INDIRECT RESPONSE TO YIELD SELECTION FOR END-USE QUALITY AND WHEAT-SENSITIVITY INDICATORS IN HARD WINTER WHEAT (*TRITICUM AESTIVUM* L.) ACROSS SIX BREEDING CYCLES AND THREE LINEAGES

By

LIZA M. VAN DER LAAN

Bachelor of Science in Plant and Soil Sciences

Oklahoma State University

Stillwater, Oklahoma

2017

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2019

INDIRECT RESPONSE TO YIELD SELECTION FOR END-USE QUALITY AND WHEAT-SENSITIVITY INDICATORS IN HARD WINTER WHEAT (*TRITICUM AESTIVUM* L.) ACROSS SIX BREEDING CYCLES AND THREE LINEAGES

Thesis Approved:

Dr. Brett Carver

Thesis Adviser

Dr. Robert Hunger

Dr. Carla Goad

ACKNOWLEDGEMENTS

At this time, I would like to thank my thesis advisor Dr. Brett Carver for all of his guidance and knowledge that got me through my graduate career thus far. I would also like to thank him for all of the advice given to me while I was still an undergraduate student working in his program. Thank you to my committee member Dr. Robert Hunger for his guidance, and to Dr. Carla Goad for all of the assistance during my statistical analysis.

I would also like to thank Nathan Stepp, Jason Ray, and Kurtis Cressman for all of the technical assistance in getting my trials planted and harvested each year, as well as being great people to work for and with for several years. Thank you to Connie Estes and Tina Johnson for all your quality analysis, and to Melanie Bayles for compiling the data. Thank you to Adrienne Blakey for generating the CORE data. Thanks also go to Dr. Guadalupe Davila El el Rassi for technical assistance and training for the FODMAP analysis. I would like to thank several fellow graduate students for their assistance and friendship: Josh, Anna, and Branden.

An additional thanks to Dr. Sergio Abit, my undergraduate advisor. Thank you for guiding my undergraduate career so well, and for always being so encouraging in my graduate career. Thank you to the Oklahoma Wheat Commission for the funding and scholarship, and to the OSU Plant and Soil Sciences department for funding. Finally, thank you to my parents, Pieter and Anita Van der Laan, for your support through all these years of school.

Name: LIZA M. VAN DER LAAN

Date of Degree: DECEMBER, 2019

Title of Study: INDIRECT RESPONSE TO YIELD SELECTION FOR END-USE QUALITY AND WHEAT-SENSITIVITY INDICATORS IN HARD WINTER WHEAT (*TRITICUM AESTIVUM* L.) ACROSS SIX BREEDING CYCLES AND THREE LINEAGES

Major Field: PLANT AND SOIL SCIENCES

Abstract:

A rising global population necessitates continued genetic improvement of wheat (*Triticum aestivum* L.), but not without oversight of intended and untended consequences along the way. This oversight is partly to blame for emergence of the bilateral "gluten crisis" spreading among users and consumers of wheat, albeit from different perspectives. The objectives were to re-establish trends of genetic progress in agronomic and milling traits using a generational yardstick as the timeline, and then use the same yardstick to measure direct and indirect responses in gluten and dough quality and in human wheat-sensitivity indicators. Randomized complete block design field experiments were conducted using 30 released and experimental cultivars spanning six breeding cycles and three distinct lineages descending from Turkey, the common ancestor for hard red winter wheat. Trials were grown in six site-years, and all reported results were derived from at minimum four site-years. Grain yield and kernel size showed a stepwise increase over cycles, with no evidence of a plateau. With the 77% total increase in grain yield, grain protein content decreased by one percentage point. Examination of protein-yield relationships indicated this protein loss was partly due to a dilution effect in higher-yielding environments. The reduced protein content, however, did not result in lower dough strength pertinent to bread baking applications. A novel method of directly testing gluten elasticity via the compression-recovery test indicated a general increase in gluten strength, though anomalies in certain cultivars were clearly evident. The ratio of total polymeric (glutenins) to total monomeric (gliadins) proteins remained stable across cycles. Also showing no change with genetic progress in yield was the level of a specific immunotoxic gluten fragment resident in α-gliadin 2, measured by the gluten G12 assay. The significant genetic variance found among contemporary cultivars signaled the possibility of further reduction without a yield penalty. The oligosaccharide, fructan, present in milled and wholemeal flours increased with increasing grain yield potential. Significant genetic variability was found among contemporary cultivars, giving breeders the necessary resource to support future fructan breeding goals. The gluten crisis appears unfounded, although the rise in fructans does implicate new dietary concerns.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Globally, common wheat (*Triticum aestivum* L.) is considered among the "big three" of cereal crops – wheat, rice, and maize - that provide the predominant sources of energy in the human diet. In many developing nations wheat constitutes the primary source of energy, plus protein, in the diet. Wheat is one of the most widely cultivated plants, extending from the Arctic Circle to the southern reaches of the Southern Hemisphere, and across large ranges of elevation (Gooding, 2009).

A rapidly growing world population is making yield gains more necessary than ever, making wheat improvement more relevant, with the added challenge of avoiding further pressure on an already strained natural resources and environment. With this issue in mind, wheat breeding programs must critically evaluate genetic gains accrued in economic traits, such as grain yield. Simultaneously, consumers are becoming more concerned than ever about the origin of and production practices attached to their food, and specifically, what is in it. This concern is manifested in an evolving consumer choice toward food products containing natural ingredients, and thus lacking oxidizing agents, dough conditioners, and other additives the baking industry would have used to compensate for inherent deficiencies in performance provided by the wheat alone.

This social movement has brought about a two-handed so-called "gluten crisis" that has gripped the entire wheat supply chain. On the one hand are consumers who hold the belief, right

or wrong, that "today's wheat is not our grandfather's wheat" (Lieberman and Segall, 2006) for various reasons, but most notably due to a perceived rise in the gluten content that is causing wheat to become less healthy to consume. On the other hand, parts of the U.S. baking industry have voiced concerns that the quality of gluten in today's bread wheat has declined, a belief only furthered by fewer options available with added ingredients (https://www.bakingbusiness.com/articles/46848-the-impending-gluten-crisis; https://www.world-grain.com/articles/11678-wands-calls-for-better-collaboration-in-wheat-industry-to-boost-quality).

Considering the latter crisis first, wheat breeders do consider end-use quality parameters along with yield and yield-formation traits such as pest resistance. Most often breeders use the mixograph, which requires less flour and time than the larger alveograph and farinograph more commonly used by the milling and baking industry. The mixograph provides multiple parameters to breeders, with the most commonly reported being the mixing tolerance score on a scale of 0 (poor tolerance) to 6 (very high tolerance). Though standard curves are used for reference, the tolerance score can be subjective depending on operator experience and bias. Fortunately, the mixograph produces two computer-generated parameters which can be used to quantify specific components of the mixing curve, although these parameters are reported less often. The first parameter, the mixograph bandwidth which is usually determined two minutes past peak dough development, has been found to have a high correlation with mixing time, tolerance, and loaf volume (Ohm and Chung, 1999; Chung et al., 2001). Generally, a higher bandwidth (typically greater than 10 to 12 mm, depending on the year of grain production) indicates a satisfactory level of mixing tolerance when flour protein levels are commensurate with the HRW class, or greater than 10% (Marburger et al., 2017). Mixing stability is the second computer-generated mixograph parameter that represents the angle of ascent and descent of the mixing curve. Opposite the bandwidth, a lower mixing stability score indicates a lower angle and thus greater

stability and tolerance, assuming flour protein content is sufficient for proper gluten development. Stability values less than 8 to 10 mm, again year-dependent, reflect a satisfactory mixing tolerance. These three parameters – tolerance score, bandwidth, and stability value – alongside an adjusted SDS sedimentation volume have formed the nucleus for quality-based advancement decisions in the OSU wheat improvement program since 1998.

The compression-recovery (CORE) test provides a direct indicator of gluten strength by measuring the degree of recovery from deformation in compressed gluten samples. Though still novel to the wheat breeding community and not yet widely used, Chapman et al. (2012) found a strong correlation of gluten elasticity with gluten strength, and concluded that the CORE test could be a viable option for measuring gluten strength. As previously mentioned, wheat breeders commonly use the mixograph parameters as a test for dough strength. The mixograph itself is a convenient test, but it does not directly measure gluten strength. The CORE analyzer is relatively quick, does not require large amounts of flour, and directly measures gluten strength by measuring gluten elasticity. This makes it a good option for breeders to incorporate into their own breeding programs, thus lending credibility of the CORE recovery index to characterize an historic wheat panel.

The second side of the gluten crisis is the perceived notion that wheat, specifically gluten, is less healthy to consume compared to wheat from a century ago. Part of this concern is fueled by the rise in celiac disease (Lohi et al., 2007; Catassi et al., 2010), yet without indisputable reasons to account for this rise. Scenarios suggested for the rise in celiac disease prevalence include environmental exposure (Lohi et al., 2007), changing clinical presentation (de Lorgeril and Salen, 2014), and better diagnostic techniques (van den Broeck et al., 2010). Wheat gluten is composed of two main fractions: the larger glutenins and the smaller gliadins. Van de Kamer et al. (1953) was the first to report that the gliadin fragment specifically elicited the negative reaction in celiac patients. In particular, the 33-mer fragment, which is predominantly found in α-

gliadin 2 peptides, causes the immunogenic reaction. Schalk et al. (2017) evaluated modern and heirloom wheat cultivars for the concerning 33-mer content. No significant trend between modern and heirloom 33-mer content could be determined.

What if gluten is not the actual causal agent for the perceived less healthy modern wheat, especially in cases of non-celiac gluten sensitivity (NCGS)? Skodje et al. (2018) found that fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) may actually be to blame for causing irritable bowel syndrome (IBS) symptoms often mistaken for NCGS. Wheat contains an oligosaccharide called fructan, which occurs in various forms of carbohydrate polymers that are almost exclusively composed of β-linked fructose (Veenstra et al., 2017). Fructans serve as a carbohydrate reserve in plants metabolic functions, and facilitate osmotic control during times of limited water availability (Hendry, 1993), and serve as osmoregulators during cold and salt stress (Pilon-Smits et al., 1995). Fructans actually have positive health effects because humans lack the enzymes to break down fructans; therefore, they are not digested in the small intestine, and instead move to the colon to selectively stimulate the growth of beneficial bifidobacteria (Gibson et al., 1995). On the other hand, poor absorption of FODMAPs allows entrance to the colon where they may cause osmotic stress (Ziegler et al., 2016). The rapid fermentation by microbes in the colon can sometimes cause the buildup of substantial amounts of gas (Gibson and Shepherd, 2005). Both the poor absorption with osmotic stress and the rapid fermentation of FODMAPs can induce luminal distension, which can result in gastrointestinal symptoms (Biesiekierski and Iven, 2015). With the rising evidence that FODMAPs, and fructans, may be causing some of the symptoms normally linked to NCGS, it is important to determine how fructan contents have changed from heirloom bread wheat cultivars to the modern-day cultivars grown and consumed today.

This study was designed to investigate multiple claims connected to the gluten crisis by quantifying historical genetic changes for numerous end-use quality parameters, α-gliadin 2

content, and fructan content. In particular, we set out to quantify the genetic changes using an historical panel of wheat organized by known parent-offspring relationships into distinct breeding cycles. This was coupled with an examination of genetic changes in traits receiving greatest selection pressure in wheat breeding programs, such as grain yield, yield components, and agronomic performance traits.

CHAPTER II

REVIEW OF LITERATURE

A growing world population has made it more important than ever to increase crop yields through both genetic improvement and improvement of agronomic management practices. Historically, genetic improvements made through breeding have been responsible for approximately half of the yield increases over the past century (Rudd, 2009). This shows the importance breeding has had so far, and demonstrates how plant breeding is more necessary than ever to meet rising demands with any assurance. In the Great Plains of the USA, one of the primary goals of wheat breeding programs has been to increase yield. Previous studies have evaluated the genetic yield gains obtained through years of breeding. The baseline study of genetic improvement in Great Plains HRW wheat showed a yield increase of 1% of the average yield of the cultivar Turkey per year up to 1987 (Cox et al. 1988). Graybosch and Peterson (2010) studied the genetic improvement of winter wheat yields in the Great Plains from 1959-2008. In the Southern Region Performance Nursery (SRPN), they found a yield gain of 1.1% relative to Kharkof yield per year. Additionally, they found no significant genetic yield progress when only considering cultivars released after 1984, indicating a yield plateau. However, Battenfield et al. (2013) also investigated genetic yield improvement for the southern Great Plains and found a yield increase of 0.9% of Kharkof yield per year, with no evidence of a yield plateau. Such discontinuity among genetic gain estimates in HRW wheat may be expected when the genotypic sample is populated with released cultivars differing widely in fitness to the environments which they were subjected.

Other studies conducted on genetic improvement of spring wheat ranging from 30 years, 50 years, and 100 years found no evidence of a yield plateau (Lopes, et al., 2012; Guzman et al., 2017; Hucl et al., 2015). The Battenfield et al. (2013) study additionally found that annual genetic yield gains decreased significantly (0.4% of TAM W-101 yield per year) when exclusively evaluating semidwarf cultivars. Hucl et al. (2015) also concluded that shorter plant heights resulted in stronger plants, thus leading to increased grain yields. Studies like this only perpetuate the Graybosch and Peterson (2010) claim that since the introduction of semi-dwarfing gene, there has been "no additional great leap forward." However, immediately subsequent to their report, U.S. wheat breeding programs received an unprecedented infusion of breeding support tools. This included a proliferation of robust molecular markers (Toth et al., 2019) further aided by a draft sequence of the wheat genome (Appels et al., 2018) and the adoption of doubled haploidy to reduce breeding cycle time (El-Hennawy et al., 2011).

Along with increasing crop yield to meet increasing global demand, wheat breeders must ensure that functionality of their released product in the wheat processing industry does not decline, even in the absence of quality standards that would hold breeding programs accountable. Cox et al. (1989) evaluated 40 HRW wheat cultivars released from 1919 to 1988 and found that flour yield showed no significant variation between heirloom and modern wheat, and the variation was highly dependent on environment. This was in agreement with Fufa et al. (2005), who evaluated 30 HRW wheat cultivars from 1874 to 2000, and Khalil et al. (2002), who evaluated 12 HRW wheat cultivars and found no trend in in flour yield over time.

Three studies also looked at trends in kernel weight over time, a key characteristic to uniformity of a grind during the initial stages of wheat processing. Khalil et al. (2002) found an increase in kernel weight of 0.05-0.06 g yr^{-1}. This agrees with the finding of Cox et al. (1988) and Fufa et al. (2005) in finding an increase in kernel weight. While weight of the kernel has

increased, no significant changes were yet to be found in the size, or diameter, of the kernel (Khalil et al., 2002).

Another important characteristic for end-use quality is the amount of protein found in the grain. Protein content generally indicates the amount of gluten present in the grain and flour, which in turn plays a crucial role in the baking process for a multitude of food products. Many studies have examined how grain and flour protein content changed through the modern breeding era. Cox et al. (1989) observed no significant change in flour protein concentration in HRW wheat germplasm, but the protein range was larger for the more modern wheat genotypes. This agrees with Khalil et al. (2002) who concluded that grain protein had not changed over 100 years of breeding. Both of these studies are in contrast with studies of winter wheat in Nebraska and Germany that observed decreases in overall wheat protein (Fufa et al., 2005; Laidig et al., 2017). Additionally, a study of spring bread wheat also showed a decrease in protein (Guzmám et al., 2017). Most importantly, the pervasive trend has been for protein concentration to decline in modern wheat genotypes, even for classes in wheat in which higher protein concentration is more desirable.

The Gluten Crisis

The term "gluten" has come to evoke a plethora of emotions among consumers, thanks to a proliferation since 2011 of its largely fear-driven misrepresentation in social media and popular literature. Even among wheat processors, reactions run at no less altitude to those of everyday consumers when gluten properties available to the food industry today are debated (https://www.bakingbusiness.com/articles/46848-the-impending-gluten-crisis; https://www.world-grain.com/articles/11678-wands-calls-for-better-collaboration-in-wheat-industry-to-boost-quality). Hence there appears to be a two-sided gluten "crisis." The first side to

the gluten crisis is the concern that modern dough and gluten are losing strength, thus forcing bakers to employ numerous additives to achieve the same product as in the past. This is costly to bakers, and also frowned upon by consumers as they become more concerned with "clean eating". Dough, consisting primarily of wheat flour and water, has the ability to trap gases during proofing and baking, which has been linked to the unique viscoelastic properties of gluten. Gluten can be broken down into two fractions: the alcohol soluble monomeric gliadin and the alcohol-insoluble polymeric glutenin (Wieser, 2007). Gliadin is more responsible for the extensibility and cohesive properties of gluten, while glutenins are more responsible for dough strength and elasticity (Wieser, 2007). The ratio in which the total polymeric proteins (TPP) to total monomeric proteins (TMP) are present can affect the rheological properties of dough, specifically the strength characteristics. A higher proportion of glutenins to gliadins generally results in stronger dough. A study between old and modern durum genotypes found that the modern genotypes had the lowest gliadin content and highest glutenin content, while the older genotypes had the opposite behavior (De Santis et al., 2018), indicating an increase in durum dough strength. This research is not representative of changes in hexaploid bread wheat though, which should be investigated.

While an increasing ratio of glutenin (TPP) to gliadin (TMP) is beneficial in terms of dough strength, a higher ratio is not unequivocally favorable for millers and bakers. Dhaka and Khatkar (2015) found that flours with a greater glutenin content were stronger, but required more mixing time. An increase in dough mixing time can add significantly more energy costs and chip away at profit margins already considered thin for the bread industry. An increase in gliadins is not desirable either, as that creates a more viscous and sticky dough (Dhaka and Khatkar, 2015); therefore, a more stable and balanced ratio of glutenin to gliadins is desired.

In addition to consideration of gluten fraction proportions, there are other tests used today to measure dough and gluten strength. One such test is the sodium dodecyl sulphate (SDS)-

sedimentation test. The SDS-sedimentation test is considered a simple and reliable method for predicting quality indices (Takata et al., 2001). The test gives an indication of the physicochemical behavior of flour and protein aggregative ability (Graybosch et al., 1996), by measuring the volume of sediment formed when a flour-water-detergent suspension is shaken. The greater the volume, the better the baking quality, and thus the strength. Hucl et al. (2015) evaluated 36 red spring wheat cultivars from the 1860s to 2001 and found the SDS sedimentation volume to increase at a rate of 0.2 mL per year. This agrees with the findings of Fufa et al. (2005), who found an increase in SDS sedimentation values in 30 HRW wheat cultivars released from 1874 to 2000. Fufa et al. (2005) also conducted a mixograph test for dough strength. They found an increase in mixing time and in mixing tolerance, indicating a more stable and stronger dough. Cox et al. (1989) found an increase in mixing time, partly indicating an increase in dough strength.

A draw-back of the mixograph tolerance score is that it is subjective in nature. A trained operator scores tolerance on a scale of 0 (poor) to 6 (very high), based on visualization of the mixograph curve properties relative to a set of standard curves. On the other hand, the mixograph test does provide some computer-generated parameters to estimate mixing tolerance and dough strength without the subjectivity, although these parameters are not as often reported. One of these parameters, the mixograph bandwidth at two minutes past peak dough development, has been found to have a high correlation with mixing time, tolerance, and loaf volume (Ohm and Chung, 1999; Chung et al., 2001). This means that bandwidth can be used to estimate multiple baking properties without human subjectivity. Generally, a higher bandwidth (typically greater than 10 mm, although the absolute value may fluctuate among wheat growing environments) indicates a greater tolerance (Marburger et al., 2017). Mixograph stability is another computer-generated parameter that can also be used to estimate dough tolerance. Unlike the subjective tolerance score, a lower mixograph stability score results from a flatter angle of ascent and

descent of the mixograph curve, which in turn indicates greater dough stability and tolerance assuming the level of flour protein is suitable for proper mixing.

The second side of the gluten crisis is the belief that today's wheat, specifically the gluten, is becoming less healthy to consume. This had led to a rise in various forms of the gluten-free diet, which gained popularity due in part to the controversial book *Wheat Belly* by cardiologist Dr. William Davis. In his book, Davis claims that wheat is now a "Frankengrain" created by genetic research and agribusiness, and blames wheat for everything from weight gain to autism and cancer. This does raise the question if modern wheat is harmful to human health in ways never considered. One component of this is the increasing frequency at which individuals are being diagnosed with celiac disease. Lohi et al., (2007) studied two samples of Finnish adults in 1978 and again in 2000. They found that the total prevalence of celiac disease has almost doubled over the two decades from 1.05% to 1.99%. The study did not conclude what was causing this rise in celiac disease incidence, although it did suggest possibilities such as increased gluten intake after infancy, as well as non-wheat related environmental causes.

Another study conducted from 1974 to 2007 found that the prevalence of celiac disease among US adults increased 5-fold over a 30-year period, or doubled every 15 years (Catassi et al., 2010). This study noted a steady rise in several autoimmune diseases in industrialized nations over the last few decades. They also concluded that the onset of celiac disease seemed to be in adulthood (Catassi et al., 2010), with no set factor being the cause of this rise. According to gastroenterologists, a possible cause for this rise in incidence is that the clinical presentation of celiac disease has changed from an intestinal disease in childhood to a disease primarily affecting adults, often without intestinal symptoms as the primary complaint (de Lorgeril and Salen, 2014). Additionally, the increase could be in part to better diagnostic techniques for the autoimmune disease (van den Broeck et al., 2010).

The apparent increased incidence of celiac disease in the U.S., along with growing concerns of gluten sensitivity, and claims that high carbohydrate intake is responsible for obesity in the USA, simply means that the changes in gluten content might be explored more broadly as a consequence of breeding advances in wheat yield. One conventional way to observe how gluten content has changed is to look at how protein content has changed. As mentioned earlier, multiple studies have found either no change in protein content, or a decrease in protein. This does not mean that the components of gluten have not changed.

A study by van de Kamer et al. (1953) was the first to report that gluten and specifically the gliadin fraction were the harmful agents in wheat flour for celiac patients. Gliadin peptides were eventually found to exert damaging effects because of their resistance to gastrointestinal enzymes (Gujral et al., 2012). The van de Kamer et al. (1953) study did not find any evidence of the glutenin fraction being detrimental to celiac patients, so the belief of it being harmless has persisted. However, our understanding of these proteins has increased, and updated studies of glutenin show that the previously held belief of its neutral effect on celiac patients may be mistaken (Howdle, 2006). As knowledge of the chemistry of these proteins has increased, features which separate the gliadin and glutenin fractions have become less distinct. With this increasing knowledge, it is important to investigate how the glutenin and gliadin fractions have changed over decades of breeding wheat, and more specifically, if certain peptides with a known linkage to digestive discomfort have changed disproportionately. The peptide in question is the α-gliadin 33-mer peptide, which is the main immunodominant toxic peptide for celiac patients (Ozuna et al., 2015). Only type 1 α-gliadins, and in particular the α-gliadin 2 form, appear to contain the 33-mer peptide, which led Ozuna et al. (2015) to conclude that it is a result of allohexaploidization events. Schalk et al. (2017) evaluated 23 modern and 15 heirloom wheat cultivars for 33-mer content. No significant trend between modern and heirloom 33-mer content could be determined. However, this lack of trend could be because of the non-uniform flour

sources used in the study. Another study investigating multiple gliadin peptides in older and modern cultivars across different wheat species found that the older cultivars had a higher gliadin peptide content (Prandi et al., 2017). Additionally, they found from all the different species, only spelt had a lower mean content (Prandi et al., 2017). This goes against the general public perception of older cereals being healthier and more nutritional.

Fructans

An increasing body of evidence indicates gluten may not necessarily be the causal agent behind wheat sensitivity, especially in cases of non-celiac gluten sensitivity (NCGS) in which symptoms may appear similar to celiac, but without intestinal damage. A recent study from Norway indicates that fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) may be to blame for many irritable bowel syndrome (IBS) symptoms often mistaken for NCGS (Skodje et al., 2018). In wheat, the principle FODMAP in question is an oligosaccharide called fructan which exists as variable-sized polymers almost exclusively composed of β-linked fructose (Veenstra et al., 2017). Fructans provide an important carbohydrate reserve supporting many plant metabolic functions. The exact reason why fructans serve the plant in this capacity is not well understood, but Hendry (1993) theorized that fructans may facilitate an osmotic control mechanism used in time of limited water availability. Additionally, fructans serve as osmoregulators under cold and salt stress (Pilon-Smits et al., 1995). This led to Veenstra et al. (2017) to hypothesize that fructans could be a potential breeding target to increase grain yields under less optimal growing conditions.

Fructans are classified based on their fructose linkages and core molecules (Verspreet et al., 2015). One type of fructan is the inulin type, which is derived most often from chicory roots (Verspreet et al., 2015). In inulin-type fructans, the fructosyl residues are linked by β-2,1-

linkages. Inulin has a wide array of applications, such as a fat or sugar replacement and dietary fiber in the food industry, as well as a stabilizer and excipient in pharmaceuticals (Barclay et al., 2010). Health effects of inulin type fructans are well defined, and it is generally recognized as a prebiotic and possessing anti-oxidant activity (Verspreet et al., 2015). However, wheat possesses the less studied graminan-type fructan, which possesses both β-2,1-linkages and β-2,6-linkages. Wheat has lower fructan content than other foods, such as garlic, artichokes, and onions (Van Loo et al., 1995). Among the cereal grains, rye contains the highest levels of fructans (Verspreet et al., 2015). Nonetheless, the wide abundance of wheat products accounts for approximately 70% of the daily fructan intake in American and Western European diets (Moshfegh et al., 1999). The health effects of the graminan-type fructan are not as well studied as the inulin type, although it is generally accepted that this type of fructan does induce prebiotic effects like that of the inulin type. These positive health effects exist because humans lack the enzymes to break down fructans; therefore, they are not digested in the small intestine and instead move to the colon to selectively stimulate the growth of beneficial bifidobacteria (Gibson et al., 1995).

Nonetheless, there is also the growing potential of some negative health effects in certain individuals with IBS. Using a double-blind placebo-controlled crossover study on individuals with self-reported NCGS, Skodje et al. (2018) found that fructans were more likely to induce IBS symptoms than gluten and that gluten alone induced no greater symptoms than the placebo. Biesiekierski et al. (2013) also conducted a study of individuals who had self-reported NCGS IBS but not celiac disease. They found that patient symptom response showed no gluten specificity (Biesiekierski et al., 2013). Many patients still displayed significant symptoms when on a gluten-free diet, but did have improvement of gastrointestinal symptoms when a low FODMAP diet was followed for two weeks (Biesiekierski et al., 2013). Because FODMAPs are often poorly absorbed in the small intestine, they enter the colon and can cause osmotic stress by drawing water into the gut lumen (Ziegler et al., 2016). Additionally, FODMAPs can be rapidly fermented

by microbes in the colon, which leads to a release of more osmotically active metabolites and substantial amounts of gas (Gibson and Shepherd, 2005). Both the poor absorption with osmotic stress and the rapid fermentation of FODMAPs can induce luminal distension, which can result in gastrointestinal symptoms (Biesiekierski and Iven, 2015). With the rising evidence that FODMAPs, and fructans, may be causing some of the symptoms normally linked to NCGS, it is important to determine how fructan contents have changed from heirloom bread wheat cultivars to the modern-day cultivars grown and consumed today.

Objectives

Numerous studies have evaluated genetic progress in wheat by selecting representative cultivars released over time and expressing progress as a function of time (years). This study aims to refine estimates of genetic change via parent-offspring relationships known to exist in consecutive breeding generations or cycles of wheat. Additionally, few studies have researched topics pertaining to wheat and human dietary issues under the same scope. With this study, I will first establish the rate of genetic gain for productivity traits, including grain yield, as a function of breeding cycle, and then explore correlated changes across a vast array of traits of direct interest to processors and consumers. Thus, in addition to protein content and protein quality, I report on the content of a specific gliadin fragment known to elicit negative responses in celiac patients, and determine how the FODMAP concentration has changed via fructan content. The overarching objective of this study is to identify and quantify characteristics of HRW wheat that have changed in response to over a century of yield improvement efforts in the southern Great Plains and recommend where future breeding priorities may be directed or re-directed.

CHAPTER III

METHODOLOGY

Field Evaluation

Thirty hard red winter wheat cultivars or experimental lines were evaluated in this study, beginning in 2014 and extending to 2018. The 2013-2014 season provided a preliminary investigation and seed increase, with only one replication at one location. That season, and through the 2015-2016 season, contained only 28 cultivars. Two additional HRW entries (Lonerider and OK12D22002-077) were added in 2016. The cultivars were selected because of their known parent-offspring relationships, along three primary lineages from the common heirloom landrace cultivar Turkey (Figure 1). Six of the selected cultivars were not publicly released, but are experimental lines from Oklahoma State University (OSU). All but one entry belonged to or were intended to be marketed in HRW class; the other entry, OK11311F, was classified as soft red winter, but with end-use characteristics aligned with the HRW class.

Based on pedigree information, the 30 cultivars used in this study were grouped for statistical analysis by their breeding cycle, or generations removed from Turkey, instead of the conventional year or decade of release. This was intentional because year of release does not always accurately reflect the age of a cultivar, as different cultivars take varying amounts of time to develop, from 8 to 16 years, depending on the number of years consumed prior to development of the inbred line and the number of years consumed to evaluate and purify it. As an extreme

example, a cultivar with a more recent year of release could have progressed through an earlier breeding cycle than a cultivar released in an earlier year. By taking into account breeding cycle, some of this temporal variability is equalized when estimating genetic gains as a function of time.

The field trials were established in a thermic Udic Paleustolls soil (Norge loam) at the Oklahoma Agricultural Experiment Station (OAES) Agronomy Research Station in Stillwater, Oklahoma (36°8'4.54"N, 97°6'12.83"W). and in a thermic Udic Argiustolls soil (Grant silt loam) at the OAES North Central Research Station near Lahoma, Oklahoma (36°23'20.80"N, 98°6'28.21"W). Sites were replanted with the same 30 cultivars for each growing season using nontreated seed produced in a prior year after light roguing to maintain purity. Each site-year was conducted as a randomized complete block design with two replications. Individual plot size was 0.91 m by 3.05 m, with five rows spaced of 0.23 m apart.

The plot area was pre-plant fertilized according to soil-test recommendations for a grain yield goal of 4000 kg ha^{-1}. All trials were planted in October at a rate of 58 kg ha^{-1}. Foliar fungicide applications were used stringently to minimize yield bias caused by greater disease susceptibility of the older cultivars. The fungicides used were Twinline (metconazole + pyraclostrobin) in April 2016 at a rate of 7 oz ac^{-1} and 9 oz ac^{-1} at Stillwater and Lahoma, respectively. Prosaro (prothioconazole + tebuconazole) was applied in April of 2017 at a rate of 8.2 oz ac^{-1} for both locations. Approach Prima (picoxystrobin + cyproconazole) was applied in April of 2018 at a rate of 6.8 oz ac^{-1} for both locations. Fungicide application was not used to control viral diseases, such as barley yellow dwarf virus, or to control aphids.

Agronomic Traits

Days to heading (DH) was recorded as the day of year at which 50% or more of the spikes in a plot had emerged completely from the boot (Feekes Stage 10.3). Plant height was

measured when all plots had reached physiological maturity (Feekes Stage 11.3). A meter stick was used to measure the distance between the base of the stem to the top of the spike, excluding awns. Two measurements were taken per plot and averaged for a final plot height.

Trials were harvested in June using a Wintersteiger Classic Plot Combine (WINTERSTEIGER AG, Innkreis, Austria). Immediately upon harvesting, grain yield was measured as the field weight of threshed grain. Cleaned samples were then run through a Dickey-John GAC500XT (DICKEY-John, Auburn, IL) grain moisture tester to obtain test weight data.

Evaluation of Milling and Kernel Characteristics

Clean grain samples, with no detectable preharvest moisture damage or postharvest insect damage, were sent to the OSU Wheat Quality Laboratory. Testing was done using approved methods and standards set by the American Association of Cereal Chemists (AACC, 2010). Wheat protein and kernel hardness were measured using near infrared reflectance spectroscopy. Kernel weight, kernel diameter, and hardness index were measured using the Perten Single Kernel Characterization System 4100 (Perten Instruments, Segeltorp, Swedem). Flour yield was evaluated using a Brabender Quadrumat Senior mill (C.W. Brabender, South Hackensack, NJ).

Evaluation of Dough and Gluten Strength Characteristics

Physical dough tests were conducted with a computer-assisted mixograph using a 10-gram bowl (method 54.40.02, AACC, 2000). Mixing tolerance measured the resistance of dough to breakdown due to continuous mixing. It was rated subjectively on a scale of 0 (very poor tolerance) to 6 (exceptional tolerance) based on comparison with standard mixograph curves. The subjective mixing tolerance scores generated in 2018 were not included in the analysis due inexperience of a different operator. The time to reach peak mixing time, when the dough reaches

maximum consistency, was measured in minutes. Mixograph bandwidth was determined as the width of the mixogram curve at 2 minutes past peak development, and the stability value was calculated from the sum of absolute values of the slopes of the ascending and descending portions of the mixogram curve. Stronger gluten flour generally has a higher peak time, greater bandwidth, and lower mixograph stability value.

The sodium dodecyl sulfate (SDS) sedimentation test was conducted to evaluate gluten swelling properties (Lorenzo and Kronstad, 1987) of two 4.3-gram flour subsamples from each plot sample. Results were reported as the specific sedimentation volume, which is the ratio of actual sedimentation volume to protein percentage (as is) in the flour.

Compression-recovery (CORE) tests were conducted to study the elastic recovery of gluten after compression, another indicator of gluten strength. Tests were performed using the same flour samples subjected to the mixograph, following the procedure described by Chapman et al. (2012). Triplicate samples of each individual flour sample were tested. Briefly, 10 grams flour were weighed and poured into a wash chamber, where 4.8 mL of a 20% NaCl aqueous solution was added. The wash chamber was loaded into a Glutomatic 2202 gluten washer (Perten Instruments AB, Huddinge Sweden), and the flour and water were mixed for 30 seconds. Following mixing, more of the 20% NaCl solution was poured into the wash chamber while the dough was continuously being mixed. This allowed the starch to be washed and drained off, thus isolating the gluten fraction. The gluten fractions were then centrifuged into a uniform cylindrical sample using a Perten Centrifuge 2015 (Perten Instruments, AB, Huddinge, Sweden) at 6000 rpm for five minutes. Shaped gluten samples were allowed to rest for one to two minutes before being loaded into the Gluten CORE Analyzer (Perten Instruments, AB, Huddinge, Sweden), then compressed for 5 seconds with a force of 8N, followed by a 55-second recovery period. The recovery index was calculated according to the equation:

$$Elastic\ Recovery\ (\%) = \frac{Height\ at\ Final\ Recovery}{Initial\ Height\ Before\ Compression}\ x\ 100$$

A further test of gluten strength was the ratio of Total Polymeric Protein (TPP) to Total Monomeric Protein (TMP), measured at the USDA-ARS Grain Quality and Structure Research Laboratory in Manhattan, KS. The quantity and polymeric composition was determined using the protein extraction procedures described by Gupta et al. (1993). A 20-mg flour sample was suspended in 1 ml of 50 mM sodium phosphate buffer (pH 6.9) containing 0.5% SDS, then sonicated for 15 seconds. The supernatant was collected and filtered using a 0.45 μm nylon centrifuge tube filter. The extract was analyzed using size exclusion chromatography with an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA) equipped with a 300 x 7.8 mm Biosep-SEC-s4000 column (Phenomenex, Torrance, CA). The chromatograms were manually integrated into two peaks, with the area of the first peak corresponding to polymeric proteins and the area of the second peak corresponding to monomeric proteins.

Sensitivity Tests

Additional flour samples were analyzed at the USDA-ARS Grain Quality and Structure Research Laboratory in Manhattan, KS to test for α-gliadin 2 content. Five grams flour were subjected to the AgraQuant Gluten G12 ELISA test (Romer Labs, Inc., Union, MO). The G12 test kit is a sandwich ELISA, and follows the protocol set forth in the AACCI 38-52.01 method. This protocol was outlined in Halbmayr-Jech et al. (2012), with a few deviations. Samples were extracted using 0.25 g homogenized flour and 2.5 mL 80% ethanol, shaking for five minutes. Extracts were incubated for 40 minutes at 50°C, then cooled to room temperature, after which 7.5

mL 80% ethanol was added and the samples were vortexed. Samples were then shaken for 60 minutes, and centrifuged for 10 minutes at 2000 x g. The aqueous supernatant was collected and diluted 1:10 with a pre-diluted sample dilution buffer, then mixed.

100 μL of each prepared sample was pipetted into a provided antibody coated microwell and allowed to incubate for 20 minutes. Microwells were then washed five times with a diluted wash buffer, then 100 μL G12-HRP conjugate was added to each well and incubated for 20 minutes. Microwells were washed five times again, then 100 μL TMB substrate was added and incubated in the dark for 20 minutes. After the final incubation, 100 μL of stop solution (0.5 M sulfuric acid) was added to each well. Strips were read with a microwell reader using a 450 nm filter.

Starting with the 2016-2017 field experiments, total fructan percentage was determined in milled flour and in ground whole-wheat flour. A 15-g sample of whole wheat kernels were milled using an UDY Cyclone Lab Sample Mill (UDY Corporation, Fort Collins, CO). Remnant flour samples previously milled for the functionality tests previously described were used for the milled flour samples. Both milled and Udy-ground whole-wheat flour samples were analyzed for fructan content at the Food and Agricultural Products (FAPC) Analytical Services Lab in Stillwater, OK. Fructan analysis was conducted using a Megazyme Fructan Assay Kit (Megazyme, Wicklow, Republic or Ireland), following procedure K-FRUC, which follows the protocol set forth in the AACCI Method 32.32. Briefly, 1.0 g flour samples were weighed into individual beakers, and 80 mL hot distilled water was added. Beakers were placed on a hot-plate magnetic stirrer for 15 minutes until the flour was dispersed throughout. The solution was allowed to cool to room temperature, and then 0.2 mL was transferred into a glass test tube. Next, 0.2 mL of a sucrase/amylase solution was added to the test tube, and the test tubes were incubated for 30 minutes at 40 °C. Next, 0.2 mL of an alkaline borohydride solution was added, and then incubated for another 30 minutes at 40 °C. Following this, 0.5 mL 200mM acetic acid was added

to the tube and stirred vigorously on a vortex machine. This was done to remove sucrose, starch, and other reducing sugars prior to the fructan analysis.

To measure the fructan content, 0.2 mL aliquots of the solution were transferred to three test tubes. One of these tubes served as a blank and had 0.1 mL of a 0.1 mM sodium acetate buffer added, and the other two had 0.1 mL of a fructanase solution added. These tubes were incubated for 30 minutes at 40 °C. Following incubation, 5.0 mL of a working reagent, provided in the kit, was added to all tubes, and incubated in a boiling water bath for six minutes. After boiling, test tubes were immediately placed in cold water for five minutes. Finally, the absorbance of each solution was measured at 410 nm against the reagent blank. Absorbance values were inputted into the Megazyme Mega-Calc™ spreadsheet to calculate the fructan content.

Statistical Analysis

Statistical analysis was conducted in SAS v9.4 (SAS Institute, Cary, NC). All traits were analyzed using linear mixed models methods. The use of other models, such as curvilinear were not explored due to the limited number of cultivars available in the earlier breeding cycles. The GLIMMIX procedure was used to account for the random effects of site-years (environments). Genetic gains or losses for all traits were estimated by linear regression of the trait of interest on breeding cycle (generations removed from Turkey). The general chi-square (GCS) score was checked to ensure there was no data overdispersion. Traits with GCS scores greater than one were subjected to square root or logarithmic transformations to reduce data overdispersion. We further analyzed the relationship between grain yield and grain protein by first fitting a linear regression using GLIMMIX. The residuals generated from this regression were then further analyzed against

breeding cycle. Residuals were treated as a trait to test for protein sensitivity to, or a metabolic cost associated with, higher grain yields.

Restricted maximum likelihood estimates (REML) of the variance components for the random effects of the environment, genotype, and genotype by environment interactions were obtained. For the α-gliadin 2 and fructan traits, an additional variance component estimation was done to partition the effects into the heirloom genotypes (breeding cycles 0-3) and the modern genotypes (breeding cycles 4-6). The primary purpose was to test for significant genetic variability in the modern era. MANOVA methods were used to estimate the covariances between particular traits, from which Pearson correlation coefficients were calculated. All tests of significance were conducted at the nominal 0.05 level.

CHAPTER IV

FINDINGS

Grain Yield and Agronomic Traits

While the central motivation behind this research was to document certain rheological and biochemical changes that might have occurred indirectly across multiple iterations of yield-focused selection, it is important to first establish any agronomic shifts as a framework for interpreting the indirect selection responses. After all, zero or miniscule changes in the primary trait (yield) would call for a different interpretation of any shift in a secondary trait such as flour sedimentation volume or flour fructan content.

Surveying the estimated variance across all lineages and breeding cycles, the genotypic effect was significant for grain yield, as well as test weight, days to heading, and plant height, (Table 1). The genotype x environment (GE) effect was highly significant and similar in magnitude among these four agronomic traits (Table 1), underscoring the importance of estimating selection response across a natural range of environmental conditions to arrive at a robust gauge of genetic change. Genotypic effects were not partitioned among the three genetic lineages considering the number of entries comprising a given lineage. Instead, the rationale was to utilize the three lineages in the analysis as they were intended in the experimental design: as an integral, ordered form of genetic replication to strengthen response estimates across multiple and consecutive parent-offspring relationships that are challenging to visualize in the vast majority of

genetic-gain experiments predicated on year of cultivar release.

The selection response for grain yield was determined by linear regression on breeding cycle (bc). The natural logarithmic transformation of the response was necessary to correct for overdispersion in the data. The slope of the regression indicated a significant increase, or genetic gain, in logarithmic yield per breeding cycle, or 0.096 kg ha^{-1} bc^{-1} (Figure 2A). In original units, the cultivar Bentley (bc 6) had the highest average grain yield, 4393 ± 342 kg ha^{-1} across all environments. Kharkof (bc 0) had the lowest average grain yield, 2197 ± 177 kg ha^{-1}. Because the response, grain yield, was transformed, the regression coefficients are virtually uninterpretable (Pek et al., 2017). Therefore, reporting the gains in grain yield per breeding cycle (a regression coefficient) relative to the baseline heirloom cultivar Turkey would be inappropriate, because regression coefficients cannot be inverse transformed (Pek et al., 2017). Only the response value for each breeding cycle can be inverse transformed. Thus grain yield increased about 77% from cycle 0 to 6, a timespan corresponding to wheat improvement in the southern Plains of the USA from the 1920s to current day. The tight-fitting linear selection response indicates genetic improvement in HRW wheat yield has occurred with no apparent genetic plateau looming. Previous studies by Cox et al. (1988), Donmez et al. (2001), Khalil et al. (2002), Fufa et al. (2005), Graybosch and Peterson (2010), Lopes et al. (2012), Battenfield et al. (2013), Hucl et al. (2015), and Laidig et al. (2017) reported an increase in grain yield on a year-of-release basis, whereas we reported on a per-breeding cycle basis. Year of cultivar release does not accurately reflect relative ages or relationships of the germplasm, as offspring in one lineage may produce commercial offspring before the parent generation in a related lineage reaches commercialization. This was the case for the cultivar Duster (Edwards et al., 2012) in bc 5 (Figure 1), which was released 12 years later than its counterpart Jagger (Sears et al., 1997). Such overlapping generations were common in the older breeding cycles 1 to 3, in which the length of a breeding cycle, or commercialization of the parent to that of its offspring, can be highly variable, but they

exist even today as wheat breeders employ conventional inbred line development strategies versus the more rapid doubled haploid strategy in the same breeding program.

Grain yield is a complex plant trait, which can be affected by many related traits, such as days to heading and plant height. Directional changes in these and other traits are not necessarily the result of the same selection strategy used for grain yield. These traits may be targeted within a certain window of acceptability for a given region or breeder, particularly in the more recent breeding cycles of 3 through 6. In agreement with previously reported results (Donmez et al., 2001; Fufa et al., 2005), we found a downward trend in heading date and plant height to reflect selection for shorter stature (almost always *Rht-B1-* or *Rht2-D1*-based) wheat lines and higher priority for earlier maturity. Using the square root transformation of the response, days to heading decreased 0.06 sqrt(days) bc^{-1}, and plant height decreased 0.11 sqrt(cm) bc^{-1}, each representing highly significant selection responses. Kharkof (bc 0) had the tallest average height (102 ± 5.7 cm) and the latest heading date (124 ± 2 days), whereas Lonerider (bc 6) was shortest (77 ± 3.5 cm) and Triumph (bc 1) was earliest (107 ± 3 days). Earlier maturity relative to the Turkey and its many re-selections was the driving motivation behind the development of Triumph wheat in the 1920s (Carver, 2009).

Grain test weight increased 0.22 kg hL^{-1} bc^{-1}, which counters reports by Khalil et al. (2002) for a similar but older genetic panel of U.S. HRW wheat and Hucl et al. (2015) for Canadian spring wheat, who both found no significant trends in grain test weight. Concomitant increases in test weight and grain yield are beneficial to wheat producers and millers. Wheat with test weight below a grain elevator threshold often gets docked, resulting in less income for the producer. On the other hand, producers may receive a premium for delivering grain with higher test weight than the norm for a given crop year, because a higher test weight could indicate a greater capacity for extractable flour (McCall and Fowler, 2009).

Future coupling of grain yield and test weight improvement appears feasible in this segment of U.S. HRW wheat. Grain yield and test weight showed no significant genetic correlation (Table 3), further supporting the opportunity to achieve significant concomitant gains in test weight and grain yield, assuming selection pressure is applied to both traits. Gursoy et al. (2010) also found no phenotypic correlation between grain yield and test weight; however, Mohammadi et al. (2012) found a weak phenotypic correlation between grain yield and test weight, but only when the crop was grown under drought conditions.

Wheat and Milling Characteristics

Against this backdrop of a stepwise, consistent rise in yielding ability since the dawn of wheat breeding in the U.S. southern Plains, any associated changes in other characteristics can be considered consequential by direct (i.e., breeders intentionally applied selection pressure though hardly ever relative to a concrete threshold) or indirect means. For the latter, selection pressure would not have been applied, but a *genetic* association with yield or other selected traits caused a correlated selection response. In some cases, wheat breeders would have been unaware of these underlying associations, such as the concentration of immunogenic protein fragments in wheat flour or FODMAP level, because the measuring tools were not widely available or there was no a posteriori reason to suspect a genetic relationship with the targeted traits. Total wheat protein content and the milling characteristics would have certainly been in breeders' sights, and thus, significant genotypic and GE effects were evident (Table 1), with one exception being the non-significant GE effect for flour yield. Kernel weight significantly increased across breeding cycles, estimated using a square root transformation of the response as 0.05 sqrt(mg) bc^{-1} (Figure 2B). Underdahl et al. (2008) and Hucl et al. (2015) reported positive gains for kernel weight in spring wheat, whereas Fufa et al. (2004) reported a positive gain for kernel weight in winter wheat.

Given that kernel weight is one component of yield, it is not surprising to see an increase in kernel weight with grain yield improvement, though wheat yield gains worldwide have not consistently shown a kernel weight dependency (Rudd et al., 2009). Genetic gains are more likely found in net weight per spike, that is, the product of kernel number per spike and kernel weight (Khalil et al., 2002). Our focus here was on kernel weight, because larger kernel weight (and size) carries obvious benefit to both producer and miller. Winter wheat breeding programs in the U.S. Great Plains tend to consider >30 mg as an acceptable target level for kernel weight. All of the 17 contemporary genotypes in the last two breeding cycles averaged 30 mg or higher across environments. For benchmarks, Turkey and Kharkof averaged 29.5 ± 0.9 mg and 29.4 ± 0.7 mg, yet what is most promising is the incrementally upward trend in kernel weight in the later breeding cycles and apparent absence of a kernel weight plateau (Figure 2b). Changes in kernel weight for any pair of consecutive generations were mirrored by changes in kernel size, measured as SKCS kernel diameter which increased at the rate 0.03 mm bc^{-1} (Table 2). The variety Lonerider (bc 6) produced the largest diameter of 2.78 ± 0.09 mm, whereas Turkey produced the smallest diameter of 2.51 ± 0.04 mm.

Kernel texture, assessed herein by the SKCS hardness index, reflects the force required to crush kernels and thus represents a key milling property. Hard winter wheat breeding programs may loosely target a window of hardness index values from 60 to 80, on a SKCS scale of -20 to 120. Selection for such minor differences in hardness within the HRW market class has not been shown to impact dough strength or protein quality, though protein quantity may be affected (Carver, 1994). Hardness index increased significantly, though erratically, across breeding cycles at the rate of 0.10 sqrt(SKCS units) bc^{-1}, an unexpected shift given the perceived lack of intra-class directional selection pressure. One explanation for the erratic nature of the hardness index could be because of different alleles of the *Puroindoline a* and *Puroindoline b* proteins. When variation occurs in either *Pin* gene sequence, or a protein is absent, then wheat kernels are hard

(Bhave and Morris, 2008). Different mutations of the *Pin* genes have been associated with different levels of kernel hardness. Bentley (bc 6) produced the highest hardness index at 76 ± 1 SKCS units, well within the target range, whereas, Nicoma (bc 2) produced the lowest at 54± 2 SKCS units. Interestingly, Turkey was in the higher range of hardness index at 73 ± 1 SKCS units. This slight uptick in hardness was not associated with a significant response in flour yield, measured with a small experimental mill. New cultivars are opportunistically selected for higher flour yield, but usually not at the expense of a competitive yield level or overall fitness. These results correspond with numerous previous reports on neutral changes in flour yield (Cox et al., 1989; Fufa et al., 2005; Hucl et al., 2015).

Most striking was the incremental decrease in wheat protein content of 0.20% per breeding cycle (Figure 2C), for a total net loss of 1.1 percentage units from bc 0 to bc 6. Other studies on trends in bread wheat protein content are mixed, with some studies indicating losses (Fufa et al., 2005), no change (Khalil et al., 2002), or gains (Cox et al., 1989; Hucl et al., 2015). All trials were managed so that higher yielding genotypes should not have had the disadvantage of insufficient nitrogen, which would have caused a negative bias in wheat protein content. Hence the loss in wheat protein with advancing breeding cycles was likely not caused by insufficient nitrogen availability for higher yielding genotypes with a higher N requirement. However, the reverse cannot be eliminated—that genotypes with a lower yield potential received an excessive N supply leading to higher protein deposition in the kernel than would be expected at the optimal level (Brown et al., 2005). Most importantly, while wheat protein content has decreased throughout the modern wheat breeding era, the current level has not fallen below the 12% threshold typically targeted by U.S. HRW wheat breeding programs (Figure 2C). In bc 6, wheat protein content varied from 12.4 ± 0.6% (Lonerider) to 13.4 ± 0.3% (OK11311F, a soft red wheat experimental line with HRW functionality). Consistent with their divergent selection responses, the genetic correlation for grain yield versus grain protein was negative (r=-0.27) (Table 3) as

reported in previous studies (Fufa et al., 2005; Graybosch et al., 1996). The inverse yield-protein relationship is well documented, and besides obvious environmental factors such as a lack of nitrogen availability not relevant to this study, genetic components (McNeal et al., 1972) and dilution of grain nitrogen due to increased grain biomass (Grant et al., 1991) are potential causes.

The loss in wheat protein content across breeding cycles leads to the question, have contemporary cultivars in the more advanced cycles become more protein-sensitive, that is, are the contemporary high yielding genotypes more inclined to lose wheat protein with increasing yield levels than foundational or heritage cultivars? To address this question, a regression of wheat protein content versus grain yield was performed for each variety, supported by replicated testing in six southern Plains site-years (for all but two entries) differing in productivity level from 2,920 kg ha^{-1} to 5,980 kg ha^{-1}. Three linear response patterns were clearly evident among cultivars: i) no change in wheat protein content across productivity levels, ii) moderate decline in wheat protein content, and iii) steep decline in wheat protein content, signifying greater protein-sensitivity to productivity. Each pattern is shown in Figure 3 for cultivars Gallagher, Iba, and OK12621, to illustrate the point that the complete spectrum of protein responses resided even within a single, and contemporary, breeding cycle, and even among closely related half selfed-sibs to the common parent, Duster.

To further explore the possibility of differential protein responses among cultivars with inherently differing yielding abilities, a linear regression was performed for site-year mean wheat protein content versus site-year mean yield among the 30 genotypes (Figure 4A), and the resulting deviations from the regression, or differentials, were then plotted against breeding cycle (Figure 4B). Cultivars with a positive deviation accumulate wheat protein at levels higher than expected for their yielding ability. Residuals from the protein-yield relationship were indeed dependent on breeding cycle. A closer examination of the residuals, however, revealed that while there has been a decline from the heirloom cultivars, the modern cultivars in breeding cycles 5

and 6 showed both positive and negative mean deviations (Figure 4B). While the modern cultivars may be at an inherent disadvantage for protein content due to their higher yield ability, it appears that any disadvantage that exists currently may be cultivar-specific and thus not a sweeping cause for alarm.

Gluten, Dough, and Flour Functional Attributes

The milling and baking industry commonly use the alveograph and farinograph to measure dough behaviors respectively as resistance to expansion and resistance to mixing. While these tests are satisfactory stalwarts in commercial practice, they lack agility for use in a wheat research programs often limited by small flour quantity or burdened by large sample load, especially in the early inbreeding generations of developing a new wheat cultivar. Instead, U.S. wheat breeders continue to rely on the less consuming mixograph, which generates multiple parameters for dough strength that would have been used in the development of most cultivars featured in breeding cycles 3 to 6. Thus our evaluation of selection response focused on the conventional mixograph parameters, plus a protein-adjusted determination of SDS sedimentation volume as an indirect measurement of the presence of larger protein components (Ross and Bettge, 2009), a direct measurement of the proportion of polymeric (larger) proteins as the TPP/TMP ratio, and a direct measurement of gluten elasticity via the CORE recovery index, all of which produced significant genotypic and GE variance components (Table 1).

Even with the previously mentioned decline in wheat protein content, dough strength has shown no change to a slight upward trend with higher yielding breeding cycles. Mixing tolerance score, on a 0-to-6 scale, increased 0.2 units bc^{-1} (Figure 2D), and the mixograph stability index decreased at a rate of 0.18 sqrt(MU) bc^{-1} (Table 2), whereas the mixograph bandwidth showed no change (Figure 2E). For both the tolerance score and bandwidth parameters, a spike occurred in

selection response at bc 3 due mostly to variety Karl 92, with a mean tolerance score of 3.1 ± 0.2 units, bandwidth of 19.5 ± 1.5 mm, and stability value of 6.3 ± 0.7. Karl 92 (Sears et al., 1997) and its immediate predecessor Karl (Sears et al., 1991) were known throughout the wheat breeding community as outstanding sources of high protein and dough strength; even long after Karl 92's success in the field, it remained a favorable procurement target by flour millers. High stability values indicative of a steeper descent in the mixograph curve, and thus poor tolerance to overmixing, were common among the older cultivars of bc's 1 and 2: Triumph (13.4 ± 1.3 MU), Triumph 64 (20.8 ± 1.0 MU) and Nicoma (13.3 ± 1.0 MU). Adjusted SDS sedimentation volume decreased at a rate of 0.07 ml bc^{-1} (Table 2). This was in contrast to Fufa et al. (2005) and Hucl et al. (2015), who reported a gain in SDS sedimentation volume.

Compression-recovery tests were conducted on the gluten fraction isolated from milled flour, as prescribed by Chapman et al. (2012). Using a square root transformation for regression, the CORE recovery index increased 0.06 sqrt(%) per breeding cycle (Figure 2F). This increase occurred solely as an indirect response to selection for mixograph, sedimentation volume, and baking characteristics, because CORE testing would not have been available for selection and development of the tested cultivars. Greater recovery indicates greater gluten elasticity conferred by polymer characteristics (Singh and MacRitchie, 2001) rather than rheological properties of dough. Nevertheless, a large spike in the recovery index occurred in bc 3 as it did for mixograph properties, where the common driver of dough strength and gluten elasticity was Karl 92 (57.2 ± 0.8% recovery). Some contemporary entries were noted for having gluten elasticity on the same level as Karl 92, including OK11231 (56.9 ± 0.7%), Spirit Rider (56.9 ± 0.9%), and Jagalene (58.2 ± 0.8%). A descendant of a Karl 92 sibling, Jagalene is known widely for having superior baking quality and is presently the HRW Wheat Quality Council check (http://www.wheatqualitycouncil.org/2018/2018-Test-Results-For-Hard-Winter-Wheat-Part-1.pdf). Chapman et al. (2012) also found Jagalene to be the variety with the highest recovery

index in their test panel. Mixograph bandwidth showed a positive genetic correlation with the recovery index, indicating that a common indicator of dough strength coincided with this less exploited indicator of gluten strength. The magnitude of the correlation (r=0.28), however, encourages positive selection for both parameters rather than one as a substitute for the other.

Knowledge of flour protein composition provides a broad sense of bread dough functionality, which must encompass a balance of elastic and viscous rheological properties. One such assessment of this balance is derived from the ratio of total polymeric proteins (TPP) to total monomeric proteins (TMP), or quasi-ratio of glutenin to gliadin contents. The monomeric gliadins mostly confer a cohesive and extensibility properties in the gluten system, whereas the polymeric glutenins confer strength and elasticity of gluten (Wrigley et al., 2009); the ratio, or balance, of the two thus confers dough strength and extensibility (Souza et al., 2019).

The TPP/TMP ratio showed no significant linear trend in Figure 2G, indicating that the balance of glutenins to gliadins has not shifted systematically in the past 100 years of HRW wheat breeding. Therefore, dough strength has not changed due to a different gluten composition patterns in HRW wheat. Research on the gluten ratio in historic wheat panels is presently limited, with the only such comparisons reported for durum wheat by De Santis et al., 2018, who found a slight increase in the glutenin fraction with breeding (De Santis et al., 2018). Hence these results help fill a gap in our knowledge of changes in bread wheat gluten composition.

The TPP/TMP ratio showed a weak negative genetic correlation with mixograph bandwidth, as did bandwidth with adjusted SDS sedimentation volume (Table 3). Both associations were unexpected, given that all three traits are general markers of dough and gluten strength, and HRW cultivars should have been selected for higher values of bandwidth (based mostly on subjective tolerance scores) and sedimentation volume. Dhaliwal et al. (1987) found a moderate to strong positive correlation between mixograph bandwidth and SDS sedimentation

volume (r=0.59 to r=0.79), though their genetic materials varied for presence or absence of T1RS.BL translocation that is readily detectable as smaller or larger SDS sedimentation volumes, respectively. For optimal mixing tolerance, an increase in TPP/TMP ratio may not always be more desirable. Further research into the TPP/TMP ratio and its implications to gluten strength and dough properties is warranted.

Sensitivity Tests

We chose two principle biochemical components most often associated with seemingly disparate but potentially convergent causal elements of human sensitivity to wheat: fructans measured herein as total concentration of fructo-oligosaccharides and fructan polysaccharide and the 33-amino acid fragment (33-mer) from α-gliadin 2. The 33-mer is frequently described as the most important celiac disease immunogenic sequence found in gluten (Arentz-Hansen et al., 2002; Shan et al., 2002), whereas fructans, which have no immunogenic potential, and besides their prebiotic role as a fermentable carbohydrate, may induce symptoms of irritable bowel syndrome (IBS) when consumed in excess amounts or by individuals with a fructan intolerance (Fedewa and Rao, 2014).

In contrast to the aforementioned agronomic and functionality traits, GE interactions were nonexistent for α-gliadin 2 content and milled flour fructan content, whereas their genotypic variances were highly significant (Table 1). Veenstra et al. (2019) also found that the genotypic effect explained most of the variance in fructan content.

Genetic trends for α-gliadin 2 and flour fructan content were again calculated by their linear regression on breeding cycle. A logarithmic transformation was necessary for α-gliadin 2, and the slope was not significant (Figure 2H), indicating no systematic change has occurred in α-gliadin 2 content, expressed in ppm of flour, in response to a definitive rise in wheat yields.

Schalk et al. (2017) found that between disconnected groups of older and modern wheat genotypes, the majority of which was registered in Germany, no difference was detected in levels of the 33-mer peptide. Escarnot et al. (2018) used A1 and G12 antibodies to assay for all copies of the 33-mer peptide, including α-gliadin 2, and reported no discernible difference among cultivars of disparate origin. While any amount of dietary gluten, and specifically α-gliadin 2 and the 33-mer peptide, is toxic to celiac patients, we show no evidence that α-gliadin 2 toxicity is an unintended consequence of decades of dedicated yield improvement and competency for leavened bread products. Continued monitoring in all market classes of U.S. wheat is needed to form the critical genetic basis to refute popular claims that modern wheat breeding has led to cultivars directly linked to our food supply with elevated levels of immunogenic proteins (Jones, 2012).

Further partitioning of the genotypic response in α-gliadin 2 content variation resident to heirloom entries (cycles 0-3) versus contemporary entries (cycles 4-6) revealed significant genetic variation in the contemporary pool (Table 4). This demonstrates that while the mean α-gliadin 2 content has not changed with each successive breeding cycle, genetic variability exists to potentially allow breeders to access germplasm with a lower α-gliadin 2 content, thus providing the necessary fuel to drive selection for reduced α-gliadin 2 content independent of yield (Table 3). Nowhere was the dispersion in α-gliadin 2 content more evident than with the closely related and widely grown (USDA-NASS, 2019) half-sib pair, Gallagher and Iba, from bc 5. Iba produced the lowest α-gliadin 2 content (103.9 ± 15.3 ppm) in the contemporary group, whereas Gallagher produced the highest and twice that level (209.1 ± 14.0 ppm), making it a less desirable resource to select for lower α-gliadin 2 content. This disparity in α-gliadin 2 content combined with their close genetic relationship supports a hypothesis worthy of testing that expression of the 33-mer peptide may be controlled by relatively few genes in HRW wheat.

In contrast to α-gliadin 2 patterns, milled flour fructan content showed a significant increase of 0.03% per breeding cycle (Figure 2I), resulting in a total proportional increase of 30% across all breeding cycles. The cultivar Jagalene in bc 5 produced the highest fructan content (1.13 ± 0.08%), and Kharkof produced the lowest (0.46 ± 0.04%). A noticeable spike again was observed in bc 3, in which all three members (Chisholm, Karl, Karl 92) produced similar fructan contents averaging 0.97%. Interestingly, Karl and thus Karl 92 are related to Chisholm via the common ancestor, Triumph (Figure 1), which produced a higher than expected fructan content relative to the selection-response regression line in Figure 2I.

Even with the anomaly of bc 3, the steady increase in fructan content cannot be overlooked for potential cause and future dietary implications. The observed selection response was clearly indirect, or an unintended consequence, because wheat breeders in the southern Plains never applied direct selection pressure for grain or flour fructans. What then would serve as a common denominator to genetic gains in grain yield and fructans deposited in the grain? At this point, one cannot rule out the mathematical aberration, or compensatory effect, of the observed decline in the protein fraction with a corresponding rise in the non-starch carbohydrate fraction. Yang et al. (2004) reported that fructans play a positive role during osmotic stress by enhancing remobilization of carbon reserves from vegetative tissues to the grain. Additionally, fructans act as osmoregulators that confer cold and salt tolerance (Hendry, 1993; Pilon-Smits et al., 1995). Thus it is plausible to observe elevated levels of fructans in germplasm developed in a region prone to periods of water stress during the nine-month winter wheat growing season of the southern Great Plains. Further research is needed to explore the relationship of grain yield with both milled flour and wholemeal fructan contents, based on genetic mapping of fructan content in winter wheat and bidirectional selection experiments establishing fructan content as the target trait.

To our knowledge, this study is the first to quantify differences in fructan content in an historic North American bread wheat panel featuring incremental changes in yielding ability, particularly at this level of environment sampling. Previous studies only reported a mean or a range of fructan contents for modern wheat cultivars. Verspreet et al. (2012) reported a fructan mean of 1.2% in milled wheat flour, whereas our highest reported fructan value was 1.1%. This difference is likely due to the inclusion of lower-fructan heirloom genotypes. Hunyh et al. (2008) and Veenstra et al. (2019) reported fructan ranges for wholemeal samples of 1.5% to 2.3% and 0.8% to 2.2%, respectively. Wholemeal samples will have a greater fructan content than milled flour, because the bran fraction contains a higher fructan concentration than the endosperm (Haska et al., 2008). More importantly, our analysis showed identical indirect selection responses for milled flour versus wholemeal fructan content (Figure 2J), lending some choice and thus a potential cost benefit to future genetic experiments involving a higher sample load.

The observed increase in fructan content in contemporary cultivars must be considered for human dietary implications. Presently, the fructan tolerance level for individuals with a FODMAP sensitivity is 0.3 grams per serving (Varney et al., 2017). In 2017, U.S. per capita consumption averaged 131.8 lbs (59.8 kg) flour per year (USDA-ERS, 2018), or 0.36 lbs (0.16 kg) per day. A total fructan content of 1% equates to 0.0036 lbs (0.0016 kg) fructans per day, or 1.63 g per day. At three meals per day, this amounts to 0.54 g fructans per meal, assuming one serving of milled wheat flour per meal. This low consumption rate is nearly two times the current purported tolerance level. The per-serving fructans consumption increases to 0.62 g considering the cultivar with the highest flour fructan content, 1.1%. For the majority of the population with no FODMAP or fructan intolerance, these escalated levels likely cause no concern. However, wheat breeders might consider the feasibility of reversing this upward trend in fructans, considering that an estimated 24% of IBS sufferers have symptoms due to a fructan sensitivity (Böhn et al., 2013).

Breeding is not the only possibility to reduce fructan content in food products for those with sensitivities to it. Baking methods can significantly reduce the amount of fructans present in the end product actually consumed. Ziegler et al. (2016) reported that longer dough proofing times before baking can significantly reduce FODMAP, and fructan, content. Menezes et al. (2019) also found that using sourdough fermentation reduced the FODMAP content, with fructans reduced by 69 to 75%. These reductions show that estimates of fructans per serving are not always accurate, as they only consider the possible amount of fructans found in milled flour, and not processed into consumer goods such as breads and pasta. Non-processed flour fructan content represents the highest potential level of fructans in each serving of food consumed daily.

In addition to the positive effects to the wheat plant, fructans have proven to be beneficial to the majority of humans. Fructans, especially the inulin-type, are generally accepted as prebiotics since their fermentation leads to favorable changes in the microbiota composition in the gastrointestinal tract, thus conferring digestive health benefits (Roberfroid et al., 2010). In addition, a high fructan diet can improve the health of patients with diabetes (Ayman et al., 2004) and reduce the risk of colonic cancers (Jacobsen et al., 2006). Couple the health benefits to humans and the stress tolerance to plants, and it becomes apparent why wheat breeders may consider breeding for increased fructan content, or maintaining current levels with further increases in grain yield.

This belief is the principle driver behind much of the wheat fructan research thus far. Hunyh et al. (2008) focused their research on discovering a QTL for fructan concentration, with the goal of using molecular markers to increase fructans. Hunyh et al. (2012) reported on the mapping and sequencing of a fructan biosynthetic gene cluster and developing single nucleotide polymorphism (SNP) markers. Veenstra et al. (2019) reported on the feasibility of using genomic selection as a tool in breeding for increase fructan content. These three studies developed tools to breed for high fructan wheat, which does have benefits that wheat breeders should carefully

evaluate. However, none of these studies considered the possible negative effect fructans can have on a portion of the population. Genomic tools can be deployed to select bidirectionally for either a high fructan wheat or a low fructan wheat, assuming the necessary diversity is available in modern germplasm. The analysis of variance in Table 4 indicated significant genotypic effects among heirloom or contemporary genotypes. This indicates variation exists in currently available germplasm to allow breeders to select for either increased or decreased fructan content. Given that the current literature on fructans in wheat is limited, it is important to have the possibility to breed for this trait in either direction. Further research on the dietary implications and benefits, and the benefits to the wheat plant itself, will enable wheat breeders to make better informed selection goals for fructans.

CHAPTER V

CONCLUSION

To our knowledge, this study was the first of its kind in quantifying genetic trends in a historic panel of wheat with known parent-offspring relationships and lineages, rather than by year of cultivar release. In particular, this study was unique in its investigation into the two-sided gluten crisis by evaluating standard and novel dough quality parameters, and two wheat sensitivity causal agents. The findings indicate that many of the claims made about the gluten crisis are unfounded, in that end-use quality has not suffered a severe decline and that the toxicity of wheat related to the 33-mer peptide has not increased. A unique finding was the observed increase in fructan content due to wheat breeding, which has multiple dietary implications, both positive and negative. Further research should investigate the trends and implications of similar traits in other market classes of wheat and developed in other regions of the country and world.

REFERENCES

American Association of Cereal Chemists. 2000. Approved methods, 10th ed. American Association of Cereal Chemists, St. Paul, MN.

Appels, R., K. Eversole, C. Feuillet, B. Keller, J. Rogers, N. Stein, et al. 2018. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361.

Arentz-Hansen, H., S.N. Mcadam, O. Molberg, B. Fleckenstein, K.E.A. Lundin, T.J.D. Jorgensen, G. Jung, P. Roepstorff, and L.M. Sollid. 2002. Celiac lesion T cells recognize epitopes that cluster in regions of gliadins rich in proline residues. Gastroenterology 123(3): 803-809.

Ayman, E.M., A.H. Sania, and M.K. Ahmed. 2004. Studies on production of soda cracker biscuits for diabetics. Ann. Agric. Sci. 49:585-595.

Barclay, T., M. Ginic-Markovic, P. Cooper, and N. Petrovsky. 2010. Inulin- a versatile polysaccharide with multiple pharmaceutical and food chemical uses. J. Excipients and Food Chem. 1(3):27-50.

Battenfield, S.D., A.R. Klatt, and W.R. Raun. 2013. Genetic yield potential improvement of semidwarf winter wheat in the Great Plains. Crop Sci. 53:946-955.

Bhave, M, and C.F. Morris. 2008. Molecular genetic of puroindoline and related genes: allelic diversity in wheat and other grasses. Plant Mol. Bio. 66(3):205-219.

Biesiekierski, J.R., S.L. Peters, E.D. Newnham, O. Rosella, J.G. Muir, and P. Gibson. 2013. No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary restrictions of fermentable, poorly absorbed short-chain carbohydrates. Gastroenterology. 145:320-328.

Biesiekierski, J.R., J. Iven. 2015. Non-coeliac gluten sensitivity: piecing the puzzle together. United European Gastro Journal. 3(2):160-165.

Böhn, L., S. Stösrud, H. Törnblom, U. Bengtsson, and M. Simrén. 2013. Self-reported food-related gastrointestinal symptoms in IBS are common and associated with more severe symptoms and reduced quality of life. Am. J. Gastroenterol. 108(5):634-641.

Brown, B., M. Westcott, N. Christensen, B. Pan, and J. Stark. 2005. Nitrogen management for hard wheat protein enhancement. Pacific Northwest Extension. PNW578.

Carver, B.F. 1994. Genetic implications of kernel NIR hardness on milling and flour quality in bread wheat. J. Sci. Food Agric. 65:125-132.

Carver, B.F. 2009. Early Triumph wheat. *In* Encyclopedia of Oklahoma History and Culture. Oklahoma Historical Society, Oklahoma City, OK.

Cattasi, C., D. Kryszak, B. Bhatti, C. Sturgeon, K. Helzlsouer, S.L. Clipp, D. Gelfond, E. Puppa, A. Sferruzza, and A. Fasano. 2010. Natural history of celiace disease autoimmunity in a USA cohort followed since 1974. Ann Med. 42(7):530-538.

Chapman, S.J., S.J. Mulvaney, R. Chinnaswamy, P. Rayas-Duarte, and B. Alvin. 2012. Large deformation stress relaxation and compression-recovery of gluten representing different wheat classes. J. Cereal Science 55: 366-372.

Chung, O.K., J.B. Ohm, M.S. Caley, and B.W. Seabourn. 2001. Prediction of baking characteristics of hard winter wheat flours using computer-analyzed mixograph parameters. Cereal Chem. 78(4):493-497.

Cox, T.S., J.P. Shroyer, L. Ben-Hui, R.G. Sears, and T.J. Martin. 1988. Genetic improvement in agronomic traits of hard red winter wheat cultivars from 1919 to 1987. Crop Sci. 28:756-760.

Cox, T.S., M.D. Shogren, R.G. Sears, T.J. Martin, and L.C. Bolte. 1989. Genetic improvement in milling and baking quality of hard red winter wheat cultivars, 1919 to 1988. Crop Sci. 29:626-631.

De Lorgeril, M., and P. Salen. 2014. Gluten and wheat intolerance today: are modern wheat strains involved? Int J Food Sci Nutr. 65(5):577-581.

De Santis, M.A., M.M. Giuliani, L. Giuzio, P. De Vita, and Z. Flagella. 2018. Assessment of grain protein composition in old and modern Italian durum wheat genotypes. Italian J. of Agronomy 13(1):40-43.

Dhaka V., and B.S. Khatkar. 2015. Effects of gliadin/glutenin and HMW-GS/LMW-GS ratio on dough rheological properties and bread-making potential of wheat varieties. J. of Food Quality 38:71-82.

Dhaliwal, A.S., D.J. Mares, and D.R. Marshall. 1987. Effect of 1B/1R chromosome translocation on milling and quality characteristics of bread wheat. Cereal Chem. 64(2):72-76.

Donmez, E., R.G. Sears. J.P. Shroyer, and G.M. Paulsen. 2001. Genetic gain in yield attributes of winter wheat in the Great Plains. Crop Sci. 41:1412-1419.

El-Hennawy, M.A., A.F. Adballa, S.A. Shafey, and I.M. Al-Ashkar. 2011. Production of doubled haplopid wheat lines (*Triticum aestivum* L.) using anther culture technique. Ann. Agric. Sci. 56(2): 63-72.

Escarnot, E., S. Gofflot, G. Sinnaeve, B. Dubois, P. Bertin, and D. Mingeot. 2018. Reactivity of gluten protein from spelt and bread wheat accessions towards A1 and G12 antibodies in the framework of celiac disease. Food Chemistry 268: 522-532.

Fedewa, A., and S.S.C. Rao. 2014. Dietary fructose intolerance, fructan intolerance and FODMAPs. Curr. Gastroenterol. Rep. 16(1):370.

Fufa, H., P.S. Baenziger, B.S. Beecher, R.A. Graybosch, K.M. Eskridge, and L.A. Nelson. 2005. Genetic improvement trends in agronomic performance and end-use quality characteristics among hard red winter wheat cultivars in Nebraska. Euphytica. 144:187-198.

Gibson, G.R., E.R. Beatty, X. Wang, and J.H. Cummings. 1995. Selective stimulation of bifidiobacteria in the human colon by oligofructose and inulin. Gasterenterology 108(4):975-982.

Gibson, P.R., and S.J. Shepherd. 2005. Personal view: food for thought- western lifestyle and susceptibility to Crohn's disease. The FODMAP hypothesis. Aliment Pharmacol Ther. 21:1399-1409.

Gooding, M.J. 2009. The wheat crop. Pages 19-49 in Wheat: Chemistry and Technology, 4th Ed. K. Khan and P.R. Shewry, eds. AACC Inernational: St. Paul, MN.

Grant, C.A., L.E. Gauer, D.T. Gehl, and L.D. Bailey. 1991. Protein and nitrogen utilization by barley cultivars response to nitrogen fertilizer under varying moisture conditions. Can J Plant Sci. 71:997-1009.

Graybosch, R.A., C.J. Peterson, D.R. Shelton, and P.S. Baenzinger. 1996. Genotypic and environmental modification of wheat flour protein composition in relation to end-use quality. Crop Sci. 36:296-300.

Graybosch, R.A., and C.J. Peterson. 2010. Genetic improvement in winter wheat yields in the great plains of North America, 1959-2008. Crop Sci. 50:1882-1890.

Gujral, N., H.J. Freeman, and A.B.R. Thomson. 2012. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. World J Gastroenterol. 18(42):6036-6059.

Gupta, R.B., K. Khan, and F. MacRitchie. 1993. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. J. Cereal Sci. 18: 23-41.

Gursoy, S., A. Sessiz, and S.S. Malhi. 2010. Short-term effects of tillage and residue management following cotton on grain yield and quality of wheat. Field Crops Research 119:260-268.

Guzmán, C., E. Autrique, S. Mondal, J. Huerta-Espino, R.P. Singh, M. Vargas, J. Crossa, A. Amaya, and R.J. Peña. 2017. Genetic improvement of grain quality traits for CIMMYT semi-dwarf spring bread wheat varieties developed during 1965-2015: 50 years of breeding. Field Crops Research 210:192-196.

Halbmayr-Jech, E., E. Hammer, R. Fielder, J. Coutts, A. Rogers, and M. Cornish. 2012. Characterization of G12 sandwich ELISA, a next-generation immunoassay for gluten toxicity. J. AOAC Int. 95(2): 372-376.

Haska, L., M. Nyman, and R. Andersson. 2008. Distribution and characterization of fructan in wheat milling fractions. J. Cereal Science 48:768-774.

Hendry, G. 1993. Evolutionary origins and natural functions of fructans- a climatological, biogeographic, and mechanistic approach. New Phytol. 123:3-14.

Howdle, P.D. 2006. Gliadin, glutenin or both? The search for the Holy Grail in coeliac disease. Eur J Gastroenerol Hepatol. 18:703-706.

Hucl, P., C. Briggs, R.J. Graf, and R.N. Chibbar. 2015. Genetic gains in agronomic and selected end-use quality traits over a century of plant breeding of Canada western red spring wheat. Cereal Chem. 92(6):537-643.

Huynh, B., H. Wallwork, J. Strangoulis, R. Graham, K. Willsmore, S. Olson, and D. Mather. 2008. Quantitative trait loci for grain fructan concentration in wheat (*Triticum aestivum* L.). Theor Appl Genet 112:701-709.

Huynh, B., D. Mather, A. Schreiber, J. Toubia, U. Baumann, Z. Shoaei, N. Stein, R. Ariyadasa, J. Stangoulis, J. Edwards, N. Shirley, P. Langridge, and D. Fleury. 2012. Clusters of genes encoding fructan biosynthesizing enzymes in wheat and barley. Plant Mol Biol 80:299-314.

Jacobsen, H., M. Poulsen, L.O. Dragsted, G. Ravn-Haren, O. Meyer, R.H. Lindecrona. 2006. Carbohydrate digestibility predicts colon carcinogenesis in azoxymethane-treated rats. Nutr. Cancer 55:163-170.

Jones, J.M. 2012. *Wheat Belly* – An analysis of selected statements and basic theses from the book. Cereal Foods World 57:177-189.

Khalil, I.H., B.F. Carver, E.G. Krenzer, C.T. MacKown, G.W. Horn, and P. Rayas-Duarte. 2002. Genetic trends in winter wheat grain quality with dual-purpose and grain-only management systems. Crop Sci. 42:1112-1116.

Konvalina, P., J. Bradova, I. Capouchova, Z. Stehno, and J. Moudry. 2013. Baking quality and high molecular weight glutenin subunit composition of emmer wheat, old and new varieties of bread wheat. Romanian Agricultural Research 30:83-89.

Laiding, F., H. Peipho, D. Rentel, T. Drobek, U. Meyer, and A. Huesken. 2017. Breeding progress, environmental variatyion and correlation of winter wheat yield and quality traits in German official variety trials and on-farm during 1983-2014. Theor. Appl. Genet. 130(1): 223-245.

Lieberman, S., and L. Segall. 2006. The gluten connection: how gluten sensitivity may be sabotaging your health- and what you can do to take control now. Rodale, New York, NY.

Lohi, S., K. Mustalahti, K. Kaukinen, K. Laurila, P. Collin, H. Rissanen, O. Lohi, E. Bravi, M. Gasparin, A. Reunanen, and M. Mäki. 2007. Increasing prevalence of coeliac disease over time. Aliment Pharmacol Ther. 26:1217-1225.

Lopes, M.S., M.P. Reynolds, Y. Manes, R.P. Singh, J. Crossa, and H.J. Braun. 2012. Genetic yield gains and changes in associated traits of CIMMYT spring bread wheat in a historic set representing 30 years of breeding. Crop Sci. 52:1123-1131.

Lorenzo, A., and W.E. Kronstad. 1987. Reliabilty of two laboratory techniques to predict bread wheat protein quality in nontraditional growing areas. Crop Sci. 27:247-252.

Marburger, D., B. Carver, C. Estes-Shelton, R. Miller, and R. Chen. 2017. Wheat and flour quality for varieties tested in the 2016 OSU wheat variety performance trials. Oklahoma Cooperative Extension Service. CR-2165.

McCall, K.L., and M.E. Fowler. Overview of wheat classification and trade. In: B.F. Carver, editor, Wheat: Science and trade. Wiley-Blackwell, Ames, IA. P. 439-454.

McNeal, F.H., M.A. Berg, C.F. McGuire, V.R. Stewart, and D.E. Baldridge. 1972. Grain and plant nitrogen relationships in eight spring wheat crosses, *Triticum aestivum* L. Crop Sci. 12:599-602.

Menezes, L., L. Molognoni, L. de Sa Plencio, F. Costa, H. Daguer, and J De Dea Lindner. 2019. Use of sourdough fermentation to reducing FODMAPs in bread. European Food Research and Technology 245:1183-1195.

Mohammadi, M.m P. Sharifi, R. Karimizadeh, and M.K. Shefazadeh. 2012. Relationships between grain yield and yield components in bread wheat under different water availability. Not Bot Horti Agrobo 40(1);195-200.

Moshfegh, A.J., J.E. Friday, J.P. Goldman, and J.K.C. Ahuja. 1999. Nutritional and health benefits of inulin and oligofructose: presence of inulin and oligofructose in the diets of Americans. J. Nutr. 129:1407S-1411S.

Ohm, J.B., and O.K. Chung. 1999. Gluten, pasting, and mixograph parameters of hard winter wheat flours in relation to breadmaking. Cereal Chem. 76(5):606-613.

Ozuna, C.V., J.C. Iehisa, M.J. Gimenez, J.B. Alvarez, C. Sousa, and F. Barro. 2015. Diversification of the celiac disease α-gliadin complex in wheat: a 33-mer peptide with six overlapping epitopes, evolved following polyploidization. The Plant Journal 82:794-805.

Pek, J., O. Wong, and C.M. Wong. 2017. Data transformations for interference with linear regression: clarifications and recommendations. Prac Assessment Research and Eval. 22(9).

Pilon-Smits, E.A.H., M.J.M. Ebskamp, M.J. Paul, M.J.W. Jeuken, P.J. Weisbeek, and S.C.M. Smeekens. 1995. Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol. 107:125-130.

Prandi, B., T. Tedeschi, S. Folloni, G. Galaverna, and S. Sforza. 2017. Peptides from gluten digestion: a comparison between old and modern wheat varieties. Food Research Int. 91:92-102.

Roberfroid, M., G.R. Gibson, L. Hoyles, A.L. McCartney, R. Rastall, I. Rowland, D. Wolver, B Watzl, H. Szajewska, B. Stahl, F. Guarner, F. Respondek, K. Whelan, V. Coxam, M.J. Davicco, L. Leotoing, Y. Wittrant, N.M. Delzenne, P.D. cani, A.M. Neyrinck, and A. Meheust. 2010. Prebiotic effects: Metabolic and health benefits. Br. J. Nutr. 104:S1-S63.

Ross, A.S. and A.D. Bettge. Passing the test on wheat end-use quality. In: B.F. Carver, editor, Wheat: Science and trade. Wiley-Blackwell, Ames, IA. P. 455-493.

Rudd, J.C. 2009. Success in wheat improvement. In: B.F. Carver, editor, Wheat: Science and trade. Wiley-Blackwell, Ames, IA. P. 387-395.

Schalk, K., C. Lang, H. Wieser, P. Koehler, and K.A. Scherf. 2017. Quantitation of the immunodominant 33-mer peptide from α-gliadin in wheat flours by liquid chromatography tandem mass spectrometry. Scientific Reports 7:45092.

Sears, R.G., E.G. Heyne, T.J. Martin, T.S. Cox, L.E. Browder, D.L. Wetzel, M.D. Shogren, L.C. Bolte, S.P. Curran, J.R. Lawless, M.D. Witt, and W.F. Heer. 1991. Registration of 'Karl' wheat. Crop Sci. 31:1386.

Sears, R.G., T.J. Martin, T.S. Cox, O.K. Chung, S.P. Curran, W.F. Heer, and M.D. Witt. 1997. Registration of 'Karl 92' wheat. Crop Sci. 37:628.

Shan, L., O. Molberg, I. Parrot, F. Hausch, F. Filiz, G.M. Gray, L.M. Sollid, and C. Khosla. 2002. Structural basis for gluten intolerance in celiac sprue. Science 297(27): 2275-2279.

Singh, H., and F. MacRitchie. 2001. Application of polymer science to properties of gluten. J. Cereal Sci. 33:231-243.

Skodje, G.I., V.K. Sarna, I.H. Minella, K.L. Rolfsen, J.G. Muir, P.R. Gibson, M.B. Veierød, C. Henriksen, and K.E.A. Lundin. 2018. Fructan, rather than gluten, induces symptoms in patients with self-reported non-celiac gluten sensitivity. Gastroenterology. 154:529-539.

Souza, T., M. de Miranda, A. Prando, M. Tilley, M. Payton, and P. Rayas-Duarte. 2019. Gluten viscoelasticity: rapid method for classification of soft-like wheat genotypes. Cereal Chemistry 96:167-181.

Takata, K., H. Yamauchi, Z. Nishio, and T. Kuwabara, T. 2001. Prediction of bread-making quality by prolonged swelling SDS-sedimentation test. Breeding Science 49:221-223.

Toth, J., S. Pandurangan, A. Burt, J.M. Fetch, and S. Kumar. 2019. Marker-assisted breeding of hexaploid spring wheat in the Canadian prairies. Can. J. Plant Sci. 99:111-127.

Underhal, J.L., M. Mergoum, and J.K. Ransom. 2008. Agronomic traits improvements and associations in hard red spring wheat cultivars released in North Dakota from 1968 to 2006. Crop Sci. 48: 158-166.

USDA-ERS. 2019. Wheat sector at a glance. Available at https://www.ers.usda.gov/topics/crops/wheat/wheat-sector-at-a-glance/#use (verified 12 November 2019).

USDA-NASS. 2019. Oklahoma wheat variety report. Available at https://www.nass.usda.gov/Statistics_by_State/Oklahoma/Publications/Oklahoma_Crop_Reports/2019/ok-wheat-variety-2019.pdf (verified 5 November 2019).

Van de Kamer, J.H., H.A. Weijers, and W.K. Dicke. 1953. Coeliac Disease: An Investigation into the Injurious Constituents of Wheat in Connection with their Action on Patients with Coeliac Disease. Acta Pediatr Scand. 42:223-231.

Van den Broeck, H.C., H.C. de Jong, E.M.J. Salentijn, L. Dekking, D. Bosch, R.J. Hamer, L.J.W.J. Gilissen, I.M. van der Meer, and M.J.M. Smulders. 2010. Presence of celiac disease epitopes in modern and old hexaploid wheat varieties: Wheat breeding may have contributed to increased prevalence of celiac disease. Theor Appl Genet. 121:1527-1539.

Van Loo, J., P. Coussement, L. De Leenheer, H. Hoebregs, and G. Smits. 1995. On the presence of inulin and oligofructose as natural ingredients in the Western diet. Crit. Rev. Food Sci. Nutr. 35:525-552.

Varney, J., J. Barrett, K. Scarkata, P. Catsos, P.R. Gibson, and J.G. Muir. 2017. FODMAPs: food composition, defining cutoff values, and international application. J. Gastroenterol. Hepatol. 32(1):53-61.

Veenstra, L.D., J. Jannink, and M.E. Sorrells. 2017. Wheat fructans: a potential breeding target for nutritionally improved, climate-resilient varieties. Crop Sci. 57:1624-1640.

Veenstra, L.D., N. Santantonio, J. Jannink, and M.E. Sorrells. 2019. Influence of genotype and environment on wheat grain fructan content. Crop Sci. 59:190-198.

Verpsreet, J., A. Pollet, S. Cuyvers, R. Vergauwen, W. Van den Ende, J.A. Delcour, and C.M. Courtin. 2012. A simple and accurate method for determine wheat grain fructan content and average degree of polymerization. J. Agric. Food Chem. 60:2102-2107.

Verspreet, J., E. Dornez, W. Van den Ende, J.A. Delcour, and C.M. Courtin. 2015. Cereal grain fructans: structure, variability and potential health effects. Trends in Food Sci and Tech. 43:32-42.

Wieser, H. 2007. Chemistry of gluten proteins. Food Microbiology. 24: 115-119.

Wrigley, C., R. Asenstorfer, I. Batey, G. Cornish, L. Day, D. Mares, and K. Mrva. The biochemical and molecular basis of wheat quality. In: B.F. Carver, editor, Wheat: Science and trade. Wiley-Blackwell, Ames, IA. P. 495-520.

Yang, J., J. Zhang, Z. Wang, Q. Zhu, and L. Liu. 2004. Activities of fructan- and sucrose-metabolizing enzymes in wheat stems subjected to water stress during grain filling. Planta 220:331-343.

Ziegler, J.U., D. Steiner, C.F.H. Longin, T. Wurschum, R.M. Schweiggert, and R. Carle. 2016. Wheat and the irritable bowel syndrome- FODMAP levels of modern and ancient species and their retention during bread making. J. Functional Foods 25:257-266.

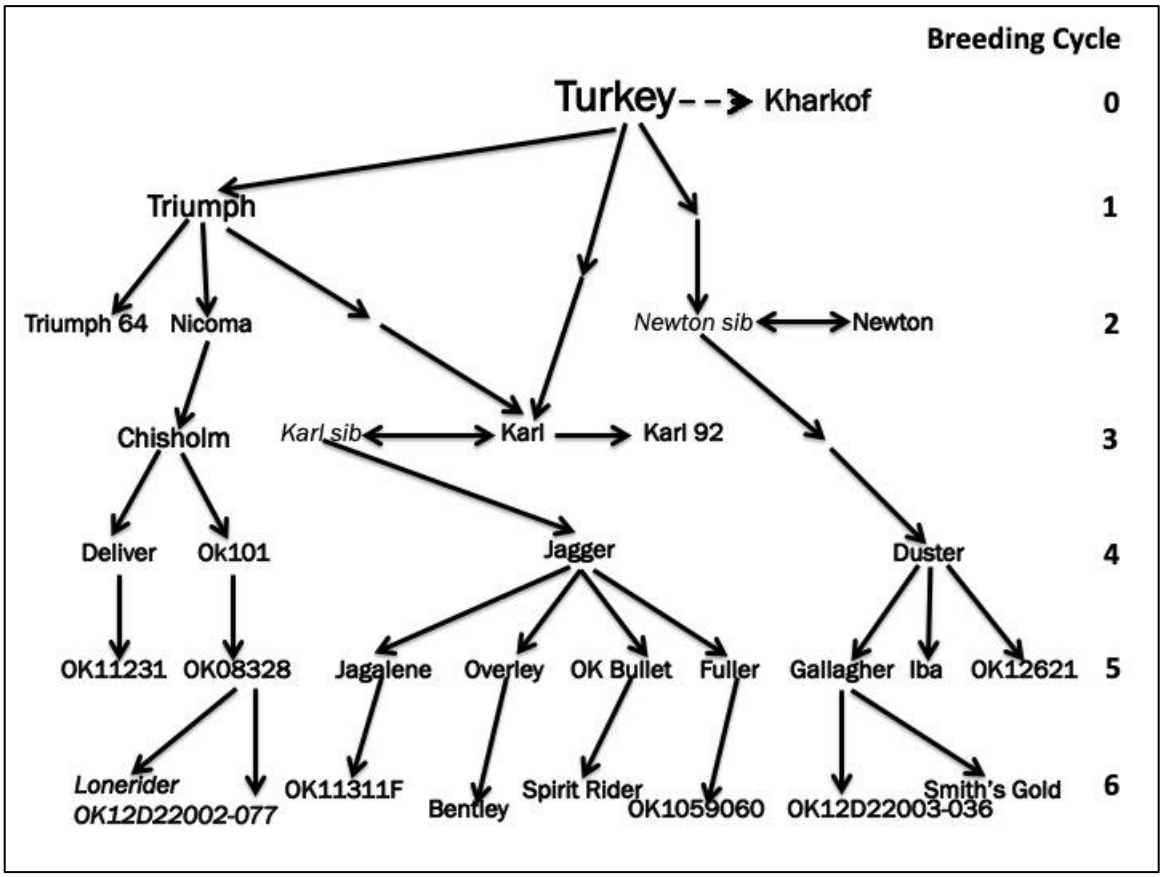


Figure 1. Three lineages of southern Plains-adapted winter wheat genotypes, arranged as six breeding cycles removed from Turkey as the primary ancestor.

Table 1. Variance component estimates from REML and significance levels for random effects of 28 to 30 genotypes evaluated for two to three years in Stillwater and Lahoma, OK.

Source of variation	**Grain yield††**	**Test weight**	**Days to heading†**	**Plant height†**	**Wheat protein**
	$(kg\ ha^{-1})^2$	$(kg\ hL^{-1})^2$	$Days^2$	cm^2	$(g\ kg^{-1})^2$
Environment (E)	0.05	1.21	0.16	0.25	1.44
Genotype (G)	0.03***	0.34*	0.02***	0.07***	0.31***
GxE	0.01***	0.74***	0.01***	0.01**	0.11***
Residual	0.02	0.61	0.02	0.04	0.20
Source of variation	**Flour yield†**	**Hardness index†**	**Kernel weight†**	**Kernel diameter†**	
	$(g\ kg^{-1})^2$		$(mg\ kernel^{-1})^2$	mm^2	
Environment	0.01	0.06	0.06	0.02	
Genotype	0.02***	0.31***	0.02***	0.004***	
GxE	0.002	0.03**	0.004*	0.001**	
Residual	0.02	0.10	0.02	0.004	
Source of variation	**Mix tolerance**	**Mix bandwidth†**	**Mix stability†**	**SDS sedimentation**	**CORE recovery index**
	$(1\text{-}10)^2$	mm^2		mL^2	$(\text{Degree of recovery (\%)})^2$
Environment	0.35	0.07	0.18	0.18	0.03
Genotype	0.46***	0.05*	0.32***	0.31***	0.17***
GxE	0.17***	0.09***	0.10***	0.10*	0.03**
Residual	0.41	0.22	0.06	0.52	0.08
Source of variation	**TPP/TMP**	**α-gliadin 2††**	**Milled flour fructans**	**Wholemeal fructans**	
		ppm^2	$(g\ kg^{-1})^2$	$(g\ kg^{-1})^2$	
Environment	0.04	0.05	0.04	0.03	
Genotype	0.005***	0.03**	0.02***	0.03***	
GxE	0.001*	0	0.003	0.01*	
Residual	0.002	0.07	0.01	0.02	

†, †† Square root and natural logarithmic transformation used on the response, respectively.

*, **, *** Significant at $p<0.05$, 0.01, and 0.001 respectively.

Table 2. Selection responses across six winter wheat breeding cycles for traits not depicted in Figure 2.

Trait (unit)	Mean of breeding cycle 0	Regression coefficient	*P > t*
Test weight (kg hL^{-1})	75.2	0.22	0.001
Heading date† (days)	10.5	-0.06	<0.001
Plant height† (cm)	9.7	-0.11	<0.001
SKCS kernel diameter (mm)	2.6	0.03	<0.001
SKCS hardness index†	7.7	0.10	<0.001
Flour yield† (g kg^{-1})	7.7	0.003	0.642
Mixograph stability†	3.3	-0.18	<0.001
Adjusted SDS sedimentation vol. (mL)	7.1	-0.07	0.014

† Square root transformation used on the response.

Table 3. Genetic correlation estimates derived from multivariate analysis of variance among 28-30 winter wheat genotypes evaluated for two to three years at Stillwater and Lahoma, OK.

	Grain yield		**Mixograph bandwidth**
Test weight	-0.04	SDS sedimentation	-0.18***
Wheat protein	-0.27***	TPP/TMP	-0.37***
α-gliadin 2	0.08	CORE recovery index	0.28***
Milled flour fructans	0.51***		
Wholemeal fructans	0.12		

*,**,*** Significantly different from zero at $p<0.05$, 0.01, and 0.001, respectively.

Table 4. Genetic variance estimates and significance levels for random effects among contemporary wheat genotypes from breeding cycles 4 through 6 and among heirloom genotypes from breeding cycles 0 through 3 for α-gliadin 2 and milled flour fructan content.

Source of variation	α-gliadin 2††		Milled flour fructans	
	Heirloom	Modern	Heirloom	Modern
	ppm^2		$(g\ kg^{-1})^2$	
Environment	0.06	0.04	0.03	0.04
Genotype	0.02	0.04**	0.04*	0.02**
GxE	0	0.001	0.01	0.001
Residual	0.07		0.01	

†† Natural logarithmic transformation applied to response.
*, **, *** Significant at $p<0.05$, 0.01, and 0.001 respectively.

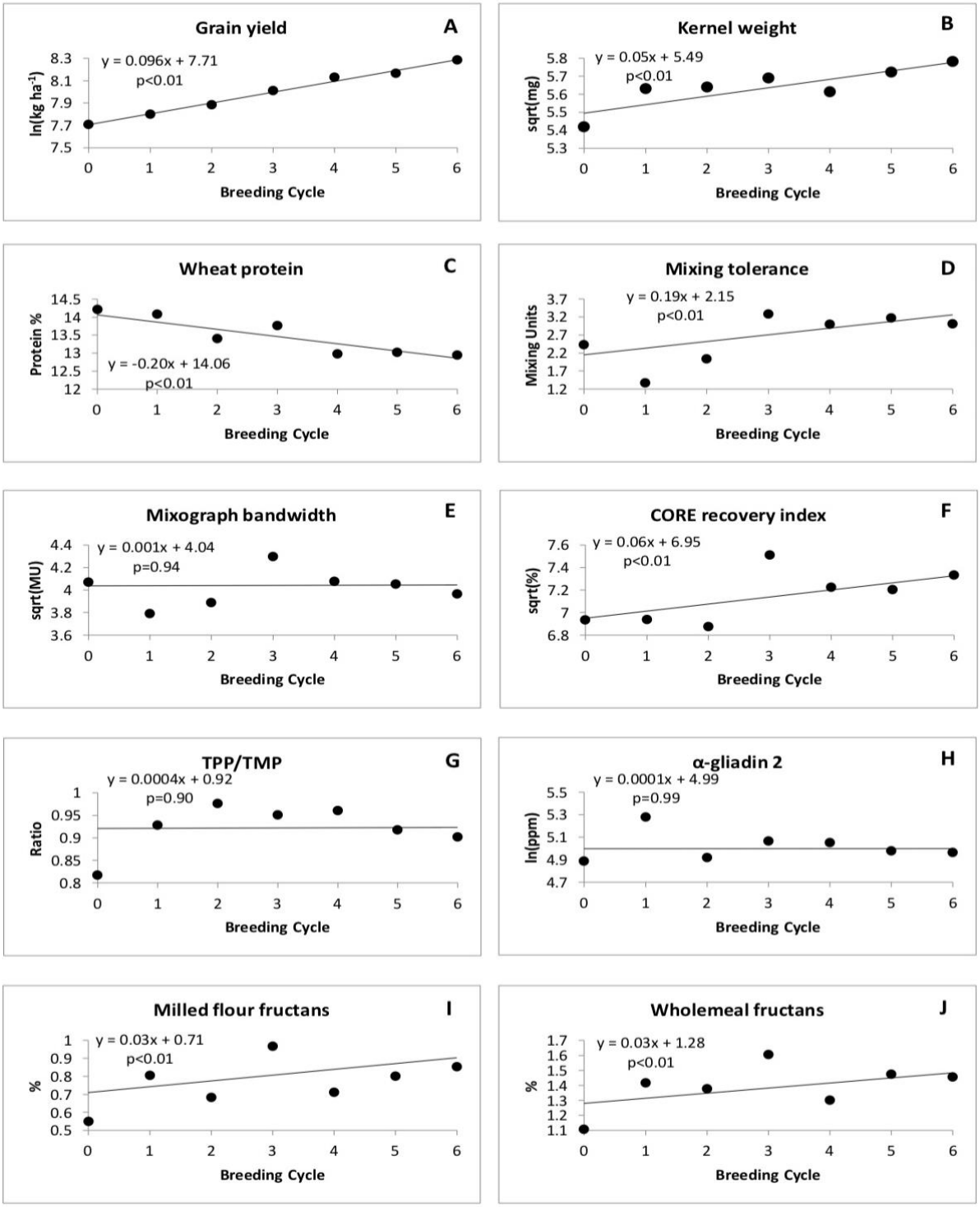
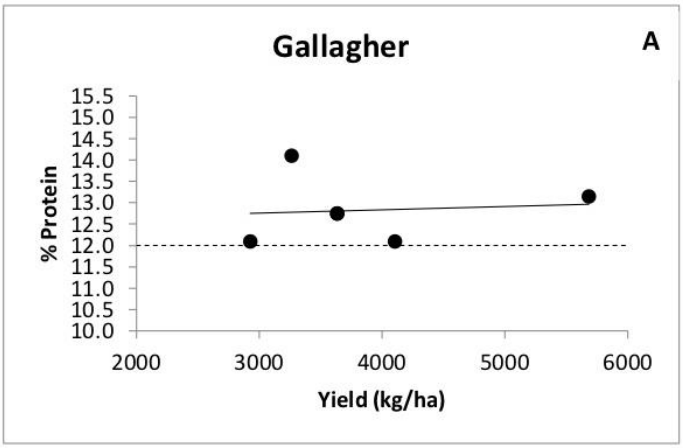
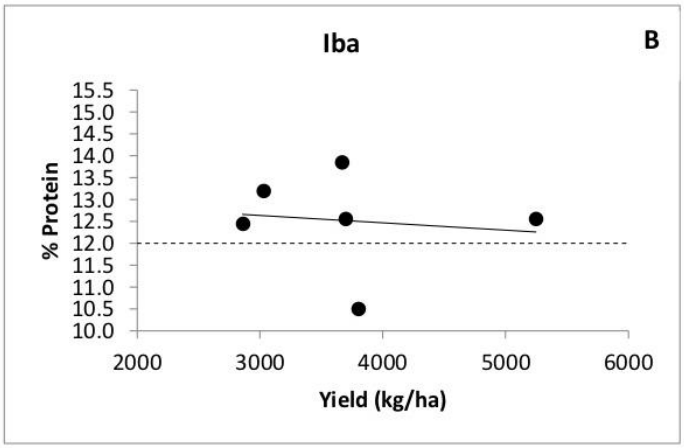


Figure 2. Selection responses for grain yield (A), kernel weight (B), wheat protein (C), mixing tolerance (D) mixograph bandwidth (E), CORE recovery index (F), TPP/TMP ratio (G), α-gliadin 2 (H), milled flour fructans (I), and wholemeal fructans (J) across six winter wheat breeding cycles.





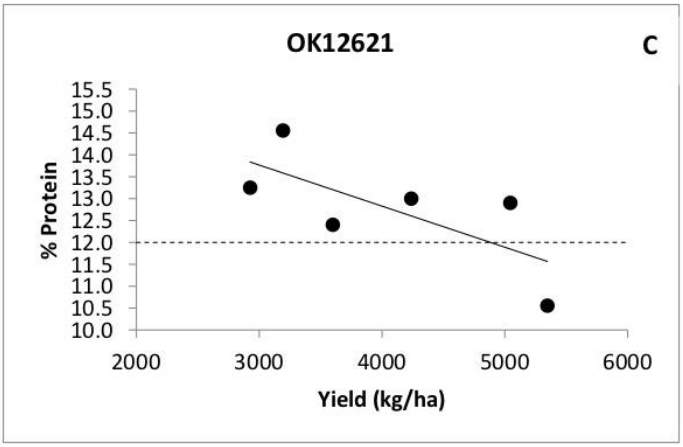


Figure 3. Representative linear response models for wheat protein versus grain yield within each of three half-sibs. Gallagher showed no change in wheat protein content over productivity levels (A), Iba showed a moderate decline (B), and OK12621 showed a steep decline (C).

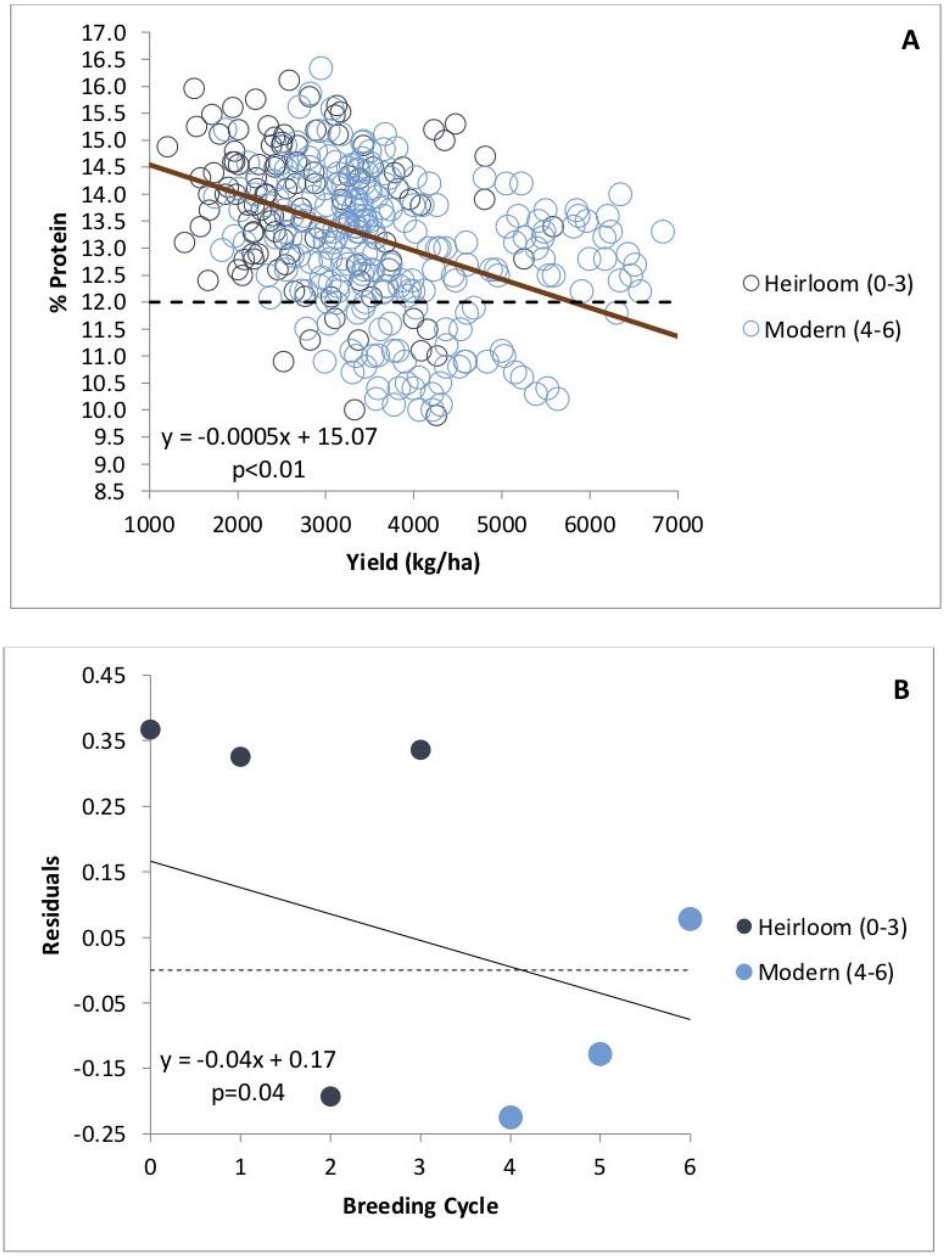


Figure 4. Residuals generated from the linear regression of wheat protein content versus grain yield for 30 winter wheat genotypes replicated within six Oklahoma environments (A), then plotted as a correlated selection response across breeding cycles (B).

APPENDIX

Table S1. Lineage and pedigrees of 30 hard winter wheat cultivars used in this study.

Entry	Name	HRW Lineage	Generations removed from Turkey	Variety type	Pedigree
1	Turkey	HRW Ancestors	0	Heirloom	
2	Kharkof		0	Heirloom	seln from Turkey
3	Triumph	Chisholm lineage	1	Heritage	Kanred/Blackhull (selns from Turkey)//Burbank Quality
4	Triumph 64 CItr 12132		2	Heritage	DANNE-BEARDLESS/BLACKHULL/3/KANRED/ BLACKHULL//FLORENCE/4/KANRED/BLACKHULL// TRIUMPH[39][533][1112][2331]
5	Nicoma		2	Heritage	Triumph/(Marquillo'/'Oro'//'Oro'/'Tenmarq)
6	Chisholm		3	Heritage	Early Sturdy/Nicoma
7	Deliver		4	Modern	OK91724(YANTAR/2*CHISOLM)/KARL
8	OK11231		5	Modern	Deliver/Farmec
9	Ok101		4	Modern	OK87W663 / Mesa //2180
10	OK08328		5	Modern	GK Keve/Ok101//Duster
11	Duster	Duster lineage	3	Modern	W0405D/NE78488//W7469C/TX81V6187
12	Gallagher		4	Modern	OK93P656-RMH3299(=Duster)/OK99711
13	Iba		4	Modern	OK93P656-RMH3299(=Duster)/OK99621
14	OK12621		4	Modern	P961341A3-2-2/OK93P656H3299-84(=Duster)
15	Smith's Gold		5	Modern	OK05511/Gallagher
16	OK12D22003-036		5	Modern	KS020638~5/Gallagher
17	Newton	Karl lineage	2	Heritage	Pitic 62 / Chris sib //2* Sonora 64 /3/ Klein Rendidor /4/ Scout (=NEBRED//HOPE/TURKEY-RED/3/CHEYENNE/PONCA)
18	Karl		2	Heritage	Plainsman V /3/ Kaw / Atlas 50 // Parker *5/ Agent (connected to Turkey via Kanred (seln from Turkey) – parent of Parker, and to Triumph via Agent (2 arrows back to each)
19	Karl 92		2	Heritage	Plainsman V /3/ Kaw / Atlas 50 // Parker *5/ Agent
20	Jagger		3	Modern	KS82W418(=Karl sib)/Stephens(=probably not)
21	Jagalene		4	Modern	Jagger/Abilene
22	OK11311F		5	Modern	Sabbe/Ok102 //Jagalene
23	Overley		4	Modern	U1275-1-4-2-2/Heyne sib//Jagger
24	Bentley		5	Modern	TAM 303/Overley
25	OK Bullet		4	Modern	Jagger//KS96WGRC39
26	Spirit Rider		5	Modern	OK Bullet/OK98680
27	Fuller		4	Modern	Jagger/Unknown
28	OK1059060		5	Modern	OK01307/Fuller
29	Lonerider	Chisholm lineage	6	Modern	Billings/OK08328
30	OK12D22002-077		6	Modern	Billings/OK08328

VITA

Liza Marie Van der Laan

Candidate for the Degree of

Master of Science

Thesis: INDIRECT RESPONSE TO YIELD SELECTION FOR END-USE QUALITY AND WHEAT-SENSITIVITY INDICATORS IN HARD WINTER WHEAT (*TRITICUM AESTIVUM* L.) ACROSS SIX BREEDING CYCLES AND THREE LINEAGES

Major Field: PLANT AND SOIL SCIENCES

Biographical:

Education:

Completed the requirements for the Master of Science in Plant and Soil Sciences at Oklahoma State University, Stillwater, Oklahoma in December, 2019.

Completed the requirements for the Bachelor of Science in Plant and Soil Sciences at Oklahoma State University, Stillwater, Oklahoma in December, 2017.

Experience:
Graduate Research Assistant (January 2018-Present)
Student Research Assistant OSU Wheat Breeding (May 2015-December 2017)

Professional Memberships:
American Society of Agronomy (2015-Present)
Crop Science Society of America (2015-Present)
Soil Science Society of America (2015-Present)