classes were found in 2011. In 2011, SW had the lowest DHDI due to drought (Table 2.7).

Trait Regression and Correlations

Plot yields each year were positively correlated with DH and TW ($p < 0.01$) and negatively correlated with GP (Table 2.8). Plant heights were negatively correlated with yield in 2011 IR, not significantly correlated with yield in 2011 DR and positively correlated with yield in 2012 IR and 2012 DR. Correlation coefficients with yield were higher in 2012 than 2011 for all traits except DH in the 2012 terminal drought treatment (Table 2.8).

GP had the highest correlation with yield in each treatment both years, ranged from -0.31 to -0.63. TW correlation with yields ranged from 0.12 in 2011 to 0.34 in 2012. DH correlation with yield was from 0.10 to 0.38. HT correlation with yield was 0.27 and 0.35 in 2012 IR and DR, respectively. Ht was negatively correlated with yields in 2011 IR and not significantly correlated with yield in 2011 DR (Table 2.8).

Regression analysis of GP, TW, DH, and Ht on yield was significant each year, except for TW in 2011 DR (Table 2.9). GP explained 23% of the yield variation in 2011 IR, 14% in 2011 DR, 14% in 2012 IR and 11% in 2012 DR. TW explained 1% of the yield variation in 2011 IR, 2011 DR, and 2012 IR trials, and 7% in 2012 DR. DH explained 3% of the yield variation in irrigated treatments and 1% in DR each year. HT explained 3% of the yield variation in 2011 and 1% to 2% in 2012. Multiple regression of all agronomic traits on yield explained 25% of the yield variation in 2011 IR, 16% in 2011 DR, 15% in 2012 IR and 19% in 2012 DR (Table 2.9). Regression of GPDI, TWDI, DHDI, and HtDI onto DSI explained 44% of the yield loss to drought in 2011 and 34% in 2012 (Table 2.9).

Status and Market Class DSI

Significant differences in yield were found for DSI groups in all improvement status groups and market classes. Individual accessions were selected for drought tolerance by DSI either zero, one or two times. In irrigated treatments, accessions that were never selected always had significantly higher yields than those selected twice and in DR treatments accessions selected twice had statistically higher yields that those never selected (Table 2.10). Between improvement status groups, LR and Adv accessions selected both years did not have significant differences in yield, protein, or test weight (Data not shown).

In total, 141 accessions had DSI values less than one in both years, 271 had DSI values below one in a single experiment and 145 did not have DSI values below one either year. Among status groups, 72 Adv (28.5%) and 42 LR accessions (23.5%) had DSI values below one. Among market classes, HR had 52 accessions (26.8%) with DSI values below one, HW had 33 (24.8%), SR had 32 (32%) and SW had 23 (21.7%) (Table 2.10).

Yield and DSI Selections

Analysis of yields and DSI found that there was a negative relationship between yields and DSI in DR treatments and a positive relationship in IR treatments. In the IR treatments, the correlation coefficient between yields and DSI was 0.54 in 2011 and 0.56 in 2012. In the DR treatments, yields were correlated with DSI at -0.48 both years (Table 2.8).

Low DSI accessions (DSI < 1.0) were further filtered to remove accessions with average yields lower than the mean yield of combined IR and DR each year. In 2011, the average yield of both IR and DR was 5586 kg/ha and in 2012 the average yield was 4100.63

kg/ha (Table 2.3). Of the 141 accessions with DSI values below one, 39 were found to yield higher than the average yields of combined treatments each year. Within this group of high yielding and drought tolerant accessions, 25 were cultivars or breeding lines, 10 were landraces and 4 were of uncertain improvement status (Supplemental Table 1).

DISCUSSION

Water stress is one of the most common and detrimental abiotic stresses to affect wheat worldwide. Here we report the difference in yield, grain protein, test weight, days to heading and plant height attributed to drought by comparing accessions grown in IR and DR treatments. Each year, significant differences were found for yield and agronomic traits between irrigated and drought treatments. The effects of drought on wheat market classes and in improvement status groups were calculated to determine if higher levels of drought were associated with different groups of wheat. No significant differences were found between improvement status groups or market classes in response to drought both years, except in Ht.

Height was not found to respond to drought conditions as expected. Treatment height averages were larger in DR than IR both years, and within LR, Adv, HR, HW, SR or SW groups. A possible explanation for this is that lodging of plots was much more prevalent in the irrigated treatments each year. When measuring heights, the lodged plots would be propped up by hand to take each measurement. In addition, plots that lodged earlier in the season may have experienced stunted growth, lowering the IR treatment averages.

It is generally accepted that there is an inverse relationship between grain yield and grain protein content. While this was found in our study, the degree of change in grain yield

and grain protein between treatments was not consistent each year. In 2011, there was a higher change in grain protein content per change in yield than in 2012. It is possible that earlier induction of terminal drought in 2012 and higher average temperatures in May, June and July could be responsible. It has been suggested that while environmental conditions affect both grain yield and grain protein, there may not be an intrinsic pleotropic interaction between grain protein and yields (Groos et al., 2003).

The DSI and average yields were used to identify high yielding drought tolerant accessions. Average yield is the average of the stressed and unstressed treatments. The DSI is able to identify lines with relatively stable yields within the context of the panel of lines that make up the trial and environments in which they are evaluated. Values below '1' indicate a specific accession lost a smaller fraction of yield due to drought stress than the average lost fraction. The drawback of using just DSI values to identify interesting lines is that there is no differentiation between high and low yielding genotypes. Average yields account for both stressed and unstressed environments and can distinguish between high and low yielding accessions, but does not account for yield loss due to drought stress. By combining these two measurements we are able to select both high yielding and drought tolerant accessions.

Correlation coefficients were unable to determine if IR or DR treatments had higher correlations between traits. There were no consistent trends within the data analysis. Regression analysis also failed to determine in which environment traits explained the largest amount of yield variation.

Status Groups and Market Classes

While drought had significant effects on grain yield, grain protein, grain test weight, days to heading and plant height each year, days to heading showed less than a single day difference between irrigated and terminal drought treatments each year. We concluded that less than a single DH difference was irrelevant on a biological level.

In general LR, Adv, as well as the four wheat market classes, had comparable yields and agronomic traits in each year and in each treatment. Adv accessions yielded more than LR each year and in each treatment, but not significantly. DSI values and percentage of yield loss were higher in breeding lines and cultivars than LR in 2011 but lower in 2012. Similarly, the protein drought index (GPDI) gave similar results with a greater increase in GP for Adv than IR in 2011, but not in 2012. TWDI and DHDI did not find LR or Adv to have higher levels of drought tolerance in both years. While TWDI did indicate LR accessions had higher levels of drought tolerance in 2011, landrace accessions showed lower levels of drought tolerance in 2012. Plant height was the only trait that indicated LR were more affected by drought than cultivars or breeding lines both years. Market classes also had similar responses to drought each year. No statistical difference between classes was found in 2012. Only GPDI and HDDI indicated that soft white spring wheat was the least susceptible to drought when compared to other market classes.

When considering the response to drought for improvement status groups and market classes, we found that there was little to no difference between groups of accessions. This result indicates that there is no advantage to using landrace or advanced accessions, nor a specific market class of wheat as sources for drought tolerance from this study. Our study

was not in agreement with previous work that have indicated landrace accessions are more likely to have higher levels of drought tolerance than cultivated lines (Blum et al,. 2005; Dencic, 2000). Dencic et al. (2000) found that cultivars were more sensitive to water stress than landrace wheat types. The cultivars used in their study showed significant differences in yield and other agronomic characteristics between irrigated and drought environments. Landraces, in contrast, did not have significant changes in yields due to drought conditions. A possible explanation for the differences between Dencic et al.(2000) and the present study is the severity of water stress. Here we induced drought conditions by denying supplemental irrigation to plots. The Dencic et al. (2000) study covered plots to prevent precipitation from reaching plots. The location of our study is naturally drought prone and requires irrigation; while the previous study had sufficient rainfall to grow plants. Each year, our trials received precipitation after induction of terminal drought conditions.

Selections

Of the 540 unique accessions evaluated in this experiment, 10 landrace accessions were found to have DSI values < 1.0 and average yields greater than the average yields of both IR and DR treatments each year. As it is assumed that these landrace accessions have not been used in modern breeding programs, there is a high probability that this group of lines contain agronomically important alleles that can help growers increase yields and dampen the effects of drought. Further study of the landrace accessions identified in this study may identify additional agronomically important alleles.

The inverse relationship between DSI and yields in irrigated treatments indicate that to select for drought tolerance in IR conditions, the lowest yielding lines are the most likely to have the highest levels of drought tolerance. However, selecting the highest yielding accessions in DR conditions is most likely to identify lines with the highest levels of drought tolerance. The contrast in selecting higher yielding accessions in drought conditions compared to lower yielding accesses in irrigated environments indicates that evaluating accessions under drought conditions may be the most efficient use of time and resources available to breeders when selecting for drought tolerance. But, when selecting for both drought tolerance and high yield additional irrigated trials are need for comparison.

CONCLUSIONS

Much work has gone into identifying and deploying drought tolerant cultivars to water limiting production areas. Here we evaluated 540 diverse spring wheat genotypes to identify accessions with high levels of drought tolerance and relatively high yields. We found when selecting for drought tolerance, that the highest yielding accessions in DR and the lowest yielding accessions in IR were the most drought tolerant. When selecting for drought tolerance and high yields, a single trial was inadequate. Comparison of improvement status groups and wheat market classes was unable to find significant differences between these groups in terms of drought tolerance or yield. This finding has both positive and negative aspects. We found that landrace and cultivar accessions had nearly identical yields and levels of drought tolerance, meaning that introducing a LR accessions into a breeding program will not negatively affect current levels of drought tolerance found in modern cultivars and would

increase the genetic diversity. But, this also indicates that improving drought tolerance through the use of landrace accessions may be difficult, unless the alleles responsible for the drought tolerance are novel. If it is found that the landrace accessions identified here have novel forms of drought tolerance, pyramiding drought tolerance loci may result in increased drought tolerance. Further characterization of the drought tolerant landrace accessions will be needed to determine if they contain new forms of drought tolerance.

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Table 2.1: Number of accessions categorized as breeding lines and cultivars, landraces, and uncertain improvement status, and the number of accessions determined to be hard red (HR), hard white (HW), soft red (SR) or soft white (SW). Three breeding lines and cultivars and four landrace accessions could not be placed in a specific quality class.

Class	Cultivars and breeding lines	Landraces	Uncertain	Total
HR	109	53	32	194
HW	51	50	32	133
SR	46	37	17	100
SW	45	39	22	106
Total	251 (3)	179 (4)	103	533

Table 2.2: Average monthly temperatures (°C) and irrigation (mm) in 2011 and 2012 experiments for early, medium, and late maturity groups of wheat accessions. Irrigation was provided for a single 12 hour increment weekly for the months indicated. Precipitation received from planting until harvest is shown in the weather column.

2011	Month:	Precipitation (mm)	May	June	July	August	Total Irrigation (mm)
Irrigated	**Early**	66.3	/	472	590	/	1063
	Medium	66.3	/	472	590	/	1063
	Late	66.3	/	472	590	/	1063
Terminal Drought	**Early**	66.3	/	472	236	/	708
	Medium	66.3	/	472	354	/	827
	Late	66.3	/	472	472	/	945
Average High Temp. (°C)			16.5	23.5	30.8	31.6	

2012	Month:	Precipitation (mm)	May	June	July	August	Total Irrigation (mm)
Irrigated	**Early**	46.2	354	590	118	/	1063
	Medium	46.2	354	590	118	/	1063
	Late	46.2	354	590	118	/	1063
Terminal Drought	**Early**	46.2	354	354	/	/	708
	Medium	46.2	354	472	/	/	827
	Late	46.2	354	590	/	/	945
Average High Temp. (°C)			19.8	25.4	31.9	31.9	

Table 2.3: Mean yield, test weight, whole grain protein, days to heading and plant height for 2011 and 2012 irrigated (IR) and terminal drought (DR) treatments. Broad sense heritability (H) is shown for each trait in each experiment.

(A) Trait	Treatment	2011 Mean	2011 H	2012 Mean	2012 H
Yield (kg/ha)	IR	5795.22a	0.64	4503.95a	0.30
	DR	5376.78b	0.54	3697.3b	0.25
Test weight (w/v)	IR	926.5a	0.9	890.0a	0.51
	DR	911.61b	0.31	871.73b	0.58
Protein (%)	IR	13.04a	0.77	15.14a	0.65
	DR	14.01b	0.77	15.46b	0.37
Days to heading (d)	IR	69.66a	0.88	70.96a	0.32
	DR	69.33b	0.78	70.03b	0.2
Plant height (cm)	IR	107.2a	0.67	105.8a	0.63
	DR	109.6b	0.69	110.0b	0.73

***Values not connected by letters indicate significant differences $p < 0.05$**
***Test weight shown as weight per volume**

Table 2.4: Correlation coefficient between 2011 and 2012 irrigated (IR) and terminal drought (DR) treatments for yield, grain protein (GP), test weight (TW), days to heading (DH) and plant height.

Yield	**2011 IR**	**2011 DR**	**2012 IR**	**2012 DR**
2011 IR	1.00			
2011 DR	0.46	1.00		
2012 IR	0.28	0.40	1.00	
2012 DR	0.20	0.27	0.36	1.00

GP	**2011 IR**	**2011 DR**	**2012 IR**	**2012 DR**
2011 IR	1.00			
2011 DR	0.67	1.00		
2012 IR	0.51	0.62	1.00	
2012 DR	0.28	0.21	0.29	1.00

TW	**2011 IR**	**2011 DR**	**2012 IR**	**2012 DR**
2011 IR	1.00			
2011 DR	0.49	1.00		
2012 IR	0.41	0.57	1.00	
2012 DR	0.47	0.25	0.52	1.00

DH	**2011 IR**	**2011 DR**	**2012 IR**	**2012 DR**
2011 IR	1.00			
2011 DR	0.85	1.00		
2012 IR	0.80	0.84	1.00	
2012 DR	0.80	0.80	0.86	1.00

HT	**2011 IR**	**2011 DR**	**2012 IR**	**2012 DR**
2011 IR	1.00			
2011 DR	0.85	1.00		
2012 IR	0.73	0.71	1.00	
2012 DR	0.68	0.70	0.64	1.00

*$p < 0.001$

Table 2.5: Comparison of yield, grain protein (GP), test weight (TW), days to heading (DH), plant height (Ht) and the percent change in each trait due to drought (Δ%) for breeding lines and cultivars (Adv) and landrace (LR) accessions.

Trait	Treatment	Adv	Δ%	LR	Δ%
Yield 2011 (Kg/ha)	**IR**	5943.45a	-8.28	5741.81a	-6.79
	DR	5489.06b		5376.51b	
Yield 2012 (Kg/ha)	**IR**	4590.56a	-22.20	4563.97a	-24.70
	DR	3756.39b		3660.07b	
GP 2011 (%)	**IR**	12.59b	7.31	13.52b	6.95
	DR	13.51a		14.46a	
GP 2012 (%)	**IR**	15.1b	2.32	15.19b	3.16
	DR	15.45a		15.67a	
TW 2011 (kg/m^3)	**IR**	929.08a	-1.88	922.4a	-1.11
	DR	911.96b		912.26b	
TW 2012 (kg/m^3)	**IR**	884.55a	-2.13	894.15a	-2.61
	DR	866.12b		871.43b	
DH 2011 (days)	**IR**	70.08a	-0.49	69.68a	-0.43
	DR	69.74a		69.38a	
DH 2012 (days)	**IR**	71.39a	-1.59	71.4a	-1.43
	DR	70.27b		70.39b	
Ht 2011 (cm)	**IR**	107.5a	2.33	107.3b	2.42
	DR	110.0a		109.9a	
Ht 2012 (cm)	**IR**	107.0b	2.71	107.9b	5.00
	DR	109.9a		113.3a	

***Values not connected by letters indicate significant differences ($p < 0.05$)**

Table 2.6: Comparison of yield, protein (GP), test weight (TW), days to heading (DH), plant height (Ht) and the percent change in each trait due to drought (Δ%) for hard red (HR), hard white (HW), soft red (SR) or soft white (SW) quality classes in irrigated and drought treatments.

Trait	TRT	SW	Δ%	SR	Δ%	HW	Δ%	HR	Δ%
Yield 2011 (Kg/ha)	**IR**	5689.08 a	-5.39	5748.07 a	-8.61	6037.75 a	-9.45	5706.95 a	-7.5
	DR	5397.99 b		5292.39 b		5516.48 b		5308.68 b	
Yield 2012 (Kg/ha)	**IR**	4607.3 a	-27.51	4342.95 a	-18.58	4643.55 a	-21.77	4430.46 a	-20.65
	DR	3613.35 b		3662.36 b		3813.44 b		3672.19 b	
TW 2011 (kg/m^3)	**IR**	919.94 a	-0.89	923.09a	-1.76	928.01a	-1.94	929.25a	-1.77
	DR	431.75b		907.1b		910.31b		913.06b	
TW 2012 (kg/m^3)	**IR**	886.34a	-2.98	885.94a	-2.01	894.51a	-2.45	889.61a	-1.43
	DR	860.72b		868.5b		873.12b		877.11b	
GP 2011 (%)	**IR**	13.02 b	5.3	13.52 b	5.92	12.88 b	8.15	12.91 b	9.06
	DR	13.71 a		14.32 a		13.93 a		14.08 a	
GP 2012 (%)	**IR**	14.97 b	4.34	15.52 a	1.1	14.89 a	2.15	15.22 a	1.45
	DR	15.62 a		15.69 a		15.21 a		15.44 a	
Ht 2011 (cm)	**IR**	110.62 a	1.72	107.95 a	1.79	105.79 a	2.84	105.89 a	2.21
	DR	112.52 a		109.88 a		108.79 a		108.23 a	
Ht 2012 (cm)	**IR**	111.63 a	2.64	106.30 b	4.56	106.05 b	4.76	105.71 b	4.02
	DR	114.58 a		111.15 a		111.10 a		109.96 a	
DH 2011 (days)	**IR**	69.8 a	0.09	69.76 a	-0.79	69.37 a	-0.51	69.73 a	-0.65
	DR	69.86 a		69.21 a		69.02 a		69.28 a	
DH 2012 (days)	**IR**	72.03 a	-1.82	70.72 a	-1.27	70.26 a	-1.22	70.95 a	-1.14
	DR	70.74 b		69.83 a		69.41 a		70.15 a	

***Values not connected by letters indicate significant differences ($p < 0.05$)**

Table 2.7: Comparison of yield based drought susceptibility index (DSI), grain protein drought index (GPDI), test weight drought index (TWDI), days to heading drought index (DH), and height drought index (HtDI), for (A) wheat improvement status groups breeding lines and cultivars (Adv) and Landraces, or (B) the wheat quality classes hard red (HR), hard white (HW), soft red (SR) or soft white (SW) quality classes.

(A) Year	Status	DSI	GPDI	TWDI	DHDI	HtDI
2011	**Adv**	0.77 a	1.08 a	1.15 a	1.16 a	0.62 b
	Landrace	0.66 a	1.05 a	0.66 b	0.88 a	1.60 a
2012	**Adv**	0.70 a	1.20 a	1.77 a	1.13 a	0.84 b
	Landrace	0.92 a	1.73 a	2.34 a	1.02 a	1.50 a

***Values not connected by letters indicate significant differences ($p < 0.05$)**

(B) Year	Class	DSI	GPDI	TWDI	DHDI	HtDI
2011	**HR**	0.63 a	1.30 a	1.09 a	1.32 ab	1.15 a
	HW	1.02 a	1.20 ab	1.14 a	1.13 ab	1.34 a
	SR	0.85 a	0.93 ab	1.07 a	1.63 a	0.91 a
	SW	0.46 a	0.79 b	0.54 a	0.07 b	0.86 a
2012	**HR**	0.72 a	0.94 a	2.41 a	0.79 a	1.18 a
	HW	0.65 a	1.28 a	2.30 a	0.86 a	1.46 a
	SR	0.57 a	0.23 a	0.93 a	0.88 a	1.31 a
	SW	0.95 a	1.78 a	2.32 a	1.33 a	0.91 a

***Values not connected by letters indicate significant differences ($p < 0.05$)**

Table 2.8: A) Correlations between yield, GP, TW, DH and Ht recorded in 2011 and 2012, irrigated and drought, treatments. B) Correlation between yields and DSI in both IR and DR each year.

2011	Trait	Yield	GP	TW	DH	Ht	2012	Trait	Yield	GP	TW	DH	Ht
IR	**Yield**	1	/	/	/	/	**IR**	**Yield**	1	/	/	/	/
	GP	-0.32	1	/	/	/		**GP**	-0.63	1	/	/	/
	TW	0.12	ns	1	/	/		**TW**	0.34	-0.23	1	/	/
	DH	0.1	-0.26	-0.16	1	/		**DH**	0.38	-0.29	ns	1	/
	Ht	-0.16	0.2	ns	0.23	1		**Ht**	0.35	-0.13	0.36	0.48	1
DR	**Yield**	1	/	/	/	/	**DR**	**Yield**	1	/	/	/	/
	GP	-0.31	1	/	/	/		**GP**	-0.43	1	/	/	/
	TW	0.32	ns	1	/	/		**TW**	0.39	-0.44	1	/	/
	DH	0.28	-0.42	0.22	1	/		**DH**	0.13	0.25	-0.36	1	/
	Ht	ns	0.24	0.18	0.16	1		**Ht**	0.27	0.09	0.1	0.33	1

***Significance levels of p < 0.01**

B) Yield	2011 DSI	2012 DSI
IR	0.54	0.56
DR	-0.48	-0.48

***Significance of p < 0.01**

Table 2.9: Regression analysis between **s**ingle and multiple traits with grain yield in irrigated (IR) and terminal drought treatments (DR) over two years. And, regression of indexed traits with the drought susceptibility index (DSI) each year.

Experiment	Trait	Regression Equation	R^2	Probability
2011 IR				
	GP	-202.3 Prot + 8249.09	0.23	<0.0001
	TW	6.83 TW + 2602.53	0.01	0.0156
	DH	45.15 DH + 2445.17	0.03	<0.0001
	HT	-18.94 HT + 6389.7	0.03	0.0001
	GP, TW, DH, HT	-177.85 Prot + 4.27 TW + 29.47 DH + -9.98 HT + 4433.48	0.25	<0.0001
2011 DR				
	GP	-139.37 Prot + 7770.88	0.14	<0.0001
	TW	3.95 TW + 4096.78	0.01	0.113
	DH	18.23 DH + 4550.59	0.01	0.026
	HT	-17.56 HT + 6572.18	0.03	<.0001
	GP, TW, DH, HT	-128.11 Prot + 6.27 TW + 12.03 DH + -7.44 HT + 4371.57	0.16	<0.0001
2012 IR				
	GP	-173.11 Prot + 6912.79	0.14	<0.0001
	TW	4.79 TW + 2283.08	0.01	0.0274
	DH	-16.34 DH + 5462.8	0.03	<0.0001
	HT	-7.76 HT + 4629.27	0.01	0.0156
	GP, TW, DH, HT	-163.08 Prot + 2.99 TW + -12.89 DH + 1.37 HT + 6357.83	0.15	<0.0001
2012 DR				
	GP	-157.59 Prot + 5912.3	0.11	<0.0001
	TW	5.05 TW + 1413.72	0.07	<.0001
	DH	-5.58 DH + 3875.2	0.01	0.0232
	HT	6.04 HT + 3220.42	0.02	0.0005
	GP, TW, DH, HT	-148.7 Prot + 3.78 TW + -4.89 DH + 6.87 HT + 4267.38	0.19	<0.0001
2011 DSI				
	GPDI, TWDI, DHDI, HtDI	-DHDI 0.03 - HtDI 0.06 - GPDI 0.57 + TWDI 0.96 + 0.51	0.44	<0.0001
2012 DSI				
	GPDI, TWDI, DHDI, HtDI	DHDI 0.07 - HtDI 0.15 + GPDI 0.09 + TWDI 0.06 + 0.66	0.34	<0.0001

*****Grain protein, GP; Test weight, TW; Days to heading, DH; Height, Ht; Indexed grain protein, GPDI; Indexed test weight, TWDI; Indexed days to heading, DHDI; and Indexed height, HtDI.

Table 2.10: (A) Number of accessions categorized as breeding lines and cultivars (Adv), landraces (LR) or of uncertain (Unc.) improvement status, and market classes: hard red (HR), hard white (HW), soft red (SR) or soft white (SW) having DSI value <1 both year (2), in one year (1) or never (0), and the percentage of accessions selected in both 2011 and 2012 (% selected). (B) Mean yields for selection groups by improvement status groups and market classes.

(A) Grouping	0	1	2	% Selected
Adv	59	122	72	28
LR	42	95	42	23
Unc	44	54	27	26
HRS	41	101	52	26
HWS	33	67	33	24
SRS	23	45	32	32
SWS	25	58	23	21

(B) Treatment	DSI Count	Adv	LR	HR	HW	SR	SW
2011 IR	**0**	6538.39 a	6095.1 a	6149.68 a	6610.8 a	6041.29 a	6542.9 a
	1	5969.32 b	5809.4 a	5806.17 a	6118.15 a	5906.83 a	5432.24 b
	2	5412.09 c	5235.66 b	5167.08 b	5301.46 b	5314.05 b	5408.71 b
2011 DR	**0**	5158.66 c	4809.43 b	4937.05 b	5061.29 b	4704.68 b	5229.9 b
	1	5485.49 b	5492.31 a	5319.1 a	5621.77 a	5307.22 a	5339.92 b
	2	5765.83 a	5681.65 a	5581.44 a	5761.09 a	5693.96 a	5727.12 a
2012 IR	**0**	5045.85 a	4556.02 a	4874.74 a	4887.21 a	4640.29 a	4994.24 a
	1	4546.52 b	4752.94 a	4386.64 b	4762.04 a	4496.04 a	4640.97 ab
	2	4292.11 b	4144.51 b	4165.27 b	4159.31 b	3913.96 b	4101.82 b
2012 DR	**0**	3089.07 c	2991.75 c	3142.12 b	3133.04 c	3041.87 c	2971.03 c
	1	3655.04 b	3623.34 b	3513.47 b	3877.4 b	3590.1 b	3598.86 b
	2	4474.97 a	4411.49 a	4398.43 a	4363.97 a	4209.95 a	4348.06 a

*Values not connected by the same letter are significantly different at $p < 0.05$.

Chapter III:

Evaluating Grain Yield in Spring Wheat with Canopy Spectral Reflectance

ABSTRACT

Worldwide, improving grain yield is the most important target for wheat (*Triticum aestivum* L.) breeders. Fast, cost-effective and non-destructive phenotyping methods for important traits are needed to increase the efficiency of cultivar development. The present study tests canopy spectral reflectance (CSR) as a potential high-throughput method for assessing wheat grain yield in a diverse set of 540 spring-habit accessions from the USDA-ARS National Small Grains Collection. Plots were grown under irrigated and terminal drought treatments over two growing seasons and CSR was measured at several growth stages in each year. CSR indices related to canopy water and nitrogen status, biomass, and photosynthetic area were evaluated for their relation to grain yield. CSR indices were significantly correlated with yield at every growth stage with anthesis of grain-filling being the most useful for predicting grain yield in irrigated and drought environments. Single CSR indices selected up to 57% of the highest 25% yielding lines in terminal drought conditions and the grain yield of accessions selected using CSR was 20% greater than randomly selected genotypes. CSR also identified up to 86% of the highest 10% yielding accessions. CSR may be valuable as high-throughput means of selecting for yield in large trials of genetically diverse wheat genotypes.

INTRODUCTION

Wheat yields have increased over the past few decades due in part to genetic improvements such as the incorporation of dwarfing genes, increased disease resistance and abiotic stress tolerance, and development of locally adapted cultivars (Reynolds et al., 2009, 2012). However, from 1959 to 2008 the estimated yield gain has been 1.1% annually, with most of the yield increase occurring before 1984 (Graybosch and Peterson, 2010; Pingali, 2012). Current estimates of annual yield increase for wheat over the past 20 years are <1% (Fischer and Edmeades, 2010) and are insufficient to meet the projected 1.7% to 2.4% increase needed to keep pace with the growing world population (Reynolds et al., 2012; Ray et al., 2013). In addition to the concern of population growth, the threat of world-wide climate change and its effects on cropping systems will be an additional challenge for breeders to overcome in efforts to improve wheat grain yield (Fedoroff et al., 2010; Malcolm et al., 2012; Stamp and Visser, 2012; Arbuckle et al., 2013). The use of novel germplasm and improved phenotyping tools would aid breeders by increasing their efficiency and widening the genetic base of wheat.

Fast, cost-effective, and non-destructive high throughput phenotyping platforms have gained interest in recent years for use by breeders to decrease the time and costs required to assess new genotypes (Cabrera-Bosquet et al., 2012). While genotyping technologies have improved significantly, in-field phenotyping tools have not kept pace (Araus and Cairns, 2014). A problem when attempting to accurately phenotype large numbers of plants is controlling the growing conditions. The use of growth chambers and greenhouses allow for the highest levels of environment control but does not reflect actual field conditions whereas

field experiments suffer from heterogeneous soil and environmental conditions (Araus and Cairns, 2014). Statistical adjustments of spatial variation found in field conditions are commonly used to reduce the effects of environmental influences on data analysis. Canopy spectral reflectance (CSR) is one of the first high-throughput phenotyping platforms applied to field assessment of crops (Aparicio et al., 1999). While the accuracy of phenotypic data taken in-field will most always be affected by heterogeneous environmental conditions to some extent, CSR indices have been used to not only assess crop characteristics, but also as means of mapping and adjusting for field heterogeneity (Araus and Cairns, 2014).

CSR is based on the differential pattern of light reflectance on leaves at photosynthetically active wavelengths (400-700 nm) and infrared wavelengths (700 - 1000 nm). CSR indices can be used to estimate plant characteristics such as leaf nitrogen content (Wright et al., 2003; 2004; Wei et al., 2008; Zhu et al., 2008; Feng et al., 2011), photosynthetic active biomass (Aparicio et al., 1999), leaf chlorophyll content, and plant water status (Penuelas et al., 1997a, b; Aparicio et al., 1999; Araus et al, 2002; Babar et al., 2006b; a; Prasad et al., 2007a; Feng et al., 2008; Gutierrez et al., 2010b).

Direct measurements of plant biomass, water status, photosynthetic capacity, and leaf nitrogen status are associated with agronomic traits such as yield and grain protein, but these conventional methods all have disadvantages. Measuring plant biomass requires destruction of the entire plant and is impractical for screening large numbers of genotypes (van Ginkel et al., 1998). Assessing plant water status through excised leaves is destructive, requires tedious measurements of small changes in leaf weight over time and sufficient time to completely dry plant tissue (McCaig and Romagosa, 1989). Similarly, plant nitrogen status measurements are

time consuming and require removal of leaf tissue and laboratory procedures to assess nitrogen content (Feng et al., 2011).

Previous studies have shown CSR indices to be predictors of yield in barley (Hansen et al., 2002), rice (Inoue and Moran, 1998), corn (Teal et al., 2006), durum wheat (Aparicio et al., 1999), winter wheat (Hansen, 2002; Prasad et al., 2007a) and bread wheat (Gutierrez et al., 2010b). Babar et al. (2006b) found indices related to photochemical, biomass, and canopy water content related indices to be highly correlated (greater than 0.80) with yields and to explain upwards of 50% of the yield variation observed across multiple years in a group of 15 high yielding CIMMYT bread wheat genotypes.

CSR has the potential to aid breeding programs where large numbers of individuals must be screened in a fast and cost effective manner. CSR could facilitate line development by identifying superior genotypes at or before anthesis, allowing for crosses to be made before grain yields have been evaluated. Previous studies on CSR relation to yields have used small groups ($n < 50$) of advanced breeding lines (Babar et al., 2006a; Gutierrez et al., 2010b), bi-parental populations (Babar et al., 2006c; Prasad et al., 2007a) or commercial cultivars (Babar et al., 2006c; Prasad et al., 2007a). For a technology to be useful to breeders, it must be applicable to a wide range of genotypes and growing conditions. While genotype selection by CSR has been successfully implemented in several wheat growing environments (Gutierrez et al., 2010a, b), screening of large, genetically diverse panels of wheat genotypes has not been reported previously.

In the present study, we used CSR as a high throughput phenotyping tool to assess grain yield in a diverse collection of genotypes accessions from the USDA-ARS National

Small Grains Collection (NSGC). Our goals were to assess CSR indices for predicting grain yield under irrigated and water-stressed conditions, and to identify high yielding germplasm in a large diverse set of wheat lines from the NSGC using CSR.

MATERIALS AND METHODS

Plant Material

The plant material used in this study consisted of 540 spring wheat accessions from the NSGC common wheat core subset. The NSGC is a component of the National Plant Germplasm System (NPGS) in the United States Department of Agriculture - Agricultural Research Service (USDA-ARS). Based on heading dates and uniformity during an initial screen in 2010, accessions were selected from the spring wheat accessions among the NSGC common wheat core subset. The 540 spring wheat accessions originated from six continents and 81 countries and included cultivars, breeding lines, landraces, and accessions of uncertain improvement status. Two cultivars, Agawam (PI648027) and Alpowa (PI566596), and three breeding lines from the University of Idaho wheat breeding program, IDO599, IDO686 and IDO702, were used as checks in 2011. PI428506 and PI520108 were chosen to replace IDO702 and PI648027 as checks to better represent the 540 lines from NSGC in the 2012 trial (Supplemental Table). Additional information on the plant material used for this study can be found at the USDA's Germplasm Resources Information Network (www.ars-grin.gov) and characteristics of the five checks can be found in Li et al. (2011).

Field Design and Experimental Conditions

Trials were arranged in an augmented complete block design 20 plots wide and 30 plots deep, as previously described (Zhang et al., 2014). In total, 540 unique accessions and 5 checks were planted in each experiment. Plots were 1.83 m long by 1.5 m wide and planted in seven rows at a rate of 364,500 kernels per hectare. Plots were divided into early, medium, and late maturity groups, each containing 180 accessions and 20 check plots for a total of 600 plots per trial. Each maturity group was further divided into four sub-blocks that contained a single plot of each of the five checks. The check lines were distributed so that each row contained two different checks and each column contained three different checks. Trials were planted in adjacent water-level treatments at the University of Idaho Aberdeen Research and Extension Center in Aberdeen, Idaho (42°57’36’’ N, 112°49’12’’ W, and elevation 1342 m). One treatment was irrigated (IR) throughout the entire growing season and the other was subjected to water stress at reproductive growth stages (terminal drought, DR). A drip tape system was employed for precise irrigation control and to allow for each plot within the treatment to receive the same amounts of water. Individual plots had three 1.83 meter sections of drip tape spaced every two rows. All plots were irrigated for a single 12 hour period each week until heading at a rate of 2.5 l/h per 30.5 meters of drip tape. Plots in the IR were irrigated until physiological maturity. Water stress conditions were induced in the DR treatment when 95% of the plots had headed.

The climate in Aberdeen, ID is conducive to terminal drought research, with annual precipitation between 20.3 cm and 27.9 cm, and mean annual temperatures between 7.2 and 8.3 °C (Li et al., 2011). The least precipitation and highest air temperatures were recorded

during June and July each year, and occurred during the heading and flowering growth stages, consistent with the onset of terminal drought conditions. The average temperatures and total precipitation from June to August were 27.2°C and 3.5 mm in 2011, and 28.7°C with 11.7 mm in 2012. Trials received 66.3 and 46.2 mm of precipitation between planting and physiological maturity (April to August) in 2011 and 2012, respectively. The field soils were Declo-loam (coarse-loamy, mixed, superactive, mesic Xeric Haplocalcids) with 0 to 2% slopes and pH of 8.1. Historical information on climate conditions for Aberdeen Idaho is available through AgriMet (http://www.usbr.gov/pn/agrimet/webarcread.html).

Agronomic Traits

In all trials, individual plots were harvested after physiological maturity with a Wintersteiger Classic small plot combine equipped with a Harvest Master system (Wintersteiger Inc., Salt Lake City, UT). Yields were calculated from raw grain weight and converted to kilograms per hectare. Days to heading were calculated from the planting date until 50% of the heads within a plot were emerged. Heights were measured from the middle rows of each plot at maturity from the soil surface to top of the spike.

Canopy Spectral Reflectance

CSR measurements were made with a portable Ocean Optics Jaz spectrometer (Ocean Optics, Dunedin, FL). This device measures the radiation reflected directly from the plot canopies. Measurements were taken between 10:00 a.m. and 3:00 p.m. on cloud-free days to minimize atmospheric interference and ensure consistent sunlight, when a majority of the

plots were at specific growth stages, heading and anthesis in 2011 and at booting, heading, anthesis, and grain-filling in 2012. The spectrometer was calibrated using a barium sulfate ($BaSO_4$) coated board to account for changes in the solar radiation intensity due to the position of the sun. New calibrations were taken every 40 plots or approximately every 20 minutes.

The spectrometer used for this experiment had three channels with gratings #3, #4 and #14 (Ocean Optics, Dunedin, FL). Across all gratings, continuous wavelengths from 339 nm to 1259 nm were recorded with an average interval of 0.324 nm. Grating #4 has an optimal range of 530 nm to 1100 nm and a full width half maximum optical resolution of 1.17 nm, which encompassed all required wavelengths except for 526 nm. Grating #4 was used in the present study because 526 nm is just slightly outside its optimal range and all other required wavelengths can be optimally measured with it.

Only the centers of plots were measured, to avoid edge effects, using a constant scanning method 50 cm above the canopy with a 25° field of view (386 cm^3 footprint). An average of 100 individual measurements was taken per wavelength recorded with a single scan of each plot used for measurements. Constant scanning method entails scanning across the center region of each plot for the duration of the measurements. The same accessions were recorded in both irrigated and terminal drought treatments within a single day and all plots were measured within a single week.

Index Calculations

The wavelengths needed for calculating CSR indices were generated by averaging the four reflectance intensity closest to the needed wavelength. In addition, while recording reflectance values the spectrometer implemented a three-step boxcar smoothing. Averaged values were used as a means of noise reduction. For example, the Water Index (WI) is calculated using the minor water absorption band at 970 nm and reference frequency 900 nm. A single value for 970 and 900 is calculated by averaging reflectance values at the frequencies shown in the equation below:

$$\text{Water Index (WI)} = \frac{\text{Average (969.89, 970.21, 970.52, 970.85)}}{\text{Average (900.16, 900.49, 900.82, 901.16)}}$$

Three general categories of CSR indices were used: water-based, vegetation-based, and nitrogen-based. The water-based and vegetation-based indices were chosen because they are correlated with yields in wheat and are indicators of canopy water status and photosynthetic biomass. The two nitrogen-based indices were chosen because they are related to plant height, leaf area, dry matter accumulation, and chlorophyll content in corn and rice (Xue et al., 2004; Zhao et al., 2003).

The water-based indices used here are Water Index (WI = 970nm / 900nm) (Penuelas et al., 1993), Normalized Water Index 1 (NWI1 = (970nm - 900nm)/(970nm + 900nm)), NWI2 ((970nm - 850nm) / (970nm + 850nm)), NWI3 ((970nm - 920nm)/(970nm + 920nm)), and NWI4((970nm - 880nm)/(970nm + 880nm)) (Babar et al., 2006c; Prasad et al., 2007a). The vegetative based indices used here are Simple Ratio (SR = 900nm / 680nm) (Tucker and Sellers, 1986), Red Normalized Difference Vegetation Index (RNDVI = (780nm - 670nm) / (780nm + 670nm)) (Raun et al., 2001), Normalized Difference Vegetation Index (NDVI =

(900nm - 680nm) / (900nm + 680nm)) (Tucker and Sellers, 1986), Photochemical Reflectance Index (PRI = (531nm - 570nm) / (531nm + 570nm)) (Penuelas et al., 1997a), and Pigment Specific Simple Ratio Chlorophyll-a (PSSRa = 800nm / 680nm) (Blackburn, 1999). PSSRa specifically measures chlorophyll-a content but has been used for estimating vegetative biomass (Babar et al., 2006c). The indices used here estimate canopy nitrogen status were the Oryza Nitrogen Index (ONI = 810nm / 560nm) (Xue et al., 2004) and the Dry Zea Nitrogen Index (DZNI = 575nm / 526nm) (Zhao et al., 2003).

Statistical Analysis

Collected data was adjusted for maturity blocks, as well as days to heading by restricted maximum likelihood (REML) models. Analysis of variance (ANOVA), Student's t-test, and Pearson's correlation coefficients were calculated using JMP Version 11 statistical software (SAS Inst, 2011). Checks were used to estimate broad sense heritability by REML models with rows, and columns as fixed effects and genotypes as random effects.

Csr Selection of High Yielding Accessions

Subsets of accessions were selected based on yields and CSR values. A subset of the 25% highest yielding accessions ($HY_{25)}$ and the 10% highest yielding accessions ($HY_{10)}$ were selected in each treatment both years. For each CSR index at each growth stage, 25% of the plots (CSR_{25}) were selected to represent accessions having values presumably associated with increased grain yield. Thus, accessions were chosen with the highest 25% index values for NDVI, RNDVI, PRI, PSSRa, SR, and ONI and the lowest 25% index values for WI, NWI1,

NWI2, NWI3, NWI4 and DZNI. Average yield from a random selection of 135 (25%) accessions, repeated 1000 times, was calculated for comparison to the CSR_{25} yield values. Bootstrapping at a 95% confidence was used to determine significance of yield increase due to selection.

RESULTS

Analysis of Grain Yield

Terminal drought conditions had significant effects on yields in both 2011 and 2012 (Table 3.1). Lines in the IR treatments produced significantly higher yields than the DR treatment and the average of yields of all lines both treatments was larger in 2011 than 2012 ($p < 0.05$) (Table 3.1). The average yields of all lines in IR were 5795.22 kg/ha in 2011 and 4503.95 kg/ha in 2012, while in DR they were 5376.78 kg/ha and 3697.30 kg/ha in 2011 and 2012, respectively. The heritability of yield was 0.64 and 0.54 in 2011 IR and DR, respectively, and 0.3 and 0.25 in 2012 IR and DR, respectively.

Csr Heritabliity and Changes by Growth Stage

Heritability (H) of CSR indices ranged from 0.0 at heading in 2012 DR to 0.68 at heading in 2011 DR. Heritabilities of RNDVI at Hd in 2011DR and at Bt in 2012 IR and DR were higher than that of yield in both years. Heritabilities of DZNI at most of growth stages over two years were higher than that of yield in 2012 (Tables 3.1 and 3.2). Heritabilities of WI and NWI1-NWI4 varied from different growth stages in the two treatments both years (Tables 3.2). All Water based CSR indices recorded in 2011 DR at HD had higher H than

yield and NWI3 had H equal to yield H in 2012 IR at anthesis. No other water based CSR heritabilities were larger than their respective yield H. In 2011, NDVI and RNDVI were the only vegetative indices to have H higher than yield H in DR at HD. All vegetative biomass and nitrogen status related indices had higher H than yield H in 2012 IR at Bt, but not at other growth stages (Tables 3.1 and 3.2). PRI, NDVI, RNDVI and DZNI had higher heritabilities than yield in 2012 DR booting and anthesis growth stages but not heading or grain filling. Overall, NDVI and RNDVI were found to have heritabilities in three of the four trials in at least one growth stage. The water based NWI3 had H greater than yield H in two trials, as did the vegetative biomass index PRI and nitrogen status based DZNI.

CSR indices of all lines showed significant differences between irrigated and terminal drought treatments in all growth stages in both years except for DZNI in 2011 at anthesis or ONI and PRI in 2011 at heading (Table 3.3). At most growth stages measured each year accessions had significantly higher values in DR versus IR for WI, NWI-1, NWI-2, NWI-3, NWI-4, and DZNI and lower values in DR than IR for PRI, NDVI, Red NDVI, PSSRa, SR and ONI. At heading in 2011, the water indices were significantly higher in the IR treatment than DR and the vegetation index PRI showed no difference between IR and DR treatments. In 2012, the highest values for the water related CSR indices (WI, NWI-1, NWI-2, NWI-3 and NWI-4) were recorded at booting and the lowest at heading, followed by an increase from heading to grain-filling. The two sets of readings taken in 2011 follow this trend with an increase in CSR values from heading to anthesis. Photochemical (PSSRa and PRI) and vegetative biomass related indices (NDVI, Red NDVI and SR) generally decreased throughout the growing season in both years. ONI increased during the early season then

decreased after heading. DZNI, in contrast, decreased during the early season then increased after heading (Table 3.3).

Correlation Between CSR Indices and Yield

Pearson's correlation coefficients between yield and CSR indices were significant ($P < 0.001$) at all growth stages in IR and DR each year (Table 3.4). WI, NWI1, NWI2, NWI3, NWI4, and DZNI were consistently negatively correlated with yield, and PRI, RNDVI, NDVI, PSSRa, SR and ONI were positively correlated with yields. No single index had consistently higher associations with yields than other indices. Water indices (WI, NWI1, NWI2, NWI3, and NWI4) showed similar correlation coefficients ranging from -0.20 at heading in 2011 IR to -0.70 at anthesis in 2012 DR. Vegetative and photochemical indices (SR, NDVI, RNDVI, PRI, PSSRa) also showed similar correlation coefficients ranging from 0.16 at heading in 2011 IR to 0.71 at grain-filling in 2012 IR (Table 3.4).

In general, correlation increased during the growing season with the most significant correlations occurring at anthesis, followed by GF in 2012. The GF growth stage had the highest correlations for RNDVI in 2012 IR and DR, for NDVI in 2012 IR, and for DZNI in 2012 DR. Water based indices were more highly correlated with yields in the irrigated treatments than the terminal drought at all readings except at booting in 2012 (Table 3.4).

CSR Based Selections

CSR_{25} selections encompassed 32% to 55% of the highest 25% yielding accessions (HY_{25}) and 37% to 86% of the top 10% yielding genotypes (HY_{10}). The proportion of

genotypes selected by both HY_{25} and CSR_{25} in DR treatments was higher than in IR treatments at both growth stages in 2011, except for PRI and ONI at anthesis. HY_{25} selections in 2012 found both terminal drought and irrigated treatments to select similar percentages by CSR_{25}. For the 10% highest yielding accessions, the DR treatment had much higher selection rates than the IR treatment at anthesis in 2011 and at all growth stages in 2012. CSR_{25} selections made after heading identified a larger proportion of HY_{25} than earlier growth stages for most indices. NWI2 at anthesis had the highest HY_{10} selection rates at 86% and PRI at grain filling had the highest HY_{10} selection rates at 85%, both in the DR treatment in 2012 (Table 3.5).

Accessions identified by CSR had average yields significantly higher than accessions selected at random. Accessions selected using CSR measured at anthesis consistently had the largest gain in yield compared to the random selections. In 2011, the yield increase of CSR_{25} at anthesis was 9.1% and 10.2% above randomly selected accessions in IR and DR, respectively. In 2012 the average yield increase for accessions selected by CSR at anthesis was 20.3% in IR and 20.8% in DR. Accessions selected based on the PRI showed the greatest yield increases in 2011, 10.7% for IR and 12.9% for DR. In 2012, accessions selected with PSSRa had 20.9% greater yield than randomly selected accessions in the IR and those selected with NWI2 had showed a 24.4% increase over the random sample.

DISCUSSION

Genetic Variation of CSR Indices

Evaluation and selection of high yielding wheat genotypes using CSR indices have been successfully applied in earlier studies (Aparicio et al., 1999; Babar et al., 2006b; a; Prasad et al., 2007a; Gutierrez et al., 2010b). Previous studies evaluated groups of fewer than 50 genotypes, consisting either of advanced breeding lines, elite cultivars or bi-parental populations. In the present study, we found that CSR indices were able to distinguish high yielding genotypes from a large and diverse collection of wheat accessions that included cultivars, breeding lines, and landraces.

Water based indices (WI, NWI1, NWI2, NWI3 and NWI4) responded as expected in each treatment and were negatively correlated with yields at all growth stages. Water indices use the 970-nm wavelength minor water absorption band and are indicators of plant water status. Increases in the water index values indicate a decrease in the amount of water within the canopy, while decreases in water indices indicate increased water status. The trend of increasing water index values from heading to grain-filling follows the expected decrease in canopy water as the growing season progressed, which has been reported in previous studies (Aparicio et al., 1999; Babar et al., 2006b; a; Prasad et al., 2007a; Gutierrez et al., 2010b). RNDVI, NDVI, SR, PRI and PSSRa, were expected to behave similarly since they are used as indicators of biomass (Aparicio et al., 1999; Babar et al., 2006b; a; Prasad et al., 2007b; Gutierrez et al., 2010b). These indices estimate vegetative biomass or photosynthesis-related chemical content through measurements of chlorophylls (RNDVI, NDVI and SR) or xanthophyll (PRI), which absorb at 670-680 nm and 531 nm wavelengths, respectively. Near

infrared wavelengths (700-1300 nm) are used in each of vegetative indices, except PRI, because NIR wavelengths are not absorbed by plant material and have a higher level of reflectance. The vegetative biomass indices assume that the leaf tissue area is related to photosynthetic tissue and, consequently, decreases as the plants mature, leaves senesce, and photochemicals are recycled.

The difference between IR and DR index values for CSR was greater in 2012 than in 2011. This result could be due to the higher precipitation in 2011, which would reduce the impact of terminal drought. In a study by Gutierrez et al., (2010b), the change in CSR index values as the season progressed was higher in water stressed treatments than well irrigated treatments, as found in the present study. Over the growing season, the more rapid and larger change in CSR indices seen in the DR treatment of this experiment indicates the water stress response. Water stressed plants need to rely almost entirely on nutrient and water reserves stored in stems and leaves, while non-stressed plants are still able to take up available water and nutrients during the reproductive growth stage. Thus stored water and nutrients are depleted more rapidly or are not as abundant in terminal drought treatments compared to irrigated treatments. The cumulative effects of lower levels of biomass, photosynthetic chemicals, and canopy are reflected in total yields (Foulkes et al., 2011).

A common finding in several past studies is the inconsistency between experiments of correlation and regression analysis of vegetative and photochemical related indices (Babar et al., 2006c; Prasad et al., 2007b; Gutierrez et al., 2010b). Gutierrez et al. (2010b) reported water indices to be more consistent in both irrigated and water stressed treatments, but suggested the use of vegetative indices in high-temperature treatments. Babar et al. (2006b)

also found that CSR indices were more associated with final yields when recorded after heading, and CSR measurements from multiple growth stages were more highly correlated with yields than each growth stage taken singly.

Here, we found CSR indices to be more highly correlated with yields in the later growth stages of anthesis and GF. But, we did not find vegetative indices to be more highly associated with yields in the DR treatments than the IR except for 2011 anthesis, and 2012booting. It is possible that the terminal drought conditions used in this study were not adequate to have this effect.

Indirect Selection of Yield Using Csr

Estimates of broad sense heritability in the present study were lower than those reported previously (Babar et al., 2006b; Prasad et al., 2007a; b). Prasad et al. (2007a) found CSR heritabilities of RNDVI, SR, WI, NWI1, NWI2, NWI3 and NWI4 to range from 0.48 to 0.78, with a majority of the heritability values exceeding 0.5 across multiple growth stages, years and environments. Similarly, Babar et al. (2006b) found heritabilities of the water indices as well as WI and NDVI of 0.6 in a majority of their trials. However, the low heritabilities in the present study might be expected when evaluating large numbers of diverse genotypes in unreplicated plots which is inherently imprecise (Federer and Ragavarao, 1975). Yet, even with low heritabilities, indirect selection tools can still be valuable to breeders if the measurement being used for indirect selection has a higher heritability than the trait of interest (Babar et al., 2006b; Prasad et al., 2007a; b). Here we found that CSR indices NDVI and

RNDVI taken at or prior to HD and at Ant in DR treatment in 2012 had higher heritabilities than yield in three of our four trials and are possibly suitable for indirect selection.

When considering correlation of yields with CSR indices, selections at Ant or GF would seem to be the most accurate as these CSR readings were more highly correlated with yield in both IR and DR each year. However, in the present study CSR selections in DR were better than in IR at identifying the highest yielding genotypes. In optimal water conditions plants are able to remain green for the maximum amount of time. Yield potential has been shown to be related to water status and biomass, but in irrigated conditions these traits could be masked by the longevity of green tissue survival. In DR, genotypes that remain green longer will have lower water index values, and higher biomass/photochemical index values at later growth stages. These genotypes will likely have the highest biomass/photochemical related yield potential. Therefore, selection using CSR indices under DR allows for more efficient selection of genotypes that remain green longer and have a higher chance of increased yields than genotypes that lose green tissue quickly in DR.

In comparison to previous studies that evaluated yield selection by CSR indices, the selections made here match closest to the high temperature water stressed treatment used by Gutierrez et al. (2010a), with ambient daily temperature at anthesis of 30^{o} to 35^{o} C. They reported average selection efficiencies of 80% and 100% using NWI1 and NWI3, respectively. Here we found the CSR indices in DR able to identify 82% of the highest 10% yielding accessions at anthesis. We observed lower selection rates of the 25% highest yielding genotypes than previously reported (Babar et al., 2006a; Prasad et al., 2007b; Gutierrez et al., 2010b), but comparable selection rates of the highest 10% yielding genotypes with water,

vegetative and photochemical based indices. In this study, CSR indices were found to be suitable for indirect selection in terminal drought environments because of their high correlation with yield, ability to identify a large proportion of the highest yielding plots at the anthesis growth stage and significantly increase average yields of selected accessions over random selection.

CONCLUSIONS

Developing improved cultivars by introducing superior traits or combinations of traits that would benefit wheat growers is the most important job for breeders worldwide. Currently, the genetic gain achieved by breeders is not adequate to keep pace with the growing world population. Some researchers have speculated that the reduction in the rate of yield gain in wheat is because the crop is approaching its theoretical yield limit (Foulkes et al., 2011; Reynolds et al., 2012; Cavanagh et al., 2013). It was shown in an analysis of multiple wheat genomes that during the domestication and subsequent development of modern cultivars the diversity of alleles was greatly reduced, creating a genetic bottle-neck for breeders (Rostoks et al., 2006; Brenchley et al., 2012; Cavanagh et al., 2013). Achieving sufficient genetic gains will require introduction of new alleles to broaden the genetic pool available to breeders. An underutilized resource that could be a source of new alleles are germplasm repositories such as the NSGC (Feuillet et al., 2008; Fischer and Edmeades, 2010; Fischer, 2011).

Identifying alleles that could increase the rate of genetic gain in wheat would require screening large numbers of wheat genotypes. Canopy spectral reflectance is a tool that could greatly decrease the time needed to screen new genotypes. Reducing the number of genotypes

early in the breeding process would significantly reduce the costs of cultivar development. Based on correlations with yield and selections made using CSR, the Ant and GF growth stages are most suitable for indirect selections. Selections made here by CSR indicate that the best results would be obtained in drought conditions. This study also suggests that additional replicated trails, using a smaller number of accessions may improve our results and is needed to validate above findings.

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Table 3.1. Grain yield, plant height, and days to heading recorded in 2011 and 2012 irrigation (IR) and terminal drought (DR) treatments, as well as the broad sense heritability (H).

A) Trait	Treatment	2011 Mean	H 2011	2012 Mean	H 2012
Yield (kg/ha)	IR	5795.22a	0.64	4503.95a	0.30
	DR	5376.78b	0.54	3697.30b	0.25
Height (cm)	IR	107.24a	0.67	105.79a	0.63
	DR	109.60b	0.69	110.00b	0.73
Days to heading (Days)	IR	69.66a	0.88	70.96a	0.32
	DR	69.33b	0.78	70.03b	0.20
Values not connected by letters indicate significant differences $p < 0.05$ within columns					

Table 3.2. Heritability of single CSR indices at each growth stage measured in 2011 and 2012 irrigated (IR) and terminal drought (DR) treatments.

	2011 IR		2011 DR		2012 IR				2012 DR			
	Hd	**Ant**	**Hd**	**Ant**	**Bt**	**Hd**	**Ant**	**GF**	**Bt**	**Hd**	**Ant**	**GF**
WI	0.28	0.44	0.57	0.27	0.27	0.20	0.27	0.26	0.21	0.05	0.17	0.14
NWI1	0.29	0.42	0.54	0.25	0.22	0.20	0.27	0.25	0.21	0.04	0.15	0.13
NWI2	0.30	0.43	0.61	0.23	0.28	0.17	0.19	0.21	0.22	0.03	0.13	0.09
NWI3	0.30	0.41	0.54	0.26	0.20	0.22	0.30	0.28	0.21	0.04	0.16	0.15
NWI4	0.32	0.44	0.59	0.26	0.26	0.19	0.23	0.24	0.21	0.03	0.14	0.11
SR	0.45	0.29	0.33	0.12	0.63	0.18	0.21	0.17	0.17	0.00	0.14	0.15
PRI	0.47	0.42	0.31	0.23	0.46	0.14	0.20	0.25	0.26	0.05	0.36	0.23
NDVI	0.25	0.44	0.65	0.22	0.51	0.24	0.21	0.22	0.61	0.07	0.28	0.11
RNDVI	0.33	0.41	0.68	0.21	0.58	0.22	0.17	0.20	0.57	0.05	0.28	0.09
PSSRa	0.45	0.31	0.34	0.11	0.60	0.17	0.19	0.15	0.15	0.00	0.13	0.18
ONI	0.22	0.31	0.27	0.19	0.66	0.20	0.29	0.24	0.16	0.04	0.20	0.19
DZNI	0.46	0.50	0.36	0.27	0.49	0.15	0.22	0.28	0.33	0.06	0.37	0.23

Table 3.3: Average CSR index value and standard deviations of measurements taken in 2011 and 2012 irrigated (IR) and terminal drought (DR) treatments.

Index	Treatment	Booting	Heading	Anthesis	Grain Filling
WI	**2011: IR**	/	0.165 ± 0.014a	0.181 ± 0.023a	/
	2011: DR	/	0.163 ± 0.018b	0.199 ± 0.027b	/
	2012: IR	0.71 ± 0.109a	0.162 ± 0.014a	0.182 ± 0.026a	0.222 ± 0.032a
	2012: DR	0.787 ± 0.058b	0.191 ± 0.025b	0.201 ± 0.027b	0.278 ± 0.038b
NWI1	**2011: IR**	/	-0.717 ± 0.021a	-0.695 ± 0.032a	/
	2011: DR	/	-0.721 ± 0.026b	-0.669 ± 0.039b	/
	2012: IR	-0.174 ± 0.078a	-0.722 ± 0.021a	-0.693 ± 0.037a	-0.638 ± 0.043a
	2012: DR	-0.12 ± 0.037b	-0.68 ± 0.035b	-0.666 ± 0.037b	-0.566 ± 0.047b
NWI2	**2011: IR**	/	-0.719 ± 0.021a	-0.694 ± 0.033a	/
	2011: DR	/	-0.722 ± 0.026b	-0.667 ± 0.042b	/
	2012: IR	-0.17 ± 0.081a	-0.717 ± 0.024a	-0.687 ± 0.042a	-0.623 ± 0.05a
	2012: DR	-0.119 ± 0.039b	-0.675 ± 0.037b	-0.657 ± 0.04b	-0.545 ± 0.049b
NWI3	**2011: IR**	/	-0.71 ± 0.021a	-0.687 ± 0.032a	/
	2011: DR	/	-0.713 ± 0.026b	-0.664 ± 0.038b	/
	2012: IR	-0.169 ± 0.075a	-0.716 ± 0.02a	-0.687 ± 0.035a	-0.635 ± 0.041a
	2012: DR	-0.117 ± 0.034b	-0.675 ± 0.035b	-0.661 ± 0.036b	-0.568 ± 0.045b
NWI4	**2011: IR**	/	-0.72 ± 0.021a	-0.697 ± 0.032a	/
	2011: DR	/	-0.724 ± 0.026b	-0.672 ± 0.04b	/
	2012: IR	-0.172 ± 0.077a	-0.722 ± 0.022a	-0.695 ± 0.038a	-0.635 ± 0.045a
	2012: DR	-0.121 ± 0.037b	-0.681 ± 0.036b	-0.666 ± 0.038b	-0.562 ± 0.047b
SR	**2011: IR**	/	20.579 ± 6.829a	12.633 ± 5.856a	/
	2011: DR	/	19.284 ± 5.105b	10.059 ± 4.139b	/
	2012: IR	11.119 ± 4.001a	12.02 ± 3.984a	6.997 ± 3.365a	3.992 ± 2.239a
	2012: DR	8.077 ± 2.782b	7.858 ± 3.486b	4.91 ± 2.191b	1.816 ± 0.865b
PRI	**2011: IR**	/	-0.023 ± 0.014a	-0.062 ± 0.026a	/
	2011: DR	/	-0.023 ± 0.016a	-0.065 ± 0.026b	/
	2012: IR	-0.145 ± 0.021a	-0.144 ± 0.021a	-0.165 ± 0.036a	-0.198 ± 0.036a
	2012: DR	-0.154 ± 0.017b	-0.162 ± 0.024b	-0.185 ± 0.028b	-0.22 ± 0.017b
NDVI	**2011: IR**	/	0.897 ± 0.032a	0.82 ± 0.086a	/
	2011: DR	/	0.892 ± 0.035b	0.785 ± 0.092b	/
	2012: IR	0.812 ± 0.082a	0.829 ± 0.063a	0.695 ± 0.143a	0.516 ± 0.199a
	2012: DR	0.744 ± 0.129b	0.732 ± 0.119b	0.613 ± 0.144b	0.243 ± 0.163b
RNDVI	**2011: IR**	/	0.861 ± 0.059a	0.808 ± 0.093a	/
	2011: DR	/	0.887 ± 0.04b	0.766 ± 0.104b	/
	2012: IR	0.817 ± 0.071a	0.809 ± 0.078a	0.653 ± 0.172a	0.444 ± 0.237a
	2012: DR	0.748 ± 0.115b	0.702 ± 0.133b	0.56 ± 0.161b	0.144 ± 0.17b
PSSRa	**2011: IR**	/	19.909 ± 6.568a	12.19 ± 5.659a	/
	2011: DR	/	18.813 ± 5b	9.544 ± 4.053b	/
	2012: IR	11.162 ± 3.845a	11.442 ± 4.054a	6.61 ± 3.373a	3.64 ± 2.207a
	2012: DR	8.122 ± 2.696b	7.413 ± 3.384b	4.486 ± 2.129b	1.569 ± 0.834b
ONI	**2011: IR**	/	9.027 ± 2.468a	6.365 ± 2.215a	/
	2011: DR	/	8.934 ± 2.028a	5.719 ± 1.706b	/
	2012: IR	5.585 ± 1.552a	6.515 ± 1.479a	4.603 ± 1.324a	3.075 ± 0.834a
	2012: DR	4.554 ± 1.164b	4.911 ± 1.412b	3.821 ± 0.933b	2.122 ± 0.458b
DZNI	**2011: IR**	/	1.098 ± 0.039a	1.199 ± 0.08a	/
	2011: DR	/	1.09 ± 0.043b	1.203 ± 0.078a	/
	2012: IR	1.52 ± 0.109a	1.479 ± 0.08a	1.578 ± 0.141a	1.701 ± 0.146a
	2012: DR	1.531 ± 0.086b	1.529 ± 0.084b	1.632 ± 0.116b	1.778 ± 0.076b

*Values not connected by the same letter are significantly different; $p < 0.05$

Table 3.4. Pearson's correlation coefficient of yield and CSR indices at different growth stages in two irrigation regimes over two growing seasons. Growth stages: Bt, booting; Hd, heading; Ant, anthesis; and GF, grain-filling.

Index	2011 IR (Hd)	2011 IR (Ant)	2011 DR (Hd)	2011 DR (Ant)	2012 IR (Bt)	2012 IR (Hd)	2012 IR (Ant)	2012 IR (GF)	2012 DR (Bt)	2012 DR (Hd)	2012 DR (Ant)	2012 DR (GF)
WI	-0.21	-0.41	-0.25	-0.39	-0.32	-0.48	-0.63	-0.58	-0.43	-0.41	-0.55	-0.45
NWI1	-0.21	-0.41	-0.25	-0.38	-0.31	-0.48	-0.63	-0.58	-0.44	-0.41	-0.56	-0.46
NWI2	-0.21	-0.42	-0.29	-0.37	-0.37	-0.52	-0.66	-0.62	-0.44	-0.44	-0.59	-0.50
NWI3	-0.20	-0.40	-0.24	-0.35	-0.30	-0.46	-0.62	-0.56	-0.42	-0.40	-0.54	-0.43
NWI4	-0.20	-0.41	-0.27	-0.35	-0.36	-0.51	-0.64	-0.60	-0.44	-0.42	-0.57	-0.47
SR	0.22	0.37	0.27	0.43	0.54	0.54	0.64	0.61	0.26	0.37	0.53	0.53
PRI	0.25	0.48	0.39	0.44	0.53	0.58	0.69	0.67	0.29	0.41	0.54	0.51
NDVI	0.25	0.46	0.28	0.39	0.57	0.56	0.69	0.70	0.26	0.39	0.49	0.45
RNDVI	0.16	0.46	0.30	0.41	0.57	0.57	0.70	0.71	0.25	0.41	0.52	0.53
PSSRa	0.22	0.38	0.28	0.44	0.56	0.55	0.65	0.62	0.26	0.39	0.56	0.55
ONI	0.26	0.40	0.30	0.34	0.56	0.51	0.59	0.50	0.22	0.32	0.40	0.28
DZNI	-0.24	-0.48	-0.38	-0.41	-0.50	-0.58	-0.70	-0.66	-0.30	-0.42	-0.40	-0.47

Growth stages: Bt, booting; Hd, heading; Ant, anthesis; and GF, grain-filling.
All values significant at $p < 0.001$

Table 3.5. Percentage of the $HY_{25\%}$ genotypes selected by $CSR_{25\%}$ and percent yield gain compared to the mean yield of a random selection of 25% (n = 135) of all genotypes ($Mean_{Rand25\%}$). Values within '( )' are the percentage of $HY_{10\%}$ genotypes selected by $CSR_{25\%}$.

Experiment	2011 IR				2011 DR				2012 IR				2012 DR			
$Mean_{Rand25\%}$ (kg/ha)	5779				5374				4508				3700			
Growth Stage	Hd	Gain %	Ant	Gain %	Hd	Gain %	Ant	Gain %	Ant	Gain %	GF	Gain %	Ant	Gain %	GF	Gain %
$WI_{25\%}$	33 (55)	6.2	40 (51)	8.8	39 (39)	5.8	45 (56)	9.9	48 (57)	20.4	48 (54)	18.8	53 (84)	22.5	48 (78)	17.6
$NWI1_{25\%}$	33 (55)	6.2	40 (51)	8.8	39 (39)	5.7	45 (56)	9.8	48 (57)	20.4	48 (54)	18.8	53 (84)	22.5	48 (78)	17.6
$NWI2_{25\%}$	32 (49)	5.3	38 (49)	8.5	41(44)	6.6	42 (54)	9.3	48 (55)	20.1	51(58)	19.9	57 (86)	24.4	49 (78)	19.2
$NWI3_{25\%}$	33 (55)	6.4	38 (49)	8.8	38 (37)	5.6	43 (54)	9.3	46 (51)	19.4	48 (52)	18.5	53 (82)	21.5	45 (71)	15.6
$NWI4_{25\%}$	32 (51)	5.9	39 (49)	8.5	41(44)	6.8	42 (54)	8.9	49 (57)	20.2	51(58)	19.3	55 (84)	23.2	47 (78)	17.6
$SR_{25\%}$	34 (49)	8.1	38 (51)	8.1	40 (49)	5.7	44 (59)	10.9	49 (58)	20.7	48 (54)	18.1	50 (84)	19.6	51(83)	15.7
$PRI_{25\%}$	32 (45)	5.1	46 (58)	10.7	46 (60)	10.5	44 (63)	12.9	47 (58)	19.8	47 (52)	17.8	42 (76)	21.4	50 (80)	19.2
$NDVI_{25\%}$	32 (51)	7.8	38 (51)	8.7	41(50)	6.2	44 (57)	10.4	49 (58)	20.7	48 (54)	18.1	50 (84)	19.6	53 (83)	15.7
$RNDVI_{25\%}$	33 (43)	5.5	46 (60)	8.8	48 (56)	6.8	49 (70)	10.4	49 (58)	20.6	46 (52)	18.2	50 (82)	21.1	53 (85)	17.8
$PSSRa_{25\%}$	35 (49)	7.6	38 (53)	8.7	39 (50)	7	44 (57)	10.6	49 (57)	20.9	48 (56)	18.2	48 (82)	21.1	50 (83)	17.1
$ONI_{25\%}$	38 (51)	8.3	44 (60)	10.1	43 (55)	7.3	41(52)	9	49 (58)	20.1	50 (56)	19.5	45 (70)	16.1	39 (68)	10.5
$DZNI_{25\%}$	35 (51)	4.8	38 (51)	10.5	37 (45)	10.1	45 (61)	11.1	49 (57)	19.9	48 (56)	18.2	48 (82)	16.4	50 (83)	19.9
Average Gain (%)		6.4		9.1		7		10.2		20.3		18.6		20.8		17
*Bootstrapped yields at a 95% confidence interval ($p < 0.0001$)																

Chapter IV:

Association Mapping of Yield, Grain Protein Content and Test Weight in Common Wheat

ABSTRACT

Genome wide association studies (GWAS) are an effective method of investigating the genetic basis underlying traits of interest in crops. In this study we identified loci significantly associated with yield, grain protein (GP) content, and test weight (TW) in a panel of spring wheat (SW) accessions from the National Small Grains Collection wheat core subset, and validate significant associations by allelic analysis in a panel of winter wheat (WW) accessions. The SW panel was evaluated in irrigated (IR) and drought (DR) treatments in 2011 and 2012, while the WW panel was evaluated in IR and DR treatments in 2013. A mixed linear model was used to identify significant associates using principal component analysis and a relationship matrix as covariates. Empirical significance thresholds were determined based on the significance of the associations between *RhtB1* and *VrnA1* with plant height and days to heading, respectively. In total, 36 loci were identified, 7 loci were associated with yield, 11 were associated with TW and 17 were associated with GP content in the SW panel. Analysis of the average yield, TW, and GP of accessions in both SW and WW panels that contained the significantly associated loci confirmed four of the yield loci, six of the TW loci, and six of the GP loci. The loci identified here should be studied further in additional germplasm to evaluate their value for breeding programs.

INTRODUCTION

Grain yield, protein content, and test weight are important agronomic traits in variety development of wheat and other crops. Selecting for these traits is confounded by environmental conditions (Reynolds et al., 2009; 2012). Identification of QTL underlying traits of interest has relied mainly on bi-parental mapping populations developed by crossing parental lines that exhibit phenotypic differences for the trait of interest. Genome wide association studies (GWAS) offer several advantages over traditional bi-parental mapping, including increased mapping resolution, decreased time requirements, and increased diversity of loci evaluated (Zhu et al., 2008).

GWAS have become the analysis of choice for investigating complex traits in many crop species, including sorghum, rice, barley, sunflower, and wheat, (Murray et al., 2009; Jin et al., 2010; Roy et al., 2010; Kloth et al., 2012; Mandel et al., 2013; Zhang et al., 2014). Association studies have been used to identify loci controlling agronomic traits in wheat. One of the first studies in wheat confirmed the locations of QTL controlling kernel morphology identified in previous bi-parental mapping studies and showed GWAS are suitable for investigating complex traits in wheat (Breseghello and Sorrells, 2006). A comprehensive analysis of quality traits in soft winter wheat found multiple significant marker-trait associations for kernel weight, grain protein content, flour sedimentation, test weight, and starch content (Reif et al., 2011a, b). Studies of pre-harvest sprouting resistance and falling numbers showed that specific traits could be targeted for analysis by GWAS (Jaiswal et al., 2012; Zhang et al., 2014). Environment-specific studies have been used in an attempt to identify QTL that influence yields in different irrigation regimes (Dodig et al., 2012).

Historic data was used in a meta-analysis study for identifying important loci associated with 20 different quality traits in a small population of bread wheat (Neumann et al., 2010). Taken together, GWAS have been applicable across a wide range of traits, mapping panels, and experimental designs

In conventional linkage mapping studies, large populations must be developed by crossing parental lines and advancing progeny for several generations before traits could be genetically mapped. In such studies the mapping resolution is often dependent on the size of the population and only two alleles per trait can be evaluated. In GWAS, population development is not required, panel size can readily be adjusted to achieve the desired resolution, and multiple alleles are evaluated in a single study (Zhu et al., 2008). GWAS exploit linkage disequilibrium to identify significant marker-trait associations, whereas bi-parental mapping relies on limited recombination events that occur during population development.

The stringency of association studies, combined with the evaluation of multiple alleles makes the identification of novel loci more difficult in GWAS than bi-parental mapping studies. This difficulty arises because the detected loci can only be found in significant proportion of the genotypes that make up the association mapping panel (Kloth et al., 2012). In practice, alleles and markers that have a minor allele frequency (MAF) within a mapping panel of less than 5% to 10% are excluded from analysis. Novel alleles, by definition, have not been knowingly used by breeders, are likely not found in a large percentage of genotypes, and may not to be found in a high enough proportion of genotypes to be detected by GWAS (Kloth et al., 2012). But, this also means that significant loci identified in GWAS will be

found in a number of genetic backgrounds equal to or greater than the MAF cut off, increasing the chance the loci identified will be applicable across a broader range of germplasm. In bi-parental mapping studies, the parental lines are often selected specifically for a single trait. Often one parent has a novel characteristic that is of interest to breeders, and the other is selected to maximize the phenotypic difference that will segregate among progeny. In this situation, bi-parental genetic mapping can identify novel rare alleles as long as it is found in one of the parental lines and there is sufficient phenotypic diversity to map the locus of interest. An additional difference between these two mapping strategies is that GWAS often underestimates the effects of identified QTLs and bi-parental mapping inflates the effects QTLs (Wang et al., 2012).

Avoiding spurious associations is a concern when conducting GWAS. Hidden population structure can significantly influence GWAS results. Factors such as allele frequencies, population admixture, population stratification, and the founder effect can influence the identification of significant marker-trait associations. While it is difficult to control those factors in panels used in GWAS without changing the panel constituents, researchers can control the molecular markers used within their study. The best markers to use in GWAS encompass the entire genetic diversity of their target organism. Molecular markers that show ascertainment bias can affect GWAS as a proportion of the genetic diversity found within the target organism is overrepresented and a proportion is underrepresented. Marker ascertainment bias can cause misrepresentation of panel structure in GWAS.

In this study we: (1) identify significant marker-trait associations for grain yield, grain protein content (GP), and test weight (TW) in a panel of spring wheat (SW) accessions; (2) evaluate the effects of high yield (HY), high test weight (HTW) and high grain protein (HGP) alleles in the SW panel; and (3) validate significant markers through allelic analysis of significant marker-trait associations in a separate panel of winter wheat (WW) accession.

MATERIALS AND METHODS

Plant Material, Field Design and Experimental Conditions

The plant materials used for this study are part of the USDA-ARS National Small Grains Collection (NSGC) wheat core subset. SW and WW GWAS panels each contained 540 individual accessions from around the world and consisted of landrace, cultivar, breeding line, and uncertain improvement status groups.

Trials were planted at the University of Idaho Aberdeen Research and Extension Center in Aberdeen, Idaho (42°57’36’’ N, 112°49’12’’ W, and elevation 1342 m). Plots were arranged in an augmented complete block design 20 plots wide and 30 plots deep for a total of 600 plots, as described by Zhang et al. (2014). Individual plots were 1.83 m long and 1.5 m wide and planted at a rate of 364,500 kernels per hectare. Irrigated (IR) and terminal drought (DR) treatments were planted adjacent to each other to minimize environmental effects each year. Each treatment was subdivided into early, medium, and late maturity blocks of 180 accessions and 20 checks based on days to heading. Maturity blocks were further divided into four sub-blocks so that each sub-block contained a single plot of each check. Across an entire

treatment, checks were arranged so that each of the 30 rows had two different checks and each of the 20 columns had three different checks.

A drip tape system was used for precise control of supplemental irrigation. Maturity blocks were independently controlled to ensure terminal drought conditions at the appropriate growth stage. Individual plots had three 1.83 meter sections of drip tape spaced every two rows. All plots were irrigated for a single 12 hour period each week until heading, at a rate of 2.5 l/h per 30.5 meters of drip tape. Information on climate conditions for Aberdeen Idaho is available through AgriMet (http://www.usbr.gov/pn/agrimet/webarcread.html).

Phenotypic Measurements

In all trials, individual plots were harvested after physiological maturity using a Wintersteiger Classic small plot combine equipped with a Harvest Master system (Wintersteiger Inc., Salt Lake City, UT). Yields were calculated from raw grain weight and converted to kilograms per hectare. Whole grain protein content (%) was measured by a Foss 6500 NIR Spectrometer with attached transport cell and test weights (kg/m^3) were measured using certified dry pint container.

Phenotypic Data for GWAS

Best linear unbiased predictors (BLUP) were calculated to account for environmental variation using JMP Version 11 statistical software (SAS Inst., 2011). Data measurements were adjusted for maturity blocks and days to heading by the restricted maximum likelihood (REML) models so that the correlation coefficients between BLUP and unadjusted values

were greater than 0.70. Both BLUP and unadjusted data were used in the analyses of marker-trait associations in the following sections.

Molecular Marker Analysis

Single nucleotide polymorphisms (SNPs) were assayed by the Illumina Infinium 9K iSelect platform (Illumina, Inc.) as part of the TCAP genotyping effort. The 9000 SNP markers used for genotyping were clustered and called using GenomeStudio as described by Cavanagh et al. (2013). SNPs were filtered to remove markers with minor allele frequencies (MAF) below 5% or more than 10% missing data. The remaining SNPs were ordered based on the most recent Consensus Wheat SNP Map (Cavanagh et al., 2013). Haplotype markers were derived by using the program TASSEL 4.0 with a sliding window of two SNPs and a single SNP step, as described by Bradbury, et al. (2007). Finally, haplotypes were filtered to remove markers with MAF below 5% or missing more than 10% data. In addition to SNP markers, a separate dataset added the RHT1 functional marker to our analysis.

Linkage disequilibrium (LD) was calculated using the 'Full Matrix LD' option in TASSEL for all marker and haplotype combinations. Significance of LD was determined using a $P < 0.05$. Marker pairs within the same linkage group that showed R^2 values higher than 0.75 were considered to have high levels of disequilibrium. The R^2 values of LD between marker pairs were plotted against genetic distance to determine the distance at which to expect linkage decay between SNPs and surrounding regions and to estimate the number of markers needed for adequate genome coverage with the accessions used in the present study

(Bradbury et al., 2007). Molecular markers not found within the consensus map were removed for estimates of LD, but were included for association analysis.

Population Structure and Kinship

Principal component analysis (PCA) was used to correct for hidden populations within our panels (Bradbury, 2007; Price et al., 2006). PCA was used as the "Q" matrix in general linear models (Q) and mixed linear models (Q+ K). Scree plots of the PCA results were used to determine number of (*K*) subpopulations present in our panel of accessions. Bi-plots of the PCA were used to visualize population structure and show the genetic variation explained by the first two principal components.

Kinship between accessions was calculated with all markers. In TASSEL the kinship matrix (K) is generated from a cladogram to visualize subpopulations, which is a measure of pairwise relatedness between accessions. Briefly, accessions were clustered through the neighbor-joining method creating a distance matrix (Saitou, 1987). The kinship matrix was derived by subtracting all distance matrix values from two, then scaling the smallest values to zero (Bradbury, 2007). The resulting matrix was used as 'K' in the mixed linear model Q + K analysis.

Association Mapping

A genome wide association study was initiated to identify loci associated with grain yield in the SW panel using single nucleotide polymorphism markers (SNPs). BLUP and unadjusted datasets were used for genome wide association analysis in TASSEL (Bradbury et

al., 2007). GLM (Q) and MLM (Q + K) were used to identify significant marker-trait associations. GLM accounted for population structure only, while MLM accounted for both population structure and relationship between accessions (Zhu, 2008). Comparison of GLM and MLM results allowed identification of significant markers found using both models.

Significance of the marker-trait associations were determined using Bonferroni adjusted *P* values and by empirically derived thresholds (Dudbrudge and Gusnanto, 2008). Empirical significant levels were determined using the reported genetic locations of *Rht-B1* dwarfing gene and *Vrn-A1* flowering time locus within the consensus SNP map constructed by Cavanagh et al. (2013). The consensus SNP map was used to assign genetic locations to all SNP markers. Traits were analyzed using both uncorrected data sets and as BLUP. Significance levels were set at $p < 0.001$ for GLM and at $p < 0.01$ for MLM. Significant SNP markers found to be associated with yield, test weight or grain protein were removed from further analysis if they did not pass both the GLM and MLM analysis significance thresholds.

Markers found to be significantly associated with yield, TW or GP were ordered and groups of significant markers were combined into loci by their genetic location. If there was a difference of greater than 3cM between adjacent significant markers, they were determined to be in separate significant loci. Loci were considered for further analysis if found in 2 or more of the SW trials.

Allelic Effects of Significant Loci in SW and WW Panels

Using the significant markers identified by GWAS in the SW panel, accessions from both the SW and WW panels were grouped into high-yielding (HY), low-yielding (LY), high-

grain protein content (HGP), low-grain protein (LGP), high-test weight (HTW), and low-test weight (LTW) allele classes. The average yield, GP, and TW of each allele class was compared in the SW and WW trials to validate the significant SW associations.

RESULTS:

Yield and Agronomic Traits

The SW panel mean yield was 5795.22 kg/ha and 5376.78 kg/ha in 2011 and 4503.95kg/ha and 3697.30 kg/ha in 2012; the WW panel mean yield was 6112.98 kg/ha and 5088.90 kg/ha in IR and DR treatments, respectively. The SW panel mean TW was 926.5 kg/m^3 and 911.61 kg/m^3 in 2011 and 890.0 kg/m^3 and 871.73 kg/m^3 in 2012; the WW panel mean TW was 881.5 kg/m^3 and 827.77 kg/m^3 in IR and DR treatments, respectively. The SW panel means for GP was 13.04% and 14.01 % in 2011 and 15.14% and 15.46 % in 2012; the WW panel means for GP was 16.58 % and 17.37 % in IR and DR treatments, respectively (Table 4.1).

Yields in the SW panel were skewed towards lower yields and TW was skewed towards higher TW in all trials (Supplemental Diagram 1). GP skewed towards higher values in the SW IR treatments in 2011 and 2012, as well as in 2012 DR, while slightly skewed towards low GP in 2012 IR. In the WW trials, yield and TW were skewed towards high TW while GP was skewed towards low in the IR treatment. In the DR treatment, TW and GP were skewed towards higher values and yield towards lower (Supplemental Diagram 1).

SNP and Haplotype Markers

Molecular markers were filtered to remove MAF below 5% (<0.05), or having greater than 10% (>0.1) missing data. After filtering, 5277 markers remained that identified 3001 unique loci across the wheat genome. Ordered markers were divided by linkage group and converted into 4658 haplotypes by TASSEL. Removal of haplotypes with MAF below 5% or missing more than 10% data left 2453 markers for further analysis. SNP markers without known chromosomal locations were included in our analysis as single markers, but were not used for designating haplotype markers.

Spring Wheat SNP Density and LD

Polymorphic SNPS were found on each of the 21 wheat linkage groups. Genome wide coverage of combined linkage groups was 3489 cM. The A genome had the largest coverage at 1404 cM, followed by the B genome at 1284 cM and the D genome at 801 cM. The B genome had the highest number of unique polymorphic markers at 1366. The A genome had 1318 polymorphic markers and the D genome had 317. The D genome had the largest mean distance between markers at 2.72 cM and the B genome the least at 0.98 cM (Supplemental Table 1).

Within the A genome, LG2A was the largest at 231 cM, LG1A had the most unique loci with 210. 3A was the shortest linkage group at 172 cM and LG4A had the lowest number of loci with 158. In the B genome, LG2B was the largest linkage group, at 272 cM, and also had the highest number of unique loci with 265. LG4B was the shortest at 124 cM and also had the smallest number of loci with 96. The D genome had several unconnected linkage

groups for LGs 3D, 5D, 6D and 7D based on the consensus SNP map constructed by Cavanagh et al. (2013). Of the three complete linkage groups, LG2D had the most loci with 76 across 192 cM and LG4D was the smallest at 102 cM and had the least number of markers with 30 (Supplemental Table 1).

Marker pairs having significant ($p < 0.05$) R^2 values higher than 0.75 were identified as having high levels of LD (Cavanagh et al., 2013). In our panel of accessions, SNP high LD blocks extended for an average of 1.09 cM and haplotype high LD blocks extended for 1.84 cM.

Population Structure and Kinship

Population structure was estimated using principal components. Scree plots of the number of principle components by eigenvalues showed two principle components were sufficient to explain most of the genotypic variance when using either SNP markers in both SW and WW panels. Bi-plots of the first two principle components also identified two clusters of genotypes that consisted mainly of either landrace accessions or breeding lines and cultivars (Fig. 4.1). Using the SNP dataset, the first principal component explained 9.6% of the genotypic variation and the second explained 4.5%.

The kinship matrix also distinguished two groups of accessions within our mapping panel (Fig. 4.2). Landrace accessions, for the most part, clustered together as did cultivars and breeding lines when the kinship distance matrix was viewed as a dendrogram. Landrace accessions clustered into three general groups based on their geographic region of origin, Africa, Southern Asia, and Western Asia. Most of the accessions of uncertain improvement

status clustered with the landraces from either Africa or Southern Asia (Fig. 4.2). Cultivars and breeding lines clustered into three groups of broader geographic regions than landraces. The first group was a mixture of accessions from North America and South America, the second was from Western Asia or Europe, and the third from Africa. A small group of accessions from multiple geographic regions clustered between the landraces and cultivars.

GWAS Significance Threshold

Bonferroni ($p < 1.89e^{-5}$) and False discovery rate (FDR) adjusted p-values did not identify any significant SNP marker-trait associations. Significance thresholds were derived using known height and flowering time related loci included within the wheat consensus linkage map. *Rht-B1* was located at 39cM on LG4B based on the consensus genetic map released by Cavanagh et al. (2013). *Rht-B1*-specific markers were significantly associated with plant height each year and in both IR and DR treatments. The p-values of *Rht-B1* specific markers ranged from $1.58e^{-12}$ in 2011 IR to $2.20e^{-7}$ in 2012 IR. The regions flanking *Rht-B1* were not significantly associated with plant height (Fig. 4.3). The most significant marker within 5cM of the *Rht-B1* locus had a p-value of $4.43e^{-3}$. Flowering time related loci *Vrn-A1* was also identified, but did not pass the Bonferroni adjusted significance thresholds. *Vrn-A1* is located on LG5A at position 120.1 cM to 124.0 cM within the wheat consensus map (Cavanagh et al., 2013). There were six SNP markers at the *Vrn-A1* locus that were consistently associated with DH in both treatments each year. P-values from this locus ranged from $3.07e^{-5}$ in 2012 DR to $6.62e^{-3}$ in 2012 IR (Fig. 4.3). Based on the significant levels of known height and flowering loci, marker-trait significance thresholds were set at 0.01, which

would encompass markers flanking *Vrn-A1*. Haplotype marker significance thresholds were treated in the same manner as single markers for subsequent analysis.

GWAS of SW Yield, GP and TW

Of the original 5277 markers, 303 markers were significant in both MLM and GLM, and were assigned genetic locations based on the wheat consensus map (Cavanagh et al., 2013). A final filtering step was used to remove loci that were not identified in multiple experiments leaving 212 significant marker trait associations with 103 markers across 35 loci. Of the loci identified by molecular markers, 24 loci were also identified by haplotype markers (Tables 4.2, 4.3 and 4.4).

Significant marker associations with yield were found on four linkage groups, LG2B, LG4A, LG5B, and LG6A (Table 4.2). On LG2B, three loci at 149cM, 157-158cM, and 160cM were found. The 2B1 locus was identified in all experiments except 2011 DR, 2B2 was found in 2012 IR and DR, and 2B3 was found in all SW trials. Significant loci on LG5B and 4A1 were found in 2011 IR and DR, while LG6A was identified in 2011 DR and 2012 IR (Table 4.2). Haplotype markers were found to be significantly associated with 6 of the 7 yield loci.

Loci on linkage groups LG2A, LG3B, LG4A, LG5A, LG5B, LG7A, and LG7B were associated with TW (Table 4.3). The 5A2 locus at 78cM was identified in all treatments each year; 2A1, 5A1, and 5B1 loci were found in 3 of the 4 SW trials; and 3B1, 5B2, 7A1, 7B1, 7B2, 4A1, 7B3 and 7B4 were found in 2 trials. Haplotype markers also identified 9 of the 11 test weight loci.

Loci associated with grain protein content were found on LG1A, LG2B, LG2D, LG3B, LG5A, LG5B, LG6A, LG6B, LG6D, and LG7A (Table 4.4). But, only 4 of the 17 loci were found to be significant in the 2012 DR treatment, 2B1, 3B1, 6A1 and 6B2. The loci 2B1, 3B1, 6B3, and 6D2 were found in 3 of the 4 SW trials. The 1A1, 2B1, 2B3, 3B2, 5A1, 6A1, 6A2, 6B2, 6B4, 6D2 AND 7A1 loci were found in 2 trials. Haplotype markers identified 9 of the 17 GP loci (Table 4.4).

Allelic Analysis of Yield, TW and GP

Comparison of the effects of the HY alleles on yield for SW and WW panels found that 5 of the 7 loci identified in the SW panel also showed higher yields associated with their respective HY allele in the WW panel, and 4 of these had yield increases in all SW and WW trials. The 2B1 locus had yield increases in the SW ranging from 2.47% in the 2011 DR to 7.17% in 2012 DR. In the WW panel, yield increases ranged from 8.18% to 39.86%. The 2B3 locus imparted increased yields from 1.9% to 16.45% in the SW panel and 2.21% to 11.74% in the WW panel. At 6A1, SW accessions with the HY allele had yield increases from 2.62% to 6.75%, while WW accessions had up to a 5.38% yield increase. The SW panel had yield increases from 8.91% to 19.36% attributable to the 6A2 HY allele, and the WW panel had 4.98% to 5.90% increases. HY loci at 2B2 and 5B1 were associated with increased grain yield in the WW panel. The single locus at 4A1 was not associated with increased yield in SW 2012 IR.

Within the SW panel, the largest increase in TW when comparing HTW and LTW alleles was associated with 7B2 with a TW increase of 3.37% followed by 5A2 in both 2011

and 2012 trials with TW gains of 2.64% and 2.69%, respectively (Table 4.3). Of the 11 significantly associated loci, six had HTW alleles that were associated with higher TW in all SW and WW trials. The HTW alleles of loci at 2A1, 3B1, 4A1, 5A1, 5B1, and 7B2 were associated with increases in TW from 0.2% to 3.37% in the SW panel and 0.76% to 4.11% in the WW trials (Table 4.3). The 5A2, 5B2, 7A1, 7B1 and 7B3 loci were found in the SW panel but were not associates with WW TW increases.

In the 2011 and 2012 SW trials, no loci were found to have significant marker trait associations with GP in both IR and DR treatments each year, but 8 of the 17 loci had HGP alleles associated with higher GP than their LGP counterparts in all SW trials (Table 4.4). In the 2011 SW trial, the largest GP increases were associated with alleles at 2B2, 5B1, 5B2 and 6D, while the 5B1 and 5B2 loci had the largest increases in GP associated with the HGP allele in 2012. In the WW panel the 5B1 HGP allele was also found to impart the largest increase in GP. Across both SW and WW trials, the HGP alleles at 1A1, 2B1, 5B1, 5B2, 6D1, 6D2, and 7A1 always had higher GP than their LGP alleles (Table 4.4). The 3B1, 6A1, and 6B1 loci did not impart increased GP.

DISCUSSION

Methodology

In this study we identify significant marker-trait associations in a diverse panel of spring wheat accessions selected from the NSGC core subset. Initial analysis of the SW molecular markers showed, on average, markers were spaced every 1.2cM across the entire genome. Analysis of genome-wide LD indicated that coverage was sufficient to identify

marker associations that were within approximately 1cM of the trait of interest (Flint-Garcia et al., 2003; Zhu et al., 2008). Population structure analysis indicated that to avoid spurious associations our analysis would need to account for two subpopulations (Zhu et al., 2008).

The criteria used here for designating significant markers attempts to reduce spurious associations or false positives. Q models and Q+K models were used together to eliminate marker-trait associations that were not significant in both analyses. The Q model is a less stringent analysis that accounts for population structure (Q) only. It is expected that Q models will have some spurious associations as it is a more sensitive method (Yu et al., 2006). The Q+K model is more stringent and accounts for both population structure (Q) and the kinship (K) within the mapping panel (Yu et al., 2006). A drawback of the Q+K model is that the stringency of the analysis decreases the ability to detect true marker-trait associations. Comparing GLM (Q model) and MLM (Q + K model) to identify true associations has been used previously in wheat association studies (Neumann et al., 2010) to designate significant loci. In addition to the two different methods of association analysis, data was analyzed as both BLUP and uncorrected. A final step used haplotype marker analysis as a way to support the significant loci identified by single markers, but not as grounds for removal from consideration. Haplotype marker analysis increased confidence that our association analysis identified true marker-trait associations. We found that using GLM or MLM, BLUP or unadjusted data, and haplotypes or single markers identified nearly identical loci associated with yield, grain protein, and grain test weight.

Significance Thresholds

To mitigate the weaknesses of MLM being too strict and GLM being too lenient, empirically derived significance thresholds were generated in the present study by using significance threshold values generated from loci with well-characterized phenotypes (plant height and flowering). FDR and Bonferroni adjustments are commonly used methods of designating significance thresholds in GWAS (Dudbrudge and Gusnanto, 2008). However, several studies have found FDR and Bonferroni adjusted p-values are often too stringent to identify marker-trait associations (Lu et al., 2010; Adhikari et al., 2011; Zhang et al., 2014; Dodig et al., 2012; Maccaferri et al., 2011). In the present study empirical thresholds were derived from plant height and heading related loci and used to identify significant associations.

Empirical significance thresholds were derived through association analysis of markers flanking *Rht-B1*and *Vrn-A1* loci. *Rht-B1* is a dwarfing gene that is found in many modern cultivars (Ellis et al., 2002). Cavanagh et al. (2013) reported that the *Rht-1B* locus exhibited high levels of geographic admixture which increased LD at this locus as a result of domestication of wheat (Flint-Garcia et al., 2003). Markers specific for *Rht-B1* (Ellis et al., 2002) were found to be highly associated with plant height in each experiment ($P < 1.0 \times e^{-6}$). In the present study, the markers flanking this dwarfing locus were not found to be significantly associated with plant height even though they were located within 1.5cM of the *Rht-B1* locus. This indicated that even with adequate genome coverage and increased LD we were unable to detect a large effect locus except through the use of gene-specific markers. Significant associations at the *Rht-B1* and *Rht-D1* locus were difficult to identify in previous

association studies in wheat (Neumann et al., 2010). Neumann et al. (2010) suggested that the inability to identify *Rht-D1* was due to low marker coverage of this linkage group in their study and that the only way to find *Rht-B1* was to raise the significance threshold to $P < 0.1$ in five of their nine years of data. But, there were still four years of data that were unable to identify *Rht-B1* at all. The study by Neumann et al. (2010) used only 96 accessions and thus likely had reduced ability to detect loci due to the small panel size. SNPs at the *Vrn-A1* locus, localized on the wheat consensus map by Cavanagh et al. (2013), were consistently associated with days to heading in all experiments. *Vrn-A1* is a vernalization response gene located on chromosome 5A that affects heading in spring wheat (Kamran et al., 2013; Zheng et al., 2013; Kiss et al., 2014). We found markers flanking *Vrn-A1* were unable to pass FDR or Bonferroni adjusted significance levels even though they were the most significant markers associated with heading. Due to the inability to detect significant associations at three large affect loci, we set significance levels to ensure inclusion of markers flanking these two loci at $p < 0.01$ for the MLM.

Analysis of Associations

The significant loci identified in this study were all located on chromosomes that have previously reported QTL for their respective trait. Previously reported QTLs related to these traits did not use the Illumina Infinium 9K iSelect platform (Illumina, Inc.), therefore it is difficult to make specific comparisons to studies that used other marker types. Regardless, the loci identified in this study may have been identified previously. Here, genetic locations are based on the consensus genetic linkage map published by Cavanagh et al. (2013) and the

location of QTL from previous studies is compared on a linkage group level. If a QLT identified in the present study is on the same linkage group as a previously identified QTL for the same trait, we conclude that it is possible we identified the same QTL. Without direct comparison of individual maps it is difficult to designate new QTL identified in this study. Here we compare studies that identified or further characterized QTL of interest on the linkage groups we identified.

Yield had the fewest significant marker-trait associations and the fewest loci identified. The six linkage groups with yield QTLs identified in the present study all had previously reported QTL. A significant locus on LG2B has been identified in two previous studies (Groos et al., 2003; Bennett et al., 2012a). Groos et al. (2003) mapped this locus in a recombinant inbred line population (F_7) consisting of 194 lines derived from a cross between a high yielding cultivar, 'Recital', and a high GP cultivar 'Renan'. In their study, the QTL identified on LG2B was located from 68cM to 162cM, on their genetic map, and explained 5.6% of the yield variation. Bennett et al. (2012a) also identified a locus on LG2B as being related to yield as it was found to affect thousand kernel weight in their double haploid mapping study. Here we found a QTL on LG2B in each of our four experiments within a smaller genetic interval. Yield related loci have been identified on LG4A in three previous studies (Groos et al., 2003; Kirigwi et al., 2007; Bennett et al., 2012a). Groos et al. (2003) identified a small effect locus in their study that explained 4-5% of the yield variation, but were unable to refine the map position on LG4A. The study by Kirigwi et al. (2007) was designed to identify drought tolerance loci in a bi-parental population. The locus on LG4A was found to influence grain yield, vegetative biomass, biomass growth rate, spike density

and drought susceptibility. A significant locus on LG5B was identified in two previous studies (Groos et al., 2003; Bennett et al., 2012a; b). The Groos et al. (2003) study found a small effect QTL that explained 4% to 7% of the yield variation located between markers at 85cM and 152cM in their genetic map. Bennett et al. (2012a) found a yield locus on LG5B that was only found in fully irrigated conditions. In the present study the locus on LG5B was identified in 2011 IR and DR environments. Yield loci on LG6A were identified in two studies previous (Groos et al., 2003; Simmonds et al., 2014). Simmonds et al. (2014) designed a study to look specifically at a locus on LG6A that had significant effects on yield and yield related traits such as spike number, spike density, and grains per spike. They determined LG6A was a major yield QTL affecting yield components and interacting with other yield or yield related QTL.

Yield related loci on LG4A and LG5B were identified in two association mapping studies (Neumann et al., 2010; Reif et al., 2011b). These loci were located at 8cM on LG4A and 68cM on LG5B in their mapping study. Both loci explained about 5% of the yield variation. The Reif et al. (2011b) study was conducted in a small set of 455 European soft winter wheat lines. Both of these yield loci were also identified by Neumann et al. (2010) that used a small mapping panel of 94 winter wheat lines. Their study indicated that the locus on LG4A was a multi-trait locus that also influenced plant height and harvest index. We did not find the locus on LG4A to be significantly associated with other traits. However, a locus influencing test weight was identified ~40cM away. The locus on LG5B was not associated with other traits, but LG5B had multiple QTL for grain protein content and a single locus for TW.

TW had the largest number of significant marker-trait associations at 84 on 11 individual loci across seven linkage groups. QTLs for test weight were identified on LG2A (Sun et al., 2008b, 2010), LG3B (McCartney and Somers, 2005; Sun et al., 2008b), LG4A (Sun et al., 2008b), LG5A (Sun et al., 2008b, 2010), LG5B (Groos et al., 2003; Sun et al., 2010), LG7A (Groos et al., 2003; Sun et al., 2010) and LG7B (Sun et al., 2008b). Each of these studies used bi-parental mapping populations. Test weight loci have been identified in a previous association study that used a panel of soft winter wheat, these include the loci identified on LG2A, LG3B, LG5A, and LG7A (Reif et al., 2011a). In the present study, individual loci did not explain more than 3% of the variation in TW.

Grain protein QTLs were identified at 17 loci on ten linkage groups. Loci were identified on LG1A (Groos et al., 2003), LG2B (Campbell et al., 2001; Prasad et al., 2003), LG2D (Prasad et al., 2003; Sun et al., 2008a), LG3B (Groos et al., 2003; Sun et al., 2008a), LG5A (Sun et al., 2008a; Xu et al., 2012), LG5B (Groos et al., 2003), LG6A (Groos et al., 2003; Sun et al., 2008a; Xu et al., 2012), LG6B (Joppa et al., 1997; Campbell et al., 2001; Prasad et al., 2003; Gupta et al., 2008) and , LG6D (Sun et al., 2008a) and LG7A (Prasad et al., 2003; Groos et al., 2003). Each of the studies used bi-parental populations to identify significant loci. The QTL identified in previous studies explained from 1% (Xu et al., 2012) to 32.44% (Prasad et al, 2003) of TW variation. The loci on LG1A1 and LG3B were identified in the Neumann et al., (2010) GWAS meta-analysis.

SW Specific Loci

Here we found several loci related to yield, TW and GP that were only found in the SW panel. Cavanagh et al. (2013) found significant differences between marker frequencies in SW and WW panels and only 11% of haplotype markers to have cross over in SW and WW populations. There were also 15 distinct genomic regions, across 6 chromosomes, which are thought to be responsible for adaption to winter and spring growth habits (Cavanagh et al., 2013). The 2B2 and 5B1 loci significantly associated with yield in our SW panel did not show an increase in yield in the WW panel, but are located 10cM and 20cM from reported growth habit adaption loci, respectively. Even more compelling, the TW loci 5A1 and 5B2 fall directly on growth habit adaption loci, and the 7B1 and 7B3 flank another with in a 29 cM interval. For the SW specific GP loci, 3B1 is within 4cM of a growth habitat adaption locus. Across all of the SW specific loci, only 6A1, 6B1 and 7A1 were not located near a growth habit adaption loci (Cavanagh et al., 2013).

Marker Annotations

Significant markers were further investigated for similarities to known genes or proteins. The USDA wheat SNP database (http://129.130.90.211/snp/) was used to query the NCBI reference protein and non-redundant nucleotide databases using the Basic Local Alignment Search Tool (BLAST, http://blast.st-va.ncbi.nlm.nih.gov/Blast.cgi). A majority of the markers hit unknown, hypothetical, predicted genes or predicted proteins from rice (*O. sativa*), corn (*Z. mays*) or sorghum (*S. bicolor*). Within the 103 individual markers identified in this study, only 12 hit annotated genes or proteins (Supplemental Table). In the group of

markers associated with grain yield, an RNA binding protein (*Rnp-1*) and PAP specific phosphatase were found on linkage groups 5B and 6A, respectively. For grain protein associated markers, a pre-mRNA splicing factor was found on LG3B, a chlorophyll a-b binding protein on LG6B, a polyadenylate-binding protein on 6D, a guanylate kinase gene on LG5B, and a putative Rh2 protein on LG6A. The significant markers associated with TW had two genes related to drought stress and starch synthesis. *LD1*, a starch de-branching enzyme that is responsible for creating starch granules from glucan was found on LG7B (Repellin et al., 2008) and an autophagy protein 9, that is expressed during drought stress, was identified on LG4A (Budak et al., 2013). In addition, a barley resistance gene analog and rice *GEP2* homolog were found on LG2A, an RND family efflux transporter on LG7A and molybdenum cofactor identified on LG7B (Supplemental Table 2).

CONCLUSIONS

Loci associated with yield, grain protein content, and test weight were discovered in the present study using a panel of spring wheat accessions selected from the NSGC core subset. Empirically derived significance thresholds, comparisons between GLM and MLM analysis methods, and comparisons between experiments were used to control for spurious associations. Using this approach, loci were identified on linkage groups with previously identified QTL for yield, grain protein content, and test weight. These loci may be the same as those identified in previous studies, and further analysis must be done to determine if the QTL identified here are novel.

Based on our GWAS results and allelic analysis, the most likely yield loci are on 2B1, 2B3, 6A1 and 6A2; the most likely TW loci are on 2A1, 3B1, 4A1, 5A1, 5B1, and 7B2; and 2A1, 5B1, 5B2, 6D1, 6D2, and 7A1 are the most likely GP loci. The present study was able to identify trait related loci that be used to improve current cultivars.

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Table 4.1: Average yields, grain test weight and grain protein content for spring wheat (SW) and winter wheat (WW) panels evaluated in irrigated (IR) and drought (DR) treatments in 2011, 2012 and 2013.

(A) Trt		SW 2011	Std Err	SW 2012	Std Err	WW 2012	Std Err
Yield (kg/ha)	IR	5795.22	50.06	4503.95	56.76	6112.98	50.06
	DR	5376.78	45.48	3697.3	49.13	5088.90	45.48
TW (kg/m^3)	IR	926.5	0.46	890.00	0.69	881.54	0.89
	DR	911.61	0.64	871.73	0.72	827.77	0.15
GP (%)	IR	13.04	0.08	15.14	0.06	16.58	0.06
	DR	14.01	0.08	15.46	0.07	17.37	0.06

Table 4.2: (A) SW trials in which loci were found to be significantly associated with yield. (B) Percent yield increase when selecting SW and WW accessions that carry the HY allele of markers significantly associated with yield in SW panel.

(A)	Linkage Group	Position cM	2011 IR	2011 DR	2012 IR	2012 DR
Yield	2B	149	X		X	X
	2B	157 - 158			X	X
	2B	160	X	X	X	X
	4A	90	X	X		
	5B	201 - 204	X	X		
	6A	110		X	X	
	6A	180		X	X	

(B)

LG	Pos cM	HY Allele	SW 11 IR	SW 11 DR	SW 12 IR	SW 12 DR	WW IR	WW DR
2B1	**149**	2	ng	ng	3.75	7.17	22.23	39.86
	149	0	5.94	2.47	3.30	2.83	8.18	ng
2B2 [h]	**157**	2	3.85	4.39	12.10	10.06	ng	ng
	158	0	6.81	6.66	14.77	8.66	ng	ng
2B3 [h]	**160**	0	4.34	1.90	4.23	0.86	11.74	11.32
	160	2	9.41	6.92	16.45	12.23	ng	2.21
	160	0	ng	3.18	5.99	8.80	2.83	7.00
	160	2	6.55	6.62	14.89	7.19	ng	ng
4A1[h]	**91**	2	1.41	0.86	ng	0.36	5.90	13.12
5B1 [h]	**202**	2	10.28	11.84	8.98	3.07	ng	ng
	204	2	9.56	9.58	10.63	5.27	ng	ng
	205	0	3.07	5.48	9.34	6.26	ng	ng
6A 1[h]	**110**	2	2.62	3.93	6.75	3.67	0.53	5.38
6A2	**180**	2	19.36	19.24	14.94	8.91	4.98	5.90

ng = no gain; ([h]) identified by haplotype markers; LG, linkage group; cM, centimorgan, SW, Spring Wheat; WW, Winter Wheat; IR, irrigated; DR, drought; and Associated allele '0' or '2'

Table 4.3: (A) SW trials in which markers were found to be significantly associated with TW. (B) Percent TW increase realized when selecting SW and WW accessions that carry the HTW allele of markers found to be associated with TW in SW.

(A)	Linkage Group	Position cM	2011 IR	2011 DR	2012 IR	2012 DR
TW	2A	30	X	X	X	
	3B	121 - 126	X	X		
	4A	50-52		X		X
	5A	11	X	X		X
	5A	78	X	X	X	X
	5B	59	X	X	X	
	5B	98	X	X	X	
	7A	51 – 53	X	X		
	7B	17		X	X	
	7B	46			X	X
	7B	55	X			X

(B)

LG	Pos cM	HTW Allele	SW 11 IR	SW 11 DR	SW 12 IR	SW 12 DR	WW IR	WW DR
2A1	**30**	2	0.22	0.84	1.64	1.21	2.3	4.11
	30	2	0.83	0.96	0.96	0.28	1.75	3.19
3B1 ^{h}	**122**	0	ng	0.77	0.81	0.13	0.4	1.62
	126	0	0.23	0.96	1.56	0.53	ng	ng
4A1 ^{h}	**50**	2	1.28	1.24	1.44	1.56	1.49	3.08
	52	0	1.53	1.50	0.78	0.82	ng	ng
5A1	**11**	2	0.29	0.30	ng	0.29	1.47	3.84
	11	0	1.33	0.78	0.15	1.50	1.33	3.15
5A2 ^{h}	**78**	0	2.64	1.06	0.84	2.33	/	/
	78	2	0.92	ng	0.65	2.69	ng	ng
5B1 ^{h}	**59**	0	0.43	0.76	ng	ng	ng	ng
	59	2	ng	ng	0.48	0.7	2.16	3.26
	59	2	0.71	1.41	0.35	ng	0.95	3.07
5B2 ^{h}	**99**	0	0.3	0.16	ng	ng	ng	ng
7A1 ^{h}	**51**	2	1.06	1.09	0.57	0.71	ng	ng
	53	2	0.79	1.46	0.51	ng	/	/
7B1 ^{h}	**17**	0	0.81	1.3	1.94	1.95	ng	ng
	17	2	0.76	1.45	1.74	1.43	ng	ng
7B2 ^{h}	**46**	2	1.12	1.36	1.26	3.37	0.76	1.32
7B3 ^{h}	**56**	2	0.51	0.3	1.02	0.64	ng	ng

ng = no gain; (^{h}) identified by haplotype markers; LG, linkage group; cM, centimorgan, SW, Spring Wheat; WW, Winter Wheat; IR, irrigated; DR, drought; and Associated allele '0' or '2'

Table 4.4: (A) SW trials in which markers were found to be significantly associated with GP. Percent GP increase realized when selecting SW and WW accessions that carry the HGP allele of markers found to be associated with GP in SW.

(A)	Linkage Group	Position cM	2011 IR	2011 DR	2012 IR	2012 DR
GP	1A	44		X	X	
	2B	158 - 163		X	X	X
	2B	211	X	X		
	2D	71	X	X		
	3B	88 - 89	X			X
	3B	127 - 128	X	X		
	5A	195	X	X		
	5B	59 - 61	X	X	X	
	5B	65		X	X	
	5B	68		X	X	
	6A	207	X			X
	6A	215	X	X		
	6B	22	X	X		
	6B	85 - 88	X		X	X
	6D	29 - 30	X	X		
	6D	116	X	X	X	
	7A	171		X	X	

Table 4.4 Continued…

(B)

LG	cM	HGP allele	SW 11 IR	SW 11 DR	SW 12 IR	SW 12 DR	WW IR	WW DR
1A1	**45**	0	2.54	3.12	2.76	1.77	2.18	1.16
2B1 [h]	**158**	2	3.46	5.91	4.15	0.54	ng	ng
	160	2	3.45	5.68	3.35	0.64	ng	ng
	163	2	1.76	0.41	0.2	2.05	1.07	1.43
2B2	**211**	0	10.64	16.03	6.24	ng	2.89	ng
2D1	**71**	2	1.79	2.8*	0.48	0.1	0.58	ng
3B1 [h]	**88**	0	5.14	3.68	2.56	0.8	ng	ng
	89	0	6.2	3.74	1.2	1.11	ng	ng
3B2	**127**	2	3.55	1.8	2.44	1.94	ng	ng
	128	0	4.33	3.0	ng	2.07	1.08	ng
5A1	**195**	0	4.51	4.34	0.99	ng	2.16	ng
5B1 [h]	**59**	2	2.74	4	ng	0.16	0.1	ng
	59	2	1.36	0.12	1.79	0.91	3.03	ng
	59	2	ng	ng	2.37	1.62	0.52	1.09
	59	0	15.17	15.7	8.11	6.62	9.48	4.08
	61	0	13.53	13.6	7.56	5.08	7.14	ng
5B2	**65**	0	14.12	14.99	6.12	2.75	4.13	2.02
5B3	**68**	2	0.58	ng	2.73	1.75	ng	1.09
6A1	**207**	0	4.15	2.93	ng	0.33	ng	ng
	207	0	3.28	1.74	ng	ng	ng	ng
6A2 [h]	**215**	2	1.73	2.33	1.64	ng	1.08	ng
6B1 [h]	**22**	2	2.5	0.49	2.48	2.06	ng	ng
6B2 [h]	**85**	2	0.39	ng	1.42	2.75	ng	0.46
	88	0	ng	ng	1.67	2.25	0.21	1.19
6D1 [h]	**29**	2	14.34	12.07	3.73	2.83	3.42	7
	30	0	9.28	8.59	2.24	0.36	1.97	5.08
6D2 [h]	**116**	0	4.59	5.48	3.9	2.48	ng	1.77
	116	2	5.22	5.86	3.83	3.01	0.77	1.81
7A1 [h]	**171**	0	8.43	7.32	1.82	1.49	1.53	0.46

Figure 4.1: Density biplots of the first two principal components of SW panel. Breeding lines and cultivars are indicated as red points, landraces are green points and accessions of uncertain origin are blue. Underlying shaded regions indicate density of accession clusters, with the darkest areas being the most dense.

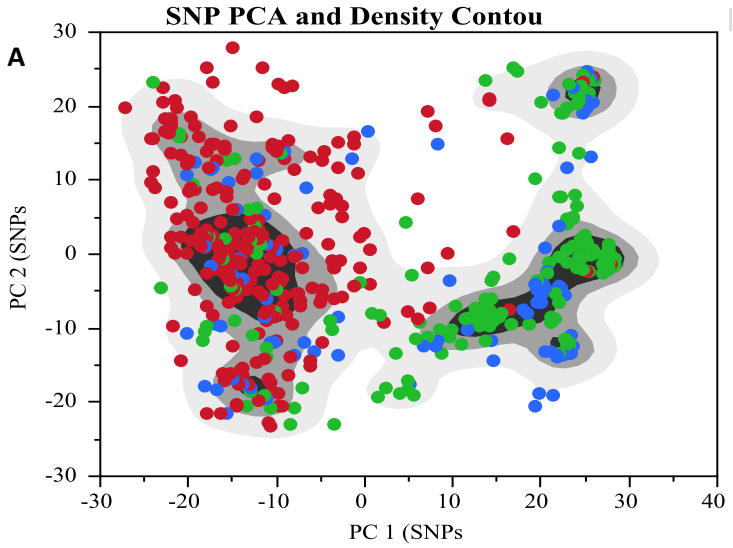


Figure 4.2: Relationship among accessions within the NSGC spring wheat core subset mapping panel using SNP markers represented by a cladogram. The indicated division is labeled as the dominant improvement status. Colored branches are labeled as the dominant region of origin.

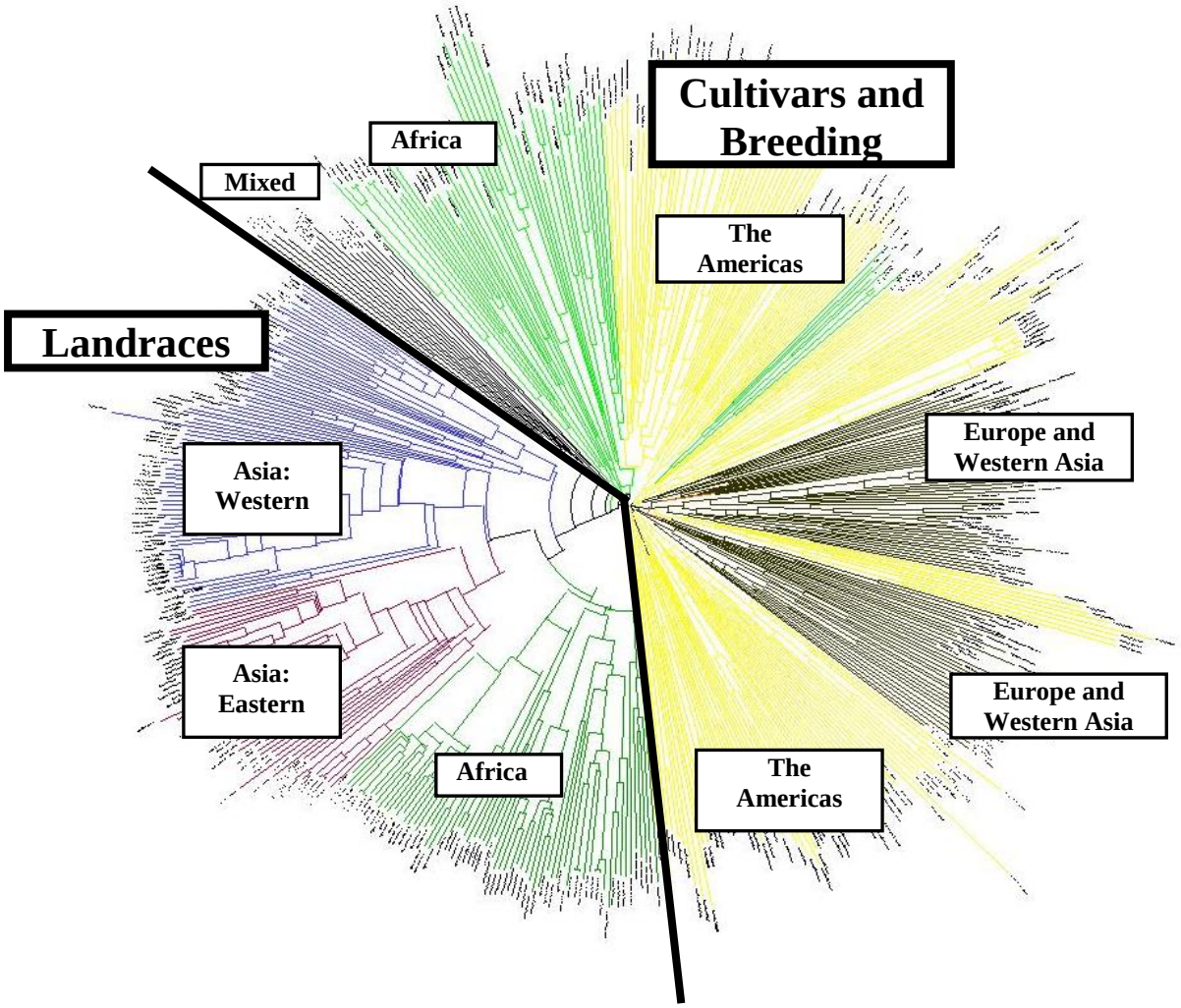
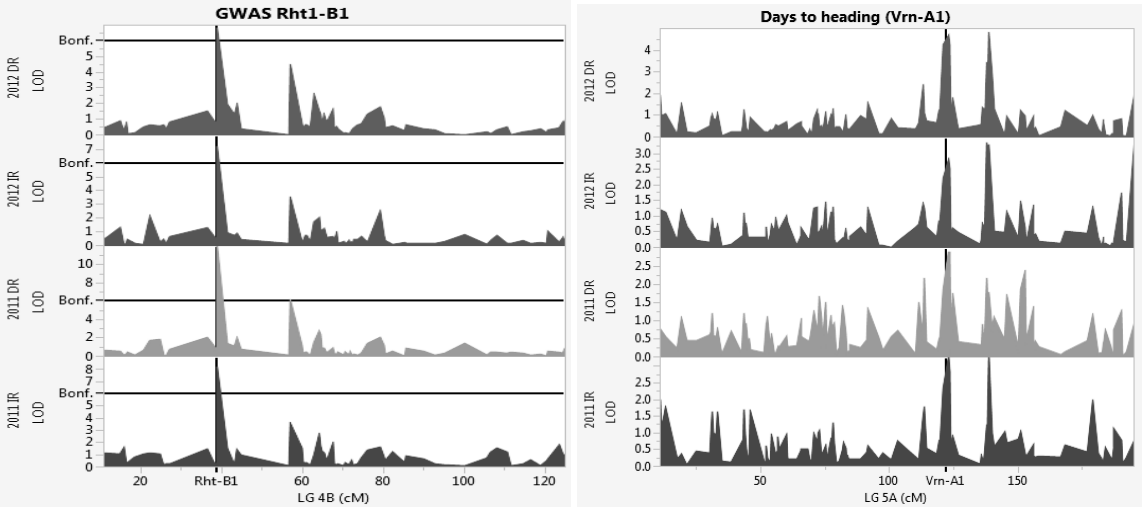


Figure 4.3: Locations of –log10 marker-trait significance levels for height across the entire length of LG4B and days to heading across LG5A. Bonferroni adjusted p-value threshold is shown (on 4B) and the locations of *Rht-B1*and *Vrn-A1*. Irrigated (IR) and drought (DR) treatments are shown for both 2011 and 2012.



Appendices

Chapter 2: Supplemental Table 1, High yielding drought tolerant accessions.

Table of yield, grain protein content (GP), and test weight (TWT) in irrigated (IR) and drought (DR) conditions for the 39 accessions found to be high yielding and drought tolerant. Drought susceptibility index = DSI Accession improvement status: Adv = cultivar or breeding lines, unknown = uncertain accessions, and LR = landrace. Wheat market classes: SRS = soft red sprng, HRS = hard red spring, HWS = hard white spring and SWS = soft white spring.

ACNO	Status	CLS	Avg Yield 2011	DSI 2011	Avg Yield 2012	DSI 2012	GP 2011 IR	GP 2011 DR	GP 2012 IR	GP 2012 DR	TWT 2011 IR	TW 2011 DR	TW 2012 IR	TW 2012 DR
PI324151	**Adv**	**srs**	6371.37	0.92	4133.66	-3.23	12.87	14.72	17.54	15.24	446.3	415.4	395.7	418.9
PI337710	**Adv**	**sws**	5598.34	-1.18	4223.17	-1.43	13.32	14.24	16.37	15.87	432.4	414	399.3	414.3
PI428528	**Adv**	**hrs**	6469.02	-2.63	4271.99	-0.11	12.35	14.48	14.76	15.29	441.5	443.6	405.7	403.6
PI519662	**Adv**	**hrs**	6794.5	-0.85	4345.23	0.32	11.26	14.65	15.67	15.16	452	447.5	420	439.9
PI268328	**Adv**	**hws**	6420.19	-1.91	4385.91	0.93	11.1	13.57	14.71	13.96	452.2	447.6	439.2	443.8
CItr14362	**Adv**	**srs**	6428.33	-1.09	4564.93	0.37	11.95	13.18	14.59	14.56	440.8	441.1	422.2	416.3
PI384036	**Adv**	**hrs**	7201.36	0.4	4743.95	-0.06	13.44	13.79	15.1	15.13	445.1	440.4	420	418.8
PI312115	**Adv**	**srs**	6102.85	-0.68	4890.41	0.68	13.69	15.05	14.97	16.37	456.2	440.3	431.6	429.7
PI520350	**Adv**	**hrs**	6045.89	-3.77	5012.47	0.42	13.37	15.49	15.93	15.34	455	445	432.9	449
PI351504	**Adv**	**hrs**	6094.71	0.33	5240.31	-1.45	14.35	12.35	14.43	13.43	441.7	448.4	418.6	415.9
CItr12881	**Adv**	**hws**	6054.02	0.87	5248.45	-0.68	11.19	13.05	14.42	12.29	437.9	443.2	432.5	434.7
PI584920	**Adv**	**sws**	6119.12	-0.45	5403.05	0.13	12.41	14.53	14.69	15.95	442.8	443.7	431.3	415.9
PI642362	**Adv**	**hrs**	6485.29	-0.82	5427.47	0.5	10.89	12.82	14.1	15.87	448.9	445.9	418.4	409.9
CItr17943	**Adv**	**hrs**	6127.26	-0.26	5476.29	0.02	14.44	15.89	14.53	15.24	443.5	447.1	434.7	437.8
PI642362	**Adv**		7071.17	-0.36	5606.48	-1.24	14.07	12.72	14.09	12.22	441	442.9	422.7	433
PI641733	**Adv**	**sws**	5801.77	-0.27	5671.58	-2.12	11.24	12.23	13.7	11.81	423.2	427.4	418	430.9
CItr14392	**Adv**	**hws**	6949.11	0.45	5704.13	0.87	10.9	12.77	13.69	14.33	438.1	441.6	429.6	426
PI428666	**Adv**	**sws**	5728.54	-0.89	5752.95	-0.76	10.36	12.24	15.08	12.97	444	439.5	416.8	426.7
PI520282	**Adv**	**hrs**	5736.68	0.12	5801.77	0.41	11.52	11.34	13.89	10.25	440.1	438.2	429.3	435.4
PI639458	**Adv**	**hrs**	6224.9	-2.57	5980.79	0.08	9.97	11.71	13.51	11.91	437.8	436.7	432.1	426.6
PI201414	**Adv**	**hws**	5940.1	-1.95	6029.61	-0.59	12.32	12.94	13.82	12.75	422.5	415	399.6	405.1
PI276705	**Adv**	**hrs**	5915.69	0.26	6119.12	-0.18	11.16	12.63	13.57	12.74	438.4	444	429.5	425.2
PI184575	**Adv**	**sws**	5964.52	-1.98	6208.63	-0.35	9.71	9.49	11.52	10.63	440.1	441.5	424.9	436
PI639455	**Adv**	**hrs**	6851.46	-1.45	6249.32	0.34	11.67	12.72	13.44	11.97	440.8	442.5	433.2	428.7
PI418575	**Adv**	**hrs**	7071.17	-2.02	7030.48	-1.33	10.7	13.85	13.53	13.8	440.1	444.5	430.4	428.2
PI625725	**LR**	**hws**	6745.68	-0.37	4426.6	0.66	13.23	12.23	13.43	14.95	408	427.3	419	395.5
PI477901	**LR**	**sws**	6290	-1	5101.98	0.65	15.75	15.63	15.99	15.53	419.9	427.9	415.3	410.5

PI624415	**LR**	**hws**	6119.12	-0.3	5150.8	0.12	12	14.84	14.12	16.84	440.9	442	431	413.2
PI173488	**LR**	**srs**	5630.89	-3.02	5256.59	-0.39	11.17	14.72	14.11	15.76	459.3	461.7	446.6	435.3
PI234385	**LR**	**srs**	6102.85	-1.15	6110.98	-0.04	15.73	15.53	16.93	13.72	426.9	442.7	428.4	441.5
PI24485	**LR**	**hrs**	6322.55	-0.32	6143.53	0.07	13.6	15.07	14.86	15.17	438	436.3	441.8	428.6
PI624426	**LR**	**srs**	6371.37	0.52	6273.73	-0.99	13.67	14.74	17.76	13.69	445.2	440.1	431.2	443.2
PI445696	**LR**	**hws**	6216.77	-2.02	6338.82	-1.26	11.11	13.35	13.66	11.45	455.3	460.1	448	453.5
PI137758	**LR**	**hws**	6737.54	-0.55	6371.37	-0.04	11.27	13.66	14.21	13.05	434	437.1	422.5	414
PI202828	**LR**	**hws**	7030.48	0.32	6932.83	-1.09	12.22	12.25	14.15	12.72	425.5	431.7	423.1	414.4
PI384374	**Unknown**	**hws**	6436.47	-0.04	4198.76	0.09	15.08	14.86	14.4	14.72	440.9	448.3	438.5	445.2
PI525284	**Unknown**	**hws**	5964.52	-1.9	4385.91	1	9.99	10.98	13.37	13.38	440.2	430.3	415.9	395.6
PI532060	**Unknown**	**sws**	6623.62	-0.91	5199.63	0.67	11.31	13.77	14.54	14.18	453.4	452	437.3	445.3
PI285819	**Unknown**	**srs**	6452.74	0.71	6542.25	0.48	13.74	13.1	14.27	12.94	450.2	450.5	439.9	441.8

Chapter 4: Supplemental Table 2: Distibution of SNP markers within the NSGC spring wheat core subset. SNP positions are based on the Consensus Wheat SNP Map by Cavanagh et al. (2013). Linkage group size, number of unique SNP loci; and largest, smallest and average gap sizes for individual linkage groups. Genetic distance units are shown in centimorgans (cM).

Linkage Groups	Size (cM)	Individual SNP loci	Mean Gap (cM)	Smallest Gap (cM)	Largest Gap (cM)
1A	183	210	0.88	0.02	10.4
2A	230.86	202	1.15	0.03	22.9
3A	172.28	192	0.9	0.06	10.89
4A	211.13	158	1.34	0.11	10.02
5A	195.83	165	1.19	0.02	10.28
6A	217.7	184	1.19	0.09	14.06
7A	193.82	207	0.94	0.02	8.96
1B	141.37	189	0.75	0.05	8.17
2B	272.12	265	1.03	0.03	10.25
3B	196.01	215	1	0.01	15.62
4B	124.94	96	1.32	0.17	11.93
5B	226.9	232	0.98	0.07	8.63
6B	154.05	206	0.75	0.05	9.36
7B	169.09	163	1.04	0.01	7.65
1D	145.36	75	1.96	0.18	40.58
2D	191.72	76	2.56	0.22	18.05
3D1	1.71	3	0.86	0.56	1.15
3D2	1.65	4	0.55	0.54	0.56
3D3	84.92	27	3.27	0.09	63.07
4D	101.5	30	3.5	0.37	31.64
5D1cult	54.01	14	4.15	0.31	39
5D2cult	15.52	3	7.76	4.5	11.02
5D3cult	48.4	17	3.03	0.29	16.36
6D1	8.49	13	0.71	0.15	2.75
6D2	77.66	24	3.38	0.04	21.45
6D3	7.78	9	0.97	0.28	1.96
7D1	6.59	4	2.2	0.53	4.94
7D2	55.34	18	3.26	0.49	8.56

Chapter 4: Supplemental Table 3

Significant markers identified for yield (Kg/ha), protein (PROT) and test weight (TWT). 'Trait _Exp' is coded as "Trait/Year/Treatment". Mixed linear model (Q + K; MLM_p) and General linear model (Q; GLM_p) significance levels, marker location (Genetic_dist) and BLAST descriptions are shown.

Trait	Trait_Exp.	Marker	MLM_p	GLM_p	ChrGen	Genetic_dist	Description (blastx refseq_protein) or (blastn nr/nt)
Kg/ha	Kg/ha2011T1	Ex_c26375_35620271	0.0067	0.000707	2B	149.356	Os04g0379900 [Oryza sativa Japonica Group]
Kg/ha	YieldBLUPT12012	Ex_c5363_9482943	0.005598	0.000112	2B	149.356	hypothetical protein SORBIDRAFT_06g013960 [Sorghum bicolor]
Kg/ha	YieldBLUPT12012	Ex_c26375_35620271	0.008984	0.000261	2B	149.356	Os04g0379900 [Oryza sativa Japonica Group]
Kg/ha	YieldBLUPT22012	be499362B_Td_2_1	0.002042	0.000549	2B	149.356	na
Kg/ha	Kg/ha2012T1	Ex_c128_255285	0.004889	0.000353	2B	157.1677	Os04g0496800 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2012T2	Ex_c128_255285	0.003816	0.000811	2B	157.1677	Os04g0496800 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2012T1	JD_c758_1132463	0.002383	2.39E-06	2B	158.0738	Os04g0503500 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2012T2	JD_c758_1132463	0.002302	7.94E-05	2B	158.0738	Os04g0503500 [Oryza sativa Japonica Group]
Kg/ha	YieldBLUPT12012	Ex_c2097_3932976	0.004082	0.000113	2B	160.0919	Os04g0507000 [Oryza sativa Japonica Group]
Kg/ha	YieldBLUPT12012	Ex_c41558_48356869	0.004082	0.000113	2B	160.0919	conserved hypothetical protein [Ricinus communis]
Kg/ha	YieldBLUPT12012	JD_c14405_14144807	0.004082	0.000113	2B	160.0919	hypothetical protein SELMODRAFT_127796 [Selaginella moellendorffii]
Kg/ha	YieldBLUPT12012	Ku_rep_c68888_68067293	0.004082	0.000113	2B	160.0919	predicted protein [Populus trichocarpa]
Kg/ha	Kg/ha2012T1	Ex_rep_c68587_67434960	0.001318	1.27E-06	2B	160.442	Os04g0503500 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2012T1	Ku_c3780_6950286	0.001374	2.02E-06	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Kg/ha	Kg/ha2012T1	Ex_c8894_14858193	0.001762	2.47E-06	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Kg/ha	Kg/ha2012T1	Ex_rep_c68386_67199155	0.001762	2.47E-06	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Kg/ha	Kg/ha2012T1	BG274019B_Ta_2_1	0.001782	1.12E-05	2B	160.442	na
Kg/ha	Kg/ha2011T2	Ku_c3780_6950286	0.003054	1.39E-05	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Kg/ha	Kg/ha2012T2	BG274019B_Ta_2_1	0.000506	0.000016	2B	160.442	na

Kg/ha	Kg/ha2011T2	Ex_c8894_14858193	0.004234	1.76E-05	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Kg/ha	Kg/ha2011T2	Ex_rep_c68386_67199155	0.004234	1.76E-05	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Kg/ha	Kg/ha2011T2	Ex_rep_c68587_67434960	0.009305	0.000068	2B	160.442	Os04g0503500 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2011T1	BG274019B_Ta_2_1	0.002744	0.000321	2B	160.442	na
Kg/ha	Kg/ha2012T2	Ex_c17700_26446810	0.000908	0.000415	2B	160.442	hypothetical protein SORBIDRAFT_06g021850 [Sorghum bicolor]
Kg/ha	YieldBLUPT22011	Ex_c8894_14858193	0.009468	2.63E-05	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Kg/ha	YieldBLUPT22011	Ex_rep_c68386_67199155	0.009468	2.63E-05	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Kg/ha	YieldBLUPT22011	Ku_c3780_6950286	0.009709	2.78E-05	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Kg/ha	YieldBLUPT22012	BG274019B_Ta_2_1	0.003911	8.48E-05	2B	160.442	na
Kg/ha	Kg/ha2011T2	Ku_c3081_5776947	0.006295	0.000821	4A	90.85943	Os03g0659400 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2011T2	Ku_c3081_5778117	0.006802	0.000992	4A	90.85943	Os03g0659400 [Oryza sativa Japonica Group]
Kg/ha	YieldBLUPT12012	Ku_c3081_5777013	0.001426	4.86E-05	4A	90.85943	Os03g0659400 [Oryza sativa Japonica Group]
Kg/ha	YieldBLUPT12012	Ku_c3081_5776947	0.001691	5.09E-05	4A	90.85943	Os03g0659400 [Oryza sativa Japonica Group]
Kg/ha	YieldBLUPT12012	Ku_c3081_5778117	0.001745	6.23E-05	4A	90.85943	Os03g0659400 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2011T2	Ra_c19660_28866961	0.008327	1.68E-05	5B	201.814	Os05g0207900 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2011T2	Ex_c7193_12354542	0.008921	1.84E-05	5B	201.814	Os05g0207900 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2011T2	Ku_c21465_31217980	0.008921	1.84E-05	5B	201.814	Os05g0207900 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2011T2	JD_c8978_9893945	0.000168	9.81E-08	5B	203.6357	RNA-binding region RNP-1 (RNA recognition motif):FAD linked oxidase, C-terminal:FAD linked oxidase, N-terminal [Fulvimarina pelagi HTCC2506]
Kg/ha	Kg/ha2011T1	JD_c8978_9893945	0.008286	2.88E-05	5B	203.6357	RNA-binding region RNP-1 (RNA recognition motif):FAD linked oxidase, C-terminal:FAD linked oxidase, N-terminal [Fulvimarina pelagi HTCC2506]
Kg/ha	YieldBLUPT22011	JD_c8978_9893945	0.001679	2.27E-06	5B	203.6357	RNA-binding region RNP-1 (RNA recognition motif):FAD linked oxidase, C-terminal:FAD linked oxidase, N-terminal [Fulvimarina pelagi HTCC2506]
Kg/ha	Kg/ha2011T2	JD_c12269_12546501	0.008597	9.25E-06	5B	204.0058	Os03g0835800 [Oryza sativa Japonica Group]

Kg/ha	YieldBLUPT22011	JD_c12269_12546501	0.005101	3.18E-06	5B	204.0058	Os03g0835800 [Oryza sativa Japonica Group]
Kg/ha	YieldBLUPT12011	Ex_c2870_5296539	0.003804	2.48E-06	5B	204.63	Os03g0836200 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2012T1	Ex_c34545_42832894	0.000682	3.40E-07	6A	110.2899	Os02g0702500 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2012T1	Ex_c34545_42833327	0.000682	3.40E-07	6A	110.2899	Os02g0702500 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2012T1	RFL_Contig2182_1514692	0.000682	3.40E-07	6A	110.2899	na
Kg/ha	Kg/ha2011T2	Ex_c34545_42832894	0.007629	3.91E-05	6A	110.2899	Os02g0702500 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2011T2	Ex_c34545_42833327	0.007629	3.91E-05	6A	110.2899	Os02g0702500 [Oryza sativa Japonica Group]
Kg/ha	Kg/ha2011T2	RFL_Contig2182_1514692	0.007629	3.91E-05	6A	110.2899	na
Kg/ha	YieldBLUPT22011	Ex_c16423_24920805	0.002012	3.63E-07	6A	180.1925	PAP-specific phosphatase [Zea mays]
Kg/ha	YieldBLUPT12012	Ex_c16423_24920805	0.001941	0.000335	6A	180.1925	PAP-specific phosphatase [Zea mays]
Prot	PROTBLUPT12012	Ku_c11769_19153951	0.000412	0.000022	1A	44.98814	hypothetical protein LOC100274440 [Zea mays]
Prot	PROTBLUPT22011	Ku_c11769_19153951	0.001343	0.000492	1A	44.98814	hypothetical protein LOC100274440 [Zea mays]
Prot	Prot2012T1	JD_c758_1132463	0.009958	3.83E-06	2B	158.0738	Os04g0503500 [Oryza sativa Japonica Group]
Prot	Prot2012T1	Ku_c3780_6950286	0.004715	9.38E-06	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Prot	Prot2012T1	Ex_c8894_14858193	0.005797	1.13E-05	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Prot	Prot2012T1	Ex_rep_c68386_67199155	0.005797	1.13E-05	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Prot	Prot2011T2	Ku_c3780_6950286	0.00589	0.000427	2B	160.442	hypothetical protein SORBIDRAFT_06g021790 [Sorghum bicolor]
Prot	Prot2012T2	Ex_c18261_27078080	0.001223	0.000415	2B	163.38	Os04g0578700 [Oryza sativa Japonica Group]
Prot	Prot2011T2	Ex_c45468_51254978	0.000329	1.75E-07	2B	211.067	hypothetical protein SORBIDRAFT_06g030930 [Sorghum bicolor]
Prot	Prot2011T1	Ex_c45468_51254978	0.001919	9.78E-06	2B	211.067	hypothetical protein SORBIDRAFT_06g030930 [Sorghum bicolor]
Prot	PROTBLUPT12011	Ex_c45468_51254978	0.000925	1.26E-07	2B	211.067	hypothetical protein SORBIDRAFT_06g030930 [Sorghum bicolor]
Prot	PROTBLUPT22011	Ex_c45468_51254978	0.001223	8.93E-07	2B	211.067	hypothetical protein SORBIDRAFT_06g030930 [Sorghum bicolor]
Prot	Prot2011T2	Ex_c1944_3664205	0.008118	0.000293	2D	71.34027	hypothetical protein SORBIDRAFT_02g041520 [Sorghum bicolor]

Prot	Prot2011T1	Ex_c1944_3664205	0.008004	0.0004	2D	71.34027	hypothetical protein SORBIDRAFT_02g041520 [Sorghum bicolor]
Prot	PROTBLUPT22012	Ex_c40060_47197713	0.002534	0.000635	3B	88.31186	Os01g0789000 [Oryza sativa Japonica Group]
Prot	PROTBLUPT22012	RFL_Contig3896_4291652	0.002534	0.000635	3B	88.31186	na
Prot	PROTBLUPT22012	JD_c19725_17732526	0.002549	0.000834	3B	88.31186	hypothetical protein LOC100275589 [Zea mays]
Prot	PROTBLUPT12011	Ex_rep_c69664_68618163	0.000534	0.000301	3B	88.51365	hypothetical protein SORBIDRAFT_03g037240 [Sorghum bicolor]
Prot	PROTBLUPT12011	Ku_c1391_2771050	0.000534	0.000301	3B	88.51365	hypothetical protein SORBIDRAFT_03g037240 [Sorghum bicolor]
Prot	Prot2011T1	JD_c2937_3905238	0.008677	0.000391	3B	127.4231	Os01g0927600 [Oryza sativa Japonica Group]
Prot	Prot2011T2	Ex_c13154_20784674	0.006303	5.03E-05	3B	127.8741	Oryza sativa (indica cultivar-group) partial mRNA for putative pre-mRNA-splicing factor cwc-22 (Os12g15420 gene)
Prot	Prot2011T2	Ex_c13154_20785032	0.006514	5.53E-05	3B	127.8741	Oryza sativa (indica cultivar-group) partial mRNA for putative pre-mRNA-splicing factor cwc-22 (Os12g15420 gene)
Prot	Prot2011T1	Ex_c2171_4074003	0.000476	4.84E-05	5A	195.0005	hypothetical protein LOC100193799 [Zea mays]
Prot	Prot2011T2	Ex_c2171_4074003	0.00546	0.000153	5A	195.0005	hypothetical protein LOC100193799 [Zea mays]
Prot	PROTBLUPT12011	Ex_c2171_4074003	0.000885	0.00068	5A	195.0005	hypothetical protein LOC100193799 [Zea mays]
Prot	Prot2012T1	Ex_c12431_19823475	0.007954	0.000839	5B	56.34129	hypothetical protein EcolC_1609 [Escherichia coli ATCC 8739]
Prot	Prot2012T1	RFL_Contig1809_946826	0.005634	0.000827	5B	59.25887	na
Prot	Prot2012T1	Ra_c2421_4647159	0.005651	0.000852	5B	59.25887	Os05g0558900 [Oryza sativa Japonica Group]
Prot	PROTBLUPT12012	RFL_Contig1809_946826	0.008368	7.31E-05	5B	59.25887	na
Prot	PROTBLUPT12012	Ra_c2421_4647159	0.008348	7.47E-05	5B	59.25887	Os05g0558900 [Oryza sativa Japonica Group]
Prot	PROTBLUPT12011	Ku_c46323_53087840	0.000374	0.000148	5B	59.25887	Oryza sativa Japonica Group Gmk gene for guanylate kinase
Prot	PROTBLUPT22011	Ku_c46323_53087840	0.003706	0.000163	5B	59.25887	Oryza sativa Japonica Group Gmk gene for guanylate kinase
Prot	PROTBLUPT12011	CAP7_c2086_1018815	0.006178	0.000412	5B	59.25887	na
Prot	PROTBLUPT12011	BF473658B_Ta_2_1	0.004215	0.000753	5B	59.25887	na
Prot	Prot2012T1	Ex_rep_c66881_65284662	0.003229	0.000451	5B	61.08055	Os07g0665700 [Oryza sativa Japonica Group]

Prot	PROTBLUPT12012	Ex_rep_c66881_65284662	0.004185	3.84E-05	5B	61.08055	Os07g0665700 [Oryza sativa Japonica Group]
Prot	Prot2011T2	Ex_c1630_3105100	0.002622	5.69E-05	5B	65.13033	na
Prot	PROTBLUPT22011	Ex_c1630_3105100	0.002035	9.31E-05	5B	65.13033	na
Prot	Prot2011T2	Ex_c6100_10676217	0.001337	6.75E-05	5B	67.53261	hypothetical protein SORBIDRAFT_02g026540 [Sorghum bicolor]
Prot	Prot2012T1	Ex_c6100_10676217	0.001888	0.000215	5B	67.53261	hypothetical protein SORBIDRAFT_02g026540 [Sorghum bicolor]
Prot	PROTBLUPT12012	Ex_c6100_10676217	0.000411	1.41E-06	5B	67.53261	hypothetical protein SORBIDRAFT_02g026540 [Sorghum bicolor]
Prot	Prot2011T1	Ex_c20352_29416468	0.009315	0.000799	6A	206.9033	Os02g0822800 [Oryza sativa Japonica Group]
Prot	PROTBLUPT22012	Ra_c39433_47141896	0.000844	0.000411	6A	207.3341	putative RH2 protein [Zea mays]
Prot	Prot2011T1	Ku_rep_c71567_71302229	0.001106	0.000016	6A	215.2325	Os02g0830100 [Oryza sativa Japonica Group]
Prot	Prot2011T2	Ku_rep_c71567_71302229	0.005186	7.90E-05	6A	215.2325	Os02g0830100 [Oryza sativa Japonica Group]
Prot	PROTBLUPT12011	Ku_rep_c71567_71302229	7.32E-05	1.58E-05	6A	215.2325	Os02g0830100 [Oryza sativa Japonica Group]
Prot	PROTBLUPT22011	Ku_rep_c71567_71302229	0.001124	0.000382	6A	215.2325	Os02g0830100 [Oryza sativa Japonica Group]
Prot	Prot2011T1	Ra_c20409_29673950	0.006177	0.000927	6B	21.7637	hypothetical protein SORBIDRAFT_04g000990 [Sorghum bicolor]
Prot	PROTBLUPT22011	Ra_c20409_29673950	0.006124	0.000203	6B	21.7637	hypothetical protein SORBIDRAFT_04g000990 [Sorghum bicolor]
Prot	PROTBLUPT12011	CAP11_c1724_940246	0.000709	1.71E-05	6B	79.99885	chlorophyll a-b binding protein 8 [Zea mays]
Prot	PROTBLUPT12011	Ex_c1319_2522682	0.001227	4.01E-05	6B	79.99885	Os02g0196300 [Oryza sativa Japonica Group]
Prot	PROTBLUPT12012	Ex_c6356_11055912	0.009554	0.000272	6B	83.88119	hypothetical protein LOC100274901 [Zea mays]
Prot	PROTBLUPT22012	Ex_rep_c69660_68614071	0.004723	0.000919	6B	84.50839	hypothetical protein SORBIDRAFT_01g021330 [Sorghum bicolor]
Prot	PROTBLUPT12012	Ex_c6356_11056222	0.00913	0.000263	6B	87.66318	hypothetical protein LOC100274901 [Zea mays]
Prot	PROTBLUPT12012	Ex_c6356_11056696	0.00913	0.000263	6B	87.66318	hypothetical protein LOC100274901 [Zea mays]
Prot	PROTBLUPT22012	Ex_c2854_5270318	0.004723	0.000919	6B	87.66318	na
Prot	Prot2011T1	Ex_c1690_3206784	0.007021	0.000653	6D	29.39409	polyadenylate-binding protein 2 [Zea mays]
Prot	PROTBLUPT22011	Ex_c1690_3206784	0.00526	0.000103	6D	29.39409	polyadenylate-binding protein 2 [Zea mays]
Prot	Prot2011T2	Ra_c13881_21836489	0.009715	3.73E-05	6D	29.78121	hypothetical protein SORBIDRAFT_04g027560 [Sorghum bicolor]

Prot	PROTBLUPT22011	Ra_c13881_21836489	0.002518	2.21E-05	6D	29.78121	hypothetical protein SORBIDRAFT_04g027560 [Sorghum bicolor]
Prot	Prot2011T2	Ex_c14691_22763609	0.009068	0.000155	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	Prot2011T1	Ex_c14691_22763753	0.00324	0.000277	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	Prot2011T1	Ex_c14691_22765150	0.00324	0.000277	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	Prot2011T1	Ex_c14691_22763609	0.003357	0.000406	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	PROTBLUPT22011	JD_rep_c50999_34772439	0.004018	2.29E-05	6D	116.253	Os02g0820000 [Oryza sativa Japonica Group]
Prot	PROTBLUPT22011	Ex_c14691_22763609	0.003083	3.18E-05	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	PROTBLUPT22011	Ex_rep_c69248_68171036	0.006546	5.45E-05	6D	116.253	Os02g0820000 [Oryza sativa Japonica Group]
Prot	PROTBLUPT12011	Ex_c14691_22763609	0.006193	9.05E-05	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	PROTBLUPT12011	Ex_c14691_22763753	0.007619	0.0001	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	PROTBLUPT12011	Ex_c14691_22765150	0.007619	0.0001	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	PROTBLUPT22011	Ex_c14691_22763753	0.007752	0.000136	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	PROTBLUPT22011	Ex_c14691_22765150	0.007752	0.000136	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	PROTBLUPT12012	JD_rep_c50999_34772439	0.001522	0.000413	6D	116.253	Os02g0820000 [Oryza sativa Japonica Group]
Prot	PROTBLUPT12012	Ex_c14691_22763609	0.002718	0.000807	6D	116.253	hypothetical protein SORBIDRAFT_04g037410 [Sorghum bicolor]
Prot	Prot2011T2	Ex_c6961_11998812	0.008319	0.000524	7A	171.3263	hypothetical protein SORBIDRAFT_10g024960 [Sorghum bicolor]
Prot	PROTBLUPT12012	Ex_c6961_11998812	0.002963	0.000503	7A	171.3263	hypothetical protein SORBIDRAFT_10g024960 [Sorghum bicolor]
TWT	TWT2012T1	Ex_rep_c67848_66550913	0.006144	4.87E-05	2A	29.57903	hypothetical protein SORBIDRAFT_06g028850 [Sorghum bicolor]
TWT	TWT2012T1	Ex_rep_c67848_66550913	0.006144	4.87E-05	2A	29.57903	hypothetical protein SORBIDRAFT_06g028850 [Sorghum bicolor]
TWT	TWT2012T1	Ku_c54793_58953037	0.006144	4.87E-05	2A	29.57903	hypothetical protein SORBIDRAFT_06g028850 [Sorghum bicolor]

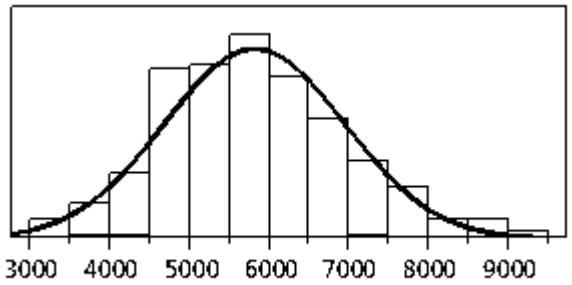
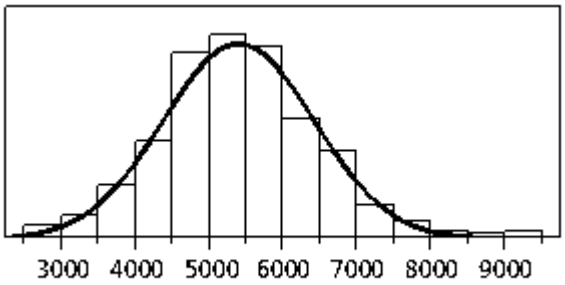
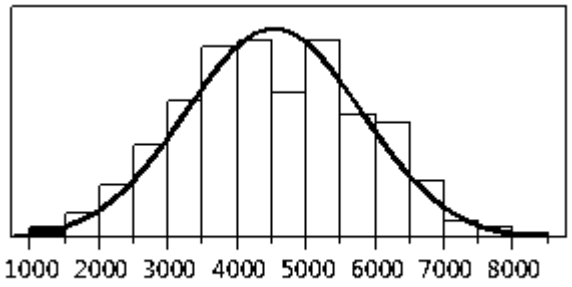
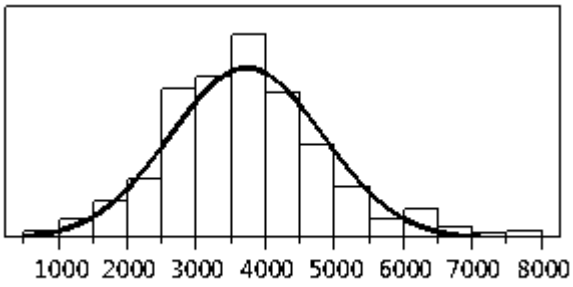
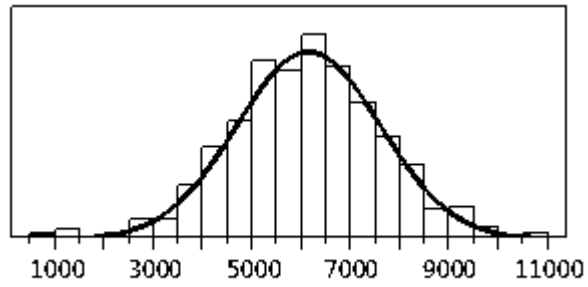
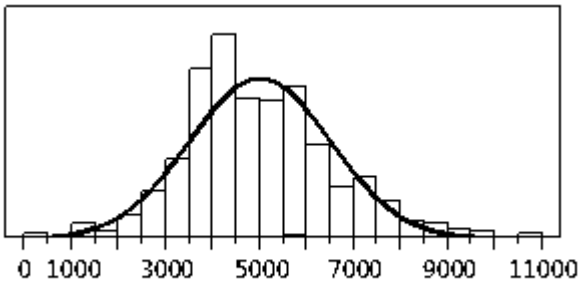
TWT	TWT2011T2	Ex_rep_c67848_66550913	0.005466	6.40E-05	2A	29.57903	hypothetical protein SORBIDRAFT_06g028850 [Sorghum bicolor]
TWT	TWT2011T2	Ku_c54793_58953037	0.005466	6.40E-05	2A	29.57903	hypothetical protein SORBIDRAFT_06g028850 [Sorghum bicolor]
TWT	TWT2012T1	Ex_c23768_33006588	0.000148	8.17E-05	2A	29.57903	Hordeum vulgare partial mRNA for NBS-LRR disease resistance protein homologue (rga S-217 gene)
TWT	TWTBLUPT22011	Ex_rep_c67848_66550913	0.001245	0.000242	2A	29.57903	hypothetical protein SORBIDRAFT_06g028850 [Sorghum bicolor]
TWT	TWTBLUPT22011	Ku_c54793_58953037	0.001245	0.000242	2A	29.57903	hypothetical protein SORBIDRAFT_06g028850 [Sorghum bicolor]
TWT	TWTBLUPT12011	Ku_c10196_16926860	0.003072	1.55E-06	3B	121.5238	Os01g0916300 [Oryza sativa Japonica Group]
TWT	TWT2011T1	Ku_c10196_16926860	0.005214	1.03E-05	3B	121.5238	Os01g0916300 [Oryza sativa Japonica Group]
TWT	TWT2011T2	Ku_c10196_16926860	0.008392	1.12E-05	3B	121.5238	Os01g0916300 [Oryza sativa Japonica Group]
TWT	TWTBLUPT22011	Ku_c10196_16926860	0.007762	5.55E-05	3B	121.5238	Os01g0916300 [Oryza sativa Japonica Group]
TWT	TWT2011T2	Ex_c11837_18996495	0.008993	5.99E-05	3B	126.2361	Os01g0923900 [Oryza sativa Japonica Group]
TWT	TWTBLUPT12011	Ex_c11837_18996495	0.004259	0.000227	3B	126.2361	Os01g0923900 [Oryza sativa Japonica Group]
TWT	TWTBLUPT22011	Ex_rep_c67799_66488792	0.002928	0.00084	4A	50.42882	Oryza sativa guanine nucleotide-exchange protein GEP2 (GEP2)
TWT	TWTBLUPT22012	Ex_c4220_7623030	0.001062	0.000019	4A	51.62826	Triticum aestivum mRNA for autophagy protein 9 (atg9 gene)
TWT	TWTBLUPT22011	Ex_c4220_7623030	0.003884	0.00037	4A	51.62826	Triticum aestivum mRNA for autophagy protein 9 (atg9 gene)
TWT	TWT2011T2	Ex_c4220_7623030	0.008194	0.000541	4A	51.62826	Triticum aestivum mRNA for autophagy protein 9 (atg9 gene)
TWT	TWT2011T1	BE399966A_Ta_2_3	0.002282	3.01E-05	5A	11.21989	na
TWT	TWTBLUPT12011	BE399966A_Ta_2_3	0.001896	9.65E-05	5A	11.21989	na
TWT	TWTBLUPT22011	Ku_c6125_10773757	0.003674	0.000142	5A	11.21989	Os02g0284500 [Oryza sativa Japonica Group]
TWT	TWTBLUPT22011	Ku_c6125_10773757	0.003674	0.000142	5A	11.21989	Os02g0284500 [Oryza sativa Japonica Group]
TWT	TWT2012T2	Ku_c6125_10773757	0.00075	0.000262	5A	11.21989	Os02g0284500 [Oryza sativa Japonica Group]
TWT	TWT2012T2	Ku_c6125_10773757	0.00075	0.000262	5A	11.21989	Os02g0284500 [Oryza sativa Japonica Group]
TWT	TWTBLUPT22011	Ex_c32825_41419391	0.000907	9.65E-06	5A	77.19494	GM11232 [Drosophila sechellia]
TWT	TWT2011T12	Ex_c32825_41419391	0.004079	3.56E-05	5A	77.19494	GM11232 [Drosophila sechellia]
TWT	TWTBLUPT12011	Ex_c32825_41419391	0.006707	0.000091	5A	77.19494	GM11232 [Drosophila sechellia]

TWT	TWTBLUPT12012	Ex_c32825_41419391	0.001211	0.000157	5A	77.19494	GM11232 [Drosophila sechellia]
TWT	TWTBLUPT22011	Ex_c3838_6981043	0.000704	1.14E-05	5A	77.52282	hypothetical protein SORBIDRAFT_02g028640 [Sorghum bicolor]
TWT	TWT2011T1	Ex_c3838_6981043	0.004534	0.000044	5A	77.52282	hypothetical protein SORBIDRAFT_02g028640 [Sorghum bicolor]
TWT	TWTBLUPT12011	Ex_c3838_6981043	0.006997	0.000118	5A	77.52282	hypothetical protein SORBIDRAFT_02g028640 [Sorghum bicolor]
TWT	TWTBLUPT12012	Ex_c3838_6981043	0.002571	0.000641	5A	77.52282	hypothetical protein SORBIDRAFT_02g028640 [Sorghum bicolor]
TWT	TWT2012T2	Ex_c3838_6981043	0.009636	0.000744	5A	77.52282	hypothetical protein SORBIDRAFT_02g028640 [Sorghum bicolor]
TWT	TWT2012T2	Ku_c14139_22353229	0.005403	3.38E-05	5A	77.85069	Triticum aestivum MBD2 mRNA
TWT	TWTBLUPT22011	Ku_c14139_22353229	0.001637	0.000783	5A	77.85069	Triticum aestivum MBD2 mRNA
TWT	TWT2011T1	Ku_c14139_22353229	0.006696	0.000951	5A	77.85069	Triticum aestivum MBD2 mRNA
TWT	TWTBLUPT22011	Ku_c6125_10773757	0.003674	0.000142	5B	31.28781	Os02g0284500 [Oryza sativa Japonica Group]
TWT	TWTBLUPT22011	Ku_c6125_10773757	0.003674	0.000142	5B	31.28781	Os02g0284500 [Oryza sativa Japonica Group]
TWT	TWT2012T2	Ku_c6125_10773757	0.00075	0.000262	5B	31.28781	Os02g0284500 [Oryza sativa Japonica Group]
TWT	TWT2012T2	Ku_c6125_10773757	0.00075	0.000262	5B	31.28781	Os02g0284500 [Oryza sativa Japonica Group]
TWT	TWTBLUPT12012	BF473658B_Ta_2_1	0.000899	1.20E-05	5B	59.25887	na
TWT	TWTBLUPT22011	CAP7_c2086_1018815	0.002108	6.48E-05	5B	59.25887	na
TWT	TWT2011T2	CAP7_c2086_1018815	0.001433	7.69E-05	5B	59.25887	na
TWT	TWTBLUPT12011	CAP7_c2086_1018815	0.006861	8.38E-05	5B	59.25887	na
TWT	TWTBLUPT22011	BF473658B_Ta_2_1	0.003179	9.16E-05	5B	59.25887	na
TWT	TWTBLUPT22011	JD_c38123_27754848	0.004492	0.000134	5B	59.25887	Os05g0334400 [Oryza sativa Japonica Group]
TWT	TWT2011T2	JD_c38123_27754848	0.001982	0.000147	5B	59.25887	Os05g0334400 [Oryza sativa Japonica Group]
TWT	TWT2012T1	BF473658B_Ta_2_1	0.008041	0.000356	5B	59.25887	na
TWT	TWTBLUPT22011	Ex_c7982_13546427	0.009828	0.000522	5B	59.25887	Os12g0502800 [Oryza sativa Japonica Group]
TWT	TWT2012T1	Ex_c47152_52446529	0.007634	0.00016	5B	98.62447	hypothetical protein SORBIDRAFT_02g029480 [Sorghum bicolor]
TWT	TWT2011T2	Ex_c47152_52446529	0.00615	0.000798	5B	98.62447	hypothetical protein SORBIDRAFT_02g029480 [Sorghum bicolor]
TWT	TWT2011T2	Ku_c29780_39658445	0.006768	0.000913	5B	98.62447	Os07g0139500 [Oryza sativa Japonica Group]
TWT	TWT2011T1	Ex_c8019_13598348	0.006065	1.78E-05	5B	181.7103	Os03g0809300 [Oryza sativa Japonica Group]

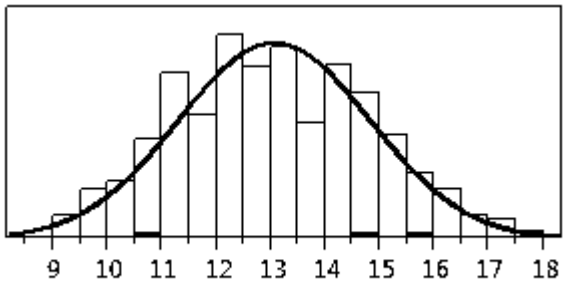
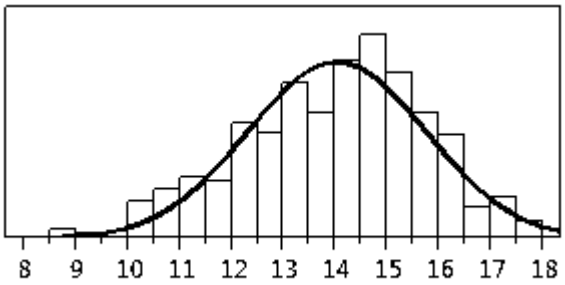
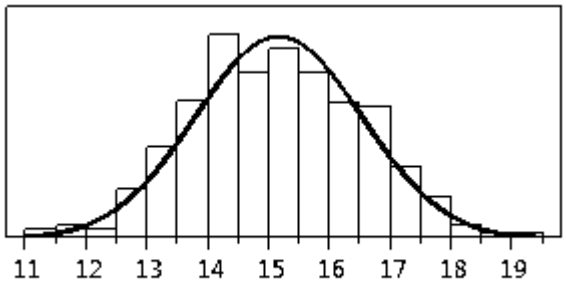
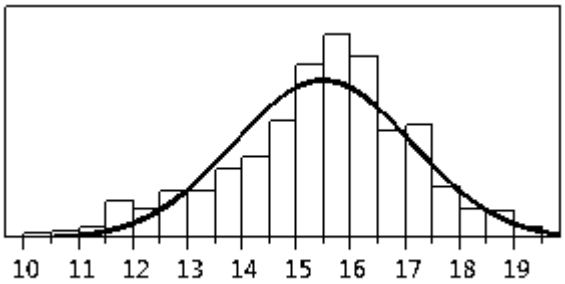
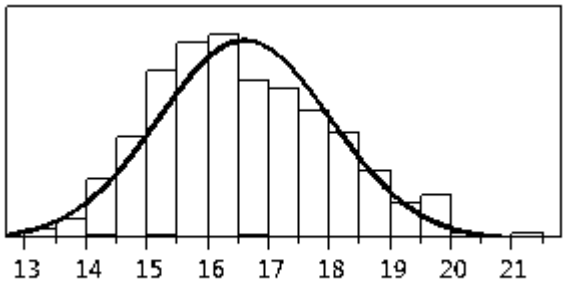
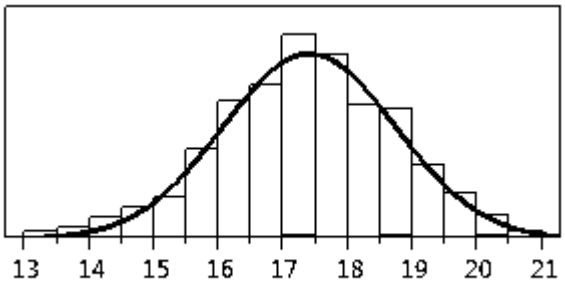
TWT	TWTBLUPT12011	Ex_c8019_13598348	0.007504	6.15E-05	5B	181.7103	Os03g0809300 [Oryza sativa Japonica Group]
TWT	TWT2012T1	Ku_c10377_17180909	0.000666	0.000485	6A	36.83932	hypothetical protein SORBIDRAFT_04g001010 [Sorghum bicolor]
TWT	TWT2011T2	Ku_c10377_17180909	0.007698	0.000975	6A	36.83932	hypothetical protein SORBIDRAFT_04g001010 [Sorghum bicolor]
TWT	TWTBLUPT12011	Ex_c20062_29096454	0.001109	6.36E-05	7A	51.17462	Os05g0255600 [Oryza sativa Japonica Group]
TWT	TWT2011T12	Ex_c20062_29096454	0.003521	0.000338	7A	51.17462	Os05g0255600 [Oryza sativa Japonica Group]
TWT	TWTBLUPT22011	CAP11_c1591_881161	0.002734	0.000137	7A	52.88501	Efflux transporter, RND family, MFP subunit [Rhodobacter sphaeroides KD131]
TWT	TWT2011T2	Ex_c24376_33618864	0.002621	4.02E-05	7B	17.07149	Triticum aestivum limit dextrinase type starch debranching enzyme (LD1)
TWT	TWT2011T2	Ex_c24376_33619527	0.008862	0.000132	7B	17.07149	Triticum aestivum limit dextrinase type starch debranching enzyme (LD1)
TWT	TWT2012T1	Ex_c24376_33618864	0.005166	0.00017	7B	17.07149	Triticum aestivum limit dextrinase type starch debranching enzyme (LD1)
TWT	TWTBLUPT22012	BE498662B_Ta_2_5	0.006769	2.33E-05	7B	46.80061	na
TWT	TWTBLUPT12012	BE498662B_Ta_2_5	0.004554	0.000143	7B	46.80061	na
TWT	TWT2011T1	Ex_c18800_27681277	0.002837	0.000199	7B	48.47886	molybdenum cofactor biosynthesis protein A [Aquifex aeolicus VF5]
TWT	TWT2011T1	JD_c9040_9947841	0.004137	0.000358	7B	48.47886	hypothetical protein SORBIDRAFT_10g014850 [Sorghum bicolor]
TWT	TWT2011T1	Ex_c27373_36578273	0.003315	0.000439	7B	48.47886	Os06g0326400 [Oryza sativa Japonica Group]
TWT	TWTBLUPT12011	Ex_c18800_27681277	0.004366	0.000963	7B	48.47886	molybdenum cofactor biosynthesis protein A [Aquifex aeolicus VF5]
TWT	TWT2011T1	RFL_Contig2167_1484520	0.003279	9.63E-06	7B	52.22267	na
TWT	TWT2012T2	RFL_Contig1735_856501	0.004438	0.000293	7B	54.0792	na
TWT	TWT2012T2	Ex_c3248_5987129	0.006799	0.000379	7B	54.0792	hypothetical protein SORBIDRAFT_05g009983 [Sorghum bicolor]
TWT	TWT2012T2	RFL_Contig3057_2961708	0.004092	0.000284	7B	55.46852	na
TWT	TWT2012T2	BE403622B_Ta_1_1	0.004438	0.000293	7B	55.46852	na
TWT	TWT2012T2	BE443396B_Ta_1_1	0.004438	0.000293	7B	55.46852	na
TWT	TWT2012T2	RFL_Contig3258_3294392	0.004438	0.000293	7B	55.46852	na
TWT	TWT2012T2	be591305B_Ta_1_1	0.00752	0.00043	7B	55.46852	na
TWT	TWT2011T1	be591305B_Ta_1_1	0.005033	0.000553	7B	55.46852	na

Chapter 4: Supplemental Diagram 1: Distribution of (A) yield, (B) grain protein and (C) test weight for spring and winter wheat panels with fitted normal distribution line.

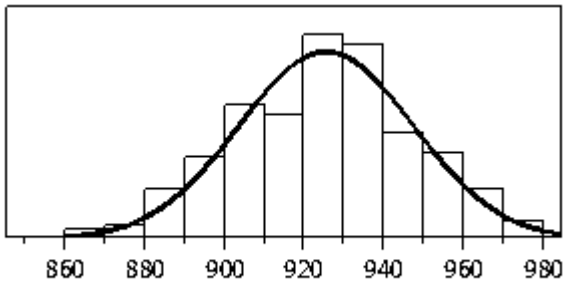
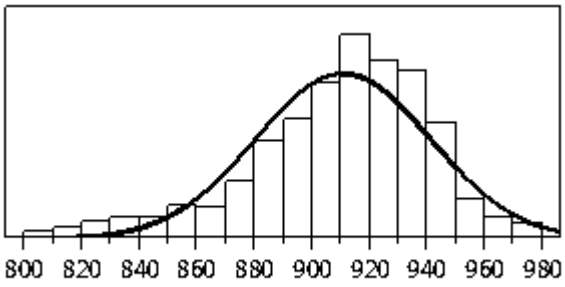
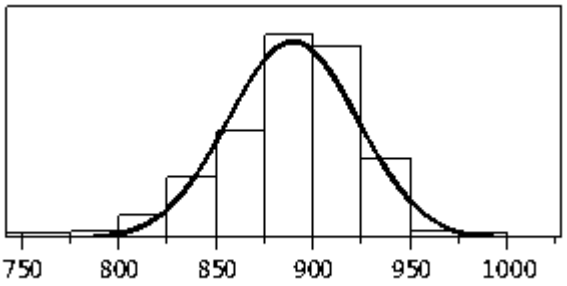
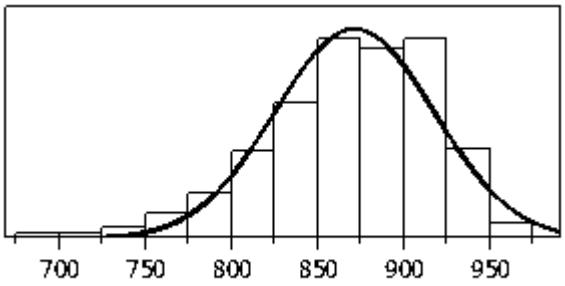
(A) Yield

Grain Yield (kg/ha) Irrigated (IR)	Grain Yield (kg/ha) Drought (DR)
2011 SW	**2011 SW**
	
2012 SW	**2012 SW**
	
WW	**WW**
	

(B) Grain Protein (%)

Grain Protein (%) Irrigated (IR)	Grain Protein (%) Drought (DR)
2011 SW	**2011 SW**
	
2012 SW	**2012 SW**
	
WW	**WW**
	

(C) Test weight (kg/m^3)

Test weight (kg/m^3) Irrigated (IR)	Test weight (kg/m^3) Drought (DR)
2011 SW	**2011 SW**
	
2012 SW	**2012 SW**
	
WW	**WW**