TRAIT IDENTIFICATION TO IMPROVE YIELD AND NITROGEN USE EFFICIENCY IN WHEAT

by

Blake Russell

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Agronomy

West Lafayette, Indiana

May 2020

THE PURDUE UNIVERSITY GRADUATE SCHOOL

STATEMENT OF COMMITTEE APPROVAL

Dr. Mohsen Mohammadi, Chair

Department of Agronomy

Dr. Jianxin Ma

Department of Agronomy

Dr. Mitchell Tuinstra

Department of Agronomy

Dr. Cankui Zhang

Department of Agronomy

Dr. Clay Sneller

Department of Horticulture and Crop Science, Ohio State University

Approved by:

Dr. Ronald F. Turco

To my family who always encouraged me and supported me.
Thank you for all the love and prayers.

ACKNOWLEDGMENTS

I wish to thank my advisor Dr. Mohsen Mohammadi for giving me this opportunity and believing in me. I am forever grateful and appreciative of your encouragement, mentorship, and guidance. Your daily pursuit on this project and enthusiasm was motivation for me. To my committee members, Drs. Jianxin Ma, Mitchell Tuinstra, Cankui Zhang, and Clay Sneller, thank you for the guidance and feedback on my projects and education. For past and present members of the Mohammadi lab, I was very fortunate and lucky to work and serve with such a diverse group of individuals. The love and support you all showed helped shape this project and my life.

I want to give thanks to the Purdue Department of Agronomy for providing the resources and environment in support of my graduate experience. The staff served humbly and invested their time and efforts for the success of graduate students. Without the assistance from Jim Beaty and the crew at the Purdue Agronomy Center for Research and Education (ACRE) farm, this project would not have been successful. I am thankful to Jason Adams at the Indiana Corn and Soybean Innovation Center (ICSC) and Eugene Glover at the ACRE farm for field assistance. I want to thank the USDA Small Grains Genotyping Facility and Dr. Gina Brown-Guedira for performing genotyping of the TCAP germplasm. I am thankful for Dr. Clay Sneller for providing the TCAP germplasm to be studied for this project. I am thankful to Dr. Shaun Casteel for providing fertilizer recommendations and Dr. Jim Camberato for soil analysis for this project. I am thankful to Dr. Carlos Guzman for wheat quality analysis and Nicole de Armond for nitrogen analysis.

Last but not least, I am thankful to my parents, wife, family, and all the friends I made along the way. To my Mom, Dad, my wife Jenny, and sister Taylor, you always believed in me and encouraged me to pursue my dreams. You were there every step of the way. I am thankful for the rest of my family for the encouragement and lessons instilled in me that led to this achievement. I am very thankful for the friendships of Rupesh Gaire, Miguel Lopez, and Seth Tolley. Rupesh, it was my humble pleasure to work alongside you every day. My friend Miguel, thank you for the encouragement and motivation. Seth, I cannot thank you enough for the help and support. I am humbled by all of the graduate students and colleagues that have developed into life-long friendships, I am blessed by all the experiences we shared and will cherish for the rest of my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Wheat is a major source of calories and protein for humans worldwide. Wheat is the most widely grown crop, with cultivation areas and production systems on every continent. The cultivated land area is vast because of its importance and adaptability to various environmental conditions. Global wheat production has not kept up with the growing population, provoking the need to develop new methods and techniques to increase genetic gains. The first research chapter of this Ph.D. dissertation involves performing genome-wide association studies (GWAS) to identify and examine transferability of marker-trait associations (MTAs) across environments. I evaluated yield and yield components traits among 270 soft red winter (SRW) wheat varieties. The population consists of experimental breeding lines adapted to the Midwestern and eastern United States and developed by public university breeding programs. Phenotypic data from a two-year field study and a 45K-SNP marker dataset were analyzed by FarmCPU model to identify MTAs for yield related traits. Grain yield was positively correlated with thousand kernel weight, biomass, and grain weight per spike while negatively correlated with days to heading and maturity. Sixty-one independent loci were identified for agronomic traits, including a region that with $-logP$ of 16.35, which explained 18% of the variation in grain yield. Using 12 existing datasets from other states and seasons, in addition to my own data, I examined the transferability of significant MTAs for grain yield and days to heading across homogenous environments. For grain yield and days to heading, I only observed 6 out of 28 MTAs to hold up across homogenous environments. I concluded that not all marker-trait associations can be detected in other environments.

In the second research chapter of this Ph.D. dissertation, I dissected yield component traits under contrasting nitrogen environments by using field-based low-throughput phenotyping. I characterized grain yield formation and quality attributes in soft red winter wheat. Using a split-block design, I studied responses of 30 experimental lines, as sub-plot, to high nitrogen and low nitrogen environment, as main-plot, for two years. Differential N environments were imposed by the application, or lack thereof, of spring nitrogen application in a field, following a previous corn harvest. In this study, I measured agronomic traits, in-tissue nitrogen concentrations, nitrogen use efficiency, nitrogen harvest index and end-use quality traits on either all or subset of the germplasm. My data showed that biomass, number of spikes and total grain numbers per unit area were most sensitive to low nitrogen while kernel weight remained stable across environments. Significant

genotype x N-environment interaction allowed me to select N-efficient germplasm, that can be used as founding parents for a potential breeding population specifically for low-N environments. I did this selection on the basis of superior agronomic traits and the presence of the desirable gluten quality alleles such as *Glu-A1b* (*2**) and *Glu-D1d* (*5+10*).

CHAPTER 1. LITERATURE REVIEW

1.1 Importance of wheat in world food security

The three major cereals that provide almost half of total plant calories consumed by humans are maize, rice, and wheat (Tweeten and Thompson, 2009). Wheat is a staple food for over 35% of the world's population (Bushuk, 1997a). The starch content and the ease of milling or grinding wheat grain into flour makes wheat a major source of carbohydrates (Enghiad et al., 2017). Gluten, which is the viscoelastic storage protein complex important in baking, makes wheat as a the main component of bread, cakes, and cookies (Pena, 2002). In 2018, 736 million metric tons of wheat was consumed globally, with China and India as top consumers. The United States consuming 30 million metric tons (~4%). In 2018-2019 season, 731 million metric tons of wheat was produced. The top producers were Europe, China, India, Russia, United States, and Canada. The United States produced 51.3 million metric tons (~7%) (USDA, 2019). The FAO reports global cereal production is expected to increase by 13% by 2027, mainly due to higher yields, and global human and animal consumption is projected to increase. For example, humans' wheat consumption is expected to increase 13% by 2027.

1.2 Wheat speciation

Wheat is estimated to have been cultivated around 7,000 – 10,000 years ago in the Fertile Crescent (Dubcovsky and Dvorak, 2007; Gill and Friebe, 2002; White and Edwards, 2007). Wheat is an annual plant from the genus *Triticum*. Based on chromosome number, the wheat species can be divided into three main ploidy levels of diploids (with one set of chromosomes 2n = 2x = 14), tetraploids (with two sets of chromosomes 2n = 2x = 28), and hexaploids (with three sets of chromosomes 2n = 6x = 42) (McFadden and Sears, 1946). The single set in the diploids is the A genome; the additional set in the tetraploids is the B genome; and the third set in the hexaploids is the D genome (McFadden and Sears, 1946). *Triticum monococcum L. spp aegilopoides* and *Triticum urartu*, also known as wild einkorn wheat, is the progenitor of cultivated diploid wheat (Feldman, 2001; Peng et al., 2011). *Triticum turgidum L. ssp. dicoccoides*, also known as wild emmer wheat, is the progenitor of cultivated tetraploid and hexaploid wheat (Feldman, 2001; J. H. Peng et al., 2011; Peng et al., 2003). Each polyploidy

species is the product of interspecies hybridization followed by chromosome doubling (Feldman, 2001). Seven chromosome pairs of the diploid wheat *T. urartu* (A genome = AA) plus seven additional chromosome pairs of the B genome constitute the 14 pairs of *T. turgidum* (AABB). These 14 pairs plus the additional 7 pairs of the D genome make up the 21 pairs of hexaploid *T. aestivum* (AABBDD) (Feldman, 2001). Therefore, the hexaploid wheat is an allopolyploid, meaning the genome contains three homologous sub-genome sets of A, B, and D.

The two primary cultivated *Triticum* species are durum wheat (*Triticum turgidum*) and bread wheat (*Triticum aestivum*). Durum wheat is tetraploid (2n=4x=28 = AABB) with 14 pairs of chromosomes (Feldman, 2001). Bread wheat is hexaploid (2n=6x=42 = AABBDD) with 21 pairs of chromosomes (Gill and Friebe, 2002) and is responsible for approximately 95% of the total world wheat production (Peng et al., 2011). Even though bread wheat is hexaploid, during meiosis it behaves as a diploid species (Feldman, 2001; Riley and Chapman, 1958). The homeologous pairing suppressor (*Ph1*) gene on chromosome 5B restricts pairing and crossovers to occur only between homologous chromosomes. Pairing and crossovers cannot occur between homeologous chromosomes (Dvorak et al., 2006; Griffiths et al., 2006).

1.3 Wheat domestication syndrome

The phenotypic differences between cultivated and wild wheat is a suite of traits, called domestication syndrome. The main domestication syndrome changes are the brittle rachis, tenacious glume and non-free threshability of wheat, which also impacted yield components (Gill et al., 2007; Peng et al., 2011). Mutations affecting primary traits were the loss of spike shattering and loss of tough glumes (Dubcovsky and Dvorak, 2007). For example, the diploid progenitors lack the free-threshing of the spike as observed in current tetraploid and hexaploid wheat (Kerber and Rowland, 1974; McFadden and Sears, 1946).

The brittle rachis trait is described as the breakage of the rachis (main stem of spike) that causes the seed to be dispersed by the shattering of the spikelet. The group 3 brittle rachis genes, *Br1, Br2,* and *Br3,* are single, dominant genes located on the short arm of chromosomes 3D, 3A, and 3B, respectively (Chen et al., 1998; Watanabe and Ikebata, 2000; Watanabe et al., 2002). Transition to non-brittle (normal) rachis (*br*) was one of the underlying genetic changes of wheat domestication. The *Q* gene in wheat, located on each of the homeologous group 5 chromosomes, confers free-threshing (Gill et al., 2007; Simons et al., 2006), and allows for grain to be

mechanically harvested. The free threshing wheats have glumes that are thinner and allow for easier release of kernels (Peng et al., 2011). The *Q* gene is a member of the *APETALA2 (AP2)*-like (WAP2) gene family (Faris et al., 2003), which is responsible for floral homeotic gene regulation. Free threshing phenotype is controlled by the dominant *Q* allele (Simons et al., 2006). The wild wheat cultivars consisted of tough glumes that were difficult to thresh for grain retrieval, while the cultivated wheats have soft glumes and free threshing ability (Peng et al., 2011). The *tenacious glumes (Tg)* gene, located on chromosome 2D, affects glume tenacity (threshability) (Jantasuriyarat et al., 2004; Kerber and Rowland, 1974). The recessive mutations at the *Tg* loci display the physical appearance of hull-less wheat spike (Dubcovsky and Dvorak, 2007). The complementary mutations of *q* to *Q* and *Tg* to *tg* give rise to the free-threshing and threshable forms of hexaploid wheat - *QQtgtg* (Dubcovsky and Dvorak, 2007; Jantasuriyarat et al., 2004; Kerber and Rowland, 1974). Based on the major gene controlling these traits, wheat chromosomes 1B, 2A, and homeologous chromosomes 3 and 5 played major roles in modification of domesticated wheat.

1.4 The underlying traits that allow wide geographical adaptation

Wheat is adapted to diverse growing regions and conditions. Wheat occupies 22% of the total cultivated area around the world (Leff, Ramankutty, & Foley, 2004). This includes the Great Plains of the United States, southern Australia, eastern Africa, southern South America, China, and throughout Europe. For example, wheat was harvested in 127 countries in 2017 (FAOSTAT) and regional distributions show wheat is the major crop in Canada, western Europe, Russia, Middle East, central Asia, and Australia (Leff et al., 2004). Wheat is widely adapted to diverse geographical regions because of the adaptive mechanisms to different seasons and temperatures. Two of these mechanisms control the cold exposure requirements before transitioning to flowering, often called vernalization requirements, and the control of flowering time via photoperiod responses.

The genetic system controlling vernalization requirements is rather complex involving epistasis. The dominant *Vrn2* allele and recessive *vrn1* allele are required for the expression of true winter growth habit, and spring wheat is confirmed by any of the dominant spring type *Vrn1* alleles that decreases vernalization requirements (Tranquilli and Dubcovsky, 2000; Yan et al., 2004) .The *Vrn1* loci are on the three homeologous chromosomes. However, *Vrn-A1* is more

potent to *Vrn-B1* and *Vrn-D1*, meaning that a recessive *vrn-A1* requires longer vernalization than any of the *vrn-B1* or *vrn-D1* does. The *Vrn1* and *Vrn2* genes are located on the long arm of chromosomes 5A, 5B, and 5D (Barrett et al., 2002; Dubcovsky et al., 1998; Nelson et al., 1995) and *Vrn3* on the short arm of chromosome 7B (Yan et al., 2006). The dominant *Vrn3* allele confers early flowering and is an orthologue to the *Arabidopsis FLOWERING LOCUS T (FT)* gene (Yan et al., 2006). Recently, it was shown that the vernalization requirements is more complex and also under the control of copy number variation (Díaz et al., 2012; Würschum et al., 2015; Zhu et al., 2014). After fulfillment of vernalization requirements, the transitioning to reproductive stage is controlled by photoperiod response genes. The transitioning to reproductive stage in wheat occurs upon extended exposure to sunlight. The major photoperiod response genes (*Ppd*) are located on the homoeologous group 2 chromosomes (Snape et al., 2001) and members of the pseudo response regulator gene family (Beales et al., 2007). Among all Ppd loci, the semi-dominant photoperiod insensitive *Ppd-D1a* allele experiences earlier spike growth and stem elongation, resulting in earlier flowering (Snape et al., 2001).

1.5 Plant architecture: drivers of source-sink and harvest index

Wheat plant height changed drastically in the 1960s, during the 'Green Revolution' which resulted in high yielding, semi-dwarf varieties. Reduction in plant height enabled applying more fertilizers and increased yield (Borlaug, 1983; Hedden, 2003). Tall wheat varieties typically fall over, or lodge, due to wind, rain, or an unsupportive stem. Peng et al. (1999) determined that interfering with plant hormone gibberellin is the mode of action of the *Reduced height-1 (Rht)* genes. Two dwarfing *Rht-B1* and *Rht-D1* loci on chromosomes 4B and 4D reduce plant height via sensitivity to gibberellin (Gale and Youssefian, 1985; Pearce et al., 2011; Peng et al., 1999). In addition to these gibberellic acid sensitive genes, there are other height reducing genes that act in gibberellic acid insensitive manner. For example, the height reducing allele *Rht8c allele* on the short arm of chromosome 2D (Guedira et al., 2010) is present in several United States soft and hard wheat breeding lines.

Decreasing plant height allowed more assimilates to be partitioned to produce more grain and increase harvest index. Harvest index is a direct measure of the source-sink ratio (Reynolds et al., 2017). Austin (1980) first proposed the theoretical limit for wheat harvest index at approximately 60%. Recent reviews have shown that genetic improvement has made minimal

progress since the 1990s, with harvest index for wheat maintaining between 50-55% (M. John Foulkes et al., 2011). In wheat, increases in harvest index is said to be driven by increases in the number of grains produced under similar canopy structures (Green et al., 2012). Genetically, the alien chromatin introgression of *Lr19* from *Agropyron elongatum* is associated with an increase in total biomass, more partitioning of biomass to spike growth, and an increase in radiation use efficiency (Reynolds et al., 2001). Harvest index is also affected by environment. For example, higher temperatures, carbon dioxide levels, and light intensity can affect photosynthetic activities and assimilates (Balota et al., 2017; Reynolds et al., 2012; Wheeler et al., 1996).

Another trait that has contributed to grain numbers per unit area is tillering. Wheat has the capacity to tiller or form new lateral branches that are independent of the main stem. The tillers can develop to grow spikes, reach maturity, and contribute to producing more spikes and grains per plant. The development of tillers is a key factor in plant architecture in wheat, as the tillers are formed by the growth of axillary buds from the basal internodes (Spielmeyer and Richards, 2004). The downside and potential drawback of increase tillering is the production of infertile tillers, because assimilates are distributed to these tillers that are competing with other sinks, but fail to contribute viable grain for increasing yield (M. John Foulkes et al., 2011). It appears that semi-dwarfism is also associated with more tillering, resulting in more grain filled heads (Borlaug, 1983). A single recessive major gene, tiller inhibition gene (*tin3*), was mapped to the long arm of chromosome 3A, which confers only one main culm, larger spikes, and greater seed size (Kuraparthy et al., 2007).

1.6 Major cropping systems worldwide

By the year 2035, wheat is predicted to have the greatest increase in global sown area in comparison to rice, maize, and soybean (W. Wu et al., 2007). Wheat cultivation occurs intensively in Europe, North America and Asia. In Europe, wheat is almost cultivated across the entire continent, most of which is winter wheat. Cropping shares for wheat are projected to increase in northern Europe (Elsgaard et al., 2012). In North America, two major wheat belts are responsible for most of the wheat production: west of the Mississippi River and spanning into southern Canada, and the Great Plains (Leff et al., 2004). Canada has a long history of growing wheat throughout the country, developed multiple classes of wheat including Canadian Western Red Spring, Canadian Western Soft White Spring, Canadian Western Amber Durum, Canadian

Prairies Spring Red, and others. Southwestern Saskatchewan practices conventional and conservative tilling practices, along with crop rotations of continuous wheat and fallow-wheat rotations (Zentner et al., 1991). In areas with high risk of soil erosion and drought, the most profitable management was a minimum or no tilling system incorporated into a fallow-wheat rotation (Zentner et al., 1991). Canada promotes organic agriculture. More research and emphasis is currently being investigated for breeding wheat in organic cropping systems (Kaut et al., 2009; Mason and Spaner, 2006). Mason et al. (2007) compared conventional management and organic management practices for Canadian Western Spring wheat cultivars grown in Alberta. They found that the major limiting factor for wheat grain yield in organic managed systems is weed pressure. In Asia, wheat is grown predominately in the Indus River valley in Pakistan, the Yellow River Valley in China, and most of central Asia. The Punjab province in Pakistan occupies 75% of the total wheat production in the country and frequent management is a rice-wheat irrigated rotation (Aujla et al., 2010). In the Indo-Gangetic Plains of South Asia, the rice-wheat agronomic system covers over 13.5 million hectares across Bangladesh, India, Nepal, and Pakistan (Ladha et al., 2009). In Chinse provinces of Jilin and Liaoning, common crop rotations are maize-wheat-soybean rotations and to the west it progresses into a maize-wheat cultivation area (Leff et al., 2004). In the Middle East, wheat is cultivated from Turkey, Iran, and along the Mediterranean coast. In this region, the main driver of yield and performance is the availability of water. An estimated 20-30% of wheat is irrigated, with the remaining in rainfed or semi-arid conditions (Pala et al., 2011). In Turkey, crop rotations with winter wheat include lentils, chickpea, sunflower, and fallow to increase productivity (Cayci et al., 2009).

Besides the intensive growing regions, wheat is also grown in South America, Africa, and Australia. In South America, wheat dominates the south and creates another wheat belt in Argentina and Chile (Leff et al., 2004). Wheat is grown in the northern parts of Africa in Morocco, Algeria, Libya, and Tunisia and in south Africa as a second crop following maize.

In Australia, wheat is the dominant crop and forms a wheat belt. In southern and western Australia, the major limiting factor for wheat yields is the availability of water, where crop experiences water stress and unfavorable high temperatures during the grain filling period (Hamblin et al., 1987; Luo et al., 2009).

1.7 Major cropping systems and market classes in the United States

In the United States, wheat production and market are based on seasonality of planting and end-uses. Five major wheat classes exist in the US including hard red winter, hard red spring, soft red winter, soft red spring, and durum wheat. Kansas dominates wheat production, followed by North Dakota and Washington. In the Northern Plains, spring wheat is predominately grown. Montana, Minnesota, North Dakota, and South Dakota account for approximately 20% of the hard red spring wheat production (USDA ERS, 2019). The primary winter wheat production region covers 16% of the United States and includes the central Midwest, the central and northern Great Plains, and the Pacific Northwest (Brown & Rosenberg, 1999). In the Great Plains, hard red winter wheat is produced, while soft red winter wheat is grown in the eastern states and along the Mississippi River. Winter wheat contributes to approximately 70% of the US wheat production, accounting for 1,100 million bushels (USDA, 2019). Winter and spring soft white wheat is a niche market predominately grown in Washington, Michigan, and New York. Durum wheat is the least produced class and accounts for only 3-5% of total wheat production and is grown mainly in Montana and North Dakota (USDA ERS, 2019). Hard grains are used for bread making, soft red grains are used for cakes and cookies, soft white grains are used for noodle products, cereals, and white breads, and durum wheat is used for pasta.

1.8 End-use quality traits

End uses are determined by grain hardness, protein content, and gluten strength. Grain hardness, or endosperm texture, defines whether the grains are for bread making or cookie making (Pasha et al., 2010). Grain hardness analysis can be performed by the Single Kernel Characterization System (SKCS) to classify wheat as soft, medium, or hard grain. In U.S. eastern soft red wheat, typical grain hardness index averages around 23-24 (Kiszonas, Fuerst, & Morris, 2013). In contrast, the hardness index of hard red winter and spring wheat averages between 58 – 70 (Martin et al., 2001; Morris et al., 1999). The variation of kernel texture is genetically controlled by the single *Hardness* (*Ha*) locus (Chantret et al., 2005), located on chromosome 5D and contains three genes i.e., puroindoline a *(Pina),* puroindoline b *(Pinb),* and grain softness protein-1 (*GSP-1*) (Pasha et al., 2010). Soft wheat, which contains minimum if any damaged

starch, is used to make cakes, cookies, and crackers where low flour water absorption is desired (Bacon, 2001).

The wheat grain can be structurally divided into three components: bran (seed coat), endosperm, and embryo. The proportion of each component on average is 14% bran, 83% endosperm, and 3% embryo (White and Edwards, 2007). The endosperm contains the storage proteins and starch that are milled for production. Gluten comprises 75-85% of the total wheat endosperm protein (Branlard and Marion, 2001; Pena, 2002) and consists of polymeric and monomeric proteins of glutenins and gliadins. Glutenins are storage proteins that are classified as high-molecular weight glutenin subunits (HMW-GS) and low-molecular weight glutenin subunits (LMW-GS). HMW-GS loci are located on the long arm of chromosome 1A, 1B, and 1D: *Glu-A1, Glu-B1,* and *Glu-D1* (Branlard and Marion, 2001). LMW-GS loci are located on the long arm of chromosome 3A, 3B, and 3D: *Glu-A3, Glu-B3,* and *Glu-D3*. Sodium dodecyl suplhate (SDS) sedimentation test is usually conducted to determine glutenin amount/strength in wheat flour.

Gliadins are alcohol-soluble proteins that represent almost 50% of gluten proteins (Branlard and Marion, 2001). In general, gliadins are less understood than glutenins. Gliadins are insignificant and non-effective for dough quality, formation, and swelling. The α-gliadins are widely known to be the most relevant for the development of celiac disease, controlled by the *Gli-2* loci on the short arm of group 6 chromosomes (Payne, 1987). Celiac disease is an autoimmune disorder that develops from the ingestions of gluten containing grains, such as wheat, barley, and rye. The disease can lead to inflammation and atrophy in the small intestine, anemia, and endocrine disorders (Briani et al., 2008; Fasano and Catassi, 2001). The prevalence of celiac disease was between 0.4 – 0.8% across the world, with significantly greater diagnosis in females than males (Singh et al., 2018). Two proteins that have been characterized for celiac disease epitopes are Glia-α9 and Glia-α20. The Glia-α9 is recognized as the major immunodominant epitope by the patients (Vader et al., 2002). Glia-α9 is more frequently recognized by the patients with celiac disease than Glia-α20, as Glia-α20 is recognized only in a minority of patients (van den Broeck et al., 2010). Wheat breeding for lower celiac disease gliadin proteins (van den Broeck et al., 2010) and biotechnology methods of silencing the gliadin proteins of wheat (Gil-Humanes et al., 2010) are being further researched for wheat varieties with lower toxicity to gluten intolerant patients.

1.9 Agronomic practices

Agronomic practices have played significant role in increasing wheat production and quality. Row spacing, seeding rate, crop rotation, and tillage practices all can have significant effects on yield and quality. Current agriculture practices have pushed decreasing row spacing with variation in seeding rate for increasing yields. The overall goal of these practices is to produce maximum tillers that can bear fertile spikes and with potential to fill the grains near the end of the season. For example, plant density, implemented by changes in seeding density, can have considerable influence on foliar coverage and radiation use efficiency, which is directly related to carbon fixation and biomass. In the northern Great Plains region, the optimal row spacing and seeding rate in hard red spring wheat yield was 15 centimeters, a deviation from the standard 30 centimeter row spacing commonly practiced in this area (Chen et al., 2008). Marshall and Ohm (1987) noticed similar trends in soft red winter wheat grown in Indiana, where a narrow row spacing than conventional practices increased yield significantly, along with a combination of a higher seeding rate.

Wheat is frequently intermixed in a two or three year crop rotation with maize and soybean in the United States. However, specific states and regions offer different crop rotations and practices based on climate and soil type. For example, Idaho performs varying tillage practices of conventional, minimum, and no-till along with rotating winter wheat between spring pea for two years or a three year winter wheat, spring barley, and spring pea rotation (Hammel, 1995). Lower midwestern regions perform intercropping of soybean and wheat to produce two crops in a fiscal year. In this system, soybean is planted in-between wheat rows at the heading stage (Reinbott & Helsel, 1987). This double-cropping system has shown increases in yields and positive crop effects on land use (Sandler et al., 2015).

A key factor in agronomic management strategies is water availability. Factors that play significantly on the watering strategies are annual precipitation and the capability of applying water through irrigation. Worldwide, 45% of wheat grown in developing countries is irrigated, and China and India have approximately 75-80% of irrigated wheat (Reynolds, et al., 1999). In the United States, irrigated wheat is not commonly practiced, with the Northwest region irrigating 20% of grown wheat in the area (USDA, 2013). Papendick (1996) described that 100 millimeters of water is required for wheat to grow to anthesis in the Pacific Northwest and water can be extracted from a depth of 1.8 meters.

A trend to decrease the environmental impact has shifted agriculture production into conservative or organic agriculture systems. Organic agriculture is dependent on nitrogen input through the use of manure, instead of synthetic fertilizers. Wheat organic systems were 35-47% more energy efficient than conventional wheat production systems in the United States (Pimentel et al., 1983). Clark and Tilman (2017) found global organic systems required more land than conventional systems per unit of food, and also used 15% less energy. Unfortunately, organic and conventional systems did not differ in their greenhouse gas emissions (Clark and Tilman, 2017).

1.10 Producing more with less resources sustainably

Nitrogen (N) is the essential element for improving crop yields and economic returns (Keeney and Hatfield, 2008). N is routinely applied as a macronutrient fertilizer for increasing photosynthetic rates of plants to grow and develop. N, as one of the main determinant of all cellular components such as protein and ultimately amino acids (Lawlor, 2002), directly impacts photosynthesis, which is required for growth of plants via storage and energy of N compounds and carbohydrates (Lawlor et al., 1989). Supplying enough nitrogen allows the plant to stimulate leaf growth, supporting tissues and enzymes e.g., Rubisco (Pask et al., 2012) and photosynthesis by cell growth, cell division, and light reactions (Lawlor, 2002). There is therefore a considerable interaction between nitrate availability and carbon dioxide fixation.

Plants also require phosphorus (P) to grow new tissue and perform cell division, as the DNA duplication and transcription are P-demanding processes (Elser, 2012). P is the least mobile and least available to plants in most conditions (Ramaekers et al., 2010). For this reason, P is applied to almost all soil types to make up for the inefficiency (Elanchezhian et al., 2015). P-efficiency is directly related to the uptake and root-to-shoot ratio (Föhse et al., 1988). Richardson et al. (2011) describes the ‘root foraging strategy’ as improving acquisition of P in the soil by the virtue of uptake by the roots. The P-efficiency is species dependent. For example, ryegrass and wheat have medium to high efficiency due to a high root to shoot ratio (Föhse et al., 1988). One way to express P-efficiency is the amount of phosphorus in the soil required to produce 80% of maximum yield (Föhse et al., 1988), which was coined as “extern phosphorus requirement”. The ability to effectively use the available P is dependent on the root capability of acquiring P that is available in the soil, also termed “phosphorus uptake efficiency”. As P-efficiency is based on P-

uptake efficiency by the roots, it was suggested that breeding for P-efficient plants should target identifying varieties that have high root-to-shoot ratio or high influx rates based on root absorption (Föhse et al., 1988). For example, Deng et al. (2018) identified significant difference among cultivars with higher phosphorus acquisition efficiency was based on variations in root morphology.

Potassium (K) is the most abundant cation in plants and is essential for photosynthesis, translocation of photosynthates into sink organs, and maintenance and activation of enzymes (Pettigrew, 2008). This macronutrient is essential for producing photosynthetic assimilates for plant growth and development (Pettigrew, 2008). White (2013) suggested K tissue concentrations must be maintained above 5 – 40 mg of potassium per gram of dry matter to avoid loss of yield. K-deficiency can be detrimental to the plant, causing leaf chlorosis, necrosis, and decreases in net photosynthesis (Cakmak, 2005). Wheat proved to be more K-efficient than barley and sugar beet, with a greater relative yield under K-deficient conditions (Dessougi et al., 2002). This was attributed mainly to the extensive root structure and producing a higher ratio of root length to shoot weight. Since K is necessary for maintaining photosynthetic carbon dioxide fixation, selecting varieties with enhanced K-efficiency can lead to more stable and adapted germplasm for diverse climatic environments. In addition, K plays a significant role in plant stress tolerance. Plants that can utilize K more efficiently have the potential to decrease production of reactive oxygen species, and thereby reducing cellular impairment under stressful conditions (Cakmak, 2005).

Increasing production efficiency and minimizing environmental footprints are important goals of modern agriculture. For wheat, in most agronomic practices two to three nutrient applications consisting of nitrogen, phosphorus, potassium are needed before planting, at the five leaf stage, and potentially near anthesis (Otteson et al., 2007). For winter wheat, up to three fertilizer applications can be performed at tillering, stem elongation, and second node stage (Hirel et al., 2007) for meeting the plant demand for nitrogen, phosphorus, and other limiting nutrients. All of the agronomic practices are to meet and surpass standards in grain yield and quality. However, this goes against production efficiency by adding potentially more fertilizer applications and nutrients that are required. For example, there is a tendency to apply excess nitrogen as “insurance” where their immediate attention is on their economic survival based on crop performance (Raun and Johnson, 1999).

One critical question is how to produce more wheat efficiently with less resources. This takes into consideration the adaptability of wheat cultivars, and also the methods of harvesting wheat with less environmental footprint. The ability to minimize fertilizer applications, utilize less water, decrease environmental contaminations, and decrease soil erosion could have long lasting effects on agricultural production systems. A current conservation effort is producing more crop residue for soil health and structure. Wheat residue could be integral in longevity for soil structure (Skidmore et al., 1979). The complexity in wheat management is the tradeoffs and conflict; increasing fertilizer applications and inputs for increasing yields has adverse impact on environmental contamination and pollution, or decreasing applications at the cost of grain yield.

Therefore, the best practices require proper stewardship to promote a sustainable and effective cropping system. The "4R" of crop nutrient stewardship are the Right source, Right rate, Right time, and Right place. The objective is to create a cropping system that matches the plant requirements in a method to reduce nutrient loss and promote sustainability. The advancement of fertilizer technology and research has included enhanced-efficiency fertilizers that are treated with nitrification or urease inhibitors to promote a controlled released of nitrogen fertilizers (Flis, 2017).

Within the 4R, the source can be a variety of chemical application, either liquid form or granular solid, for a desired nutrient. For example, the source for nitrogen can include anhydrous ammonia, urea, and ammonium nitrate, or for phosphorus the source could be diammonium phosphate or monoammonium phosphate, and potassium chloride as a source for potassium (Heffer et al., 2015). The rate of application is dependent on crop necessities and management objectives. This can vary tremendously depending on limiting factors such as climate, soil type, and agronomic practices and many different rates and recommendations are discussed in other reviews (Ladha et al., 2005; Zhang et al., 1993). The timing of fertilizer is as equally importance as the source and rate. In maize and wheat, multiple applications of nitrogen fertilizer are routinely applied before planting, during vegetative growth, and occasionally during the grain filling period (Heffer et al., 2015). Lastly, the placement of fertilizer will depict how the nutrient is available to the crop. Common practices of fertilizer placement include broadcast over the top applications, or applying fertilizer directly to the top soil or deeper banding for targeted root zones (Heffer et al., 2015).

Another critical question is how to increase fertilizer recovery and how to reduce the environmental footprints. Of the three important nutrients mentioned above, nitrogen is the most prevalent. Erisman et al. (2008) estimated that in 2008, nitrogen fertilizers were responsible for feeding 48% of the world's population. In the past four decades, the doubling of agricultural food production has come at a seven fold increase in nitrogen fertilizer use (Hirel et al., 2007). While the result of increasing nitrogen use is producing more crops for food, a large source of the nitrogen escapes into the environment. Raun and Johnson (1999) reported worldwide nitrogen use efficiency for cereal production was approximately 33%. Just a 1% increase in the efficiency of nitrogen could lead to saving over $230 million in nitrogen fertilizer costs.

With crop fertilizer recovery estimated below 50% (Kanampiu, Raun, & Johnson, 1997), the unaccounted nitrogen is lost through leaching, volatilization, combustion, and runoff in the water. Nitrogen fertilizer applications are also prone to emission losses of ammonia and nitrous oxide, or losses on the surface and groundwater as nitrate (Flis, 2017). Developing crops with more N use efficiency can lead to decreasing the environmental footprints.

1.11 Breeding for high- and low-input systems

Most breeding programs are historically performed under optimal conditions and yield potential, where selection is routinely performed under abundance of nitrogen with sufficient water availability. This practice has resulted in continuous genetic gains. Heritability was shown to be higher for grain yield, nitrogen use efficiency, grain quality, and other yield and nitrogen component under optimal conditions (Brancourt-Hulmel et al., 2005; Cormier et al., 2013; Laperche et al., 2006). In maize, broad sense heritability was decreased approximately 29% in a low nitrogen environment compared to a high nitrogen environment due to the lower genetic variance in the low nitrogen environment (Bänziger et al., 1997).

There is little evidence on effectively matching germplasm performance and fertilizer application for breeding purposes. Germplasm selection under higher nitrogen conditions may not be the best performers under lower N conditions. Brucker and Morey (1988) examined cost-effectiveness in relation to maximum grain yield in wheat and fertilizer application, where they concluded a moderate application of 67 kg per hectare nitrogen application produced 96% of the maximum grain yield and did not adversely affect grain protein.

In general, most focus on low input cropping systems is based on the management of nitrogen, which is the most widely used fertilizer and accounted for 57% of the total fertilizer utilized in the United States (EPA, https://www.epa.gov/roe/). Low input management systems can be advantageous for producers due to reducing nitrogen fertilizer applications, therefore, providing a more cost-effective system with less environment impact. One obvious drawback is the decrease in grain yield with reduced nitrogen supplied (Gaju et al., 2011), although strategies for how this could be improved upon are not currently in place. Barraclough et al. (2010) proposed that the only way to produce a high yielding and high quality nitrogen efficient wheat variety is through increasing uptake of nitrogen. Dhugga and Waines (1989) stated that under increasing soil N levels, uptake is more important than utilization, which is in agreement with Ortiz-Monasterio et al. (1997) and Le Gouis et al. (2000) under high nitrogen conditions. In maize, a 38% reduction in grain yield was observed by reduction of nitrogen uptake of 50% at silking under low nitrogen conditions (Gallais and Coque, 2005), while utilization efficiency decreased with increasing nitrogen levels

Barraclough et al., (2010) described four key traits for evaluating wheat nitrogen efficiency: grain yield, grain nitrogen percent, total nitrogen uptake, and nitrogen harvest index. These traits are constrained by the "law of conservation of matter" and that there is a physiological limit on crop nitrogen requirements. Nitrogen use efficiency (NUE), defined as the grain yield per unit of nitrogen available in the soil (Moll et al., 1982) is divided into two components: nitrogen uptake efficiency (NUpE), or the efficiency of absorbing nitrate and ammonium from the soil, and nitrogen utilization efficiency (NUtE), or the efficiency that the absorbed nitrogen is utilized to produce grain (Moll et al., 1982). Harper et al. (1987) determined that nitrogen uptake by the plant continues until maturity, even during the transition from vegetative to reproductive growth. The nitrogen remobilized from the vegetative tissues is one of the predominant sources of nitrogen for the grain (Pask et al., 2012).

In 225 winter wheat varieties that represent 25 years of European winter wheat breeding, the additive genetic effect of nitrogen use efficiency increased 0.33% per year based on the progression of nitrogen utilization increasing 0.20% per year (Cormier et al., 2013). A current challenge is to improve NUE in wheat to produce more with less N input. Kanampiu et al. (1997) described varieties with a high harvest index and low forage (biomass) yield had lower plant nitrogen loss, and could be targeted traits for nitrogen use efficient wheat varieties. One

challenge is said to be the negative correlation observed between nitrogen uptake and utilization (Gallais and Coque, 2005). Nitrogen uptake efficiency accounted for 54% and 72% of the genotypic variation in nitrogen use efficiency soft red winter wheat grain yield and grain protein, respectively (Van Sanford and MacKown, 1986).

1.12 Traits with influence on yield: prospects of further selection

Crop improvement for high- or low-input environments require detailed information about contribution of yield-contributing traits in the final harvestable organs. In very applied and practical breeding programs, most of the focus is on genetic gains for yield. However, dissecting grain yield in wheat into the contributing traits in a target environment will identify targets of further improvements (Figure 1).

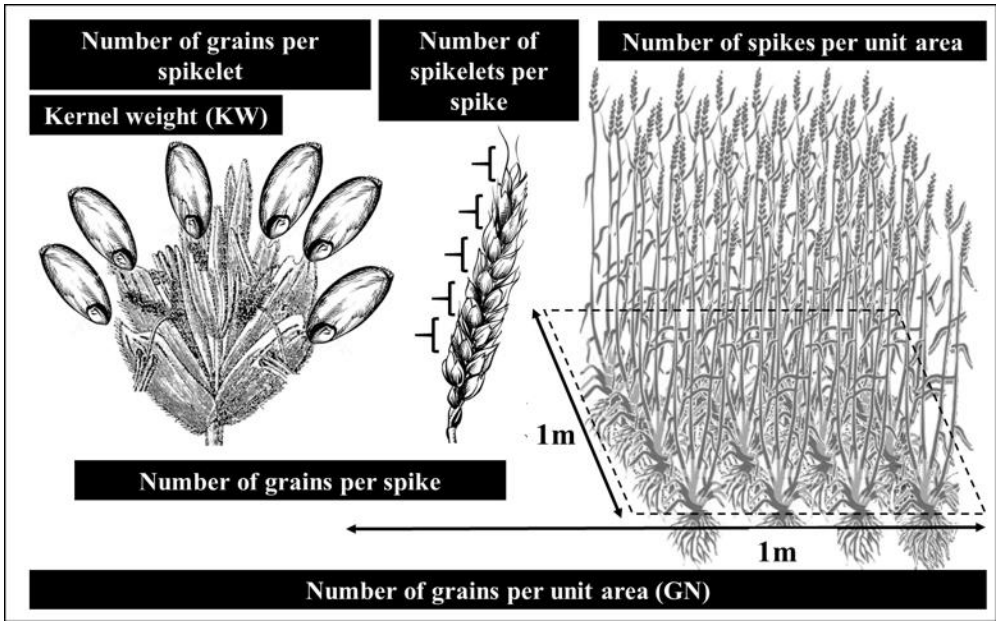

Figure 1. Grain yield can be divided into the two main components of grain number (GN) and kernel weight (KW). Grain number is further dissected into number of spikes per unit area (on the far right), number of spikelets in each spike (middle sketch), and number of grains per each spikelet. The kernel weight (KW) is the contribution of each individual grain to total yield. This figure is adapted from a manuscript from major advisor lab that is under evaluation for publication.

Yield can be defined in many different ways. For example, grain yield can be defined as the product of the number of grains per unit area and kernel weight (Abbate et al., 1998).

Alternatively, grain yield can be presented by the product of tiller density, kernels per head, and kernel weight (Brucker and Morey, 1988). Dhugga and Waines, (1989) proposed that grain yield can be defined as the reproductive sink capacity for dry matter, since more than 80% of the post-anthesis dry matter is deposited into the grain.

Previous studies have shown grain yield has been more impacted by the changes in the number of grains per area than kernel weight (Abbate et al., 1998), under both low nitrogen and high nitrogen environments (Le Gouis et al., 2000). The relationship between grain number and grain weight can be described as competitive, where an increase in the number of grains led to a decrease in thousand kernel weight, and vice versa (Le Gouis et al., 2000). However, alternative and contradicting results from Miralles and Slafer (1995) and Ugarte et al. (2007) describe this relationship as non-competitive for the available assimilates. The grain number per unit area was shown to be strongly correlated with the grain to spike weight ratio, and contributed to yield improvement in Argentina (Abbate et al., 1998).

Evidence shows that exotic translocations such as the 1B/1R rye translocation onto the short arm on chromosome 1B (Rayburn and Carver, 1988), has contributed to grain yield by increasing the number of spikes, thousand kernel weight, and test weight (Villareal et al., 1995). The same translocation was shown to confer delayed leaf senescence, or the stay-green phenotype (Chen et al., 2010). Chen et al. (2010) performed an experiment where two genotypes with the 1BL/1RS translocation experienced higher values of photosynthesis than the controls without the translocation, resulting in producing larger grains by extending the flag leaf photosynthetic competence.

Gaju et al. (2014) suggest delayed senescence would extend the grain filling period to allow for more photosynthesis for higher grain yields. The stay-green trait is the ability to retain green leaf area during grain filling. Delayed senescence, or promoting the stay green phenotype, allows crops to use up more agriculture inputs, extending the life span of individual leaves, and increase canopy duration (Thomas and Smart, 1993). The more time is allowed for photosynthesis to occur in the leaves, the more assimilates can be supplied to the grain. By further postponing senescence, more time can be allocated for grain filling and overall yield improvement by producing more carbohydrates for above ground growth and increasing the source to sink ratio.

For soft red winter wheat, the number of spikes per square meter and 500-kernel weight were highly significant and contributed to yield variation (Green et al., 2012). For grain size and weight, several important genes have been identified. The sucrose synthase 2 gene (*TaSus2*) contains a single nucleotide polymorphism for two distinct haplotypes, *Hap-H* and *Hap-L*, and both haplotypes were significantly associated with thousand grain weight (Jiang et al., 2011). The gene associated with grain weight, *TaGW2*, was identified in homologous group 6 chromosomes and the favorable haplotype on 6A (*TaGW2-6A*) confirmed wider grains and higher grain weight (Su et al., 2011). Other genes including *TaGS5-3A* and *TaCKX6-D1* were found to be positive regulators of grain size and weight in wheat (Ma et al., 2016; Zhang et al., 2012).

In wheat, an increase in yield is not the result from an increase in biomass production, but from increasing grain number and size (Green et al., 2012; Lawlor, 2002). This indirectly increases harvest index. Austin (1980) hypothesized that the theoretical limit for harvest index for wheat is 60%. Most studies report harvest index to be between 0.40 – 0.50 (Green et al., 2012), even under contrasting nitrogen environments (Cormier et al., 2013; Gaju et al., 2011; Hitz et al., 2017). Therefore, increases in biomass can lead to higher grain yield if harvest index stays unchanged.

1.13 Conclusion

Trait improvement for wheat can be dissected for yield potential in a high input environment and also for adaptability in a low input environment. The benefits of high inputs are associated with increasing genetic gains and yield performance. However, examining traits and breeding under less inputs and resources can be beneficial for selecting germplasm that maximize resource utilization under limited environment. The ***goal*** of this dissertation is to dissect the roles of yield and quality contributing traits under low input and high input environments. The first objective of this dissertation is to identify genomic regions associated with yield determining traits in soft red winter wheat population. The ability to utilize the wheat reference genome along with an elite population of diverse soft red wheat breeding lines from different breeding programs allowed identification of marker trait associations and potential quantitative trait loci for yield improvement. The second objective of this dissertation is to identify wheat traits, cultivars, and management adapted to a low-N environment. The

interaction of wheat cultivars, nitrogen applications, and environment was studied to determine germplasm and traits that can be used as founders for a new breeding population.

CHAPTER 2. TRANSFERABILITY OF MARKER TRAIT ASSOCIATIONS IN WHEAT IS DISTURBED MAINLY BY GENOTYPE BY ENVIRONMENT INTERACTIONS

A version of this chapter was submitted to Functional & Integrative Genomics

Data for the transferability testing for this chapter comes from the Triticeae CAP project, funded by NIFA-AFRI Grant no. 2011-68002-30029 from the USDA National Institute of Food and Agriculture.

2.1 Introduction

Over 35% of the world's population relies on wheat as a main source of food (Bushuk, 1997a). Wheat is widely cultivated around the world for its adaptability to diverse growing regions and environmental conditions. The United States produced 51.3 million metric tons of wheat during the 2018-2019 season (USDA, 2019), where most wheat harvested occurs west of the Mississippi River and in the Great Plains (Leff et al., 2004). However, soft red winter (SRW) wheat is grown mainly in the Midwestern and eastern United States, accounting for 15-20% of US wheat production (USDA, 2019). Specifically, the growing region extends from 30°N to 45°N in latitude and about 73°E to 96°W in longitude (Bacon, 2001). In the Midwest and eastern wheat breeding region, grain yield and resistance to Fusarium head blight disease are the underlying traits for profitability. The soft wheat products require minimal gluten protein, and lower protein levels than hard wheats (Kiszonas et al., 2013). Therefore, producing more grain is the first focus of most SRW wheat breeding programs.

Global demand for wheat is growing faster than genetic gains in yield potential (Reynolds et al., 1999). In the Great Plains region, the annual rate of genetic gain was estimated at 0.44%, mainly due to traits contributing to an increase in grain number (Donmez et al., 2001). The USDA winter wheat regional performance nurseries for the Great Plains region displayed similar results over a 50-year period, with estimated genetic gain for grain yield at 0.79% per year. From 1919 to 2008, the genetic gains in SRW wheat in multiple environments ranged from 0.56% to 1.41% (Green et al., 2012).

Much of hereto forth yield increases were due to increases in the number of spikes per area, the number of seeds per spike and spikelet, and harvest index - producing more grain from increasing yield components but maintaining the same biomass (Green et al., 2012). With harvest index approaching its theoretical maximum biologic limits (Austin et al., 1980), increasing biomass can provide an opportunity to increase the photosynthetic tissues for fixing carbon and a productive canopy to capture radiation energy and convert it into dry matter. Reynolds et al. (2005) reported that an increase in radiation use efficiency, grain number, and grain yield were positively associated with an increase in above ground plant biomass.

Breeding methodologies and techniques have changed drastically over the years. Further advances in statistical methodology and molecular markers led to the construction of genetic maps, evaluating complex traits, and associating the phenotypic variation with molecular markers (Devos et al., 1992; Helentjaris et al., 1986). The genetic maps facilitated the identification of quantitative trait loci (QTL) - the genomic region responsible for trait variation (Doerge, 2002). In wheat, QTL mapping has been performed for traits including yield components (Kumar et al., 2007), plant height (Cui et al., 2011), heat tolerance (Paliwal et al., 2012), grain quality (Olmos et al., 2003), and disease tolerance (Löffler et al., 2009; Shen et al., 2003; Zwart et al., 2010), among others. The identification of QTL has led to the use of molecular markers in screening germplasm for trait improvement (Anderson et al., 2001; Kirigwi et al., 2007). Bi-parental mapping is a powerful mapping tool. However, the limited number of recombination events in bi-parental populations are limited, which restricts the allelic diversity (Doerge, 2002; Myles et al., 2009) and leads to a low mapping resolution (Zhu et al., 2008).

The need to dissect complex traits within a large, diverse population led to the development of statistical methods that gave rise to genome-wide association studies (GWAS). Unlike bi-parental mapping, GWAS consists of genetically diverse germplasm that harbor many historical and ancestral recombination events. GWAS is based on the strength of linkage disequilibrium (LD) between the markers and the polymorphisms controlling the observable phenotypes in a population (Yu and Buckler, 2006; Zhu et al., 2008). The statistical power to detect causal polymorphisms is based on the extend of LD in the population (Ersoz et al., 2007). Wheat, being a self-pollinating species, experiences relatively slow LD decay. Selection on wheat, as it is practiced in breeding prorams, leads to relatively slower rates of LD decay, as Liu et al. (2018) displayed that the extent

of high LD islands is much greater in cultivars (1,053kb) than landraces (785kb) due to the effect of artificial selection.

GWAS has been used previously to study wheat for kernel size and milling quality (Breseghello and Sorrells, 2006; Daba et al., 2018; Gaire et al., 2019), spike traits (Liu et al 2018), root traits (Beyer et al., 2019), and grain yield and yield components traits (Sukumaran et al., 2015; Lozada et al., 2018). These studies implemented the various GWAS mapping approaches such as mixed linear model (MLM) (Yu et al., 2006) and compressed mixed linear model (CMLM) (Z. Zhang et al., 2010) to appropriately account for the underlying population structure and kinship. Recent studies have shown that single locus models, such as MLM and CMLM, generate more false negatives due to overfitting (Kaler et al., 2020; Wen et al., 2018). The multi-locus Fixed and Random Model Circulating Probability Unification (FarmCPU) model was shown to better control false positives and false negatives (Kaler et al., 2020; Liu et al., 2016), improving statistical power to identify true marker trait associations (MTAs).

In this study, our goal was to identify MTAs for yield and yield component traits in an elite SRW winter wheat population developed by eastern and midwestern public breeding programs. Previous work by Gaire et al. (2019) in this population identified MTAs concerning SRW wheat end use quality traits in this population, but no work to date has explored yield related traits in the context of GWAS. We achieve this goal by field-based phenotyping and high-throughput genotyping.

2.2 Materials and Methods

2.2.1 Experimental design

The Triticeae Coordinated Agricultural Project (TCAP) population consists of lines developed from breeding programs in Illinois, Kentucky, Maryland, Missouri, New York, Ohio, Indiana, and Virginia. The pedigree of lines are detailed in Huang et al (2016). The germplasm were grown in two growing seasons 2016-17 (WL17) and 2017-18 (WL18) at the Purdue Agronomy Center for Research and Education (ACRE) in West Lafayette, IN (40.43° N, 86.99° W) after a previous soybean crop. Similar field layouts and germplasm were planted in both years. Trials were planted in late September and harvest in late June of the following year. The

experiments were planted using a Hege (Wintersteiger, Australia) drill planter and harvested with a Wintersteiger plot harvester at physiological maturity. In each year, two replications were planted. Each replicate was a 13-row x 24-column layout, consisting of eight incomplete blocks, each accommodating 39 plots. Each plot measured 1.22m x 1.22m and we planted 20 grams seed per plot, which amounts to approximately plant density of 370 - 420 seeds per square meter. Before planting, 336 kg ha^{-1} of mono-ammonium phosphate (11-52-0) was applied. A spring nitrogen top-dress application of 112 kg ha^{-1} in the form of liquid urea ammonium nitrate (28-0-0) was applied as recommended by crop management practices in the region. Trials were rainfed and did not rely on any form of irrigation. Monthly precipitation and temperature obtained from iClimate (2019) are detailed in Table 1.

Table 1. Monthly precipitation and temperature in West Lafayette, Indiana, for the two cropping seasons of the study.

	Temperature (°C)		**Precipitation (mm)**	
Month	**2016-2017**	**2017-2018**	**2016-2017**	**2017-2018**
September	20.8	19.3	81.0	50.5
October	15.1	14.5	32.8	68.1
November	8.3	5.3	135.9	125.8
December	-1.9	-1.4	58.9	20.6
January	-0.2	-4.7	111.7	39.9
February	4.7	0.4	19.9	139.8
March	5.6	2.8	109.1	79.1
April	13.5	6.8	108.7	73.7
May	15.7	21.1	175.3	93.7
June	22.2	22.8	135.4	157.8

2.2.2 Trait measurements

We measured grain yield (YLD), days to heading (HD), days to maturity (MD), thousand kernel weight (TKW), biomass (BIO), number of spikes per area (NS), number of grains per spike

(GPS), grain weight per spike (GWS), and plant height (PH). YLD was measured at harvest, adjusted for 13% seed moisture, and was expressed as kg ha^{-1}. HD was determined by complete emergence of heads (Feekes 10.5, Zadoks 58) in more than 50% of individual plants in a plot and expressed as the number of days after January 1^{st}. Similarly, MD was determined when more than 50% of plot reached physiological maturity (Feekes 11.3, Zadoks 91) and expressed as the number of days after January 1^{st}. At maturity, PH was recorded by four random measurements per plot, from the ground to the top spikelet, excluding the awns, and expressed in centimeter (cm). Yield components were evaluated by measuring traits from an area of 0.25m x 0.3048m that was cut from the ground level after physiological maturity. First aboveground BIO was dried to constant weight, measured and expressed in grams (g). Next effective tiller numbers per unit area were counted from the cut sample and represented as number of spikes (NS). Then, five random spikes were randomly sampled from the total cut area to measure the number of grains per spike (GPS), and grain weight per spikes (GWS) – also expressed in grams. TKW was measured by counting and weighing 1,000 kernels, which was expressed in grams.

2.2.3 Description of genotypic data

This population was initially genotyped by using the 90K SNP chip array (Wang et al., 2014), and the marker density was later increased by completing genotyping-by-sequencing method, as explained in Poland et al. (2012). Briefly, reduced genomic libraries were created using *Pst1-Msp1* restriction enzyme combination consistent with Poland et al. (2012). The samples were pooled together at 96-plex to create libraries and each library was sequenced on a single lane of Illumina Hi-Seq 2500. Variant calling was performed using the TASSEL 5 GBSv2 pipeline (Bradbury et al., 2007) with 64 base k-mer length and minimum k-mer count of five. Reads were aligned to the wheat genome sequence assembly IWGSCv1.0 (Appels et al., 2018), using aln method of Burrows-Wheeler aligner (BWA) version 0.7.10 (Li and Durbin, 2009). For filtering of both 90K SNP chip array and GBS markers, we excluded any markers missing ≥ 10% data and those with minor allele frequency less than 0.05. We then used Linkage Disequilibrium K-number neighbor imputation (LD-kNNi) algorithm (Money et al., 2015) implemented in TASSEL 5 (Bradbury et al., 2007) to impute the missing markers. Markers that were not mapped to any specific chromosome were excluded from further analysis. The final genotypic dataset that was used in this

study consisted of 45K variants of which 13K were produced from the 90K SNP chip array pipeline and 32K were produced from GBS pipeline.

2.2.4 Statistical analysis of phenotypic data

In order to test the significance of genotypes, year, and genotype x year interaction, analysis of variance (ANOVA) was performed in R environment (R Core Team, 2019). For each trait, the following ANOVA model was fitted:

$$[\mathbf{1}]\ Y_{ijkl} = \mu + G_i + Y_j + R_k(Y_j) + GY_{ij} + B_l(RY_{kj}) + \varepsilon_{ijkl}$$

Where the response variable Y_{ijkl} is the observed phenotypic value of the ith genotype, in the jth year, in the kth replicate, and the lth incomplete block; μ is the overall mean, G_i is the effect of the ith genotype, Y_j is the effect of the jth year, $R_k(Y_j)$ is the effect of the kth replicate within the jth year, GY_{ij} is the interaction effect of the ith genotype by the jth year, and $B_l(RY_{kj})$ is the effect of the lth incomplete block within the kth replicate and the jth year. The ε_{ijkl} represents the residual error.

To produce phenotypic values of each line for GWAS analysis, the best linear unbiased estimate (BLUE) values were derived by implementing a mixed model (Yu et al., 2006) using the '*lme4*' package (Bates et al., 2015) in R environment (R Core Team, 2019) in equation [1], where genotype was considered as fixed effect and other terms were considered as random effects. The Pearson correlation coefficient was calculated by *cor* function in R by using BLUE values. Path analysis was performed on BLUE values by using the latent variable analysis '*lavaan*' package (Rosseel, 2012) in R environment (R Core Team, 2019).

2.2.5 Estimating heritability estimates

Estimation of heritability based on experimental design requires a balanced design where all experimental entries are included in each replicate. Therefore, for producing variance components for estimating the broad sense heritability (H^2), we used a reduced model as follows:

$$[\mathbf{2}]\ Y_{ijkl} = \mu + G_i + Y_j + R_k(Y_j) + GY_{ij} + \varepsilon_{ijkl}$$

Where the response variable Y_{ijkl} is the observed phenotypic value of the ith genotype, in the jth year, in the kth replicate, and the lth incomplete block; μ is the overall mean, G_i is the effect of the ith genotype, Y_j is the effect of the jth year, $R_k(Y_j)$ is the effect of the kth replicate within the jth year, and GY_{ij} is the interaction effect of the ith genotype by the jth year. The ε_{ijkl} represents the residual error. In this model all terms were considered as random effect. The broad sense heritability (H^2) on an entry-mean basis was estimated following the equation (Nyquist, 1991; Piepho & Möhring, 2007a):

[3] $$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{gy/y}^2 + \sigma_{\varepsilon/yr}^2}$$

where σ_g^2 is the genetic variance, σ_ε^2 is the error variance, and *y* is the number of years ($y = 2$), and r is the number of replications per year ($r = 2$).

2.2.6 Genome-wide association studies (GWAS)

Principal component analysis (PCA) of marker data was used to visualize the underlying population structure. We used the first three principal components (PCs) to produce a 3D scatter plot. Pair-wise LD estimates between adjacent markers were calculated, as the squared coefficient of correlation (r^2), using TASSEL 5 (Bradbury et al., 2007) with a sliding window of 1000 markers. The pairwise LD estimates were plotted against the physical distance to determine the decay of LD against physical range on each chromosome, and in particular around the regions, where marker-trait associations were identified in GWAS. LD decay plots generated in R using the Hill and Weir (1988) method and loess regression with assessment at r^2 value of 0.2 (Edae et al., 2014; Vos et al., 2017).

GWAS was performed using the GAPIT software (Lipka et al., 2012) in R for each trait using the Fixed and Random Model Circulating Probability Unification (FarmCPU) model (Liu et al., 2016) and first 3 PCs were used to control the population structure (Price et al., 2006). We reported MTAs that were identified at $-logP \geq 4.0$ (pvalue $\leq$ 0.0001). If a genomic region was identified with multiple MTAs close to each other, we only report a representative MTA. We also identified MTAs that passed a 5% false discovery rate (FDR) for controlling multiple testing

(Benjamini and Hochberg, 1995). The coefficient of determination (R_2) for each identified MTA was determined by fitting a linear model in R environment with the contrasting alleles of the marker and the 3 PCs as the covariates using an ordinary least squares regression.

2.2.7 Transferability and validation of GWAS results

YLD and HD data obtained from trials conducted in previous years and other states during the Triticeae CAP project were used to validate the transferability in other environments of the MTAs we identified in Indiana. This data comes from diverse environments i.e., five different locations and two growing seasons 2011-12 and 2012-13, as described by (Huang et al., 2016). These environments are: moderate nitrogen in Kentucky 2011-12 (KYM12), moderate nitrogen in Maryland 2011-12 (MDM12), moderate nitrogen in Missouri 2011-12 (MOM12) and 2012-13 (MOM13), low nitrogen in Ohio 2011-12 (OWL12) and 2012-13 (OWL13), moderate nitrogen in Ohio 2011-12 (OWM12) and 2012-13 (OWM13), low nitrogen in Virginia 2011-12 (VAL12) and in 2012-13 (VAL13), and moderate nitrogen in Virginia 2011-12 (VAM12) and 2012-13 (VAM13). We abbreviated grain yield and heading date we obtained from our 2016-17 and 2017-18 seasons as WL17 and WL18. In total, we assembled data from 14 environments for validation and transferability examination. For WL17 and WL18 environments, we first accounted for incomplete block design and then included the data in the multi-environment data analysis. Multi-dimensional scaling and linear discriminant analysis were used to cluster environments into seemingly homogeneous groups based on YLD or HD data. Then the accuracy of grouping was examined by cross validation. The *cmdscale* function in R was used to perform multidimensional scaling with Euclidean distances extracted using the *dist* function. Eigenvalues from three dimensions were extracted and incorporated into the *lda* function in the *MASS* package (Ripley et al., 2019) for linear discriminant analysis and cross validation by setting CV=TRUE. Upon confirmation of groupings, BLUEs were obtained for each homogeneous group following the same model [2] and GWAS analysis was completed for each homogeneous group. We considered a MTA as validated or transferable if identified with a $-logP > 1.3$ (pvalue < 0.05) in another homogenous group of environments. We chose this threshold because for validation, we are only interested in one specific marker and there is no need to control for testing of multiple hypotheses.

2.3 Results

2.3.1 Phenotyping analysis and relationship among traits

We evaluated grain yield and yield components of a soft red winter wheat population in West Lafayette, Indiana for two years. For all traits, the effect of genotype was significant at 0.001, indicating the presence of noticeable genetic variation in the germplasm. In addition, the effect of year, and replicate within years were significant at 0.001 for all traits except for GPS, where the effect of year, and rep within years were significant at 0.01. More importantly, the genotype x year interaction effect was significant at 0.001 for YLD, BIO, PH, HD, and MD, at 0.01 for NS, and at 0.05 for GWS, but not significant for TKW and GPS (Table 2). The significant effect of genotype x year interaction will be further discussed in the GWAS section.

Table 2: Analysis of Variance.

		Trait								
Source of variance	df	YLD	TKW	BIO	NS	GPS	GWS	PH	HD	MD
Genotype	269	***	***	***	***	***	***	***	***	***
Year	1	***	***	***	***	***	***	***	***	***
Genotype x Year	269	***	ns	***	**	ns	*	***	***	***
Rep(Year)	2	***	***	***	***	**	***	***	***	***
Block(Rep x Year)	28	**	ns	***	***	ns	ns	***	***	***

Significant values: *** < 0.001, ** < 0.01, * < 0.05, ns > 0.05

df: degrees of freedom

YLD: grain yield; TKW: thousand kernel weight; BIO: biomass; NS: number of spikes; GPS: grain per spike; GWS: grain weight per pike; PH: plant height; HD: days to heading; MD: days to maturity

Grain yield ranged from 3,900 – 7,500 kg ha^{-1} with a mean of 5,830 kg ha^{-1} and heritability of 0.50 (Table 3). The top 10% highest yielding lines in the population averaged at 6,940 kg ha^{-1}, while the 10% lowest yielding lines averaged 4,650 kg ha^{-1}- a 1.5-fold difference. Not all of the

10% highest yielding entries were developed by one breeding program, indicating a potential for achieving genetic gains via germplasm exchange. Among these, high yielding lines developed from public breeding programs at Purdue (10 lines), Illinois (7 lines), Missouri (3 lines), Ohio State (2 lines), Kentucky (2 lines), and Maryland (2 lines) were identified.

Table 3: Summary statistics and heritabilities (H_2) based on WL1718.

Trait	**Unit**	**Mean**	**SD**	**Minimum**	**Maximum**	**H_2**
Grain Yield	kg ha^{-1}	5827	678	3905	7500	0.50
Thousand Kernel Weight	grams	32	1.79	27	38	0.49
Biomass	grams	201	20	151	255	0.21
Number of Spikes	count	108	13	74	152	0.48
Grain per spike	count	37	4	25	50	0.44
Grain weight per spike	grams	0.98	0.14	0.68	1.38	0.41
Plant Height	centimeters	90	6	76	111	0.84
Days to Heading	Julian Days (from Jan 1)	133	2.01	128	139	0.69
Days to Maturity	Julian Days (from Jan 1)	171	1.28	168	175	0.62

The traits with the greatest and significant positive phenotypic correlation to YLD were BIO (r = 0.29***), TKW (r = 0.29***), and GWS (r = 0.29***) (Table 4). BIO had an average of 201 grams per cut area and heritability of 0.21. TKW ranged from 27.8 – 38.8 grams with a mean of 32.3 grams and heritability of 0.49 (Table 3). The three lines with the greatest kernel weight were MD04W249-11-7, 04702A1-18, and MD03W64-10-3 and the three lines with the smallest kernel weight were OH08-178-52, VA09W-188WS, and MO080584. However, looking at the top 10% high yielding entries, the range of thousand kernel weight was narrower (30-35 grams), and around the average value for kernel weight. Total grain number in wheat is the cumulative effect of spike number per unit area and the number of grains per spike. The NS per measured area ranged from

74 to 152 spikes. The lines with greatest number of spikes were OH08-172-42, TRIBUTE, and IL08-12174 and the lines with lowest number of spikes per measured area were INW1021, 0566A1-3-1-67, and 05251A1-1-136-9-5. GPS and GWS had a mean of ~37 grains per spike and 0.98 grams, respectively and similar heritability estimates (Table 3). A significant negative correlation (r = -0.22***) was observed between NS and GPS (Table 4). This negative correlation has been observed previously in multiple experiments (Kotal et al., 2010; Philipp et al., 2018). PH had the highest heritability of 0.84, averaged at 90 cm, and showed a standard deviation of 6.2 cm. The tallest lines were CAYUGA, MO101329, and MO100647 while the shortest lines were 03207A1-7-3-1, 9346A1-2-5-5-2-1, and MD03W665-10-5. The height of the 10% shortest lines averaged 80 cm. Lastly, the HD and MD had a mean of 133 and 171 days, respectively, and were highly correlated with one another (r=0.68***) (Table 4). Lines that headed later (>138 days) and matured later (> 173 days) included NY103-208-7263, NY99066-3444, CAYUGA, NY96009-3037, and MEDINA, all varieties developed in New York and adapted to the eastern climate region. Both traits were significant and negatively correlated with YLD (r = -0.19**, r = -0.18**) (Table 4) as this relationship has been documented previously (Addison et al., 2016).

Table 4: Phenotypic correlations of BLUEs of nine measured traits.

Trait	TKW	BIO	NS	GPS	GWS	PH	HD	MD
GY	0.29***	0.29***	0.04	0.07	0.29***	0.05	-0.19**	-0.18**
TKW		0.04	-0.18*	-0.16**	0.28***	0.01	-0.08	-0.16**
BIO			0.47***	0.24***	0.22***	0.31***	0.09	0.13*
NS				-0.22***	-0.31***	-0.14*	-0.16**	-0.10
GPS					0.72***	0.28***	0.22***	0.24***
GWS						0.26***	0.12*	0.10
PH							0.38***	0.28***
HD								0.68***
MD								

Significance: < 0.001 = ***, <0.01 = **, < 0.05 = *.

YLD: grain yield; TKW: thousand kernel weight; BIO: biomass; NS: number of spikes; GPS: grain per spike; GWS: grain weight per pike; PH: plant height; HD: days to heading; MD: days to maturity

2.3.2 Path coefficient analysis

The correlation magnitudes were further broken down by using path analysis, following Dewey and Lu (1959). Path analysis parses out the correlation magnitude to direct and indirect components of influence (Dewey and Lu, 1959). In Figure 2, the single arrow lines indicate direct influence as measured by path coefficients (P_{xx}) and the indirect effects are the association between variables measured by correlation coefficients (r_{xx}). The indirect effects are the product of the path coefficients and correlation coefficients. The sum of the path coefficients and indirect effects of correlation coefficients equal the phenotypic correlations, thus breaking down the reasoning for positive and negative correlations observed.

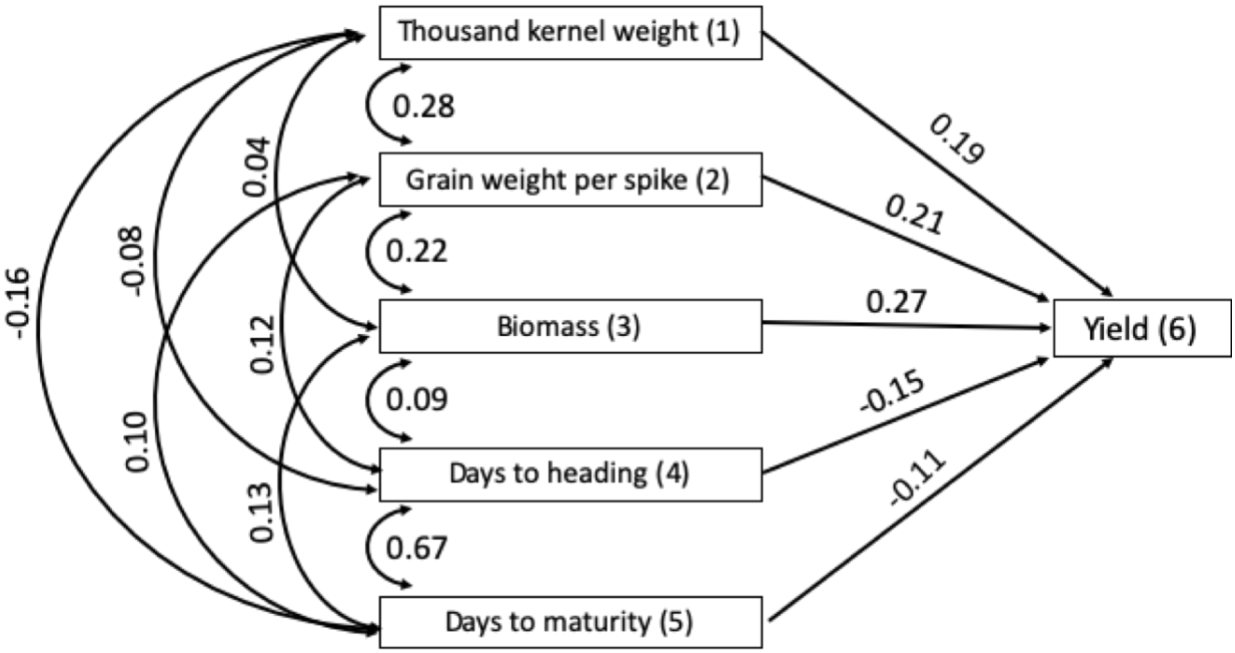

Figure 2: Path coefficient analysis diagram.

In the a priori model, grain yield is directly affected by traits with significant phenotypic correlation (Table 4). These traits are thousand kernel weight, grain weight per spike, biomass, heading date, and maturity date. Biomass had the largest direct path coefficient of 0.27, followed by grain weight per spike and thousand kernel weight coefficients of 0.21, and 0.19, respectively (Table 5). The indirect effect of thousand kernel weight on grain weight per spike represents almost

one-sixth of the phenotypic correlation (Table 4) and direct path coefficient between grain weight per spike and yield (Table 5. Biomass and grain weight per spike are correlated (r=0.22; Table 3) and positively contribute to correlations with grain yield. Days to heading showed a negative direct effect on grain yield with path coefficient of -0.15 (Table 5), consistent with its negative correlation with grain yield (r = -0.19; Table 4). Similar patterns were observed for days to maturity.

Table 5: Path coefficients for direct and indirect effects

Path	**Effects**		
Thousand kernel weight → Yield			Coefficients
	P_{16}	Direct effect	0.19
	$P_{16} x r_{12}$	Indirect effect via grain weight per spike	0.06
	$P_{16} x r_{13}$	Indirect effect via biomass	0.01
	$P_{16} x r_{14}$	Indirect effect via days to heading	0.01
	$P_{16} x r_{15}$	Indirect effect via days to maturity	0.02
		Total	0.29
Grain weight per spike →Yield			
	P_{26}	Direct effect	0.21
	$P_{26} x r_{21}$	Indirect effect via thousand kernel weight	0.05
	$P_{26} x r_{23}$	Indirect effect via biomass	0.06
	$P_{26} x r_{24}$	Indirect effect via days to heading	-0.02
	$P_{26} x r_{25}$	Indirect effect via days to maturity	-0.01
		Total	0.29
Biomass →Yield			
	P_{36}	Direct effect	0.27
	$P_{36} x r_{31}$	Indirect effect via thousand kernel weight	0.01
	$P_{36} x r_{32}$	Indirect effect via grain weight per spike	0.05
	$P_{36} x r_{34}$	Indirect effect via days to heading	-0.01
	$P_{36} x r_{35}$	Indirect effect via days to maturity	-0.01
		Total	0.29

Table 5 continued

Days to heading → Yield			
	P_{46}	Direct effect	-0.15
	$P_{46} \times r_{41}$	Indirect effect via thousand kernel weight	-0.01
	$P_{46} \times r_{42}$	Indirect effect via grain weight per spike	0.02
	$P_{46} \times r_{43}$	Indirect effect via biomass	0.02
	$P_{46} \times r_{45}$	Indirect effect via days to maturity	-0.07
		Total	-0.19
Days to maturity → Yield			
	P_{56}	Direct effect	-0.11
	$P_{56} \times r_{51}$	Indirect effect via thousand kernel weight	-0.03
	$P_{56} \times r_{52}$	Indirect effect via grain weight per spike	0.02
	$P_{56} \times r_{53}$	Indirect effect via biomass	0.04
	$P_{56} \times r_{54}$	Indirect effect via days to heading	-0.10
		Total	-0.18

2.3.3 Genome-wide association studies

The objectives of this study were to identify MTAs that control grain yield and other agronomic traits in this population in the Indiana environment and examine the transferability of MTA results across other environments. Of the 45K variants used in this study, approximately 17K, 22K, and 5.7K were located on sub-genome A, B, and D, respectively. The first three principal components (PCs) of all marker data explained 6.5%, 5.2%, and 3.8% of the total variation (Figure 3). Consistent with the reports of Gaire et al. (2019) and Huang et al. (2016), PCs separated two distinct groups, which were previously attributed to whether germplasm is progeny, close relative, or descendants of the soft red winter wheat variety ‘Truman’ or not (Huang et al., 2016). Linkage disequilibrium persisted variably across different chromosomes and the half decay distance (in base pairs) are presented in Table 6 for each chromosome. For example, LD persisted the longest physical range on chromosomes 2B (~125 mega base pairs Mbp) and 7D (109 Mbp). In contrast, chromosomes 5D (0.74 Mbp) and 6D (0.71 Mbp) displayed the fastest LD decay.

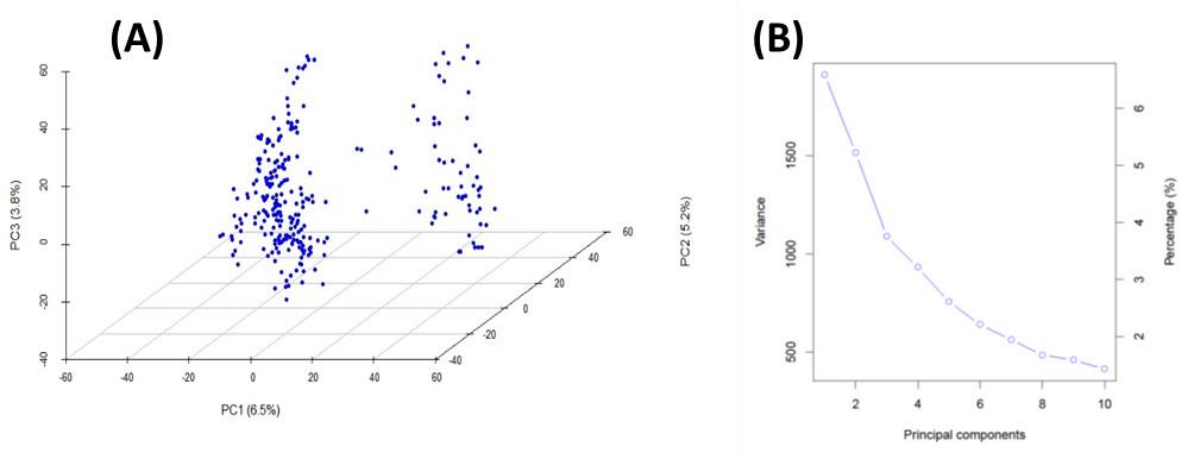

Figure 3: Principal component analysis based on all SNPs for 270 lines. (A) 3D scatterplot of first three principal components (PCs). (B) Scree plot describing the amount of variation by each principal component.

Table 6: LD decay half distance per chromosome and genome.

Chromosome	Half Decay Distance (in base pairs)
1A	2,110,000
1B	12,150,000
1D	6,320,000
2A	3,640,000
2B	124,980,000
2D	6,460,000
3A	1,290,000
3B	2,290,000
3D	2,420,000
4A	1,680,000
4B	4,990,000
4D	1,090,000
5A	3,470,000
5B	3,410,000
5D	740,000
6A	1,320,000
6B	2,670,000
6D	710,000
7A	1,260,000
7B	2,260,000
7D	108,980,000
GENOME	1,052,196

We used the first 3 PCs to account for the underlying population structure in GWAS analysis for all traits evaluated in West Lafayette, IN, USA. For GWAS we used estimates of phenotypic data based on two years of study i.e., WL17 and WL18, termed WL1718 throughout, and 45K genome-wide variants for GWAS. In this study, we reported and discussed MTAs that were identified at $-logP \geq 4.0$ (pvalue ≤ 0.0001) threshold. A total of 62 MTAs were identified for eight traits in WL1718 except for NS on 20 chromosomes (all excluding 3D). Based on their physical distances and the LD decay, the 62 MTAs were resolved in 59 independent loci (Figure 4). Of the 59 loci, 11 passed the 5% FDR threshold for grain yield, days to heading, days to maturity, and plant height. Chromosome 3B showed the highest number of loci. Regions on chromosome 5A were found to be associated with four phenotypic traits including grain weight per spike, grain per spike, days to maturity, plant height, and thousand kernel weight (Figure 4; Table 7). Plant height showed maximum number of MTAs among traits. None of the MTAs were associated with multiple traits.

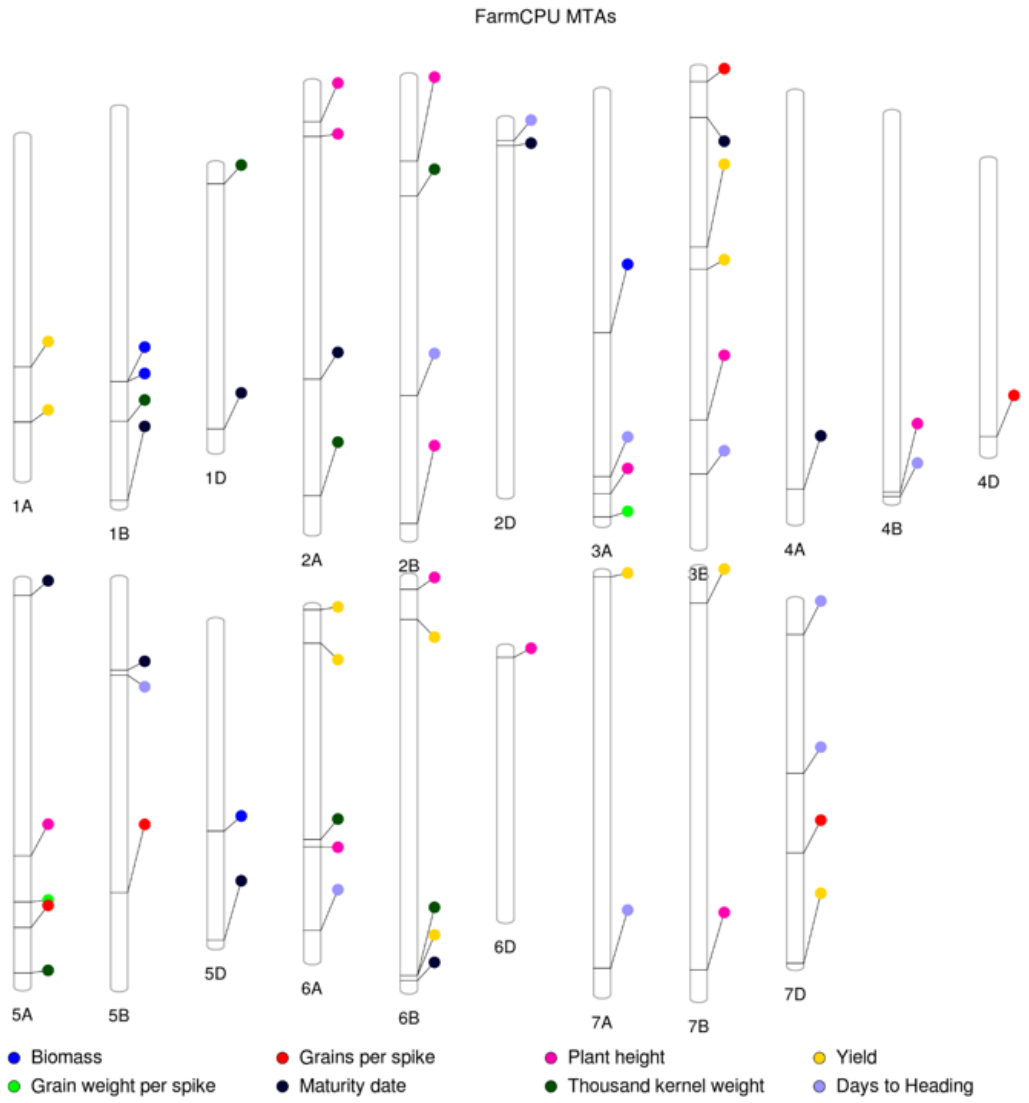

Figure 4: Genetic map of all significant MTAs identified by FarmCPU method for yield and yield component traits.

For YLD, eleven MTAs were reported on chromosomes 1A, 3B, 6A, 6B, 7A, 7B, and 7D (Figure 7). The MTA with the largest -*logP* value of 16.35 on chromosome 7D located at 633,027,374 base pairs (bp) explained 18% of phenotypic variation for grain yield. The next largest signal on chromosome 1A of -*logP* = 8.27 had allele effect of 174 kg ha^{-1} (Table 7).

Five MTA were identified for GPS on 3B, 4D, 5A, 5B, and 7D (Figure 5). These marker effects accounted for approximately 2 grain per spike and explained 4 – 7% of the phenotypic variation (Table 7). One MTA for GWS were found on chromosome 3B. Marker

gbs_3A_739555657 explained 8% of the phenotypic variation and accounted for an allele effect of 63 milligrams of grain weight per spike (Table 7). Lastly, TKW had 7 MTAs on chromosomes 1D, 2A, 3B, 5A, 6A, and 6B (Figure 5). The strongest signal for TKW was identified on 5A at position 685,795,509 bp. This region exerted an effect of 540 mg and covered 10% of total phenotypic variation. The next largest signal was observed at position 206,962,855 bp on chromosome 2B with an effect of 690 mg and phenotypic explanation of 8%.

For BIO, we identified 4 MTAs on chromosomes 1B, 3A, and 5D (Figure 5, Table 7). The largest signal for biomass was identified on chromosome 5D at position 365,732,020 bp with –*logP* of 5.65 that explained 9% of variation observed in biomass. The next large signal for biomass was -*logP* of 4.69 on chromosome 3A. Independent MTAs for BIO represented 4-9% of the phenotypic variation with positive allele effects between 8.00 – 13.41 grams.

Ten MTAs were identified for days to heading for WL1718 across nine chromosomes (Figure 8). Two MTAs on 7D had -*logP* values of 5.77 and 8.38 with allele effects of 0.74 and 0.58 earlier heading date, respectively. Ten MTAs were identified with –*logP* up to 9.41 for days to maturity (Table 7). The most significant signal was identified at 44,485,665 bp position of chromosome 2D, which explained 16% of variation. Eleven MTAs were identified with –*logP* up to 9.90 for PH. One marker on 6D explained 16% of the phenotypic variation for plant height and had a minor allele frequency of 0.07 (Table 7).

Table 7: MTAs for yield and yield component traits in WL1718 environment.

Trait	Chr	Marker	Position	Alleles[a]	MAF	*-logP*	Effect	R^2	Units
Biomass	1B	IWA6758	474118005	A/G (226/20)	0.12	4.07	12.30	0.04	grams
	1B	IWB72708	473529137	T/C (227/19)	0.11	4.36	12.80	0.05	grams
	3A	3A_419257151	419257151	A/G (241/9)	0.07	4.69	13.41	0.07	grams
	5D	5D_365732020	365732020	A/C (212/51)	0.21	5.65	8.00	0.09	grams
Grain per spike	3B	3B_22698880	22698880	A/G (246/20)	0.08	4.07	1.99	0.06	count
	4D	4D_479593371	479593371	G/A (229/39)	0.15	4.33	1.75	0.07	count
	5A	5A_606524326	606524326	C/G (221/44)	0.17	4.08	1.67	0.06	count
	5B	5B_546826603	546826603	G/A (242/24)	0.1	4.61	2.33	0.04	count
	7D	7D_440881288	440881288	G/A (233/34)	0.13	4.04	1.87	0.05	count
Grain weight per spike	3A	3A_739555657	739555657	T/C (238/31)	0.12	4.60	0.063	0.08	grams
Maturity date	1B	1B_680465515	680465515	G/A (241/6)	0.06	4.92	0.64	0.05	Julian days
	1D	1D_458723021	458723021	G/A (247/21)	0.08	4.30	0.48	0.03	Julian days
	2A	2A_515253009	515253009	T/C (230/3)	0.08	6.24	0.59	0.03	Julian days
	2D	2D_44485665	44485665	A/G (237/31)	0.12	9.41	0.74	0.16	Julian days
	3B	3B_85344544	85344544	C/T (238/7)	0.07	6.46	0.71	0.04	Julian days

Table 7 continued

	4A	4A_688222191	688222191	A/G (244/7)	0.06	4.72	0.49	0.03	Julian days
	5A	5A_26153196	26153196	G/A (135/126)	0.48	4.28	0.21	0.02	Julian days
	5B	5B_158399441	158399441	G/A (239/28)	0.11	4.91	0.32	0.02	Julian days
	5D	IWB54292	556553226	T/G (173/96)	0.36	4.31	0.19	0.05	Julian days
	6B	IWA3268	705159045	T/C (232/35)	0.14	4.09	0.33	0.07	Julian days
Plant height	2A	2A_66985350	66985350	C/A (248/19)	0.08	4.24	2.39	0.06	centimeters
	2A	IWB51951	92797308	T/G (227/43)	0.16	5.01	1.46	0.08	centimeters
	2B	2B_146441175	146441175	A/G (230/34)	0.14	7.60	1.77	0.04	centimeters
	2B	2B_776795892	776795892	G/A (215/5)	0.11	9.90	3.00	0.04	centimeters
	3A	3A_699195908	699195908	T/C (233/6)	0.08	5.41	2.28	0.07	centimeters
	3B	IWB9589	611497265	T/C (211/52)	0.21	6.49	1.30	0.10	centimeters
	4B	IWB43355	657825660	A/G (150/114)	0.43	7.59	1.45	0.07	centimeters
	5A	5A_480705790	480705790	C/A (255/13)	0.05	6.45	2.64	0.03	centimeters
	6A	6A_419959989	419959989	T/G (149/113)	0.43	8.09	1.29	0.07	centimeters
	6B	6B_21208064	21208064	G/A (239/29)	0.11	5.08	1.51	< 0.01	centimeters
	7B	IWA4750	701186266	A/G (172/93)	0.35	6.67	1.20	0.01	centimeters

Table 7 continued

Thousand kernel weight	1B	1B_542725487	542725487	A/C (212/53)	0.21	4.77	0.37	0.03	grams
	1D	1D_32441418	32441418	T/C (232/27)	0.12	5.08	0.49	0.02	grams
	2A	2A_718459754	718459754	T/G (253/16)	0.06	4.37	0.57	0.04	grams
	2B	2B_206962855	206962855	A/G (236/14)	0.09	5.89	0.69	0.08	grams
	5A	5A_685795509	685795509	C/A (226/42)	0.16	6.30	0.54	0.10	grams
	6A	6A_406733069	406733069	A/G (247/8)	0.06	4.68	0.82	0.07	grams
	6B	6B_695913077	695913077	G/A (225/44)	0.16	5.67	0.45	0.02	grams
Grain Yield	1A	IWA5011	400311021	T/C (235/33)	0.13	5.90	191	0.04	kg ha^{-1}
	1A	1A_496309488	496309488	G/A (199/59)	0.24	8.27	174	0.06	kg ha^{-1}
	3B	IWB32652	349636369	A/G (172/96))	0.36	4.00	97	0.03	kg ha^{-1}
	3B	3B_310333182	310333182	G/A (221/19)	0.13	5.56	213	0.03	kg ha^{-1}
	6A	IWB26414	5326425	A/G (236/30)	0.12	7.73	224	0.03	kg ha^{-1}
	6A	IWB63176	63563014	A/G (193/74)	0.28	7.30	163	0.15	kg ha^{-1}
	6B	IWB38887	696150409	A/G (143/126)	0.47	4.41	92	0.05	kg ha^{-1}
	6B	6B_73187805	73187805	G/A (251/15)	0.06	4.07	250	0.14	kg ha^{-1}
	7A	IWB59141	6499010	A/C (194/69)	0.27	4.00	117	0.11	kg ha^{-1}
	7B	IWB6720	59632081	A/C (227/39)	0.15	5.28	154	0.05	kg ha^{-1}
	7D	7D_633027374	633027374	C/T (236/17)	0.09	16.35	492	0.18	kg ha^{-1}

Table 7 continued

Days to Heading	2B	IWB34502	553613770	T/C (144/126)	0.44	6.62	0.44	0.01	Julian days
	2D	2D_35683268	35683268	G/A (172/90)	0.35	4.81	0.37	0.02	Julian days
	3A	IWB6009	669524837	T/G (144/126)	0.47	6.03	0.39	0.05	Julian days
	3B	3B_705185712	705185712	C/T (142/121)	0.46	5.98	0.39	0.02	Julian days
	4B	4B_665871684	665871684	C/T (241/10)	0.07	6.92	1.11	0.02	Julian days
	5B	5B_167440402	167440402	A/G (241/26)	0.10	4.33	0.42	0.02	Julian days
	6A	6A_565344991	565344991	C/A (151/110)	0.42	7.39	0.46	0.02	Julian days
	7A	7A_690860911	690860911	G/A (185/66)	0.28	4.86	0.38	0.05	Julian days
	7D	7D_301325415	301325415	G/T (243/21)	0.09	5.77	0.74	0.10	Julian days
	7D	7D_58927880	58927880	C/T (204/61)	0.24	8.38	0.58	0.08	Julian days

[a]The underlined nucleotide represents the favorable allele. For days to heading, days to maturity, and plant height, the favorable allele was reducing whereas all other traits the favorable allele was considered as increasing.

Chr: chromosome

MAF: minor allele frequency

R^2: coefficient of determination

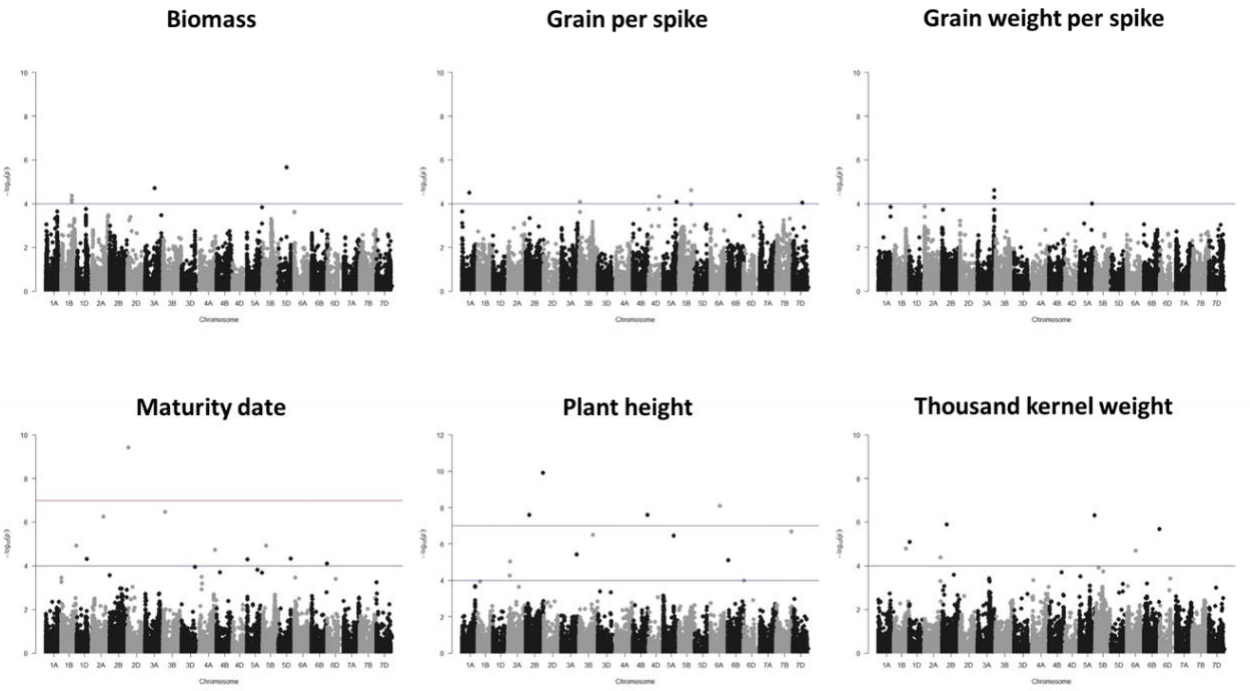

Figure 5: Manhattan plots of traits based on FarmCPU method. Blue horizontal line indicates -*logP* = 4.0, and red horizontal line indicates 5% FDR threshold.

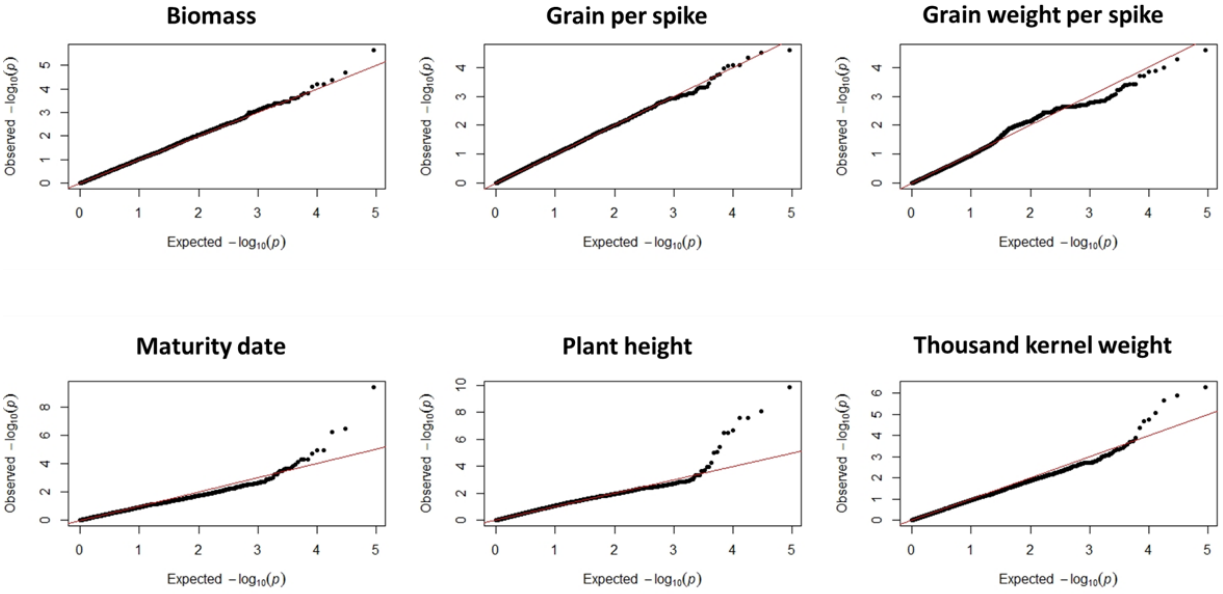

Figure 6: Q-Q plots from Manhattan plots of traits in Figure 5.

2.3.4 Transferability of GWAS results

We used existing YLD and HD data that were generated from the same germplasm in other states and seasons. Altogether, we assembled 14-environment datasets, of which WL17 and WL18 are from our field testing in Indiana. Linear discriminant analysis (LDA) on grain yield resulted in three homogeneous groups (Figure 7A) and on heading date resulted in four homogenous groups (Fig. 7B). Strikingly, we observed that year-to-year variations resulted in different groupings in some cases (Table 8). For example, for grain yield, LDA group 1 included WL17, KYM12, MDM12, MOM12, and MOM13, group 2 included WL18, OWL12, OWM12, VAL12, OWL13, and OWM13, and group 3 consisted of VAL13 and VAM13. We observed that for example, VAL12 and VAL13 are categorized in different groups (Figure 7A). Similar observation was true for WL17 and WL18. In addition, we noticed that groupings were different for grain yield and heading date. LDA for grain yield and heading date had a percent separation above 87% for each discriminant function and cross-validation confirmed successful separation of environments.

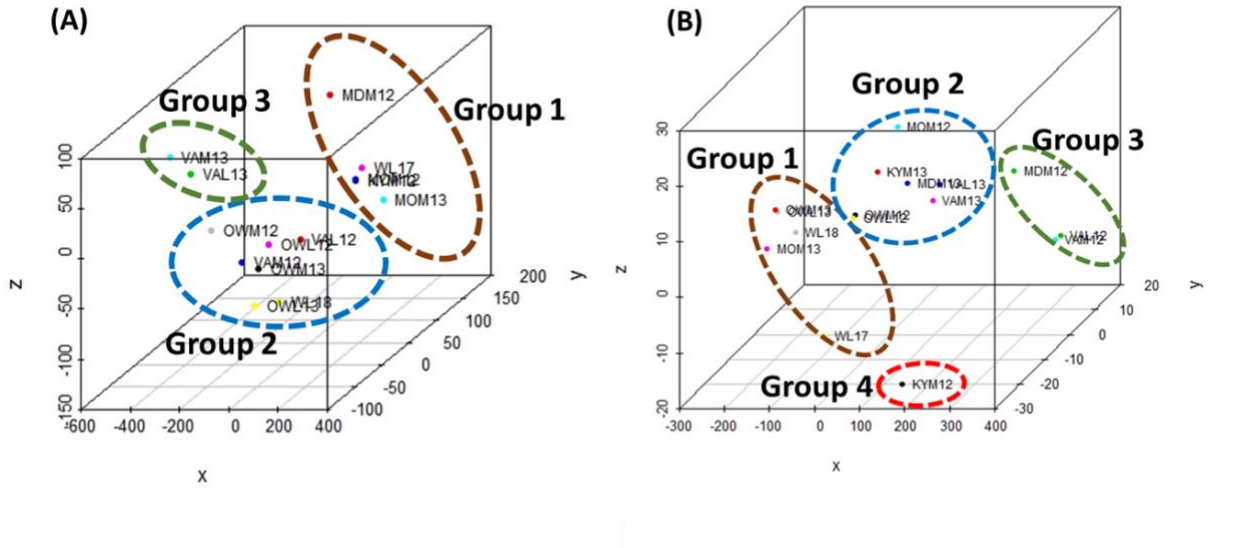

Figure 7: Grouping of environment and year based on linear discriminant analysis. 3D plot of multi-dimensional scaling to visually observe groupings based on (A) grain yield and (B) heading date.

Table 8: Grouping of environments from linear discriminant analysis for GWAS based on grain yield and heading date.

	Grouping	
Environment	Grain yield	Heading date
WL17	1	1
WL18	2	1
KYM12	1	4
MDM12	1	3
MOM12	1	2
OWL12	2	2
OWM12	2	2
VAL12	2	3
VAM12	2	3
MOM13	1	1
OWL13	2	1
OWM13	2	1
VAL13	3	2
VAM13	3	2

We performed GWAS for YLD and HD based on phenotypic observations from four environments: WL1718, Group 1, Group 2, and Group 3 (Figure 8 and Figure 10). In Group 1, twelve MTAs were identified in chromosomes 1B, 2B, 5A, 5B, 6A, 6B, 7A, 7B, and 7D (Figure 8). Three MTAs were present on chromosome 6B and two MTAs on 7A. For Group 3, eight MTAs were identified on chromosomes 3B, 5B, and 7B, however, applying the same standard for markers in LD as above, resolved to five independent MTAs. No MTAs were identified in Group 2. When we compared YLD signals among the three homogenous groups, there was not any MTA identified in more than one group, indicating that QTL are specific to each group.

A total of 28 independent MTAs were identified across environmental groupings for YLD but we only noticed seven MTAs that were identified in at least two environments which are indicative of transferability across environments. Two of these MTAs are located on chromosomes 6B and 7D. The MTA on chromosome 6B for YLD at position 73,187,805 bp was identified in WL1718, Group 1, and Group 3 environments with -*logP* of 4.07, 7.75, and 2.38, respectively (Table 9). The marker effect for this validated MTA showed an effect size of 238 – 250 kg ha^{-1} across environments. The MTA on chromosome 7D for YLD is at 633,027,374 bp, and was identified in WL1718, Group 1, and Group 3 environments with -*logP* of 16.35, 20.87, and 1.64,

respectively (Figure 8; Table 9). The marker effect of this MTA was approximately 492 kg ha^{-1} in WL1718, 393 kg ha^{-1} in Group 1, and 184 kg ha^{-1} in Group 3 (Table 9).

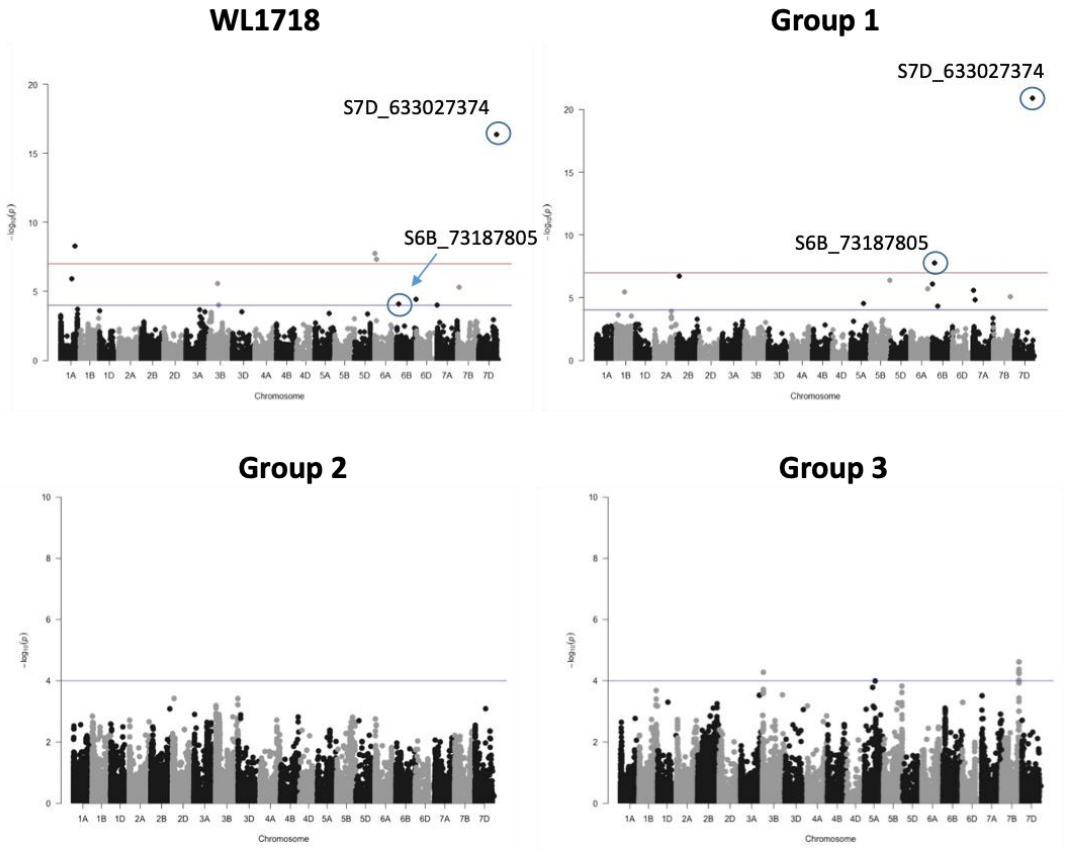

Figure 8: Manhattan plots of Grain yield based on FarmCPU method. Blue horizontal line indicates *–logP* = 4.0, and red horizontal line indicates 5% FDR threshold. Blue circles indicate markers present in multi-environments.

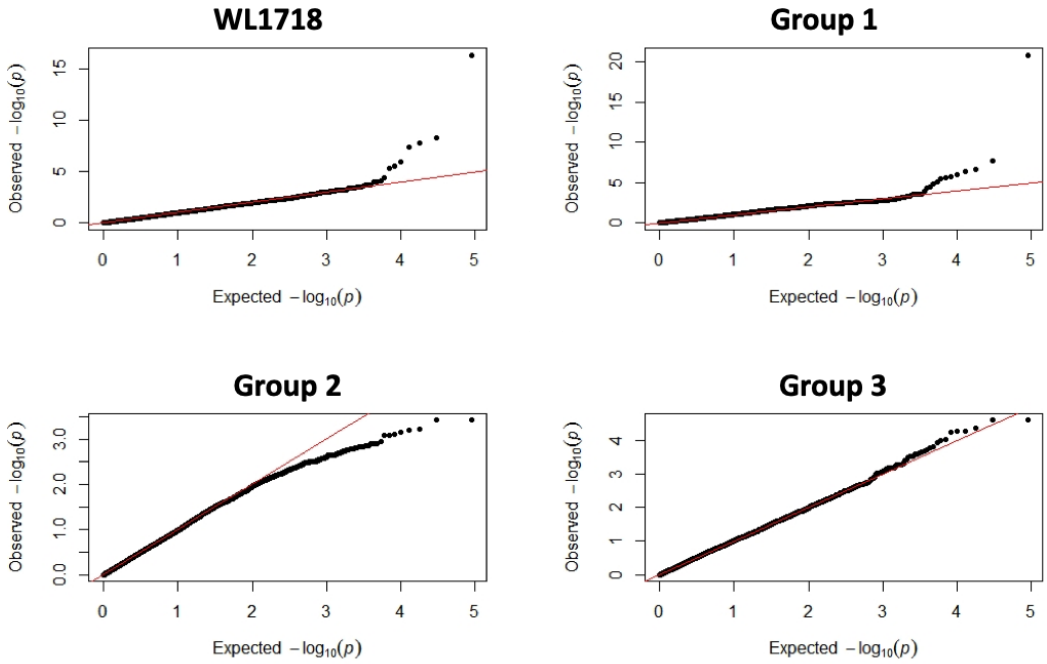

Figure 9: Q-Q plots from Manhattan plots of grain yield from Figure 8.

For HD, LDA grouping clustered environments into four groups. LDA group 1 included WL17, WL18, MOM13, OWL13, and OWM13, group 2 included MOM12, OWL12, OWM12, VAL13, and VAM13, and group 3 consisted of MDM12, VAL12, and VAM12 (Table 8). KYM12 was a singleton Group 4, with no other group member (Figure 7B), and was left out of the analysis. GWAS was performed for these three groups. Group 1 had 35 MTAs (Figure 10), that were grouped into 26 independent loci. Eleven of these loci were located on chromosome 7A and five were located on chromosome 7D. For Group 2, eleven MTAs were identified on chromosomes 1A, 1B, 3A, 4B, 5B, 6A, 6B, 7A, 7B, and 7D (Figure 10). Lastly, Group 3 did not show any significant MTAs for HD. When we compared HD signals among the three homogenous groups, only one MTA, marker 7D_301325415 on chromosome 7D, was present in more than one group.

A total of 47 MTAs were detected for heading date across environments but we only noticed eight MTAs that were identified in at least two environments which are indicative of transferability across environments. These MTAs were identified on chromosomes 2B, 3A, 3B, 4B, 5B, 7A, and 7D (Table 9). For HD, one marker from the SNP chip array, IWB34502 located at 553,613,770 bp on chromosome 2B was associated with days to heading (Table 9), in WL1718,

Group 1, and Group 2 environments with allele effect of 0.44, 0.29, and 0.30 days, respectively. A marker with similar effects in the same environments was identified at 690,860,911 bp on chromosome 7A with -*logP* of 4.86, 9.10, and 2.23 in WL1718, Group1, and Group 2, respectively (Table 9).

Chromosome 7D contained two markers significant for days to heading. The positive allele associated with this marker (301,325,415 bp) on 7D showed effect sizes of 0.74, 0.68, and 0.98 days for WL1718, Group 1, and Group 2, respectively (Table 9). The marker at position 58,927,880 bp on chromosome 7D was found to be associated with heading date in environment WL1718, Group 1, and Group 2 with -*logP* of 8.38, 4.44, and 1.48 (Table 9).

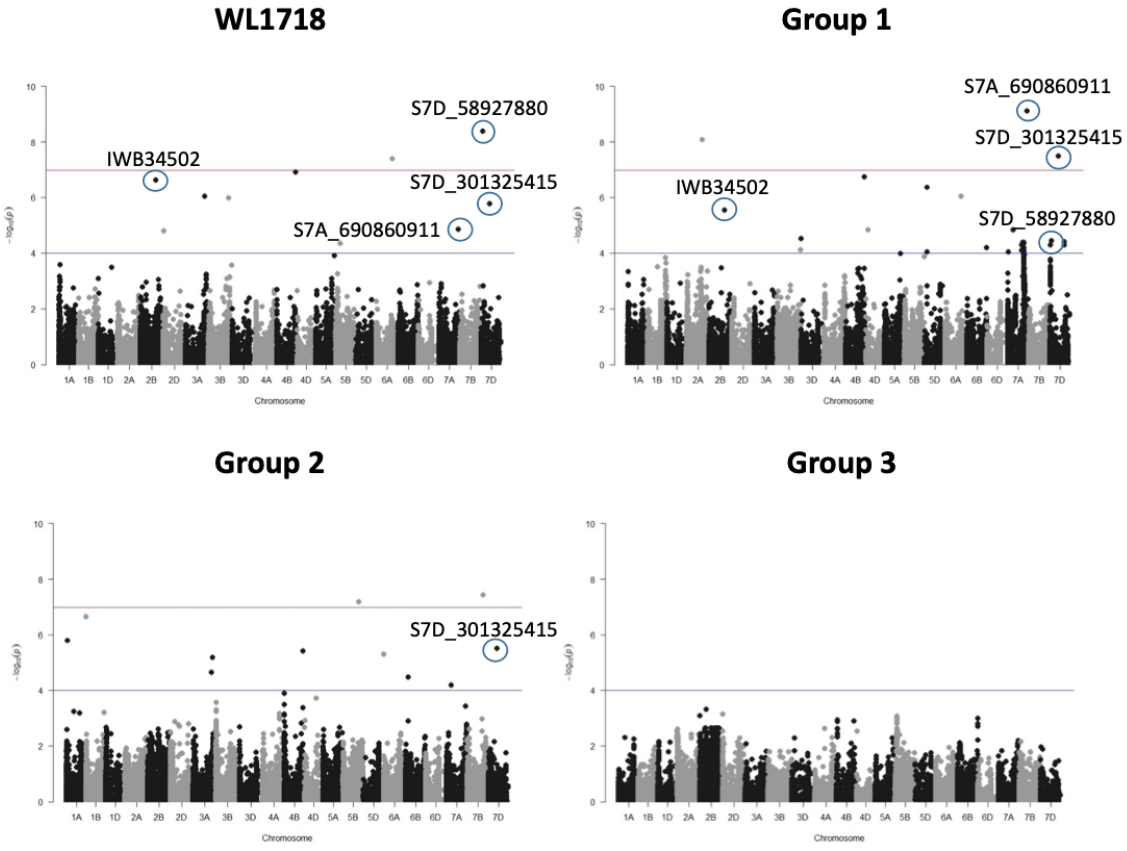

Figure 10: Manhattan plots of days to heading based on FarmCPU method. Blue horizontal line indicates –*logP* = 4.0, and red horizontal line indicates 5% FDR threshold. Blue circles indicate markers present in multi-environments.

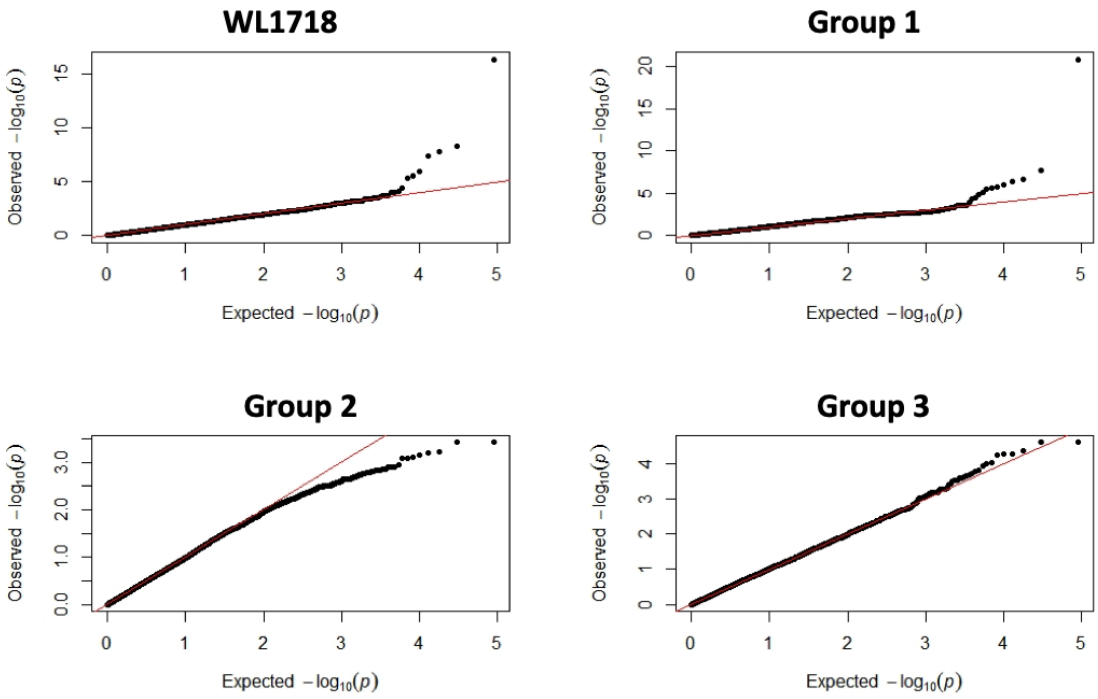

Figure 11: Q-Q plots from Manhattan plots of heading date from Figure 10.

Table 9: -*logP* value and marker effect for significant multi-environment MTAs.

			Environment							
			WL1718		Group 1		Group 2		Group 3	
Trait	Chr	SNP	-*logP*	Effect	-*logP*	Effect	-*logP*	Effect	-*logP*	Effect
Grain yield	1A	IWA5011	5.90	191	1.55	64	1.68	96	-	-
	1A	1A_496309488	8.27	174	2.73	71	-	-	-	-
	3B	3B_310333182	5.56	213	-	-	-	-	1.69	170
	6A	IWB63176	7.30	163	-	-	-	-	1.57	94
	6B	6B_73187805	4.07	250	7.75	238	-	-	2.38	235
	7A	IWB59141	4.00	117	1.72	56	-	-	3.04	150
	7D	7D_633027374	16.35	492	20.87	393	-	-	1.64	184
Days to heading	2B	IWB34502	6.62	0.44	5.53	0.29	1.94	0.30	-	-
	3A	IWB6009	6.03	0.39	1.85	0.16	-	-	-	-
	3B	3B_705185712	5.98	0.39	-	-	1.40	0.24	-	-
	4B	4B_665871684	6.92	1.11	6.74	0.84	2.08	0.81	-	-
	5B	5B_167440402	4.33	0.42	1.46	0.22	-	-	-	-
	7A	7A_690860911	4.85	0.38	9.10	0.40	2.23	0.33	-	-
	7D	7D_301325415	5.77	0.74	7.48	0.68	5.50	0.98	-	-
	7D	7D_58927880	8.38	0.57	4.44	0.30	1.48	0.29	-	-

Represented MTAs based on the accepted threshold (-*logP* value > 1.3).

For YLD, seven out of 28 MTAs and for HD, eight out of 47 MTAs were found to be transferable across seemingly homogenous environments. Therefore, we concluded that not all marker-trait associations are transferable and MTAs are often environment specific.

2.4 Discussion

Wheat provides approximately 20% of the protein and calories for human consumption worldwide (M. Reynolds et al., 2012). In order to meet the needs of the growing population, food supplies from major cereals such as maize, rice and wheat will need to increase by 2-3% annually, and wheat has shown the lowest rate of increases (Hawkesford et al., 2013). Ray et al. (2013) estimated wheat yields are increasing at 0.9% per year, much less than the 2.4% required to double global production by 2050. With future food security and climate challenges ahead, wheat breeding efficiency and genetic gains must improve significantly to develop stable, adapted, and high-yielding wheat varieties.

In this study, we analyzed associations between genotypes and phenotypes in a US SRW wheat elite population, consisting of breeding lines that were developed by breeding programs in the Midwest and east. Marker-trait associations for this population have been previously identified for *Fusarium* head blight (Arruda et al., 2016), days to heading (Huang et al., 2018), and grain quality (Gaire et al., 2019) from plants grown in Ohio and Virginia. We dissected the genetic architecture of this population for grain yield and related traits based on phenotypes observed in Indiana. In addition, we examined the transferability of SNPs across environments for the traits of YLD and HD.

Phenotypic correlations among traits and deciphering their relationship can give insight into identifying selection criteria for improving traits of interest. Our study showed that grain components including TKW, BIO, and GWS were significantly and positively correlated with YLD. Previous studies have documented positive relationship between TKW and YLD as well (Arguello et al., 2016; Sharma et al., 2008). In wheat breeding research, biomass is often referred to as the whole above ground plant parts. The pre-Green Revolution wheat germplasm were tall, and their height was the driver of plant aboveground weight. Therefore, during the Green Revolution the main force that led to increases in harvest index and productivity was only reducing plant height. In this population, although variation in biomass was observed, we think that in this era a “useful biomass” is one that can lead to non-competing multiple well-grown culms (tillers)

with the potential to lead to a fertile spike. Increasing tiller numbers or protecting tillers in soft red winter wheat is one approach that can produce useful biomass. Our data showed that NS and BIO were significantly correlated (r=0.47) and that NS is distributed in a wide range from 74 to 152. For example, the varieties OHO8-172-42, IL08-12174, MD05W1292-11-1, 05264A1-1-3-2, and IL07-20728 showed averages above 240 grams for BIO and 134 NS. Other traits that can lead to useful biomass are smaller leaves with enhanced photosynthetic capacity and the levels of spike fertility, among others.

While TKW, BIO, and GWS showed positive correlation with YLD, the duration of vegetative growth period, indicated by days to heading (and similarly days to maturity) negatively correlated with YLD. Similar negative correlation was reported by Addison et al., (2016). Addison et al. (2016) noticed this trend in a SRW wheat recombinant inbred line (RIL) population across nine environments in the southern US, with the population segregating for photoperiod and vernalization loci. Grain number is the main driver of grain yield but no correlation was observed. This is a population of elite lines therefore; loci influencing traits relating to grain number could be potentially fixed in the population of elite germplasm.

There are reports in the literature that shows positive correlations between days to heading and grain yield, (Godoy et al., 2018), especially under cooler temperatures for hard red spring wheat (Lanning et al., 2010). The primary reason for the observed negative correlation between days to heading and yield in this population could be that most of the late heading germplasm were developed by and adapted to the state of New York. Therefore, a hidden G x E interaction works contrary to the yield formation. Path analysis affirms the consequence of heading later is indirectly decreasing grain development. Therefore, a practical consideration for future characterization of populations that are mixture of germplasm from multiple crop breeding programs is that the experimenters can use days to heading as biomarkers because a shit in phenology could mask yield traits. When a drastic change between native germplasm and others is observed, yield differences are likely expected. Tessmann et al. (2019) used QTL markers for plant height, vernalization, and photoperiod genes along with the actual heading date trait as covariates in the GWAS model to account for the latitude differences. This method is also routinely performed for maize but including flowering time (days to anthesis) as covariates (Bian et al., 2014; Poland et al., 2011).

One major concern in GWAS discoveries is marker density. Wheat is a self-pollinated crop and the germplasm has been under selection. Therefore, in the beginning of the experiment, 45K

markers seemed unnecessarily dense. We found evidence to the contrary. Increasing the marker density increased the probability of finding more MTAs which could have been missed. Significant MTAs with SNPs from GBS were 32 for yield components measured in WL1718 and 10 multi-environment MTAs for GY and HD. In contrast, markers from the SNP chip array contributed 8 MTA for yield components and 5 multi-environment MTA for GY and HD. This data indicates that MTAs were identified from both sets of SNP markers. In addition to this, we examined the inter-marker spaces for SNP chip markers located between two GBS markers. For example, the SNP IWB72708 (identified for biomass at $-logP = 4.36$) is located 897,950 bp downstream of gbs_1B_472631187 while 296,024 bp upstream of gbs_1B_473,825,161. The SNP IWB51951 (identified for plant height at $-logP = 5.01$) is located 458,542 bp downstream of gbs_2A_92,338,766 while 1,095,722 bp upstream of gbs_2A_93,893,030. While these distances must be judged based on the basis of the local LD decay rates in each region, our conclusion is that the 45K marker set, combined from chip array and GBS methods, is not in excess for this germplasm and the combination of both marker sets can be complementary in GWAS applications.

FarmCPU is a multiple loci linear mixed model that eliminates confounding effects between markers and kinship by iterating between both fixed and random effect models. In the fixed effect model, individual SNPs are tested while using pseudo-QTNs as covariates to control false positives. The FarmCPU model controls false positives, false negatives, and provides greater statistical power than alternative models used for association mapping (Kaler et al., 2020; Liu et al., 2016). Based on quantile-quantile (Q-Q) plots, FarmCPU effectively controlled false positives and false negatives based on the population structure and significant associations (Supplemental Figure S3, S4, S5). The Q-Q plot line holds close to the 1:1 line of expected versus observes association probabilities, with a slight upward tail indicating deviation from expected distribution. A deviation in the tail area indicates properly controlling false positives and false negatives, where any inflation line upward would indicate false positives or downward indicate false negatives (Kaler et al., 2020). Other researchers reported similar claims. Xu et al. (2018) and Vanous et al (2019) concluded the multi-locus model of FarmCPU provided more statistical power than single locus models with less over or under fitting. One potential drawback of FarmCPU is that the model identifies the most significant single SNP at a specific genomic location instead of a large peak of SNPs with other MLM models (Kaler et al., 2020).

In the target environment of Indiana, several loci affected yield and yield components traits were identified. Germplasm were also identified that harbor those favorable alleles. The 17 lines that harbored the favorable yield QTL for the region on 7D were all developed by Purdue's small grains breeding program (Figure 12). It is possible that all 17 of these lines contain a 7E translocation for resistance to barley yellow dwarf and cereal yellow dwarf virus, and are descendants of the Purdue line "P107" (Ohm et al., 2005). However, we could identify the 7E translocation harboring line in the pedigrees of only 11 out of the 17 lines. This translocation could explain slow LD decay rate in over 100 Mbp on chromosome 7D.

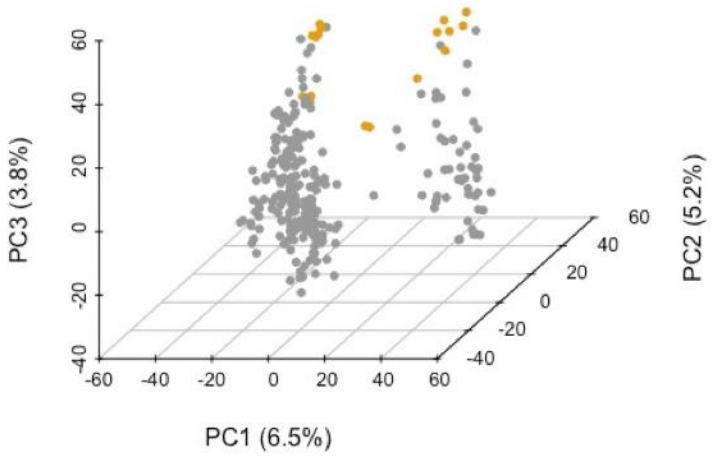

Figure 12: Principal component analysis based on all SNPs for 270 lines. A 3D scatterplot of first three principal components (PCs) where gold indicates the 17 Purdue breeding lines.

QTL expressed in one environment may not equally or ever be expressed in other environments. To a large degree, this can be associated with the key environmental clues that are critical regulatory event for the mode of action and expression of traits and QTL. For example, if the mode of action of a growth QTL is via tiller development before winter that are later on sensitive to freezing temperature, then two environments differing in winter temperature would results in different number of tillers that are counted in the spring. Therefore, QTL could go unnoticed in the colder environment. Similar examples can be given for kernel weight QTL expression under two hot and mild grain-fill period temperatures. Such QTL by environment interaction effects can vary depending on the location and specific year. To identify stable QTLs,

GWAS on the basis of combined analysis of years and locations is suggested, which is often known as multi-environment GWAS (Gutiérrez et al., 2015; Sukumaran et al., 2018) for future QTL implementation in. marker assisted selection (Collard and Mackill, 2008; Ribaut and Ragot, 2007). However, our results showed that majority of MTAs are environment specific. Even when we contained GWAS analysis within homogenously environments, the majority of MTAs we identified in WL site for YLD and HD and were not observed in other environments. Even when markers were significant across environments, there was differences in phenotypic variation explained by each marker and the size of marker effect. For some traits such as grain yield the magnitude of variance component due to G x Y was 20% greater than the magnitude of variance component due to G. Since winter wheat is grown over nine months, variation in climate and weather can directly impact the year to year variability and effect of the environment. For example, the WL site in 2017 showed significantly higher monthly temperature than WL18 site from February through April (Table 1), which is a critical time in winter wheat development. With the increase in temperature, the vernalization period for 2017 was shorter than 2018, resulting in a decrease in yield. This could be one potential reason for the difference in classifying the WL17 and WL18 site into different groups for YLD. Previous work is a mix of success and failure in the transferability of QTL across environments. Guan et al. (2018) identified 226 QTL controlling yield component traits and heat susceptibility in a Chinese elite double haploid winter wheat population. Across the 12 environments in northern China, only 39 of these QTL were deemed "stable" based on detection in at least three individual environments. Further explanation could be the significant source of variance based on effect of environment and effect of genotype by environment on all measured traits. In the United Kingdom, a double haploid population was developed from favorable bread making hexaploid winter wheat cultivars to detect QTL controlling yield variation. The population was evaluated and phenotyped at five field trials across multiple years in England, Scotland, Germany, and France. Two QTL were mapped on chromosome 6B for grain size and yield, *Qtgw-jic.6A* and *Qyld-jic.6A*, that were stable across nine of the twelve environments (Simmonds et al., 2014). These favorable QTL validated with near isogenic lines displayed improvements of 5.5% and 5.1% for grain yield and grain weight.

2.5 Conclusion

Seeking stable QTLs for yield determining traits may not be the most thoughtful approach to improve stability and genetic gains for wheat breeding. QTL transferability is challenging, and we suggest proceeding with caution to identify QTLs across multiple environments. In our case, detecting MTAs in homogenous environments showed minimal opportunities for making progress across regions or even or for developing biomarkers for marker assisted selection. We suggest performing GWAS and evaluating MTAs in the targeted breeding environment. The ability to utilize past data is powerful for predictability and examining transferability, however, the effect of the environment could be the leading issue in non-transferable QTLs controlling significant MTAs.

CHAPTER 3. CULTIVAR, TRAIT AND MANAGEMENT SYSTEM SELECTION TO IMPROVE SOFT-RED WINTER WHEAT PRODUCTIVITY IN THE EASTERN UNITED STATES

The end use quality data in this chapter was generated by the wheat quality laboratory in CIMMYT (Dr. Carlos Guzman). CG is a co-author in a paper we published from a version of this chapter in *Frontiers Plant Science*: https://doi.org/10.3389/fpls.2020.00335

3.1 Introduction

Wheat cultivation occupies 22% of the major croplands globally, and covers the temperate latitude of both hemispheres, consisting of the Great Plains in US, Canadian Prairie Provinces, western Europe, the Indus and the upper Ganges valleys, southern South America, eastern Africa, eastern China, southern Australia, and along the Kazakhstan and Russia border (Leff et al., 2004). Wheat grown throughout the world consists of either spring or winter wheat. Winter wheat requires a vernalization period to transition from vegetative to reproductive stage (Jorge Dubcovsky et al., 2006). The vernalization requirement is genotype specific, with variations in time (15-45 days) and temperature (0-5°C) (Crofts, 1989). Some wheat producing regions manage autumn-grown wheat that are not considered winter types. These regions use the mild but elevated winter temperatures to grow wheat for higher yield potential. Examples of these locations are Mexico, California, and parts of the Middle East. Winter wheat is typically not viewed as a cover crop but has dual grain and grazing purposes in targeted regions such as Oklahoma and Texas (Maulana et al., 2019).

A key characteristic of wheat is the unique properties of forming dough from flour (Shewry, 2009). Quality is indicated by the performance of a cultivar at specific protein levels for defined end use products (Bushuk, 1997b) and viscoelastic properties (Shewry, 2009). Wheat classes are defined by grain hardness, protein content, and growth habit. Hard wheat has hard endosperm texture and higher protein content. Soft wheat has soft endosperm texture, low levels of damaged starch granule upon milling, and weaker dough strength that is suitable to make biscuits, cookies, and cakes (Bushuk, 1997b). Protein composition in the endosperm is made of monomeric gliadins and polymer glutenins subunits (Porceddu et al., 1997). Glutenins are further divided into high molecular weight (HMW) and low molecular weight (LMW) subunits. The composition of high and low molecular weight glutenin subunits is the key quality determinant for dough (Bushuk,

1997b). In addition to genetics, protein quantity and quality is dependent on environmental conditions (Cooper et al,, 2001; Luo et al., 2000).

Management practices in wheat have substantial impacts on crop productivity and environmental stewardship. In both winter and spring wheat cropping systems, nitrogen (N) fertilizer applications are routinely applied pre-planting or during leaf formation (Zadoks 15) with additional N top-dress application in the stem elongation stage (Zadoks 30) or post-anthesis (Zadoks 69) (Otteson et al., 2007; Woodard and Bly, 1998). Developing a site-specific understanding for fertilizer expenses, environmental impacts such as leaching and volatilization, and efficient use of N by crops are pillars of crop profitability in relation to N management. Previous work by Koch et al. (2004) described the economic benefits for site-specific and environment-specific management practices for variable rate nitrogen applications, but further research is needed in the area of targeted genotype by environment by management practices for improved economic and environmental outcomes.

N is necessary for growth of canopy, intercepting solar radiation, and photosynthesis in green tissues (Barraclough et al., 2014). Nitrogen use efficiency (NUE) is the amount of grain produced per unit of N available in the soil (Moll et al., 1982). In other words, the ability to increase grain yield per N applied. The two main components of NUE are uptake efficiency and utilization efficiency. Nitrogen uptake efficiency (NUpE) is the plant's ability to absorb N available in the soil, and nitrogen utilization efficiency (NUtE) is the efficiency of which the absorbed N is utilized to produce grain (Moll et al., 1982). NUtE is also described as the ratio between crop yield and total N absorbed by the plant (Todeschini et al., 2016), indicative of the output of grain yield based on the amount of N taken up by the plant.

It is nearly impossible to identify and recommend a single variety that is the "best" across multiple environments due to the infinite interactions that can cause unstable phenotypic characteristics (Allard and Bradshaw, 1964). Yield is the most economically important trait, making both pre-planting and in-season crop management (Kirkegaard and Hunt, 2010) critical to maximize this market for growers and suppliers. The end-use quality traits such as protein content and endosperm texture are also influenced by N availability during plant growth. Farm profitability is primarily dependent on grain yield and quality. With approximately 7.8 million metric tons of soft-red winter wheat produced in the US in 2018, accounting for ~15% of total wheat production, it is paramount to strategically manage the cost and benefits to increase yields. The goal of our

study was to identify traits responsive to N in a typical soft-red winter wheat breeding population under two contrasting N management and identify potential useful genetic solutions for the long term goal of managing wheat with reduced nitrogen fertilizer. To accomplish our goal, we evaluated grain yield, yield determining traits and N components under low N and high N environments and assessed protein quality.

3.2 Materials and Methods

3.2.1 Field experiments and nitrogen management

Thirty experimental breeding lines, designated as PU01-PU30, from Purdue University's soft-red winter wheat breeding program were selected based on their variation in grain yield (from 3,500 to 6,500 kg ha^{-1}). These 30 lines were planted in the Purdue Agronomy Farm (40.43° N, 86.99°W) for two seasons: 2016-2017 and 2017-2018. The experimental layout included two N rates arranged in a split plot design with 4 blocks, where N rate was main-plot and line was sub-plot. Each experimental unit measured 1.22 m x 3.05 m, with 7 rows spaced 15 cm apart with a targeted planting density of 370 seeds m^{-2}. The soil type at the Agronomy Research Farm is a combination of Rockfield silt loam (fine-silty, mixed, superactive, mesic Oxyaquic Hapludalfs), Fincastle silt loam (fine-silty, mixed, superactive, mesic Aeric Epiaqualfs), and Toronto silt loam (fine-silty, mixed, superactive, mesic Udollic Epaqulafs) (USDA Web Soil Survey). Experiments were planted in late September following corn and harvested late June of the following year. The experiments were planted using a Hege (Wintersteiger, Austria) drill planter and plots harvested with a Wintersteiger (Wintersteiger, Austria) plot harvester at physiological maturity.

In the fall, 224 kg ha^{-1} of mono-ammonium phosphate (11-52-0) was applied based on soil test (Mehlich-3) recommendations. The plot area was then chisel cultivated. Approximately 100 kg ha^{-1} of potassium chloride was added to the entire experimental area as recommended by soil analysis. Emergence began approximately six days after planting. Spring nitrogen applications of 112 kg N ha^{-1} of urea (46-0-0) was broadcast applied to the main plots, designed as high-N treatment, at stem elongation (Zadoks 30) growth stage. Prior to application, urea was treated with Limus (BASF, Germany), a urease inhibitor which prevents urea from being broken down via urease enzymes and lost through volatilization. The main plots, designated for low-N treatment, received zero spring N. Herbicide (Harmony Extra [thifensulfuron + tribenuron], DuPont, 35 g ha-

1) was applied in mid-April to minimize weed pressure. Weather information including average monthly precipitation and temperature, as per iClimate (2019), are shown in Table 10.

Table 10: Average temperature and precipitation for the duration of the study. Historical averages based on previous 30 years from the National Weather Service.

	Temperature (°C)			Precipitation (mm)		
Month	**2016- 2017**	**2017-2018**	**Historical**	**2016- 2017**	**2017-2018**	**Historical**
September	20.8	19.3	19.4	81.0	50.5	79.5
October	15.1	14.5	12.8	32.8	68.1	79.2
November	8.3	5.3	6.5	135.9	125.8	94.0
December	-1.9	-1.4	-0.2	58.9	20.6	80.5
January	-0.2	-4.7	-2.2	111.7	39.9	67.6
February	4.7	0.4	0.1	19.9	139.8	58.9
March	5.6	2.8	5.7	109.1	79.1	90.4
April	13.5	6.8	11.7	108.7	73.7	96.8
May	15.7	21.1	17.1	175.3	93.7	128.3
June	22.2	22.8	22.2	135.4	157.8	107.9

3.2.2 Agronomic traits

Days to heading (HD) and days to physiological maturity (MD) were recorded when 50% of the plot showed head emergence and maturity, respectively, and expressed as the number of days from January 1 of the current year. Plant height (PLH), from the ground to the top of the uppermost spikelet, was measured at four locations within the plot at physiological maturity. Thousand kernel weight was measured and the average weight for a single kernel was calculated (KW). Grain yield (YLD) was measured on a whole plot basis, corrected for 13% moisture.

The aboveground biomass (BIO) was estimated by cutting 0.25 m x 0.30 m (2 rows) from the middle of each plot for all treatments at heading (Zadoks 58), anthesis (Zadoks 60-68), and maturity (Zadoks 91) and dried to constant weight. Number of spikes per cut area (NS) was estimated by averaging the count of spikes at heading, anthesis, and maturity from the samples of cut area (0.25 m x 0.30 m). Yield component traits were measured from the same cut area sample at physiological maturity. Five random spikes were chosen to measure spike length (SPL), and hand-threshed to obtain the number of kernels per spike (KNS), kernel weight per spike (KWS),

and grain number per cut area (GN). Fruiting efficiency (FE) was calculated by the number of kernels produced by each spike divided by the spike weight at anthesis. Lastly, harvest index (HI) was determined by the dividing the grain yield by the aboveground biomass at maturity.

We chose 5 out of 30 lines, based on earlier yield data, to analyze N concentration in biomass and grain. These lines showed a range of grain yield over five years and three locations in Indiana. The entire aboveground biomass (phytomass) was analyzed at heading and anthesis. At maturity once leaf senescence was complete, plant biomass was divided into grain and leaves plus straw. All samples were dried for 72 hours at 49°C.

Plant samples were ground with cutting mill (Model E3703, Eberbach Corp, Bellevile, MI) and UDY grinder (Udy Corp, Fort Collins, CO) and passed through a 1.0 mm screen. Thirty milligrams of each sample were sent for flash combustion analysis (Flash EA 112 Series, CE Elantech, Lakewood, NJ). The N concentration of phytomass at heading (NCPH) and anthesis (NCPA) were measured on whole plant samples. The nitrogen concentration of phytomass at maturity (NCPM) was measured on leaf and straw tissues. The nitrogen concentration of grains at maturity (NCGM) was measured on the grain samples.

For NUE measurement, we adopted the methods presented by Foulkes et al., (2009), and Moll et al., (1982).

$$NUE = \frac{Grain\ dry\ matter}{available\ N}$$

where $Grain\ dry\ matter$ is the grain yield (kg ha^{-1}) of plots atmaturity (Zadoks 92), and $available\ N$, based on the formula, is the nitrogen available from the soil and fertilizer. Residual N was not tested and is not included in the study and calculation of NUE. In this estimation, instead of available N, we used the amount of N applications in each treatment. Both low-N and high-N environments received the same fall N application of 25 kg N ha^{-1} as monoammonium phosphate. A spring N application of 112 kg ha^{-1} N was applied to the high-N environment only. The total N supplied in low-N environment was 25 kg ha^{-1} N, while the total N supplied in the high-N environment was 137 kg ha^{-1} N. N uptake was calculated as the total nitrogen in the aboveground biomass including grain. NUtE was measured as grain dry matter produced per gram of plant N

uptake. Nitrogen harvest index (NHI) was estimated as amount of nitrogen that was recovered in grains relative to overall N uptake of the plants.

3.2.3 Phenotyping grain and flour characterization

A subsample of grains from each N environment were subjected to Single Kernel Characterization System 4100 (SKCS) (Perten Instruments, Sweden) analysis. A single replicate was performed for each linein each N environment. The SKCS weighs and crushes individual kernels and converts the force-crush profile to a unit-less Grain Hardness Index (GHI). Whole-meal flour samples were also prepared with a UDY Cyclone mill (Udy Corp, Fort Collin, CO) with a 0.5 mm screen. Sodium dodecyl sulfate (SDS) sedimentation volume was carried out according to the modified protocol described in Peña et al. (1990) using 1 g of flour.

3.2.4 Glutenin subunits and the rye translocation

Allelic variation of glutenin subunits and the presence or absence of the rye translocation were evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for all thirty lines following method described by (Peña et al., 2004).

3.2.5 Statistical analysis

Combined year analysis of variance (ANOVA) was performed with PROC GLM in SAS 9.4 (SAS Institute, Cary NC) similar to the model presented by Iannucci et al., (2008), where sources of variations are year, nitrogen, year x nitrogen interaction, genotype, year x genotypes, nitrogen x genotypes, and year x nitrogen x genotype interaction effects, each tested against appropriate error term (Table 11).

$$\textbf{[1]}\ Y_{ijkl} = \mu + Yr_i + \underline{rep(Yr)}_{li} + N_j + NYr_{ji} + \underline{rep*N(Yr)}_{lji} + G_k + GYr_{ki} + GN_{kj} + GNYr_{kji} + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the phenotypic observation of the l_{th} replicate of the k_{th} genotype, in the j_{th} nitrogen treatment, observed in the i_{th} year. μ is the grand mean, Yr_i is the effect of i_{th} year, $\underline{rep(Yr)}_{li}$ is the effect of the l_{th} replicate in the i_{th} year. The effect of year was tested against $\underline{rep(Yr)}_{li}$. N_j is the effect of the j_{th} nitrogen treatment and NY_{ji} is the interaction effect of the j_{th} nitrogen level with the

i_{th} year. These two terms were tested against the interaction effect of nitrogen by replicate within the year ($\underline{rep*N(Yr)}_{lji}$). G_k represents the effect of the k_{th} genotype. Remaining interactions were tested against the residual error. Tukey's studentized range test (HSD) was implemented for comparison of means using the MEANS statement in PROC GLM (SAS 9.4) and significant differences reported with $p < 0.05$.

Table 11: ANOVA for year (Y), nitrogen level (N), and genotype (G).

		Grain Yield (YLD)		
Source of Variation	d.f.	Mean Square (x 10_4)	F value	Pr > F
Year (Y)	1	1286	5.23	ns
Residual 1	6	246		
N levels (N)	1	11144*	12.75*	*
Y x N	1	40.4	0.05	ns
Residual 2	6	874		
Genotype (G)	29	281***	8.31***	***
Y x G	29	132***	3.89***	***
N x G	29	53.5*	1.58*	*
Y x N x G	29	43.7	1.29	ns
Residual	348	33.9		
Total	479			

Significance levels: <0.001 = ***, <0.01 = **, <0.05 = *, and > 0.05 = ns
d.f: degrees of freedom

Least squares means was estimated using '*lsmeans*' package (Lenth, 2016) in R environment (R Core Team, 2019) for genotypes and N levels with combining years and implemented for phenotypic analysis. Heritability, in the broad sense (H_2) (Nyquist, 1991; Piepho & Möhring, 2007b), was estimated for each nitrogen environment by restricted maximum likelihood (REML) variance and covariance components using PROC MIXED (SAS Institute Inc., 2013) with random effect model in equation 2.

$$\textbf{[2]}\ H^2 = \frac{\sigma_g^2}{\sigma_g^2 + + \frac{\sigma_{gy}^2}{y} + \frac{\sigma_\varepsilon^2}{yr}}$$

With σ_g^2 representing variance component of genotype (genetic variance), σ_{gy}^2 the variance component of genotype x year interaction, and finally σ_ε^2 the residual error. Denominators represent years (*y*=2), and replications (*r=4).* Pearson's correlations were calculated for low-N and high-N environments separately using *cor* function in R environment (R Core Team, 2019). The linear relationship among measured traits was evaluated by Pearson's correlation coefficient (r). Principal component biplot analysis was used to visualize relationships among traits and lines by using the *'factoextra'* (Kassambara & Mundt, 2016) package and *'factoMineR'* (Lê et al., 2008) package in R environment (R Core Team, 2019).

3.3 Results

3.3.1 Agronomic traits

On average, the lines took approximately 130 days (from first of January) to head, and 168 days to reach physiological maturity (Table 12). N effect was significant on biomass accumulated at physiological maturity (Table 13). For example, biomass at maturity (BIO_{MD}) was ~22% greater in high N compared with low N.

The effects of G and N x G were significant for number of spikes (NS) (Table 13). We observed correlations of r ≥ 0.21 between NS and BIO_{MD} in both N treatments (Table 14), as more tillers produces more biomass. The lines showed variations in their number of tillers and biomass (Table 12). PU10 and PU14 showed an average of approximately 60 NS across both N treatments, and BIO_{MD} greater than 95 g. In comparison, PU21 and PU29 averaged 43 NS and BIO_{MD} of 87 and 88 g, respectively, showing a difference of 20 spikes and 10 g of biomass per cut area.

Number of spikes had the highest significant positive correlation observed with yield (r = 0.64* in low N; r = 0.36* in high N). On average, 8 more effective spikes per sampled area were observed in high N compared to low N, which resulted in 275 more kernels per sampled area in high N compared to low N (Table 12). The grain number per unit area was a result of NS and effective tillers, which in our study, was significantly impacted by N. However, the weight of individual kernels was unaffected by N treatment (Table 13). The mean KW was 36 mg, with a

range of 25 – 47 mg across lines and environments (Table 12). PU14 was the only line to have a KW above 40 mg in low N and high N. We observed a negative correlation between GN and KW under both treatments (r = -0.34 low-N; r = -0.30 high-N) (Table 14).

Table 12: Mean, standard deviation (sd), and range of 14 agronomic traits and 7 in tissue nitrogen analysis traits in both environments. Heritability (H_2) calculated for all 30 genotypes per nitrogen treatment.

	High-N			Low-N		
Trait – All 30 Genotypes	**Mean ± sd**	**Range**	**H_2**	**Mean ± sd**	**Range**	**H_2**
Plant Development						
Days to Heading (HD)	130 ± 5.53	119 - 137	0.66	130 ± 5.57	119 - 137	0.70
Days to Maturity (MD)	168 ± 3.53	162 - 175	0.55	167 ± 3.4	162 - 174	0.71
Biomass at Maturity (g) (MD_{BIO})	109.02 ± 28.57	46.86 - 193.59	0.19	87.51 ± 28.07	21.09 - 157.49	0.20
Plant height (cm) (PLH)	89.11 ± 9.43	67.25 - 112.75	0.77	81.42 ± 9.95	54.50 - 104.75	0.62
Yield Components						
Yield (kg ha^{-1}) (YLD)	6,335 ± 824.04	3,799 – 8,090	0.46	5,359 ± 888.4	2,965 – 7,640	0.41
NUE (kg ha^{-1} grain / kg ha^{-1} N supply)	46.05 ± 6.70	27.73 – 59.05	0.46	209.92 ± 46.67	118.62 – 305.61	0.41
Grain Number per area (GN)	1,312 ± 317.99	538 – 2,128	0.23	1,037 ± 340.94	297 – 1,842	0.27
Number of Spikes per area (NS)	58 ± 11.24	32 - 100	0.48	50 ± 11.03	25 - 94	0.55
Kernel Weight (mg) (KW)	36 ± 4.4	25 - 46	0.88	36 ± 3.9	28 - 47	0.89
Spike length (cm) (SPL)	8.4 ± 0.8	6.3 - 10.5	0.75	7.8 ± 0.7	5.9 - 10.0	0.63
Kernel number per spike (KNS)	32 ± 6.08	15 - 49	0.52	29 ± 6.18	14 - 48	0.52
Kernel weight per spike (g) (KWS)	1.02 ± 0.21	0.51 - 1.66	0.51	0.91 ± 0.17	0.47 - 1.34	0.38
Fruiting efficiency (grains g^{-1}) (FE)	87 ± 37.93	21 - 186	0.57	85 ± 39.56	23 - 210	0.56
Harvest index (HI)	0.44 ± 0.05	0.27 - 0.55	0.22	0.38 ± 0.07	0.21 - 0.55	0.15
Trait – 5 Subset Genotypes						
Nitrogen Analysis						
Nitrogen concentration of Phytomass at Heading (NCPH) (mg g^{-1})	15.8 ± 1.9	11.0 - 20.8	-	11.1 ± 2.3	7.9 - 17.9	-
Nitrogen concentration of Phytomass at Anthesis (NCPA) (mg g^{-1})	12.1 ± 2.7	8.1 – 17.8	-	8.8 ± 1.8	6.3 – 16.6	-
Nitrogen Concentration of Phytomass at Maturity (NCPM) (mg g^{-1})	4.7 ± 1.6	2.6 – 10.7	-	3.5 ± 0.8	2.4 – 6.4	-
Nitrogen concentration of Grains at Maturity (NCGM) (mg g^{-1})	18.7 ± 2.6	13.5 – 23.4	-	16.9 ± 2.1	12.8 – 20.3	-
N uptake (g g^{-1})	1.42 ± 0.34	0.69 - 2.62	-	0.87 ± 0.29	0.42 - 1.53	-
NUtE (g g^{-1})	34.13 ± 5.99	18.10 - 45.72	-	39.78 ± 6.14	24.76 - 51.58	-
NHI (%)	63 ± 7	42 - 72	-	66 ± 6	46 - 75	-

Table 13: ANOVA for year (Y), nitrogen level (N), genotype (G), and interactions for measured traits. ANOVA performed on all 30 lines except for last 7 traits relating to N analysis.

Trait – All 30 genotypes	**Y**	**N**	**Y x N**	**G**	**Y x G**	**N x G**	**Y x N x G**
Days to Heading (HD)	***	ns	ns	***	***	ns	ns
Days to Maturity (MD)	***	***	ns	***	**	ns	ns
Biomass at Maturity (g) (MD_{BIO})	**	*	ns	*	ns	ns	*
Yield (kg ha^{-1}) (YLD)	ns	*	ns	***	***	*	ns
NUE (kg ha^{-1} grain / kg ha^{-1} N supply)	ns	***	ns	***	***	***	*
Spike length (cm) (SPL)	**	***	ns	***	*	ns	ns
Kernel number per spike (KNS)	***	***	ns	***	ns	ns	ns
Kernel weight per spike (g) (KWS)	***	***	*	***	**	ns	ns
Grain Number per area (GN)	ns	**	ns	***	ns	*	*
Number of Spikes per area (NS)	ns	ns	ns	***	ns	*	*
Kernel Weight (mg) (KW)	***	ns	ns	***	***	ns	ns
Fruiting efficiency (grains g^{-1}) (FE)	***	ns	ns	***	***	ns	ns
Harvest index (HI)	***	ns	ns	***	*	ns	ns
Plant height (cm) (PLH)	***	**	ns	***	ns	ns	ns
Trait – 5 subset genotypes							
Nitrogen Concentration of Phytomass at Heading (NCPH) (mg g^{-1})	*	***	ns	ns	ns	ns	ns
Nitrogen Concentration of Phytomass at Anthesis (NCPA) (mg g^{-1})	***	***	ns	ns	ns	ns	ns
Nitrogen Concentration of Phytomass at Maturity (NCPM) (mg g^{-1})	**	**	ns	**	*	ns	ns
Nitrogen Concentration of Grains at Maturity (NCGM) (mg g^{-1})	***	***	*	***	**	ns	ns
N uptake (g)	ns	**	ns	ns	ns	ns	*
Nitrogen utilization efficiency (NUtE) (g g^{-1})	***	***	ns	***	***	ns	ns
Nitrogen harvest index (NHI) (%)	ns	*	ns	***	**	*	ns

Significance: < 0.001 = ***, <0.01 = **, < = 0.05*, and > 0.05 = ns.

Table 14: Correlation table of Pearson correlation coefficients and significant p-values of correlations. Upper right triangle represents low-N and lower left triangle represents high-N environment.

	KW	PLH	HI	KWS	NS	MDBIO	SPL	FE	NUE	YLD	GN	KNS
KW		0.45*	-0.45***	0.09	-0.09	0.25*	-0.26	-0.75*	-0.16	-0.16	-0.34	-0.54
PLH	0.55		-0.57	0.19	-0.06	0.43	0.11	-0.52*	0.10	0.10	-0.03	-0.19
HI	-0.32***	-0.52		0.00	0.04	-0.33	0.13	0.51**	0.05	0.05	0.31	0.28
KWS	0.27	0.32	-0.09		-0.11	0.3	0.46	0.66	0.10	0.05	0.23	0.68
NS	-0.25	-0.22	0.03	-0.31		0.51	-0.02	0.02	0.64*	0.64*	0.60	-0.04
MDBIO	0.35	0.53	-0.53	0.14	0.21		0.18	-0.32	0.44	0.44	0.71	0.04
SPL	-0.18	-0.12	0.22	0.37	-0.09	0.01		0.31	0.09	0.09	0.32	0.59
FE	-0.80*	-0.54	0.34**	-0.07	0.18	-0.39	0.22		0.10	0.10	0.23	0.66
NUE	-0.11	-0.12	0.22	-0.05	0.36*	0.10	0.02	0.16		1.00	0.56	0.10
YLD	-0.11	-0.12	0.22	-0.05	0.36*	0.10	0.02	0.16	1.00		0.56	0.10
GN	-0.30	0.01	0.06	-0.09	0.42	0.66	0.12	0.21	0.34	0.34		0.40
KNS	-0.43	-0.13	0.14	0.63	-0.09	-0.15	0.53	-0.07	-0.05	0.06	0.14	

Significance: <0.001 = ***, < 0.01 = **, and <0.05 = *.

KW: kernel weight; PLH: plant height; HI: harvest index; KWS: kernel weight per spike; NS: number of spikes; MDBIO: biomass at maturity; SPL: spike length; FE: fruiting efficiency; NUE: nitrogen use efficiency; YLD: grain yield; GN: grain number; KNS: kernel number per spike

The effect of N, G, Y x G, and N x G were significant on YLD (Table 11) and the interaction of Y x N was not significant. On average, YLD was 976 kg ha^{-1} less in low N compared to high N (Table 12). In the high-N treatment, YLD had a mean of 6,335 kg ha^{-1} and ranged between 3,799 – 8,090 kg ha^{-1}. Difference in YLD resulted from producing more GN per treatment based on NS where N, G, N x G, and Y x N x G had significant effects on GN (Table 13). Y, G, and G x Y had significant effects on HI. Across genotypes in environments, HI ranged from 0.21 – 0.55 (Table 12). The 5 lines selected for in-tissue N analysis revealed a range of grain yield. For example, PU08, PU10, and PU15 exhibited YLD greater than the mean across both environments, and PU17 and PU21 exhibiting less YLD than average (Table 15).

Table 15: Nitrogen analysis and grain quality assessment of 5 subset lines.

	Germplasm				
	PU08	PU10	PU15	PU17	PU21
Low-N					
Yield (kg ha^{-1})	5,698	5,527	5,874	4,696	4,928
NUE (kg ha^{-1} grain / kg ha^{-1} N supply)	227.96	221.10	235.01	187.84	197.12
NCPH (mg g^{-1})	10.5	11.0	11.4	12.4	9.9
NCPA (mg g^{-1})	8.5	8.4	9.0	8.8	8.5
NCPM (mg g^{-1})	3.4	3.3	3.7	4.3	3.3
NCGM (mg g^{-1})	16.1	16.2	16.0	18.1	18.3
N uptake (g)	0.83	0.85	1.00	1.00	0.63
NUtE (g g^{-1})	42.84	41.47	42.97	34.56	37.53
NHI (%)	69	66	67	62	68
GHI	14	14	13	24	16
SDS-Sed	4.8	4.0	4.3	5.0	5.0
High-N					
Yield (kg ha^{-1})	7,391	7,320	7,098	5,483	5,567
NUE (kg ha^{-1} grain / kg ha^{-1} N supply)	53.95	53.43	51.81	40.03	40.64
NCPH (mg g^{-1})	15.7	15.6	15.8	16.3	15.6
NCPA (mg g^{-1})	11.8	11.3	12.1	12.1	12.9
NCPM (mg g^{-1})	4.5	3.7	5.8	5.2	4.1
NCGM (mg g^{-1})	18.9	18.4	17.9	19.1	19.6
N uptake (g)	1.56	1.29	1.53	1.30	1.35
NUtE (g g^{-1})	35.11	36.76	33.45	31.24	34.22
NHI (%)	65	68	57	58	66
GHI	20	17	9	17	19
SDS-Sed	4.8	5.5	5.3	4.8	5.3

Nitrogen concentration at heading (NCPH; mg g^{-1}), anthesis (NCPA; mg g^{-1}), maturity (NCPM; mg g^{-1}), in grains (NCGM; mg g^{-1}), nitrogen uptake (N uptake; g), nitrogen utilization (NUtE; g g^{-1}), and nitrogen harvest index (NHI; %) determined from in season tissue analysis for 5 lines.

Grain hardness index (GHI) based on single kernel characterization (SKCS).

SDS Sedimentation (SDS-Sed) based on whole grain flour meal.

Spike traits were investigated by measuring SPL and the KNS in both environments. The effect of N and G were significant on SPL and KNS (Table 13). SPL ranged from 5.9 - 10.5 cm (Table 12). The mean SPL was 7.8 cm in low N and 8.4 cm in high N. Positive correlation was

observed between SPL and KNS at 0.53 in high N and 0.59 in low N, respectively (Table 14). The mean KNS in high N was 32, in comparison to the mean KNS of 29 in low N. However, the range was similar under both N levels, from 20 to 50 KNS. PU28 produced the most KNS in high N with average of 41, and PU15 produced the most KNS under low N. The percent reduction of SPL and KNS from high-N to low-N treatments were, on average, 7.7% and 10.3%, respectively. In most cases, larger SPL values were associated with larger KNS values, suggesting that the length of the spike could be a primary determinant of the number of kernels per spike.

Lines were significantly different for fruiting efficiency (FE) (Table 13); however, N did not affect FE. FE was highly heritable across environments (H_2 >0.50) (Table 12). In high N, FE showed a mean of 87 kernels per gram of dry matter spike at anthesis (range 21 – 186) (Table 12). Genotypes PU02 and PU20 had the lowest FE of 57 and 62 in high-N environment, well below the average. PU07 and PU19 showed FE above 100 in both low-N and high-N treatment.

3.3.2 In-tissue nitrogen analysis

N treatment had significant effects on N concentration in phytomass at heading, anthesis, and maturity, as well as in grains for the 5 subset genotypes (Table 13). On average, N concentration in biomass at heading was 11.1 mg g^{-1} in low N (Table 12) where genotype PU17 showed the maximum in-biomass N concentration (Table 15). In high N, plants were able to accumulate N concentration of 15.8 mg g^{-1} in biomass at heading (Table 12). The amount of in-biomass N concentration decreased to 8.8 mg g^{-1} and 12.1 mg g^{-1} by anthesis in low-N and high-N treatments and in-phytomass N concentration decreased to 3.5 mg g^{-1} and 4.7 mg g^{-1} by maturity in low-N and high-N treatments, respectively (Table 12).

From anthesis to maturity, the amount of N in phytomass decreased. The effect of N and Y was significant for N concentration at anthesis and maturity (Table 13) where PU21 displayed the largest loss of 8.8 mg g^{-1} N from anthesis to maturity in high N, while PU15 lost 5.3 mg g^{-1} in low N (Table 15). This signifies the translocation of N into the grains. Genotypes were only significantly different at maturity stage for N concentration in phytomass and in grains (Table 13). The maximum NHI of 69% was observed in PU08 in low N. While the minimum NHI of 57% was observed in PU15 in high N (Table 15). The sum of N in phytomass and grain at maturity was approximately 22.0 mg g^{-1}, on average (Table 13). The total N at anthesis was approximately 10.5

mg g^{-1} across environments. We observed that pre-anthesis N concentration was correlated with grain N concentration ($r = 0.51$; p-value < 0.001) among the 5 lines.

3.3.3 Nitrogen use efficiency

Nitrogen use efficiency was estimated for all 30 lines across N treatments. N, G, Y x G, N x G, and Y x N x G were significant for NUE (Table 13). Due to the level of N application, and method of calculation, NUE estimates were higher in low N (Table 12). For example, NUE averaged 209.92 kg ha^{-1} grain per kg ha^{-1} N supplied in low-N environment. PU03 had the lowest NUE of 179.78 kg ha^{-1} grain per kg ha^{-1} N, with PU13 the highest at 243.62 kg ha^{-1} grain per kg ha^{-1} N. In high N, NUE averaged 46.05 kg ha^{-1} N. PU08, PU10, and PU15 had the greatest NUE in high N (Table 15). We further quantified N uptake, NUtE, and NHI in 5 selected genotypes in this study (Table 15). The effect of N was significant on N uptake (Table 13). N uptake average 1.42 g and 0.87 g in high N and low N, respectively (Table 12). This was a 38% reduction in whole plant N uptake. However, the effect of G and G x N was not significant, indicating that lines responded similarly to their N uptake across the two environments (Table 13). The effects of Y, N, G, and Y x G were significant on NUtE (Table 13). NUtE was significantly greater in low N (compared to high N) by 14% (Table 12). The effects of N, G, Y x G, and N x G was significant on NHI (Table 13). NHI ranged from 42 – 75% across years and environments.

3.3.4 Glutenin subunits and the rye translocation

Loci for HMW glutenin subunits *Glu-A1*, *Glu-B1*, and *Glu-D1* and LMW subunits *Glu-A3*, *Glu-B3*, and *Glu-D3* and presence of 1B/1R translocation (Table 16) were characterized (Figure 13). In the thirty lines tested, the common *Glu-A1* allele was the *1* subunit with only six genotypes possessing the *2** allele. The variants observed in *Glu-B1* locus were *7*, *7+8*, *7+9*, *13+16* and *32+33* subunits. Two alleles *2+12* and *5+10* were found for *Glu-D1* locus at almost equal frequency. For LMW, the *Glu-A3c* subunit and *Glu-D3a* subunit were the most frequent (Table 16), while *Glu-B3* showed a wide allelic variation. The 1B/1R rye translocation was identified in 17 out of 30 genotypes. When we compared genotypes with translocation with those without the translocation by using two-sample t test, the difference was not significant (p value > 0.05).

Genotypes with the 1B/1R translocation varied in allelic variation for HMW and LMW subunits (Table 16).

Table 16: Allelic variation of HMW and LMW glutenin subunits and presence of 1B/1R translocation for each line.

	Low-N	High-N	HMW			LMW				Low-N		High-N	
Germplasm	Yield	Yield	Glu-A1	Glu-B1	Glu-D1	Glu-A3	Glu-B3	Glu-D3	Translocation	GHI	SDS-Sed	GHI	SDS-Sed
PU01	5,001	6,296	1	7	2+12	f	j	a	1B/1R	10	4.0	13	4.8
PU02	5,405	6,292	2*	32+33†	5+10	c	j	b	1B/1R	6	4.8	12	5.5
PU03	4,494	5,842	1	7	5+10	c	f,g	a	-	9	4.8	12	5.8
PU04	5,340	5,900	1	7	5+10	d†	b	a	-	9	4.0	13	4.3
PU05	5,426	6,780	1	7+9	2+12	d	f,g,j†	c/b	1B/1R	2	6.0	4	6.3
PU06	5,571	6,485	1	13+16†	2+12	c	f,g	a	-	7	4.3	13	5.8
PU07	4,668	6,328	1	7	2+12	f	j	a	1B/1R	13	4.3	16	5.0
PU08	5,699	7,392	2*	7	2+12	g	j	a	1B/1R	14	4.8	20	4.8
PU09	5,875	6,479	1	7	2+12	c	b	a	1B/1R	10	5.0	12	6.3
PU10	5,528	7,320	2*	7+9	2+12	g	j	a	1B/1R	14	4.0	17	5.5
PU11	5,269	6,105	1'±	13+16	5+10	c	h	a	-	20	6.3	17	7.3
PU12	5,270	5,656	1	7	5+10†	c	f†	b†	1B/1R	12	5.3	17	6.3
PU13	6,090	6,817	1'±	13+16	5+10	c	h	a	-	16	5.5	17	7.0
PU14	4,917	6,151	0	7+8	2+10.1±	c	g	a	-	19	5.5	20	7.0
PU15	5,752	7,099	1	7	2+12	c	b	a	1B/1R	13	4.3	9	5.3
PU16	5,638	6,710	1	7+9†	2+12†	c	j†	c†	1B/1R	13	3.8	16	4.0
PU17	4,696	5,484	1	7	2+12	c	j	a	1B/1R	24	5.0	17	4.8
PU18	5,870	6,707	1	7+8	2+12/5+10	c	b	b	-	12	4.5	9	5.0
PU19	5,650	6,148	2*	7+9	2+12	c	j	c	1B/1R	23	4.8	29	6.3
PU20	5,742	6,676	2*†	7+9	2+12†	c	h†	a	-	11	3.8	16	5.0
PU21	4,928	5,568	1	7†	2+12	f	j†	a	1B/1R	16	5.0	19	5.3
PU22	5,617	6,242	1	7+8	2+12	c	b'	a	-	22	4.8	18	5.8
PU23	5,619	6,402	1	7	2+12	d	b'	a	-	12	5.0	14	5.3
PU24	4,719	5,851	1'±	13+16†	2+12	c	h/b	a	-	25	5.3	31	4.8
PU25	5,979	6,866	2*	7	2+12	g	j	a	1B/1R	17	4.3	21	5.3
PU26	5,802	6,170	1	7+9†	2+12†	c	j	c†	1B/1R	19	3.8	25	4.3
PU27	5,358	6,230	1	7+8	5+10†	c	b	b ±†	-	15	5.0	16	5.3
PU28	4,901	5,938	1	7+8/32+33	5+10/2+12	c	f,g,j†	b†	1B/1R	6	4.8	9	4.8
PU29	5,040	6,059	1	7+9	2+12	c	j	c†	1B/1R	16	5.3	19	4.8
PU30	5,080	6,065	1	7+8	5+10/2+12	d	b'	b	-	7	4.5	12	5.0

± indicates similar to the allele showed but not confirmed with a proper check

† indicates that the allele was not identified with certainty

Grain hardness index (GHI) and SDS-Sedimentation (SDS-Sed) evaluated under both nitrogen environments for each line.

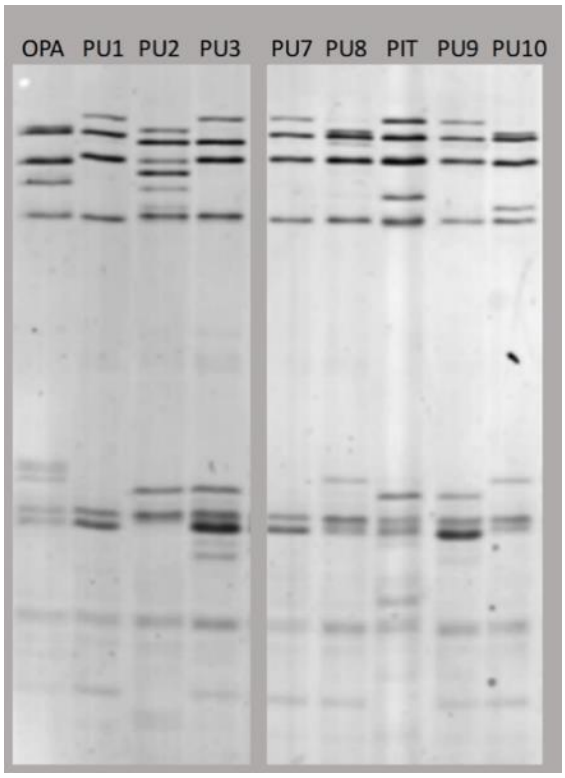

Figure 13: Separation of glutenin subunits with SDS-PAGE. Varieties include Purdue Germplasm PU1-3, PU7-9, and OPA (Opata) and PIT (Pitic) were checks for reference. HMW glutenin subunits: OPA (*Glu-A1 2**; *Glu-B1 13+16*; *Glu-D1 2+12*); PU1 (*Glu-A1 1*; *Glu-B1 7*; *Glu-D1 2+12*); PU2 (*Glu-A1 2**; *Glu-B1 32+33*; *Glu-D1 5+10*); PU3 (*Glu-A1 1; Glu-B1 7*; *Glu-D1 5+10*); PU7 (*Glu-A1 1*; *Glu-B1 7*; *Glu-D1 2+12*); PU8 (*Glu-A1 2**; *Glu-B1 7*; *Glu-D1 2+12*); PIT (*Glu-A1 1; Glu-B1 7+8*; *Glu-D1 2+12*); PU9 (*Glu-A1 1*; *Glu-B1 7*; *Glu-D1 2+12*); PU10 (*Glu-A1 2**; *Glu-B1 7+9*; *Glu-D1 2+12*). LMW glutenin subunits: OPA (*Glu-A3 b*; *Glu-B3 i*; *Glu-D3 a*); PU1(*Glu-A3 f*; *Glu-B3 j*; *Glu-D3 a*); PU2 (*Glu-A3 c*; *Glu-B3 j*; *Glu-D3 b*); PU3 (*Glu-A3 c*; *Glu-B3 f,g*; *Glu-D3 a*); PU7 (*Glu-A3 f*; *Glu-B3 j*; *Glu-D3 a*); PU8 (*Glu-A3 g*; *Glu-B3 j*; *Glu-D3 a*); PU9 (*Glu-A3 c*; *Glu-B3 b*; *Glu-D3 a*); PU10 (*Glu-A3 g*; *Glu-B3 j*; *Glu-D3 a*).

3.3.5 Grain quality indicators

The GHI values greater than 59 are indicative of hard while GHI values less than 33 specify soft endosperms. Because we analyzed only single replicate grains with SKCS, we could not perform ANOVA or any significance test among genotypes. GHI averaged 13.8 ± 1.03 (standard error of the mean) in low N. In high N, GHI averaged 16.1 ± 1.05 (Table 16). PU24 showed maximum GHI values of 25 and 31 in low N and high N, respectively. In contrast, PU05 showed the minimum GHI values less than five in both treatments.

For SDS-sedimentation, higher values indicate better bread-making quality (Moonen et al., 1982). SDS tested whole meal flour samples of each line performed in duplicate showed

sedimentation mean of 5.4 ± 0.15 in high N in contrast to 4.7 ± 0.12 sedimentation mean observed in low N (Table 16). PU16 showed minimum SDS-sedimentation while PU11 showed the maximum.

Germplasm with the 1B/1R translocation showed a lower grain hardness and lower SDS-sedimentation (Table 16). For example, PU05 and PU16 had the minimum GHI and the minimum SDS-sedimentation across environments, respectively, while PU11 and PU24 which do not carry the translocation show maximum GHI and SDS-sedimentation for whole grain flour meal. PU10 and PU15 exhibit the translocation and were among the highest yielding lines in high N and low N, with lower protein in both environments and a lower SDS-sedimentation score than average in low N (Table 16).

3.3.6 Nitrogen x genotype interaction

Five traits including grain yield, grain number, number of spikes, nitrogen use efficiency, and nitrogen harvest index showed significant N x G interaction effect (Table 13), indicating that lines performed differently in response to nitrogen environments. In particular, when we assessed grain yield with ranks, a cross over interaction was observed for lines PU08 and PU13. PU08 was the first rank line in the high-N environment while PU13 was the first rank in the low-N environment (Figure 14). The change was evident as only 4 of 30 genotype held the same rank across environments. One specific genotype, PU26, is an example of the importance of phenotyping in low input environments. Under high N, PU26 yielded 6,170 kg ha^{-1}, below average, and ranked as the 18^{th} best genotype based on yield performance. However, in low N, PU26 yielded 5,802 kg ha^{-1}, above average, and moved up twelve spots to the 6^{th} best yielding genotype. The change in ranking was indicative of genotype by nitrogen interaction.

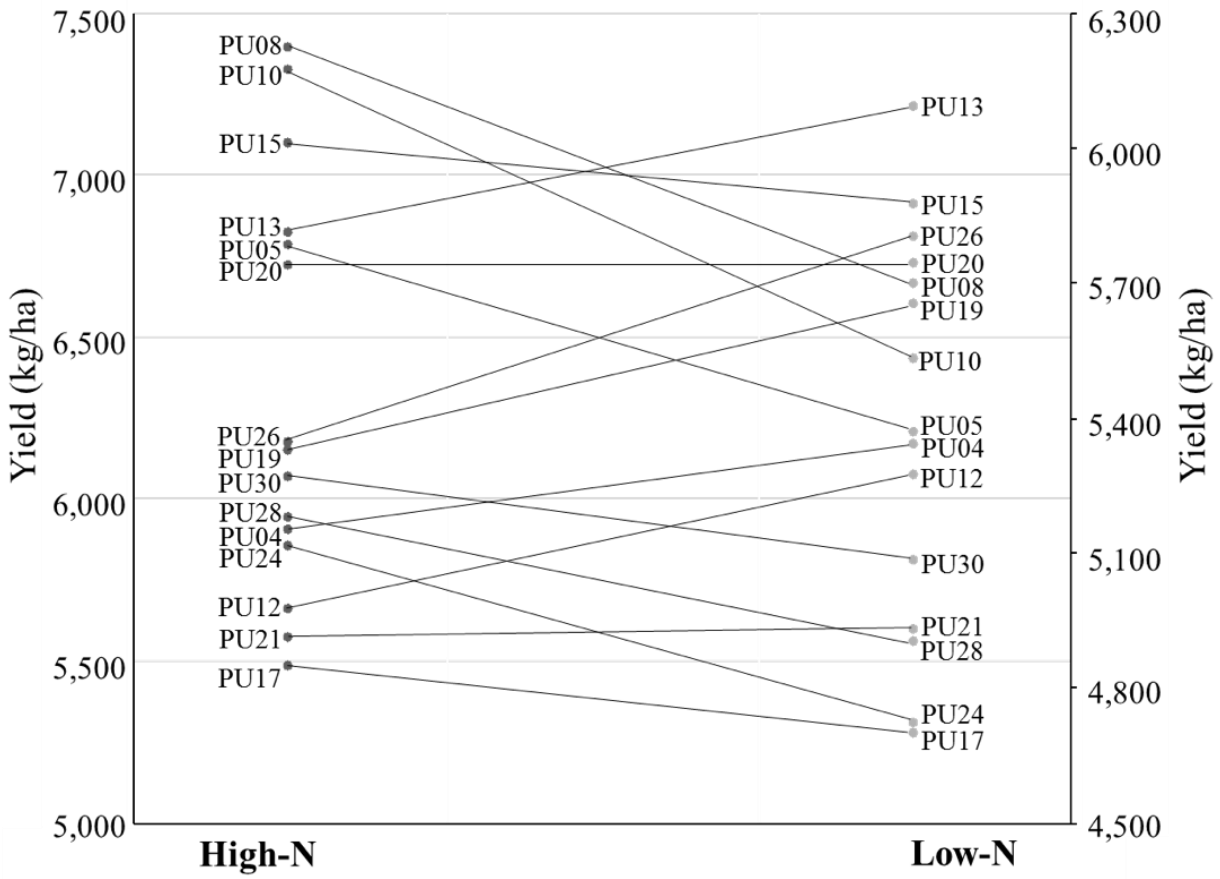

Figure 14: Genotype ranking and interactions based on grain yield in low-N and high-N environment for 15 out of 30 genotypes.

3.3.7 PCA – biplot analysis

The interrelationship among traits and genotypes in the form of biplots in each environment is shown in Figure 15. Principal component analysis (PCA) was performed on the 12 traits measured and all 30 lines in both environments. In low N, PC1 and PC2 explained 34.8 and 32.5% of phenotypic variations, respectively. In high N, PC1 and PC2 explained 32.6 and 22.0% of phenotypic variation, respectively. The number of spikes was significantly and positively associated with grain yield in both environments (Figure 15; Table 14). Kernel weight was not positively associated with any other trait but had significant negative correlations with harvest index and fruiting efficiency. Lines are also visually shown in PCA-biplot. Two high yielding lines in both environments, PU08 and PU10, were in the same direction as grain yield and number of spikes.

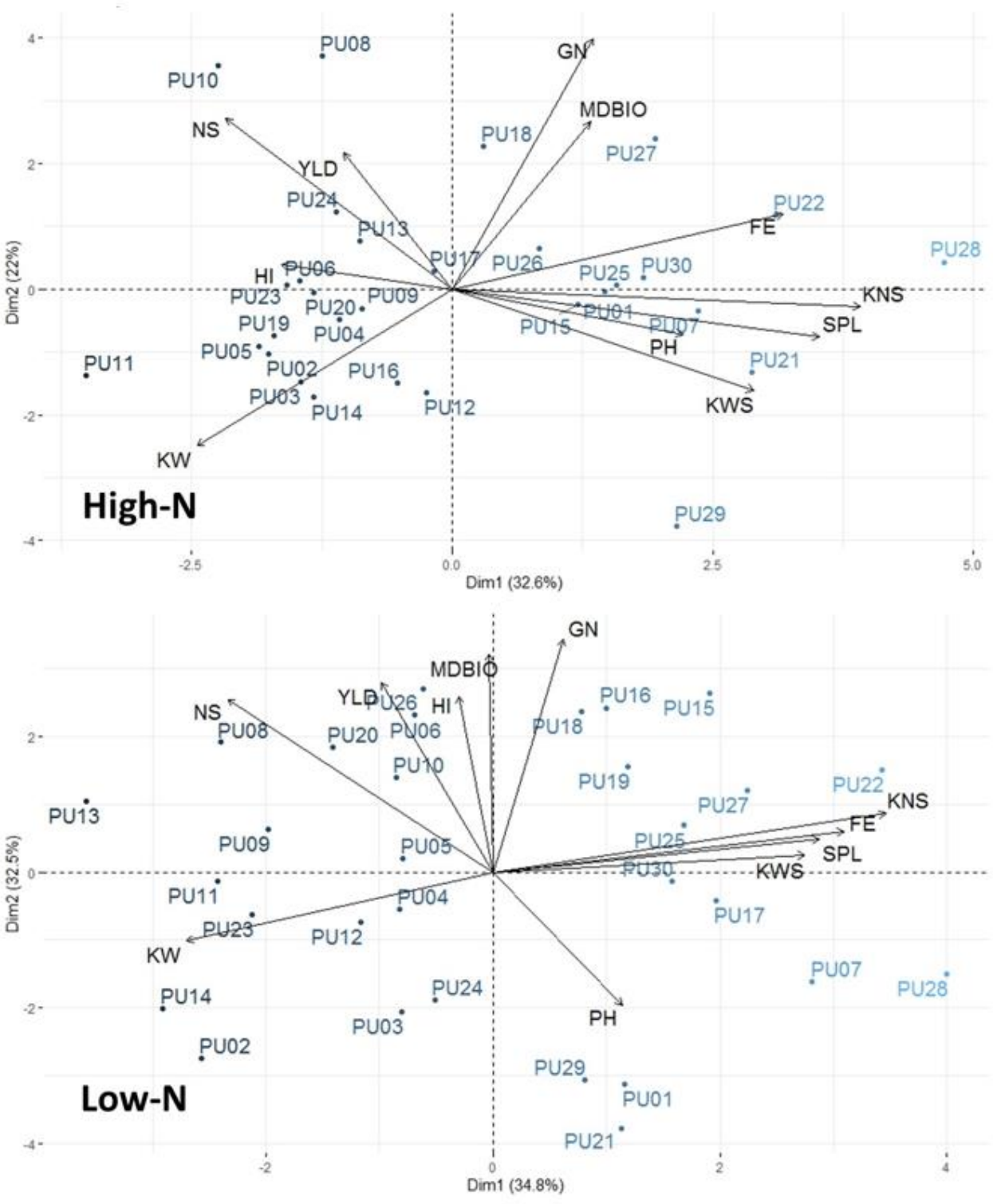

Figure 15: PCA-biplot analysis among 12 agronomic traits and 30 genotypes. PCA-biplots were performed in both high-N and low-N environments.

3.4 Discussion

Of the estimated 31.8 million acres of winter wheat planted in 2019, approximately 5.54 (~17%) million acres are estimated to be planted as soft-red winter wheat in the eastern USA. A record low harvest area is expected in New Jersey, Ohio, and Virginia (USDA, 2019). The decline in wheat cultivation area in the US is due to an increase in acreage and production of maize and soybean. In maize, nitrogen dynamics and optimizations under varying environments have been studied extensively to increase productivity with efficient fertilization, management, and less environmental footprint (Bänziger et al., 1997; Ciampitti and Vyn, 2012). Studies in wheat took a variety of objectives from improving wheat for low-nitrogen input in order to reduce environmental impacts (Brancourt-Hulmel et al., 2005; Delogu et al., 1998; Le Gouis, Béghin, Heumez, and Pluchard, 2000; Ortiz-Monasterio et al., 1997), breeding for productivity gains and cost-effectiveness under low input environments (Bänziger and Cooper, 2001), and nitrogen use efficiency in soft-red winter wheat (Brasier et al., 2018; Hitz et al., 2017; Van Sanford and MacKown, 1986).The ability to identify nitrogen efficient soft-red winter wheat germplasm will have the potential to reduce N applications, therefore saving time, resources, and management costs.

3.4.1 Yield and yield component responses

The rank change of lines across environments, e.g., from high N to low N (Figure 14), can indicate the potential profit loss or gain. For example, the profit made by PU17, which yielded 4,696 and 5,484 kg ha^{-1} under low N and high N, would be below the average profit margins across all 30 lines and displays the potential loss in comparison to other higher yielding lines. This data seems to suggest breeding specifically for separate environments by using beneficial founder individuals for each environment. A PCA-biplot that shows trait and line associations (Figure 15), can be useful for shortlisting of founder individuals. For example, in low N, unlike in high N, the biomass at maturity has a close association and higher correlation (Table 14) with grain yield, showing that, under limited nitrogen, the decreases of biomass (tillers and leaves), is the bottleneck for grain production later in the season. Therefore, it seems that the negative effect of low N is

through reduction in canopy size and radiation use. Yield potential is expressed as a function of light interception, radiation use efficiency, and harvest index, where the critical underlying trait common to all three components is above-ground plant biomass. An increase in biomass is associated with an increase in radiation use efficiency, grain number, and ultimately grain yield (Reynolds et al., 2005). In spring wheat, Caviglia and Sadras (2001) observed nitrogen deficiency reduced light interception and radiation use efficiency, ultimately because of smaller leaf area index due to decrease tillering and less shoot dry matter (biomass). Calderini et al., (1997) identified wheat cultivars reached a maximum leaf area index between the booting and terminal spikelet growth stage, implying the importance of establishing a wheat canopy earlier in the growth season as leaf area index and dry matter decreases post-anthesis when the wheat transitions from vegetative growth to reproductive growth for grains.

In our study, the difference in spike number can be attributed to the lack of tiller initiation in the spring or the loss of an emerging tiller in winter. The decreases in biomass due to low-N treatment resulted in reduction of grain number via decreases of number of spikes, and kernel per spike, similar to previously reported observations (Le Gouis et al., 2000; Terrile et al., 2017). Grain number, as an important yield component, is positively related to pre-anthesis dry matter accumulation (Duan et al., 2018) and was shown to respond directly to N supply to the spike (Abbate, Andrade, & Culot, 1995). Our results indicate grain number and biomass are highly correlated (Table 14) and are associated with genotypes producing more grain in low and high N (Figure 15). Despite responsiveness of grain number, our study indicated that kernel weight is more stable under environmental conditions with higher heritability (H_2 = 0.88 and 0.89), implying that the physiological mechanisms that control grain filling are able to fill the number of grains that were determined earlier. Even though a contradicting report of kernel weight was described as the main determinant of grain yield (Major et al., 1988), we observed grain number as the primary contributor for grain yield. Similar to our observation, other physiological studies reported similar behavior for environmental responsiveness of grain number and kernel weight (Ferrante et al., 2017; Sadras and Slafer, 2012; Slafer et al., 2014).

3.4.2 End-use quality determinants

One aspect of genotypic differences in responses to low N is end-use quality traits. Protein content, gluten quality, and endosperm texture in wheat are the driver of end-use products. Several

studies evaluated the relationship between grain yield to protein content and quality. For example, experimental evidence is indicative of a negative correlation between grain yield and protein (Cooper et al., 2001; Magallanes-López et al., 2017). We used several measures to understand the dynamics of protein quality under the two contrasting N regimes.

Contrary to changes that we observed for grain yield under different N management, our study only indicated a slight decrease in SDS-sedimentation and grain hardness index. This is an opportunity for developing low-N efficient soft-red winter wheat breeding because these traits were minimally affected by the lack of sufficient N. Contrary to our results of soft-red winter wheat, N fertilizer was previously shown to have significant effect on SDS sedimentation in hard wheat (C. Luo et al., 2000; Saint Pierre et al., 2008).

Gluten quality is a function of allelic variation of HMW and LMW subunits. For example, *Glu-A1(2*)* and *Glu-D1(5+10)* HMW subunits are considered high gluten quality alleles. Line PU02 revealed high yield and possessed *Glu-A1(2*)* and *Glu-D1(5+10)* HMW subunits. One of the highest yielding lines under low N, PU15, possessed *Glu-A1(1)* and *Glu-D1(2+12)* subunits, which are not considered the highest glutenin quality alleles. Selection of lines as breeding parents with reasonable yield under low N condition and high glutenin subunits as parents of breeding populations, may be a way to maintain the quality under low N in the breeding population.

Germplasm with the 1B/1R translocation showed a lower grain hardness and lower SDS-sedimentation. For example, PU05 and PU16 had the minimum GHI and the minimum SDS-sedimentation across environments, respectively, while PU11 and PU24 which do not carry the translocation show maximum GHI and SDS-sedimentation for whole grain flour meal. PU10 and PU15 exhibit the translocation and were among the highest yielding lines in high N and low N (Figure 14), with lower protein in both environments and a lower SDS-sedimentation score than average in low N (Table 16).Morris & Paulsen (1985) analyzed hard winter wheat under two contrasting treatments. In deficient N, the low levels of vegetative N resulted in a significant decreased in total grain N after anthesis. In comparison, high N maintained 37 mg N plant^{-1} throughout grain filling but increased grain N dramatically (Morris & Paulsen, 1985). Parts of the N that is in the grain comes from senescence of leaves (remobilization of existing N compounds) (Hawkesford, 2014). Tolley and Mohammadi (2020), showed significant differences for grain N at maturity in seven diverse wheat accessions. The grain N in low-N treatment was 23.3 mg g^{-1} while grain N in high-N environment was 27.8 mg g^{-1}. Our study did not detect any significant

genotypic variation of N uptake in spite of previous studies showing genetic variation in nitrogen uptake and assimilation previously described in wheat (Cox et al.,1985; Le Gouis et al., 2000; Ortiz-Monasterio et al., 1997).

3.4.3 Breeding for low-N environments

A comparative view of the crop produced per nitrogen used in this study indicates that breeding and selection for performance under low-N environment has the potential for minimizing N use and environmental impacts. In our study each additional kg ha^{-1} of spring N fertilizer resulted in a grain yield increase of 9 kg ha^{-1}, with the G x N effect for grain yield being significant, indicating that lines responded differently (Table 11). For example, PU10 responded maximally and PU04 responded minimally by increasing 16 and 5 kg ha^{-1} of yield per each kg ha^{-1} of nitrogen applied.

Most breeding programs and variety testing are historically performed under optimal conditions and sufficient N applications for evaluating yield potential. N applications have the negative environmental impact of leaching, pollution, and runoff into the water, as nitrate is the most commonly detected agricultural chemical in the water. Wu et al., (1996) estimated an average annual runoff and leaching of 4.47 kg N ha^{-1} and 4.57 kg N ha^{-1}, respectively, in the midwestern and northern plain regions under corn, sorghum, soybean, wheat, or legume hay cultivation, accounting for about 5.5% and 5.6% of N applied.

This result indicates that establishing breeding and selection for specifically performance under low-N cropping systems has the potential to produce reasonably well under low-N conditions while decreasing the environmental footprint. The former was evident by changes in rank analysis of lines in both environments (Figure 14). Change of rank in differential environments was previously used in drought (Li et al., 2011; Lopes et al., 2014), salinity (Chamekh et al., 2015; Salam et al., 1999), and other nutrient deficiencies (Murphy et al., 2008; Torun et al., 2000; Zhao et al., 2018), to postulate a need for environment specific management and breeding practices. For example, van Bueren and Struik (2017) described breeding for grain crops and vegetables under diverse N management for genotype adaptation and interaction with availability of N.

Our data seems to suggest that the lines PU05, PU08, PU10, PU13, PU15, PU19, PU20, and PU26 have the potential to be the founder of a breeding population for low-N environment (Figure 14).

For this selection we used criteria such as higher ranks in low-N conditions, higher kernel per spike in low-N, superior *Glue-A1* (*2**) allele, the rye 1B/1R translocation, and higher NHI and FE. Another related trait that can help wheat breeding for low-N system is the use higher grain protein content trait. It has been shown that greater translocation of nitrogen to grains from increased fertilizer N results in a higher grain protein concentration (Delogu et al., 1998; Saint Pierre et al., 2008). A grain protein content (GPC) locus, *GPC-B1*, has been identified on chromosome 6B in wheat (Distelfeld et al., 2006). *Gpc-B1* increases protein content via N remobilization from leaves and senescence (Uauy et al., 2006).

3.5 Conclusion

In conclusion, we propose the first ideotype for breeding N-efficient cultivars specifically for the US midwest wheat. In soft-red winter wheat, where grain yield and relatively lower grain protein content is desired, we believe that in-tissue concentration of nitrogen, which traditionally represents uptake and utilization of N, may not be a good indicator of nitrogen use efficiency.

In fact, a superior and N-efficient genotype is one which uses the available N to produce a canopy allowing for maximum radiation use efficiency, producing dry matter that is required for fertile tiller and grain numbers. Therefore, for a grain crop where protein content is not critical, a good indicator of nitrogen use efficiency is fixation of carbon, efficient use of radiation, and developing a productive canopy, per unit of nitrogen used. The rank differences among lines in contrasting environments is a testament to the opportunity to select and breed for more crop per same N (or same crop with less or optimized N). In this context, the success of wheat breeding for N-deficient environments needs management strategies that enable supplying continuous availability of N in the field post-anthesis and during grain fill.

Our study resulted in identification of traits and variants that will lead to increases of yield and maintaining of yield under lower nitrogen conditions, and therefore can be regarded as “the breeder’s toolkit for developing N-efficient soft-red winter wheat varieties”. For breeding soft-red winter wheat for high-N environment, PU08, PU10, and PU15 would be advantageous due to responsiveness to N with significant increases in grain number, biomass, and number of spikes, which led to the increase in grain yield. Since N treatment did not significantly impact end-use quality of the grains, N management in soft-red winter wheat can focus on the best practices for canopy enhancement, grain number per unit area, and yield.

CHAPTER 4. CONCLUSION

Wheat will continue to be a major and important cereal for meeting the future food demands. Continual progress in US wheat production and genetic gains is critical for domestic and export markets for products such as cakes, crackers, and pastries. SRW is the highest yielding class of wheat and accounted for 15% of total wheat production in 2018-2019. A system based approach by aligning the right genetic materials to the right management practices is required for further increases in wheat yield and competitiveness given the competing US row crops i.e. corn and soybean. My ***goal*** was to identify traits in two different breeding populations and two different production systems that can allow informed decisions on which traits to select for further genetic improvement for each production system.

In the first research chapter, investigating a diverse population of elite breeding lines from multiple public breeding programs provided the opportunity to investigate important characteristics of high yielding SRW wheat lines in the United States. Varieties exhibiting better yield performance in Indiana were identified. Detailed yield component traits, such as kernel weight, number of spikes, and biomass were examined. Further genetic mapping identified MTAs and QTL regions for each trait with the effect sizes and coefficient of determination were discussed. While several MTAs were identified in Indiana environment, the genotype x environment interaction greatly limited the transferability of grain yield and days to heading MTAs across different environments in the SRW regions. Homogenous environments did not share MTAs, indicating the lack of stable QTL for grain yield and days to heading. My data emphasized that the quest for stable QTL across environment may not be a successful strategy and breeding must be targeted to specific environments.

Next, in the second research chapter, I researched wheat traits, cultivars, and management practices on yield determining traits and grain quality. Precision management idealizes providing crops with the proper nutrients and minimizing the environmental impact. For wheat, intensive management practices bring into consideration fertilizers source, rate, timing, and placement for a cropping system to be effective. The genetic and physiological adaptations to agronomic fertilizer management is the main reason for past wheat yield gains. The best practices must consider stewardship of the environment and planning for future generations of agricultural production.

This objective classified Purdue breeding lines based on phenotypic performance and grain quality parameters in high and low nitrogen environments. Evaluating varieties under limiting nitrogen allowed identifying varieties with advanced performance and quality under low nitrogen management. My work established that under limiting nitrogen management, grain yield is reduced mainly due to reduction in biomass, tillering, and grain number. Varieties that exhibited higher above ground biomass were also higher yielding under nitrogen limitations. Grain number was the most sensitive yield component to limited N environment. My collaborations with CIMMYT Quality Laboratory allowed profiling of glutenin subunits and assessment of grain hardness and SDS-sedimentation. Lower N input may result in lower N concentration, I hypothesize that this deficit could be compensated by enriching the germplasm with alleles that confer higher proteins with more quality.

Wheat breeding must continue to become more precise and adapted to the future management practices and needs in order to be competitive. For example, the data allowed me to design and propose two continuation populations to emerge from my thesis, which have the potential to advance wheat breeding efforts. In continuation of the first research chapter, the highest 10% yielding lines (n = 26) were selected for further multi-environment trials and crosses to enhance genetic diversity of Purdue soft red winter wheat germplasm. From my second research chapter, I selected ten lines with desirable traits of grain yield, grain number, kernel weight, favorable gluten allele, and presence of the 1B/1R translocation. A follow-up work could be producing a base breeding population by these founders targeted to low nitrogen management practices. A greater understanding of yield formation and nitrogen responses was accomplished for SRW wheat in this dissertation and data-driven breeding suggestions were proposed.

REFERENCES

Abbate, P. E., Andrade, F. H., & Culot, J. P. (1995). The effects of radiation and nitrogen on number of grains in wheat. *The Journal of Agricultural Science*, *124*(3), 351–360. https://doi.org/10.1017/S0021859600073317

Abbate, P. E., Andrade, F. H., Lázaro, L., Bariffi, J. H., Berardocco, H. G., Inza, V. H., & Marturano, F. (1998). Grain yield increase in recent Argentine wheat cultivars. *Crop Science*, *38*(5), 1203–1209. https://doi.org/10.2135/cropsci1998.0011183X003800050015x

Addison, C. K., Mason, R. E., Brown-Guedira, G., Guedira, M., Hao, Y., Miller, R. G., … Harrison, S. A. (2016). QTL and major genes influencing grain yield potential in soft red winter wheat adapted to the southern United States. *Euphytica*, *209*(3), 665–677. https://doi.org/10.1007/s10681-016-1650-1

Allard, R. W., & Bradshaw, A. D. (1964). Implications of Genotype-Environmental Interactions in Applied Plant Breeding1. *Crop Science*, *4*(5), 503. https://doi.org/10.2135/cropsci1964.0011183x000400050021x

Anderson, J. A., Stack, R. W., Liu, S., Waldron, B. L., Fjeld, A. D., Coyne, C., … Frohberg, R. C. (2001). DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical and Applied Genetics*, *102*(8), 1164–1168. https://doi.org/10.1007/s001220000509

Appels, R., Eversole, K., Feuillet, C., Keller, B., Rogers, J., Stein, N., … Wang, L. (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science*, *361*(6403). https://doi.org/10.1126/science.aar7191

Arguello, M. N., Mason, R. E., Roberts, T. L., Subramanian, N., Acuña, A., Addison, C. K., … Gbur, E. (2016). Performance of soft red winter wheat subjected to field soil waterlogging: Grain yield and yield components. *Field Crops Research*, *194*, 57–64. https://doi.org/10.1016/j.fcr.2016.04.040

Arruda, M. P., Brown, P., Brown-Guedira, G., Krill, A. M., Thurber, C., Merrill, K. R., … Kolb, F. L. (2016). Genome-Wide Association Mapping of Fusarium Head Blight Resistance in Wheat using Genotyping-by-Sequencing. *The Plant Genome*, *9*(1), plantgenome2015.04.0028. https://doi.org/10.3835/plantgenome2015.04.0028

Aujla, khalid mahmood, Taj, S., Mahmood, K., & Akmal, N. (2010). Wheat Management practices and factors of yield decline in the punjab province of pakistan. *Pakistan J. Agric. Res.*, *23*(1–2), 17–24.

Bacon, R. K. (2001). US Soft Winter Wheat Pool. In A. P. Bonjean & W. J. Angus (Eds.), *The World Wheat Book: A History of Wheat Breeding* (pp. 469–478).

Balota, M., Green, A. J., Griffey, C. A., Pitman, R., & Thomason, W. (2017). Genetic gains for physiological traits associated with yield in soft red winter wheat in the Eastern United States from 1919 to 2009. *European Journal of Agronomy*, *84*, 76–83. https://doi.org/10.1016/j.eja.2016.11.008

Bänziger, M., Betrán, F. J., & Lafitte, H. R. (1997). Efficiency of high-nitrogen selection environments for improving maize for low-nitrogen target environments. *Crop Science*, *37*(4), 1103–1109. https://doi.org/10.2135/cropsci1997.0011183X003700040012x

Bänziger, Marianne, & Cooper, M. (2001). Breeding for low input conditions and consequences for participatory plant breeding: Examples from tropical maize and wheat. *Euphytica*, *122*(3), 503–519. https://doi.org/10.1023/A:1017510928038

Barraclough, P. B., Howarth, J. R., Jones, J., Lopez-Bellido, R., Parmar, S., Shepherd, C. E., & Hawkesford, M. J. (2010). Nitrogen efficiency of wheat: Genotypic and environmental variation and prospects for improvement. *European Journal of Agronomy*, *33*(1), 1–11. https://doi.org/10.1016/j.eja.2010.01.005

Barraclough, P. B., Lopez-Bellido, R., & Hawkesford, M. J. (2014). Genotypic variation in the uptake, partitioning and remobilisation of nitrogen during grain-filling in wheat. *Field Crops Research*, *156*, 242–248. https://doi.org/10.1016/j.fcr.2013.10.004

Barrett, B., Bayram, M., & Kidwell, K. (2002). Identifying AFLP and microsatellite markers for vernalization response gene Vrn-B1 in hexaploid wheat using reciprocal mapping populations. *Plant Breeding*, *121*(5), 400–406. https://doi.org/10.1046/j.1439-0523.2002.732319.x

Bates, D., Boker, B. M., Machler, M., & Walker, S. C. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software2*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Beales, J., Turner, A., Griffiths, S., Snape, J. W., & Laurie, D. A. (2007). A Pseudo-Response Regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *115*(5), 721–733. https://doi.org/10.1007/s00122-007-0603-4

Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc Ser B*, *57*, 15780-15785. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Beyer, S., Daba, S., Tyagi, P., Bockelman, H., Brown-Guedira, G., & Mohammadi, M. (2019). Loci and candidate genes controlling root traits in wheat seedlings—a wheat root GWAS. *Functional and Integrative Genomics*, *19*(1), 91–107. https://doi.org/10.1007/s10142-018-0630-z

Bian, Y., Yang, Q., Balint-Kurti, P. J., Wisser, R. J., & Holland, J. B. (2014). Limits on the reproducibility of marker associations with southern leaf blight resistance in the maize nested association mapping population. *BMC Genomics*, *15*(1). https://doi.org/10.1186/1471-2164-15-1068

Borlaug, N. E. (1983). *Contributions of Conventional Plant Breeding to Food Production Author (s): Norman E . Borlaug Source : Science , New Series , Vol . 219 , No . 4585 , Biotechnology (Feb . 11 , 1983), pp . 689-693 Published by : American Association for the Advancemen*. *219*(4585), 689–693.

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics*. https://doi.org/10.1093/bioinformatics/btm308

Brancourt-Hulmel, M., Heumez, E., Pluchard, P., Beghin, D., Depatureaux, C., Giraud, A., & Le Gouis, J. (2005). Indirect versus direct selection of winter wheat for low-input or high-input levels. *Crop Science*, *45*(4), 1427–1431. https://doi.org/10.2135/cropsci2003.0343

Branlard, G., & Marion, D. (2001). Proteins and Lipids for the 21st Century. In A. P. Bonjean, W. J. Angus, & M. van Ginkel (Eds.), *World Wheat Book* (pp. 981–1012).

Brasier, K. G., Tamang, B. G., Carpenter, N. R., Fukao, T., Reiter, M. S., Pitman, R. M., … Griffey, C. A. (2018). Photoperiod response gene Ppd-D1 affects nitrogen use efficiency in soft red winter wheat. *Crop Science*, *58*(6), 2593–2606. https://doi.org/10.2135/cropsci2018.03.0207

Breseghello, F., & Sorrells, M. E. (2006). Association Mapping of Kernel Size and Milling Quality in Wheat (Triticum aestivum L.) Cultivars. *Genetics*, 1165–1177. https://doi.org/10.1534/genetics.105.044586

Briani, C., Samaroo, D., & Alaedini, A. (2008). Celiac disease: From gluten to autoimmunity. *Autoimmunity Reviews*, *7*(8), 644–650. https://doi.org/10.1016/j.autrev.2008.05.006

Brown, R. A., & Rosenberg, N. J. (1999). Climate change impacts on the potential productivity of corn and winter wheat in their primary united states growing regions. *Climatic Change*, *41*(1), 73–107. https://doi.org/10.1023/A:1005449132633

Brucker, P. L., & Morey, D. D. (1988). Nitrogen Effects on Soft Red Winter Wheat Yield, Agronomic Characteristics, and Quality. *Crop Science*, *28*(1), 152. https://doi.org/10.2135/cropsci1988.0011183x002800010033x

Bushuk, W. (1997a). Wheat breeding for end-product use. *Euphytica*, *100*, 203–211. https://doi.org/10.1007/978-94-011-4896-2_27

Bushuk, W. (1997b). *Wheat breeding for end-product use*. 203–211. https://doi.org/10.1007/978-94-011-4896-2_27

Cakmak, I. (2005). The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *Journal of Plant Nutrition and Soil Science*, *168*(4), 521–530. https://doi.org/10.1002/jpln.200420485

Calderini, Da. F., Dreccer, M. F., & Slafer, G. A. (1997). Consequences of breeding on biomass, radiation interception and radiation-use efficiency in wheat. *Field Crops Research*, *52*, 271–281.

Caviglia, O. P., & Sadras, V. O. (2001). Effect of nitrogen supply on crop conductance, water- and radiation-use efficiency of wheat. *Field Crops Research*, *69*(3), 259–266. https://doi.org/10.1016/S0378-4290(00)00149-0

Cayci, G., Heng, L. K., Öztürk, H. S., Sürek, D., Kütük, C., & Sağlam, M. (2009). Crop yield and water use efficiency in semi-arid region of Turkey. *Soil and Tillage Research*, *103*(1), 65–72. https://doi.org/10.1016/j.still.2008.09.004

Chamekh, Z., Karmous, C., Ayadi, S., Sahli, A., Hammami, Z., Belhaj Fraj, M., … Slim-Amara, H. (2015). Stability analysis of yield component traits in 25 durum wheat (Triticum durum Desf.) genotypes under contrasting irrigation water salinity. *Agricultural Water Management*, *152*, 1–6. https://doi.org/10.1016/j.agwat.2014.12.009

Chantret, N., Rô Me Salse, J., Ois Sabot, F., Rahman, S., Bellec, A., Laubin, B., … Chalhoub, B. (2005). Molecular Basis of Evolutionary Events That Shaped the Hardness Locus in Diploid and Polyploid Wheat Species (Triticum and Aegilops) W. *The Plant Cell*, *17*, 1033–1045. https://doi.org/10.1105/tpc.104.029181

Chen, C., Neill, K., Wichman, D., & Westcott, M. (2008). Hard red spring wheat response to row spacing, seeding rate, and nitrogen. *Agronomy Journal*, *100*(5), 1296–1302. https://doi.org/10.2134/agronj2007.0198

Chen, J., Liang, Y., Hu, X., Wang, X., Tan, F., Zhang, H., … Luo, P. (2010). Physiological characterization of "stay green" wheat cultivars during the grain filling stage under field growing conditions. *Acta Physiologiae Plantarum*, *32*(5), 875–882. https://doi.org/10.1007/s11738-010-0475-0

Chen, Q. F., Yen, C., & Yang, J. L. (1998). Chromosome location of the gene for brittle rachis in the Tibetan weedrace of common wheat. *Genetic Resources and Crop Evolution*, *45*, 407–410. https://doi.org/10.1023/A:1008738423817

Ciampitti, I. A., & Vyn, T. J. (2012). Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. *Field Crops Research*, *133*, 48–67. https://doi.org/10.1016/j.fcr.2012.03.008

Clark, M., & Tilman, D. (2017). Comparative analysis of environmental impacts of agricultural production systems, agricultural input efficiency, and food choice. *Environmental Research Letters*, *12*(6). https://doi.org/10.1088/1748-9326/aa6cd5

Collard, B. C. Y., & Mackill, D. J. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1491), 557–572. https://doi.org/10.1098/rstb.2007.2170

Cooper, M., Woodruff, D. R., Phillips, I. G., Basford, K. E., & Gilmour, A. R. (2001). Genotype-by-management interactions for grain yield and grain protein concentration of wheat. *Field Crops Research*, *69*(1), 47–67. https://doi.org/10.1016/S0378-4290(00)00131-3

Cormier, F., Faure, S., Dubreuil, P., Heumez, E., Beauchêne, K., Lafarge, S., … Beauchêne, K. (2013). A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (Triticum aestivum L.). *Theor Appl Genet*, *126*, 3035–3048. https://doi.org/10.1007/s00122-013-2191-9

Cox, M. C., Qualset, C. O., & Rains, D. W. (1985). Genetic Variation for Nitrogen Assimilation and Translocation in Wheat. III. Nitrogen Translocation in Relation to Grain Yield and Protein. *Crop Science*, *25*, 435–440. https://doi.org/10.2135/cropsci1986.0011183x002600040022x

Crofts, H. J. (1989). On defining a winter wheat. *Euphytica*, *44*(3), 225–234. https://doi.org/10.1007/BF00037529

Cui, F., Li, J., Ding, A., Zhao, C., Wang, L., Wang, X., … Wang, H. (2011). Conditional QTL mapping for plant height with respect to the length of the spike and internode in two mapping populations of wheat. *Theoretical and Applied Genetics*, *122*(8), 1517–1536. https://doi.org/10.1007/s00122-011-1551-6

Daba, S. D., Tyagi, P., Brown-Guedira, G., & Mohammadi, M. (2018). Genome-wide association studies to identify loci and candidate genes controlling kernel weight and length in a historical United States wheat population. *Frontiers in Plant Science*, *9*(August), 1–14. https://doi.org/10.3389/fpls.2018.01045

Delogu, G., Cattivelli, L., Pecchioni, N., De Falcis, D., Maggiore, T., & Stanca, A. M. (1998). Uptake and agronomic efficiency of nitrogen in winter barley and winter wheat. *European Journal of Agronomy*, *9*(1), 11–20. https://doi.org/10.1016/S1161-0301(98)00019-7

Deng, Y., Teng, W., Tong, Y. P., Chen, X. P., & Zou, C. Q. (2018). Phosphorus efficiency mechanisms of two wheat cultivars as affected by a range of phosphorus levels in the field. *Frontiers in Plant Science*, *871*(November), 1–12. https://doi.org/10.3389/fpls.2018.01614

Devos, K. M., Atkinson, M. D., Chinoy, C. N., Liu, C. J., & Gale, M. D. (1992). RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. *Theoretical and Applied Genetics*, *83*(8), 931–939. https://doi.org/10.1007/BF00232953

Dewey, D. R., & Lu, K. H. (1959). A Correlation and Path-Coefficient Analysis of Components of Crested Wheatgrass Seed Production 1 . *Agronomy Journal*, *51*(9), 515–518. https://doi.org/10.2134/agronj1959.00021962005100090002x

Dhugga, K. S., & Waines, J. G. (1989). Analysis of Nitrogen Accumulation and Use in Bread and Durum Wheat. *Crop Science*, *29*(5), 1232. https://doi.org/10.2135/cropsci1989.0011183x002900050029x

Díaz, A., Zikhali, M., Turner, A. S., Isaac, P., & Laurie, D. A. (2012). Copy number variation affecting the photoperiod-B1 and vernalization-A1 genes is associated with altered flowering time in wheat (Triticum aestivum). *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0033234

Distelfeld, A., Uauy, C., Fahima, T., & Dubcovsky, J. (2006). Physical map of the wheat high-grain protein content gene Gpc-B1 and development of a high-throughput molecular marker. *New Phytologist*, *169*(4), 753–763. https://doi.org/10.1111/j.1469-8137.2005.01627.x

Doerge, R. W. (2002). Mapping and analysis of quantitative trait loci in experimental populations. *Nature Reviews. Genetics*, *3*(1), 43–52. https://doi.org/10.1038/nrg703

Donmez, E., Sears, R. G., Shroyer, J. P., & Paulsen, G. M. (2001). Genetic gain in yield attributes of winter wheat in the Great Plains. *Crop Science*, *41*(5), 1412–1419. https://doi.org/10.2135/cropsci2001.4151412x

Duan, J., Wu, Y., Zhou, Y., Ren, X., Shao, Y., Feng, W., … Guo, T. (2018). Grain number responses to pre-anthesis dry matter and nitrogen in improving wheat yield in the Huang-Huai Plain. *Scientific Reports*, *8*(1), 1–10. https://doi.org/10.1038/s41598-018-25608-0

Dubcovsky, J., & Dvorak, J. (2007). Genome Plasticity a Key Factor. *Science*, *316*(OCTOBER), 1862–1866. https://doi.org/10.1126/science.1143986

Dubcovsky, J., Lijavetzky, D., Appendino, L., & Tranquilli, G. (1998). Comparative RFLP mapping of Triticum monococcum genes controlling vernalization requirement. *Theoretical and Applied Genetics*, *97*(5–6), 968–975. https://doi.org/10.1007/s001220050978

Dubcovsky, Jorge, Loukoianov, A., Fu, D., Valarik, M., Sanchez, A., & Yan, L. (2006). Effect of photoperiod on the regulation of wheat vernalization genes VRN1 and VRN2. *Plant Molecular Biology*, *60*(4), 469–480. https://doi.org/10.1007/s11103-005-4814-2

Dvorak, J., Deal, K. R., & Luo, M.-C. (2006). Discovery and Mapping of Wheat Ph1 Suppressors. *Genetics Society of America*. https://doi.org/10.1534/genetics.106.058115

Edae, E. A., Byrne, P. F., Haley, S. D., Lopes, M. S., & Reynolds, M. P. (2014). Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. *Theoretical and Applied Genetics*, *127*(4), 791–807. https://doi.org/10.1007/s00122-013-2257-8

El Dessougi, H., Claassen, N., & Steingrobe, B. (2002). Potassium efficiency mechanisms of wheat, barley, and sugar beet grown on a K fixing soil under controlled conditions. *Journal of Plant Nutrition and Soil Science*, *165*(6), 732–737. https://doi.org/10.1002/jpln.200290011

Elanchezhian, R., Krishnapriya, V., Pandey, R., Rao, A. S., & Abrol, Y. P. (2015). Physiological and molecular approaches for improving phosphorus uptake efficiency of crops. *Current Science*, *108*(7), 1271–1279.

Elser, J. J. (2012). Phosphorus: A limiting nutrient for humanity? *Current Opinion in Biotechnology*, *23*(6), 833–838. https://doi.org/10.1016/j.copbio.2012.03.001

Elsgaard, L., Børgesen, C. D., Olesen, J. E., Siebert, S., Ewert, F., Peltonen-Sainio, P., … Skjelvåg, A. O. (2012). Shifts in comparative advantages for maize, oat and wheat cropping under climate change in Europe. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *29*(10), 1514–1526. https://doi.org/10.1080/19440049.2012.700953

Enghiad, A., Ufer, D., Countryman, A. M., & Thilmany, D. D. (2017). An Overview of Global Wheat Market Fundamentals in an Era of Climate Concerns. *International Journal of Agronomy*, *2017*. https://doi.org/10.1155/2017/3931897

Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z., & Winiwarter, W. (2008). How a century of ammonia synthesis changed the world. *Nature Geoscience*, *1*(10), 636–639. https://doi.org/10.1038/ngeo325

Ersoz, E. S., Yu, J., & Buckler, E. S. (2007). Applications of linkage disequilibrium and association mapping in crop plants. *Genomics-Assisted Crop Improvement*, *1*, 97–119. https://doi.org/10.1007/978-1-4020-6295-7_5

Faris, J. D., Fellers, J. P., Brooks, S. A., & Gill, B. S. (2003). A bacterial artificial chromosome contig spanning the major domestication locus Q in wheat and identification of a candidate gene. *Genetics*, *164*(1), 311–321.

Fasano, A., & Catassi, C. (2001). Current approaches to diagnosis and treatment of celiac disease: An evolving spectrum. *Gastroenterology*, *120*(3), 636–651. https://doi.org/10.1053/gast.2001.22123

Feldman, M. (2001). Origin of Cultivated Wheat. In *The World Wheat Book: A History of Wheat Breeding* (pp. 3–56).

Ferrante, A., Cartelle, J., Savin, R., & Slafer, G. A. (2017). Yield determination, interplay between major components and yield stability in a traditional and a contemporary wheat across a wide range of environments. *Field Crops Research*, *203*, 114–127. https://doi.org/10.1016/j.fcr.2016.12.028

Flis, S. (2017). The 4Rs in crop nitrogen research. *Crops & Soils*, *50*(2), 18–20. https://doi.org/10.2134/cs2017.50.0209

Föhse, D., Claassen, N., & Jungk, A. (1988). Phosphorus efficiency of plants - I. External and internal P requirement and P uptake efficiency of different plant species. *Plant and Soil*, *110*(1), 101–109. https://doi.org/10.1007/BF02143545

Foulkes, M. J., Hawkesford, M. J., Barraclough, P. B., Holdsworth, M. J., Kerr, S., Kightley, S., & Shewry, P. R. (2009). Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. *Field Crops Research*, *114*(3), 329–342. https://doi.org/10.1016/j.fcr.2009.09.005

Foulkes, M. John, Slafer, G. A., Davies, W. J., Berry, P. M., Sylvester-Bradley, R., Martre, P., … Reynolds, M. P. (2011). Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. *Journal of Experimental Botany*, *62*(2), 469–486. https://doi.org/10.1093/jxb/erq300

Gaire, R., Huang, M., Sneller, C., Griffey, C., Brown-Guedira, G., & Mohammadi, M. (2019). Association analysis of baking and milling quality traits in an elite soft red winter wheat population. *Crop Science*, *59*(3), 1085–1094. https://doi.org/10.2135/cropsci2018.12.0751

Gaju, O., Allard, V., Martre, P., Snape, J. W., Heumez, E., LeGouis, J., … Foulkes, M. J. (2011). Identification of traits to improve the nitrogen-use efficiency of wheat genotypes. *Field Crops Research*. https://doi.org/10.1016/j.fcr.2011.05.010

Gaju, Oorbessy, Allard, V., Martre, P., Le Gouis, J., Moreau, D., Bogard, M., … Foulkes, M. J. (2014). Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. *Field Crops Research*. https://doi.org/10.1016/j.fcr.2013.09.003

Gale, M. D., & Youssefian, S. (1985). Dwarfing genes in wheat. In G. E. Russell (Ed.), *Progress in Plant Breeding I* (pp. 1–28). Butterworths.

Gallais, A., & Coque, M. (2005). Genetic variation and selection for nitrogen use efficiency in maize: A synthesis. *Maydica*, *50*(3–4), 531–547.

Gil-Humanes, J., Pistón, F., Tollefsen, S., Sollid, L. M., & Barro, F. (2010). Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(39), 17023–17028. https://doi.org/10.1073/pnas.1007773107

Gill, B.S., & Friebe, B. (2002). Cytogenetics, phylogeny and evolution of cultivated wheats. In B. C. Curtis, S. Rajaram, & H. Gomez Macpherson (Eds.), *Bread Wheat: Improvement and Production*. Food and Agriculture Organization of the United Nations (FAO).

Gill, Bikram S., Li, W., Sood, S., Kuraparthy, V., Friebe, B. R., Simons, K. J., … Faris, J. D. (2007). Genetics and genomics of wheat domestication-driven evolution. *Israel Journal of Plant Sciences*, *55*(3–4), 223–229. https://doi.org/10.1560/IJPS.55.3-4.223

Godoy, J., Gizaw, S., Chao, S., Blake, N., Carter, A., Cuthbert, R., … Talbert, L. (2018). Genome-wide association study of agronomic traits in a spring-planted north american elite hard red spring wheat panel. *Crop Science*, *58*(5), 1838–1852. https://doi.org/10.2135/cropsci2017.07.0423

Green, A. J., Berger, G., Griffey, C. A., Pitman, R., Thomason, W., Balota, M., & Ahmed, A. (2012). Genetic yield improvement in soft red winter wheat in the Eastern United States from 1919 to 2009. *Crop Science*, *52*(5), 2097–2108. https://doi.org/10.2135/cropsci2012.01.0026

Griffiths, S., Sharp, R., Foote, T. N., Bertin, I., Wanous, M., Reader, S., … Moore, G. (2006). *Molecular characterization of Ph1 as a major chromosome pairing locus in polyploid wheat*. https://doi.org/10.1038/nature04434

Guan, P., Lu, L., Jia, L., Kabir, M. R., Zhang, J., Lan, T., … Peng, H. (2018). Global QTL analysis identifies genomic regions on chromosomes 4A and 4B harboring stable loci for yield-related traits across different environments in wheat (Triticum aestivum L.). *Frontiers in Plant Science*, *9*(April). https://doi.org/10.3389/fpls.2018.00529

Guedira, M., Brown-Guedira, G., van Sanford, D., Sneller, C., Souza, E., & Marshall, D. (2010). Distribution of Rht genes in modern and historic winter wheat cultivars from the eastern and central USA. *Crop Science*, *50*(5), 1811–1822. https://doi.org/10.2135/cropsci2009.10.0626

Gutiérrez, L., Germán, S., Pereyra, S., Hayes, P. M., Pérez, C. A., Capettini, F., … Castro, A. J. (2015). Multi-environment multi-QTL association mapping identifies disease resistance QTL in barley germplasm from Latin America. *Theoretical and Applied Genetics*, *128*(3), 501–516. https://doi.org/10.1007/s00122-014-2448-y

Hadjichristodoulou, A. (1989). Environmental correlations among grain yield and other important traits of wheat in drylands. *Euphytica*, *44*(1–2), 143–150. https://doi.org/10.1007/BF00022609

Hamblin, A., Tennant, D., & Perry, M. W. (1987). Management of soil water for wheat production in Western Australia. *Soil Use and Management*, *3*(2), 63–69. https://doi.org/10.1111/j.1475-2743.1987.tb00712.x

Hammel, J. E. (1995). Long-term tillage and crop rotation effects on winter wheat production in northern Idaho. *Agronomy Journal*, *87*(1), 16–22. https://doi.org/10.2134/agronj1995.00021962008700010004x

Harper, L. A., Sharpe, R. R., Langdale, G. W., & Giddens, J. E. (1987). Nitrogen Cycling in a Wheat Crop: Soil, Plant, and Aerial Nitrogen Transport1. *Agronomy Journal*, *79*(6), 965. https://doi.org/10.2134/agronj1987.00021962007900060004x

Hawkesford, M. J. (2014). Reducing the reliance on nitrogen fertilizer for wheat production. *Journal of Cereal Science*, *59*(3), 276–283. https://doi.org/10.1016/j.jcs.2013.12.001

Hawkesford, M. J., Araus, J. L., Park, R., Calderini, D., Miralles, D., Shen, T., … Parry, M. A. J. (2013). Prospects of doubling global wheat yields. *Food and Energy Security*, *2*(1), 34–48. https://doi.org/10.1002/fes3.15

Hedden, P. (2003). The genes of the Green Revolution. *Trends in Genetics*, *19*(1), 5–9. https://doi.org/10.1016/S0168-9525(02)00009-4

Heffer, P., Magen, H., Mikkelsen, R., & Wichelns, D. (2015). Managing Water and Fertilizer for Sustainable Agricultural Intensification. In *International Fertilizer Industry Association*.

Helentjaris, T., Slocum, M., Wright, S., Schaefer, A., & Nienhuis, J. (1986). Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. *Theoretical and Applied Genetics*, *72*(6), 761–769. https://doi.org/10.1007/BF00266542

Hill, W. G., & Weir, B. S. (1988). Variances and covariances of squared linkage disequilibria in finite populations. *Theoretical Population Biology*, *33*(1), 54–78. https://doi.org/10.1016/0040-5809(88)90004-4

Hirel, B., Le Gouis, J., Ney, B., & Gallais, A. (2007). The challenge of improving nitrogen use efficiency in crop plants: Towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany*, *58*(9), 2369–2387. https://doi.org/10.1093/jxb/erm097

Hitz, K., Clark, A. J., & Van Sanford, D. A. (2017). Identifying nitrogen-use efficient soft red winter wheat lines in high and low nitrogen environments. *Field Crops Research*, *200*, 1–9. https://doi.org/10.1016/j.fcr.2016.10.001

Huang, M., Cabrera, A., Hoffstetter, A., Griffey, C., Van Sanford, D., Costa, J., … Sneller, C. (2016). Genomic selection for wheat traits and trait stability. *Theoretical and Applied Genetics*, *129*(9), 1697–1710. https://doi.org/10.1007/s00122-016-2733-z

Huang, M., Mheni, N., Brown-Guedira, G., McKendry, A., Griffey, C., Van Sanford, D., … Sneller, C. (2018). Genetic analysis of heading date in winter and spring wheat. *Euphytica*, *214*(8), 1–18. https://doi.org/10.1007/s10681-018-2199-y

Iannucci, A., Fares, C., & Codianni, P. (2008). Influence of nitrogen levels on bio-agronomic and quality traits of tetraploid wheats under organic farming. *Annals of Applied Biology*, *173*(1), 1–15. https://doi.org/10.1111/aab.12429

Jantasuriyarat, C., Vales, M. I., Watson, C. J. W., & Riera-Lizarazu, O. (2004). Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *108*(2), 261–273. https://doi.org/10.1007/s00122-003-1432-8

Jiang, Q., Hou, J., Hao, C., Wang, L., Ge, H., Dong, Y., & Zhang, X. (2011). The wheat (T. aestivum) sucrose synthase 2 gene (TaSus2) active in endosperm development is associated with yield traits. *Functional and Integrative Genomics*, *11*(1), 49–61. https://doi.org/10.1007/s10142-010-0188-x

Kaler, A. S., Gillman, J. D., Beissinger, T., & Purcell, L. C. (2020). Comparing Different Statistical Models and Multiple Testing Corrections for Association Mapping in Soybean and Maize. *Frontiers in Plant Science*, *10*(February), 1–13. https://doi.org/10.3389/fpls.2019.01794

Kanampiu, F. K., Raun, W. R., & Johnson, G. V. (1997). Effect of nitrogen rate on plant nitrogen loss in winter wheat varieties. *Journal of Plant Nutrition*, *20*(2–3), 389–404. https://doi.org/10.1080/01904169709365259

Kashif, M., & Khaliq, I. (2004). Heritability , correlation and path coefficient analysis for some metric traits in wheat. *Inter. J Agric. Bio.*, *6*(1), 138–142. https://doi.org/1560–8530/2004/06–1–138–142

Kassambara, A., & Mundt, F. (2016). *Factoextra: Extract and Visualize the Results of Multivariate Data Analyses*. R Package.

Kaut, A. H. E. E., Mason, H. E., Navabi, A., O'Donovan, J. T., & Spaner, D. (2009). Performance and stability of performance of spring wheat variety mixtures in organic and conventional management systems in western Canada. *Journal of Agricultural Science*, *147*(2), 141–153. https://doi.org/10.1017/S0021859608008319

Keeney, D. R., & Hatfield, J. L. (2008). The Nitrogen Cycle, Historical Perspective, and Current and Potential Future Concerns. *Nitrogen in the Environment*, 1–18. https://doi.org/10.1016/B978-0-12-374347-3.00001-9

Kerber, E. R., & Rowland, G. G. (1974). Origin of the free threshing character in hexaploid wheat. *Canadian Journal of Genetics and Cytology*, *16*(1), 145–154. https://doi.org/10.1139/g74-014

Kirigwi, F. M., Van Ginkel, M., Brown-Guedira, G., Gill, B. S., Paulsen, G. M., & Fritz, A. K. (2007). Markers associated with a QTL for grain yield in wheat under drought. *Molecular Breeding*, *20*(4), 401–413. https://doi.org/10.1007/s11032-007-9100-3

Kirkegaard, J. A., & Hunt, J. R. (2010). Increasing productivity by matching farming system management and genotype in water-limited environments. *Journal of Experimental Botany*, *61*(15), 4129–4143. https://doi.org/10.1093/jxb/erq245

Kiszonas, A. M., Fuerst, E. P., & Morris, C. F. (2013). A comprehensive survey of soft wheat grain quality in U.S. germplasm. *Cereal Chemistry*, *90*(1), 47–57. https://doi.org/10.1094/CCHEM-06-12-0073-R

Koch, B., Khosla, R., Frasier, W. M., Westfall, D. G., & Inman, D. (2004). *Economic Feasibility of Variable-Rate Nitrogen Application Utilizing Site-Specific Management Zones*. 1572–1580.

Kotal, B. D., Das, A., & Choudhury, B. K. (2010). Genetic variability and association of characters in wheat (Triticum aestivum L.). *Asian Journal of Crop Science*, Vol. 2, pp. 155–160. https://doi.org/10.3923/ajcs.2010.155.160

Kumar, N., Kulwal, P. L., Balyan, H. S., & Gupta, P. K. (2007). QTL mapping for yield and yield contributing traits in two mapping populations of bread wheat. *Molecular Breeding*, *19*(2), 163–177. https://doi.org/10.1007/s11032-006-9056-8

Kuraparthy, V., Sood, S., Dhaliwal, H. S., Chhuneja, P., & Gill, B. S. (2007). Identification and mapping of a tiller inhibition gene (tin3) in wheat. *Theoretical and Applied Genetics*, *114*(2), 285–294. https://doi.org/10.1007/s00122-006-0431-y

Ladha, J.K., Kumar, V., Alam, M. M., Sharma, S., Gathala, M. K., Chandna, P., … Balasubramanian, V. (2009). Integrating crop and resource management technologies for enhanced productivity, profitability, and sustainability of the rice-wheat system in South Asia. In J. Ladha, Yadvinder-Singh, O. Erenstein, & B. Hardy (Eds.), *Integrated Crop and Resource Management in the Rice-wheat System of South Asia* (pp. 69–108). Phillipines: International Rice Research Institute.

Ladha, Jagdish K., Pathak, H., Krupnik, T. J., Six, J., & van Kessel, C. (2005). Efficiency of Fertilizer Nitrogen in Cereal Production: Retrospects and Prospects. *Advances in Agronomy*, *87*(05), 85–156. https://doi.org/10.1016/S0065-2113(05)87003-8

Lammerts van Bueren, E. T., & Struik, P. C. (2017). Diverse concepts of breeding for nitrogen use efficiency. A review. *Agronomy for Sustainable Development*, *37*(5). https://doi.org/10.1007/s13593-017-0457-3

Lanning, S. P., Kephart, K., Carlson, G. R., Eckhoff, J. E., Stougaard, R. N., Wichman, D. M., … Talbert, L. E. (2010). Climatic change and agronomic performance of hard red spring wheat from 1950 to 2007. *Crop Science*, *50*(3), 835–841. https://doi.org/10.2135/cropsci2009.06.0314

Laperche, A., Brancourt-Hulmel, M., Heumez, E., Gardet, O., & Le Gouis, J. (2006). Estimation of genetic parameters of a DH wheat population grown at different N stress levels characterized by probe genotypes. *Theoretical and Applied Genetics*, *112*(5), 797–807. https://doi.org/10.1007/s00122-005-0176-z

Lawlor, D. W., Kontturi, M., & Young, A. T. (1989). Photosynthesis by Flag Leaves of Wheat in Relation to Protein, Ribulose Bisphosphate Carboxylase Activity and Nitrogen Supply. *Journal of Experimental Botany*, *40*, 43–52.

Lawlor, David W. (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of Experimental Botany*, *53*(370), 773–787. Retrieved from https://academic.oup.com/jxb/article-abstract/53/370/773/2908378

Le Gouis, J., Béghin, D., Heumez, E., & Pluchard, P. (2000). Genetic differences for nitrogen uptake and nitrogen utilisation efficiencies in winter wheat. *European Journal of Agronomy*, *12*(3–4), 163–173. https://doi.org/10.1016/S1161-0301(00)00045-9

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software*, *25*(1), 1–18. https://doi.org/10.18637/jss.v025.i01

Leff, B., Ramankutty, N., & Foley, J. A. (2004). Geographic distribution of major crops across the world. *Global Biogeochemical Cycles*, *18*(1), n/a-n/a. https://doi.org/10.1029/2003GB002108

Lenth, R. V. (2016). Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, *1*, 1–33. https://doi.org/10.18637/jss.v069.i01

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, P., Chen, J., & Wu, P. (2011). Agronomic characteristics and grain yield of 30 spring wheat genotypes under drought stress and nonstress conditions. *Agronomy Journal*, *103*(6), 1619–1628. https://doi.org/10.2134/agronj2011.0013

Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., … Zhang, Z. (2012). GAPIT: genome association and prediction integrated tool. *Bioinformatics (Oxford, England)*, *28*(18), 2397–2399. https://doi.org/10.1093/bioinformatics/bts444

Liu, J., Xu, Z., Fan, X., Zhou, Q., Cao, J., Wang, F., … Wang, T. (2018). A genome-wide association study of wheat spike related traits in China. *Frontiers in Plant Science*, *871*(October), 1–14. https://doi.org/10.3389/fpls.2018.01584

Liu, X., Huang, M., Fan, B., Buckler, E. S., & Zhang, Z. (2016). Iterative Usage of Fixed and Random Effect Models for Powerful and Efficient Genome-Wide Association Studies. *PLoS Genetics*, *12*(2). https://doi.org/10.1371/journal.pgen.1005767

Löffler, M., Schön, C. C., & Miedaner, T. (2009). Revealing the genetic architecture of FHB resistance in hexaploid wheat (Triticum aestivum L.) by QTL meta-analysis. *Molecular Breeding*, *23*(3), 473–488. https://doi.org/10.1007/s11032-008-9250-y

Lopes, M. S., Rebetzke, G. J., & Reynolds, M. (2014). Integration of phenotyping and genetic platforms for a better understanding of wheat performance under drought. *Journal of Experimental Botany*, *65*(21), 6167–6177. https://doi.org/10.1093/jxb/eru384

Lozada, D. N., Mason, R. E., Sukumaran, S., & Dreisigacker, S. (2018). Validation of grain yield QTLs from soft winter wheat using a CIMMYT spring wheat panel. *Crop Science*, *58*(5), 1964–1971. https://doi.org/10.2135/cropsci2018.04.0232

Luo, C., Branlard, G., Griffin, W. B., & McNeil, D. L. (2000). The effect of nitrogen and sulphur fertilisation and their interaction with genotype on wheat glutenins and quality parameters. *Journal of Cereal Science*, *31*(2), 185–194. https://doi.org/10.1006/jcrs.1999.0298

Luo, Q., Bellotti, W., Williams, M., & Wang, E. (2009). Adaptation to climate change of wheat growing in South Australia: Analysis of management and breeding strategies. *Agriculture, Ecosystems and Environment*, *129*(1–3), 261–267. https://doi.org/10.1016/j.agee.2008.09.010

Ma, L., Li, T., Hao, C., Wang, Y., Chen, X., & Zhang, X. (2016). TaGS5-3A, a grain size gene selected during wheat improvement for larger kernel and yield. *Plant Biotechnology Journal*, *14*(5), 1269–1280. https://doi.org/10.1111/pbi.12492

Magallanes-López, A. M., Ammar, K., Morales-Dorantes, A., González-Santoyo, H., Crossa, J., & Guzmán, C. (2017). Grain quality traits of commercial durum wheat varieties and their relationships with drought stress and glutenins composition. *Journal of Cereal Science*, *75*, 1–9. https://doi.org/10.1016/j.jcs.2017.03.005

Major, D. J., Blad, B. J., Bauer, A., Hatfield, J. L., Hubbard, K. G., Kanemasu, E. T., & Reginato, R. J. (1988). Winter wheat grain yield response to water and nitrogen on the north american great plains*. *Agricultural and Forest Meterology*, *44*, 141–149.

Marshall, G. C., & Ohm, H. W. (1987). Yield Responses of 16 Winter Wheat Cultivars to Row Spacing and Seeding Rate 1 . *Agronomy Journal*, *79*(6), 1027–1030. https://doi.org/10.2134/agronj1987.00021962007900060015x

Martin, J. M., Frohberg, R. C., Morris, C. F., Talbert, L. E., & Giroux, M. J. (2001). Milling and bread baking traits associated with puroindoline sequence type in hard red spring wheat. *Crop Science*, *41*(1), 228–234. https://doi.org/10.2135/cropsci2001.411228x

Mason, H. E., & Spaner, D. (2006). Competitive ability of wheat in conventional and organic management systems: A review of the literature. *Canadian Journal of Plant Science*, *86*(2), 333–343. https://doi.org/10.4141/P05-051

Mason, Heather E., Navabi, A., Frick, B. L., O'Donovan, J. T., & Spaner, D. M. (2007). The weed-competitive ability of Canada western red spring wheat cultivars grown under organic management. *Crop Science*, *47*(3), 1167–1176. https://doi.org/10.2135/cropsci2006.09.0566

Maulana, F., Anderson, J. D., Butler, T. J., & Ma, X.-F. (2019). Improving Dual-Purpose Winter Wheat in the Southern Great Plains of the United States. In *Global Wheat Production* (pp. 1–15). Retrieved from http://dx.doi.org/10.5772/intechopen.86417

McFadden, E. S., & Sears, E. R. (1946). The origin of triticum spelta and its free-threshing hexaploid relatives. *Journal of Heredity*, *37*(3), 81–89. https://doi.org/10.1093/oxfordjournals.jhered.a105590

Miralles, D. J., & Slafer, G. A. (1995). Individual grain weight responses to genetic reduction in culm length in wheat as affected by source-sink manipulations. *Field Crops Research*, *43*(2–3), 55–66. https://doi.org/10.1016/0378-4290(95)00041-N

Moll, R. H., Kamprath, E. J., & Jackson, W. A. (1982). Analysis and Interpretation of Factors Which Contribute to Efficiency of Nitrogen Utilization1. *Agronomy Journal*, *74*, 562–565. https://doi.org/10.2134/agronj1982.00021962007400030037x

Money, D., Gardner, K., Migicovsky, Z., Schwaninger, H., Zhong, G. Y., & Myles, S. (2015). LinkImpute: Fast and accurate genotype imputation for nonmodel organisms. *G3: Genes, Genomes, Genetics*, *5*(11), 2383–2390. https://doi.org/10.1534/g3.115.021667

Moonen, J. H. E., Scheepstra, A., & Graveland, A. (1982). Use of the SDS-sedimentation test and SDS-polyacrylamidegel electrophoresis for screening breeder's samples of wheat for bread-making quality. *Euphytica*, *31*(3), 677–690. https://doi.org/10.1007/BF00039206

Morris, C. F., DeMacon, V. L., & Giroux, M. J. (1999). Wheat grain hardness among chromosome 5D homozygous recombinant substitution lines using different methods of measurement. *Cereal Chemistry*, *76*(2), 249–254. https://doi.org/10.1094/CCHEM.1999.76.2.249

Morris, C. F., & Paulsen, G. M. (1985). Development of Hard Winter Wheat after Anthesis as Affected by Nitrogen Nutrition1. *Crop Science*, *25*(6), 1007. https://doi.org/10.2135/cropsci1985.0011183x002500060026x

Murphy, K. M., Reeves, P. G., & Jones, S. S. (2008). Relationship between yield and mineral nutrient concentrations in historical and modern spring wheat cultivars. *Euphytica*, *163*(3), 381–390. https://doi.org/10.1007/s10681-008-9681-x

Myles, S., Peiffer, J., Brown, P. J., Ersoz, E. S., Zhang, Z., Costich, D. E., & Buckler, E. S. (2009). Association Mapping: Critical Considerations Shift from Genotyping to Experimental Design. *The Plant Cell*, *21*, 2194–2202. https://doi.org/10.1105/tpc.109.068437

Nelson, J. C., Sorrells, M. E., Van Deynze, A. E., Yun Hai Lu, Atkinson, M., Bernard, M., … Anderson, J. A. (1995). Molecular mapping of wheat: Major genes and rearrangements in homoeologous groups 4, 5, and 7. *Genetics*, *141*(2), 721–731.

Nyquist, W. E. (1991). Estimation of Heritability and Prediction of Selection Response in Plant Populations. *Critical Reviews in Plant Sciences*, *10*(3), 235–322. https://doi.org/10.1080/07352689109382313

Ohm, H.W., Anderson, J.M, Sharma, H.C., Ayala, L., Thompson, N., and Uphaus, J.J. (2005). Registration of Yellow Dwarf Viruses Resistant Wheat Germplasm Line P961341. Crop Science, 45, 805-806. https://doi.org/10.2135/cropsci2005.0805

Olmos, S., Distelfeld, A., Chicaiza, O., Schlatter, A. R., Fahima, T., Echenique, V., & Dubcovsky, J. (2003). Precise mapping of a locus affecting grain protein content in durum wheat. *Theoretical and Applied Genetics*, *107*(7), 1243–1251. https://doi.org/10.1007/s00122-003-1377-y

Olsen, K. M., & Wendel, J. F. (2013). *A Bountiful Harvest: Genomic Insights into Crop Domestication Phenotypes*. https://doi.org/10.1146/annurev-arplant-050312-120048

Ortiz-Monasterio, J. I., Sayre, K. D., Rajaram, S., & McMahon, M. (1997). Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. *Crop Science*, *37*(3), 898–904. https://doi.org/10.2135/cropsci1997.0011183X003700030033x

Otteson, B. N., Mergoum, M., & Ransom, J. K. (2007). Seeding rate and nitrogen management effects on spring wheat yield and yield components. *Agronomy Journal*, *99*(6), 1615–1621. https://doi.org/10.2134/agronj2007.0002

Pala, M., Oweis, T., Benli, B., De Pauw, E., El Mourid, M., Karrou, M., … Zencirci, N. (2011). Assessment of wheat yield gap in the Mediterranean: case studies from Morocco, Syria and Turkey. In *International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria. iv*.

Paliwal, R., Röder, M. S., Kumar, U., Srivastava, J. P., & Joshi, A. K. (2012). QTL mapping of terminal heat tolerance in hexaploid wheat (T. aestivum L.). *Theoretical and Applied Genetics*, *125*(3), 561–575. https://doi.org/10.1007/s00122-012-1853-3

Papendick, R. I. (1996). Farming systems and conservation needs in the Northwest Wheat Region. *American Journal of Alternative Agriculture*, *11*(2–3), 52–57. https://doi.org/10.1017/S0889189300006767

Pasha, I., Anjum, F. M., & Morris, C. F. (2010). Grain Hardness: A Major Determinant of Wheat Quality. *Food Science and Technology International*, *16*(6), 511–522. https://doi.org/10.1177/1082013210379691

Pask, A. J. D., Sylvester-Bradley, R., Jamieson, P. D., & Foulkes, M. J. (2012). Quantifying how winter wheat crops accumulate and use nitrogen reserves during growth. *Field Crops Research*. https://doi.org/10.1016/j.fcr.2011.09.021

Payne, P. I. (1987). Genetics of Wheat Storage Proteins and the Effects of Allelic Variation on Bread-Making Quality. *Ann. Rev. Plant Physiol.*, (28), 141–153.

Pearce, S., Saville, R., Vaughan, S. P., Chandler, P. M., Wilhelm, E. P., Sparks, C. A., … Thomas, S. G. (2011). Molecular characterization of Rht-1 dwarfing genes in hexaploid wheat. *Plant Physiology*, *157*(4), 1820–1831. https://doi.org/10.1104/pp.111.183657

Pena, R. J. (2002). Wheat for bread and other foods. In B. C. Curtis, S. Rajaram, & H. Gomez Macpherson (Eds.), *Bread Wheat: Improvement and Production*. Retrieved from http://www.fao.org/3/y4011e0w.htm#bm32

Pena, R. J., Amaya, A., Rajaram, S., & Mujeeb-Kazi, A. (1990). Variation in quality characteristics associated with some spring 1B/1R translocation wheats. *Journal of Cereal Science*, *12*(2), 105–112. https://doi.org/10.1016/S0733-5210(09)80092-1

Pena, R. J., Gonzalez-Santoyo, H., & Cervantes, F. (2004). Relationship between Glu-D1/Glu-B3 allelic combinations and bread-making quality parameters commonly used in wheat breeding. In *The Gluten Proteins* (pp. 156–157).

Peng, J. H., Sun, D., & Nevo, E. (2011). Domestication evolution, genetics and genomics in wheat. *Molecular Breeding*, *28*(3), 281–301. https://doi.org/10.1007/s11032-011-9608-4

Peng, Jinrong, Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., … Harberd, N. P. (1999). "Green revolution" genes encode mutant gibberellin response modulators. *Nature*, *400*(6741), 256–261. https://doi.org/10.1038/22307

Peng, Junhua, Ronin, Y., Fahima, T., Röder, M. S., Li, Y., Nevo, E., & Korol, A. (2003). Domestication quantitative trait loci in Triticum dicoccoides, the progenitor of wheat. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(5), 2489–2494. https://doi.org/10.1073/pnas.252763199

Pettigrew, W. T. (2008). Potassium influences on yield and quality production for maize, wheat, soybean and cotton. *Physiologia Plantarum*, *133*(4), 670–681. https://doi.org/10.1111/j.1399-3054.2008.01073.x

Philipp, N., Weichert, H., Bohra, U., Weschke, W., Schulthess, A. W., & Weber, H. (2018). Grain number and grain yield distribution along the spike remain stable despite breeding for high yield in winter wheat. *PLoS ONE*, *13*(10). https://doi.org/10.1371/journal.pone.0205452

Piepho, H. P., & Möhring, J. (2007a). Computing heritability and selection response from unbalanced plant breeding trials. *Genetics*, *177*(3), 1881–1888. https://doi.org/10.1534/genetics.107.074229

Piepho, H. P., & Möhring, J. (2007b). Computing heritability and selection response from unbalanced plant breeding trials. *Genetics*, *177*(3), 1881–1888. https://doi.org/10.1534/genetics.107.074229

Pimentel, D., Berardi, G., & Fast, S. (1983). Energy efficiency of farming systems: Organic and conventional agriculture. *Agriculture, Ecosystems and Environment*, *9*(4), 359–372. https://doi.org/10.1016/0167-8809(83)90021-X

Poland, J. A., Bradbury, P. J., Buckler, E. S., & Nelson, R. J. (2011). Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(17), 6893–6898. https://doi.org/10.1073/pnas.1010894108

Poland, J. A., Brown, P. J., Sorrells, M. E., & Jannink, J. L. (2012). Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS ONE*, *7*(2). https://doi.org/10.1371/journal.pone.0032253

Porceddu, E., Turchetta, T., Masci, S., D'Ovidio, R., Lafiandra, D., Kasarda, D. D., … Nachit, M. M. (1997). *Variation in endosperm protein composition and technological quality properties in durum wheat*. 263–271. https://doi.org/10.1007/978-94-011-4896-2_36

Prasad, M., Varshney, R. K., Kumar, A., Balyan, H. S., Sharma, P. C., Edwards, K. J., … Gupta, P. K. (1999). A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. *Theoretical and Applied Genetics*, *99*(1–2), 341–345. https://doi.org/10.1007/s001220051242

Price, A. L., Patterson, N. J., Plenge, R. M., Weinblatt, M. E., Shadick, N. A., & Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies. *Nature Genetics*, *38*(8), 904–909. https://doi.org/10.1038/ng1847

R Core Team. (2019). *R: A language and environment for statistical computing*. Retrieved from http://www.r-project.org/

Rafalski, J. A. (2010). Association genetics in crop improvement. *Current Opinion in Plant Biology*, *13*(2), 174–180. https://doi.org/10.1016/j.pbi.2009.12.004

Ramaekers, L., Remans, R., Rao, I. M., Blair, M. W., & Vanderleyden, J. (2010). Strategies for improving phosphorus acquisition efficiency of crop plants. *Field Crops Research*, *117*(2–3), 169–176. https://doi.org/10.1016/j.fcr.2010.03.001

Raun, W. R., & Johnson, G. V. (1999). Review and interpreation : Improving nitrogen use efficiency for cereal production. *Agronomy Journal*, *91*(3), 357–363. https://doi.org/10.2134/agronj1999.00021962009100030001x

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). kuYield Trends Are Insufficient to Double Global Crop Production by 2050. *PLoS ONE*, *8*(6). https://doi.org/10.1371/journal.pone.0066428

Rayburn, A. L., & Carver, B. F. (1988). Cytological identification of 1B/1R wheat-rye translocations in winter wheat breeding lines. In *Euphytica* (Vol. 38).

Reinbott, T., & Helsel, Z. (1987). Intercropping soybean into standing green wheat. *Agronomy Journal*, *79*(5), 886–891. https://doi.org/10.2134/agronj1987.00021962007900050026x

Reynolds, M., Foulkes, J., Furbank, R., Griffiths, S., King, J., Murchie, E., … Slafer, G. (2012). Achieving yield gains in wheat. *Plant, Cell and Environment*, *35*(10), 1799–1823. https://doi.org/10.1111/j.1365-3040.2012.02588.x

Reynolds, M. P., Calderini, D. F., Condon, A. G., & Rajaram, S. (2001). Physiological basis of yield gains in wheat associated with the LR19 translocation from Agropyron elongatum. *Euphytica*, *119*(1–2), 137–141. https://doi.org/10.1007/978-94-017-3674-9_44

Reynolds, M. P., Pellegrineschi, A., & Skovmand, B. (2005). Sink-limitation to yield and biomass: A summary of some investigations in spring wheat. *Annals of Applied Biology*, *146*(1), 39–49. https://doi.org/10.1111/j.1744-7348.2005.03100.x

Reynolds, M. P., Rajaram, S., & Sayre, K. D. (1999). Physiological and genetic changes of irrigated wheat in the post-green revolution period and approaches for meeting projected global demand. *Crop Science*, *39*(6), 1611–1621. https://doi.org/10.2135/cropsci1999.3961611x

Reynolds, Matthew P., Pask, A. J. D., Hoppitt, W. J. E., Sonder, K., Sukumaran, S., Molero, G., … Joshi, A. K. (2017). Strategic crossing of biomass and harvest index—source and sink—achieves genetic gains in wheat. *Euphytica*, *213*(11). https://doi.org/10.1007/s10681-017-2040-z

Ribaut, J. M., & Ragot, M. (2007). Marker-assisted selection to improve drought adaptation in maize: The backcross approach, perspectives, limitations, and alternatives. *Journal of Experimental Botany*, *58*(2), 351–360. https://doi.org/10.1093/jxb/erl214

Richardson, A. E., Lynch, J. P., Ryan, P. R., Delhaize, E., Smith, F. A., Smith, S. E., … Simpson, R. J. (2011). Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil*, *349*(1–2), 121–156. https://doi.org/10.1007/s11104-011-0950-4

Riley, D. R., & Chapman, V. (1958). Genetic Control of the Cytologically Diploid Behaviour of Hexaploid Wheat. *Nature*.

Rosseel, Y. (2012). lavaan: An R Package for Structural Equation Modeling. *Journal of Statistical Software*, *48*(2).

Sadras, V. O., & Slafer, G. A. (2012). Environmental modulation of yield components in cereals: Heritabilities reveal a hierarchy of phenotypic plasticities. *Field Crops Research*, *127*, 215–224. https://doi.org/10.1016/j.fcr.2011.11.014

Saint Pierre, C., Peterson, C. J., Ross, A. S., Ohm, J. B., Verhoeven, M. C., Larson, M., & Hoefer, B. (2008). Winter wheat genotypes under different levels of nitrogen and water stress: Changes in grain protein composition. *Journal of Cereal Science*, *47*(3), 407–416. https://doi.org/10.1016/j.jcs.2007.05.007

Salam, A., Hollington, P. A., Gorham, J., Wyn Jones, R. G., & Gliddon, C. (1999). Physiological genetics of salt tolerance in wheat (Triticum aestivum L.): Performance of wheat varieties, inbred lines and reciprocal F1 hybrids under saline conditions. *Journal of Agronomy and Crop Science*, *183*(3), 145–156. https://doi.org/10.1046/j.1439-037X.1999.00361.x

Sandler, L., Nelson, K. A., & Dudenhoeffer, C. (2015). Winter wheat row spacing and alternative crop effects on relay-intercrop, double-crop, and wheat yields. *International Journal of Agronomy*, *2015*. https://doi.org/10.1155/2015/369243

SAS Institute Inc. (2013). *SAS 9.4*. Retrieved from https://www.sas.com/en_us/software/sas9.html

Sharma, R. C., Tiwary, A. K., & Ortiz-Ferrara, G. (2008). Reduction in kernel weight as a potential indirect selection criterion for wheat grain yield under terminal heat stress. *Plant Breeding*, *127*(3), 241–248. https://doi.org/10.1111/j.1439-0523.2007.01460.x

Shen, X., Zhou, M., Lu, W., & Ohm, H. (2003). Detection of Fusarium head blight resistance QTL in a wheat population using bulked segregant analysis. *Theoretical and Applied Genetics*, *106*(6), 1041–1047. https://doi.org/10.1007/s00122-002-1133-8

Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany*, *60*(6), 1537–1553. https://doi.org/10.1093/jxb/erp058

Simmonds, J., Scott, P., Leverington-Waite, M., Turner, A. S., Brinton, J., Korzun, V., … Uauy, C. (2014). Identification and independent validation of a stable yield and thousand grain weight QTL on chromosome 6A of hexaploid wheat (Triticum aestivum L.). *BMC Plant Biology*, *14*(1), 1–13. https://doi.org/10.1186/s12870-014-0191-9

Simons, K. J., Fellers, J. P., Trick, H. N., Zhang, Z., Tai, Y. S., Gill, B. S., & Faris, J. D. (2006). Molecular characterization of the major wheat domestication gene Q. *Genetics*, *172*(1), 547–555. https://doi.org/10.1534/genetics.105.044727

Singh, P., Arora, A., Strand, T. A., Leffler, D. A., Catassi, C., Green, P. H., … Makharia, G. K. (2018). Global Prevalence of Celiac Disease: Systematic Review and Meta-analysis. *Clinical Gastroenterology and Hepatology*, *16*(6), 823-836.e2. https://doi.org/10.1016/j.cgh.2017.06.037

Skidmore, E. L., Kumar, M., & Larson, W. E. (1979). Crop residue management for wind erosion control in the Great Plains. *Journal of Soil and Water Conservation*, *34*(2), 90–94.

Slafer, G. A., Savin, R., & Sadras, V. O. (2014). Coarse and fine regulation of wheat yield components in response to genotype and environment. *Field Crops Research*, *157*, 71–83. https://doi.org/10.1016/j.fcr.2013.12.004

Smith, N., Guttieri, M., Souza, E., Shoots, J., Sorrells, M., & Sneller, C. (2011). Identification and validation of QTL for grain quality traits in a cross of soft wheat cultivars pioneer brand 25r26 and foster. *Crop Science*, *51*(4), 1424–1436. https://doi.org/10.2135/cropsci2010.04.0193

Snape, J. W., Butterworth, K., Whitechurch, E., & Worland, A. J. (2001). Waiting for fine times: Genetics of flowering time in wheat. *Euphytica*, *119*(1–2), 185–190. https://doi.org/10.1023/A:1017594422176

Spielmeyer, W., & Richards, R. A. (2004). Comparative mapping of wheat chromosome 1AS which contains the tiller inhibition gene (tin) with rice chromosome 5S. *Theoretical and Applied Genetics*, *109*(6), 1303–1310. https://doi.org/10.1007/s00122-004-1745-2

Su, Z., Hao, C., Wang, L., Dong, Y., & Zhang, X. (2011). Identification and development of a functional marker of TaGW2 associated with grain weight in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *122*(1), 211–223. https://doi.org/10.1007/s00122-010-1437-z

Sukumaran, S., Lopes, M., Dreisigacker, S., & Reynolds, M. (2018). Genetic analysis of multi-environmental spring wheat trials identifies genomic regions for locus-specific trade-offs for grain weight and grain number. *Theoretical and Applied Genetics*, *131*(4), 985–998. https://doi.org/10.1007/s00122-017-3037-7

Talukder, S. K., Babar, M. A., Vijayalakshmi, K., Poland, J., Prasad, P. V. V., Bowden, R., & Fritz, A. (2014). Mapping QTL for the traits associated with heat tolerance in wheat (Triticum aestivum L.). *BMC Genetics*, *15*(1), 1–13. https://doi.org/10.1186/s12863-014-0097-4

Terrile, I. I., Miralles, D. J., & González, F. G. (2017). Fruiting efficiency in wheat (Triticum aestivum L): Trait response to different growing conditions and its relation to spike dry weight at anthesis and grain weight at harvest. *Field Crops Research*, *201*, 86–96. https://doi.org/10.1016/j.fcr.2016.09.026

Tessmann, E. W., Dong, Y., & Van Sanford, D. A. (2019). GWAS for fusarium head blight traits in a soft red winter wheat mapping panel. *Crop Science*, *59*(5), 1823–1837. https://doi.org/10.2135/cropsci2018.08.0492

Thomas, H., & Smart, C. M. (1993). Crops that stay green. *Annals of Applied Biology*, *123*(1), 193–219. https://doi.org/10.1111/j.1744-7348.1993.tb04086.x

Todeschini, M. H., Milioli, A. S., Trevizan, D. M., Bornhofen, E., Finatto, T., Storck, L., & Benin, G. (2016). Nitrogen use efficiency in modern wheat cultivars. *Bragantia*, *75*(3), 351–361. https://doi.org/10.1590/1678-4499.385

Tolley, S. A. (2019). Biomass Allocation Variation Under Different Nitrogen and Water Treatments in Wheat. *Master's Thesis*.

Torun, B., Bozbay, G., Gultekin, I., Braun, H. J., Ekiz, H., & Cakmak, I. (2000). Differences in shoot growth and zinc concentration of 164 bread wheat genotypes in a zinc-deficient calcareous soil. *Journal of Plant Nutrition*, *23*(9), 1251–1265. https://doi.org/10.1080/01904160009382098

Tranquilli, G., & Dubcovsky, J. (2000). Epistatic interaction between vernalization genes Vrn-Am1 and Vrn-Am2 in diploid wheat. *Journal of Heredity*, *91*(4), 304–306. https://doi.org/10.1093/jhered/91.4.304

Tweeten, L., & Thompson, S. (2009). Long-term global agricultural output supply-demand balance and real farm and food prices. *Farm Policy Journal*, *6*(1), 1–16.

Uauy, C., Brevis, J. C., & Dubcovsky, J. (2006). The high grain protein content gene Gpc-B1 accelerates senescence and has pleiotropic effects on protein content in wheat. *Journal of Experimental Botany*, *57*(11), 2785–2794. https://doi.org/10.1093/jxb/erl047

Ugarte, C., Calderini, D. F., & Slafer, G. A. (2007). Grain weight and grain number responsiveness to pre-anthesis temperature in wheat, barley and triticale. *Field Crops Research*, *100*(2–3), 240–248. https://doi.org/10.1016/j.fcr.2006.07.010

Vader, W., Kooy, Y., Van Veelen, P., De Ru, A., Harris, D., Benckhuijsen, W., … Koning, F. (2002). The Gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. *Gastroenterology*, *122*(7), 1729–1737. https://doi.org/10.1053/gast.2002.33606

van den Broeck, H. C., de Jong, H. C., Salentijn, E. M. J., Dekking, L., Bosch, D., Hamer, R. J., … Smulders, M. J. M. (2010). Presence of celiac disease epitopes in modern and old hexaploid wheat varieties: Wheat breeding may have contributed to increased prevalence of celiac disease. *Theoretical and Applied Genetics*, *121*(8), 1527–1539. https://doi.org/10.1007/s00122-010-1408-4

Van Sanford, D. A., & MacKown, C. T. (1986). Variation in nitrogen use efficiency among soft red winter wheat genotypes. *Theoretical and Applied Genetics*, *72*(2), 158–163. https://doi.org/10.1007/BF00266987

Villareal, R. L., del Toro, E., Mujeeb-Kazi, A., & Rajaram, S. (1995). The 1BL/1RS chromosome translocation effect on yield characteristics in a Triticum aestivum L. cross. *Plant Breeding*, *114*(6), 497–500. https://doi.org/10.1111/j.1439-0523.1995.tb00843.x

Vos, P. G., Paulo, M. J., Voorrips, R. E., Visser, R. G. F., van Eck, H. J., & van Eeuwijk, F. A. (2017). Evaluation of LD decay and various LD-decay estimators in simulated and SNP-array data of tetraploid potato. *Theoretical and Applied Genetics*, *130*(1), 123–135. https://doi.org/10.1007/s00122-016-2798-8

Wang, Z., Wu, X., Ren, Q., Chang, X., Li, R., & Jing, R. (2010). QTL mapping for developmental behavior of plant height in wheat (Triticum aestivum L.). *Euphytica*, *174*(3), 447–458. https://doi.org/10.1007/s10681-010-0166-3

Watanabe, N., & Ikebata, N. (2000). The effects of homoeologous group 3 chromosomes on grain colour dependent seed dormancy and brittle rachis in tetraploid wheat. *Euphytica*, *115*(3), 215–220. https://doi.org/10.1023/A:1004066416900

Watanabe, N., Sugiyama, K., Yamagishi, Y., & Sakata, Y. (2002). Comparative telosomic mapping of homoeologous genes for brittle rachis in tetraploid and hexaploid wheats. *Hereditas*, *137*(3), 180–185. https://doi.org/10.1034/j.1601-5223.2002.01609.x

Wen, Y. J., Zhang, H., Ni, Y. L., Huang, B., Zhang, J., Feng, J. Y., … Wu, R. (2018). Methodological implementation of mixed linear models in multi-locus genome-wide association studies. *Briefings in Bioinformatics*, *19*(4), 700–712. https://doi.org/10.1093/bib/bbw145

Wheeler, T. R., Hong, T. D., Ellis, R. H., Batts, G. R., Morison, J. I. L., & Hadley, P. (1996). The duration and rate of grain growth, and harvest index, of wheat (Triticum aestivum L.) in response to temperature and CO 2 . *Journal of Experimental Botany*, *47*(5), 623–630. https://doi.org/10.1093/jxb/47.5.623

White, J., & Edwards, J. (Eds.). (2007). *Wheat Growth and Development*. NSW Department of Primary Industries.

White, P. J. (2013). Improving potassium acquisition and utilisation by crop plants. *Journal of Plant Nutrition and Soil Science*, *176*(3), 305–316. https://doi.org/10.1002/jpln.201200121

Woodard, H. J., & Bly, A. (1998). Relationship of nitrogen management to winter wheat yield and grain protein in South Dakota. *Journal of Plant Nutrition*, *21*(2), 217–233. https://doi.org/10.1080/01904169809365397

Wu, J., Lakshminarayan, P. G., & Babcock, B. A. (1996). *Impacts of Agricultural Practices and Policies on Potential Nitrate Water Pollution in the Midwest and Northern Plains of the United States*. *148*, 1–36.

Wu, W., Shibasaki, R., Yang, P., Tan, G., Matsumura, K. ichiro, & Sugimoto, K. (2007). Global-scale modelling of future changes in sown areas of major crops. *Ecological Modelling*, *208*(2–4), 378–390. https://doi.org/10.1016/j.ecolmodel.2007.06.012

Würschum, T., Boeven, P. H. G., Langer, S. M., Longin, C. F. H., & Leiser, W. L. (2015). Multiply to conquer: Copy number variations at Ppd-B1 and Vrn-A1 facilitate global adaptation in wheat. *BMC Genetics*, *16*(1), 1–8. https://doi.org/10.1186/s12863-015-0258-0

Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., … Dubcovsky, J. (2006). The wheat and barley vernalization gene VRN3 is an orthologue of FT. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(51), 19581–19586. https://doi.org/10.1073/pnas.0607142103

Yan, Liuling, Loukoianov, A., Blechl, A., Tranquilli, G., Ramakrishna, W., SanMiguel, P., … Dubcovsky, J. (2004). The Wheat VRN2 Gene Is a Flowering Repressor Down-Regulated by Vernalization. *Science*, *303*(5664), 14. https://doi.org/10.1126/science.1094305

Yu, J., & Buckler, E. S. (2006a). Genetic association mapping and genome organization of maize. *Current Opinion in Biotechnology*, *17*(2), 155–160. https://doi.org/10.1016/j.copbio.2006.02.003

Yu, J., & Buckler, E. S. (2006b, April). Genetic association mapping and genome organization of maize. *Current Opinion in Biotechnology*, Vol. 17, pp. 155–160. https://doi.org/10.1016/j.copbio.2006.02.003

Yu, J., Pressoir, G., Briggs, W. H., Vroh Bi, I., Yamasaki, M., Doebley, J. F., … Buckler, E. S. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *NATURE GENETICS*, *38*(2). https://doi.org/10.1038/ng1702

Zentner, R. P., Tessier, S., Peru, M., Dyck, F. B., & Campbell, C. A. (1991). Economics of tillage systems for spring wheat production in southwestern Saskatchewan (Canada). *Soil and Tillage Research*, *21*(3–4), 225–242. https://doi.org/10.1016/0167-1987(91)90022-P

Zhang, F., Smith, D. L., & Mackenzie, A. F. (1993). Corn yield and shifts among corn quality consti-tuents following application of different nitro-gen fertilizer sources at several times during corn development. *Journal of Plant Nutrition*, *16*(7), 1317–1337. https://doi.org/10.1080/01904169309364615

Zhang, L., Zhao, Y. L., Gao, L. F., Zhao, G. Y., Zhou, R. H., Zhang, B. S., & Jia, J. Z. (2012). TaCKX6-D1, the ortholog of rice OsCKX2, is associated with grain weight in hexaploid wheat. *New Phytologist*, *195*(3), 574–584. https://doi.org/10.1111/j.1469-8137.2012.04194.x

Zhang, Z., Ersoz, E., Lai, C. Q., Todhunter, R. J., Tiwari, H. K., Gore, M. A., … Buckler, E. S. (2010). Mixed linear model approach adapted for genome-wide association studies. *Nature Genetics*, *42*(4), 355–360. https://doi.org/10.1038/ng.546

Zhao, D. Y., Zheng, S. S., Naeem, M. K., Niu, J. Q., Wang, N., Li, Z. J., … Ling, H. Q. (2018). Screening wheat genotypes for better performance on reduced phosphorus supply by comparing glasshouse experiments with field trials. *Plant and Soil*, *430*(1–2), 349–360. https://doi.org/10.1007/s11104-018-3739-x

Zhu, C., Gore, M., Buckler, E. S., & Yu, J. (2008). Status and Prospects of Association Mapping in Plants. *The Plant Genome Journal*, *1*(1), 5. https://doi.org/10.3835/plantgenome2008.02.0089

Zhu, J., Pearce, S., Burke, A., See, D. R., Skinner, D. Z., Dubcovsky, J., & Garland-Campbell, K. (2014). Copy number and haplotype variation at the VRN-A1 and central FR-A2 loci are associated with frost tolerance in hexaploid wheat. *Theoretical and Applied Genetics*, *127*(5), 1183–1197. https://doi.org/10.1007/s00122-014-2290-2

Zwart, R. S., Thompson, J. P., Milgate, A. W., Bansal, U. K., Williamson, P. M., Raman, H., & Bariana, H. S. (2010). QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. *Molecular Breeding*, *26*(1), 107–124. https://doi.org/10.1007/s11032-009-9381-9