Effects of wild emmer introgression on hard winter wheat grain quality and use of the GlutoPeak to assess wheat quality characteristics

by

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Abstract

Among the most widely grown cereal crops, bread wheat (*Triticum aestivum* L.) is a vital source of nutrients and calories for many people across the world. Due to its unique grain quality characteristics, wheat is among the most versatile crops, producing a vast range of products that vary widely in formulation and among cultures. Wheat grain quality characteristics have profound influence on end product type and quality. Progress in breeding hard winter wheat for quality is limited by the long growing season and the inverse relationship between grain yield and grain protein concentration. Wheat breeders are faced with the uniquely difficult task of finding effective methods to sustain and improve grain protein concentration without compromising yield, and without increasing externalized production costs for fertilizer. One approach is to introgress wild emmer, *(Triticum turgidum* L. subsp. *dicoccoides*) into bread wheat. As an ancestral relative to wheat, it contains genes that have potential to improve wheat quality. The focus of this research is to evaluate the effects of wild emmer introgression on both the agronomic traits and grain quality of hard winter wheat. Accessions of winter wheat were planted at four locations for two growing seasons. The 94 winter wheat accessions in the study have a theoretical genetic percentage of 25% *T. dicoccoides.* Using several testing procedures, including solvent retention capacities (SRC) and the GlutoPeak, quality characteristics were assessed for each accession in each growing environment. Protein concentration, gluten strength, and protein quality score were highly heritable among the accessions. Relationships between quality traits and GlutoPeak parameters were also explored to determine the utility of the GlutoPeak as an early generation quality testing method, and several parameters were found to have correlations with quality traits. Maximum torque time was found to be highly correlated with the results of a hybrid lactic acid-sodium dodecyl sulfate SRC, which yields a measure of

gluten strength ($r = 0.63$, $P < 0.001$). Maximum torque was found to be highly correlated with grain protein concentration ($r = 0.76$, $P < 0.001$). This research complements previous research on the utilization of wild emmer to improve modern wheat and contributes to modern research on the applicability of the GlutoPeak for early generation quality evaluation.

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Chapter 1 - Introduction and Review of Literature

Introduction

Among the cereal crops, wheat plays an extensive role as a source of nutrients and daily calorie intake for billions of people across the world. With over an estimated 50 million metric tons harvested in the United States alone for the 2023-2024 growing season, it is a staple in almost every American's diet (Noltemeyer, 2024). The global harvest of wheat for this year was over 790 million metric tons, with the top producing countries being China, Russia, and the United States (USDA Foreign Agriculture Services, 2025). Wheat is one of the most versatile of the cereal crops, capable of making a wide range of food products across many cultures. Despite this, wheat has one of the lowest rates of yield gains of the commercial grain crops, likely because it requires not only breeding for increased yield but also sustaining grain quality. It is also often grown in environments with abiotic stress pressure, which can narrow the effect of yield gain for each accession (Bell et al., 1995).

Wheat-based products consumed by Americans every day include breads, cakes, pastries, tortillas, pastas, among many more. Each of these products requires specific flour parameters that are met by the flour mills. The presence of gluten forming proteins in wheat is what sets it apart from other cereal grains and is one of the components that lends itself to the versatility of the flour produced from wheat. Glutenin and gliadin are the two proteins that form the gluten network, which is fully developed after hydration and mixing of the flour.

The milling industry takes several grain parameters into account when formulating flours for bakeries, including wheat type, protein concentration, gluten strength, and more. The desired end product will dictate the flour requirements provided by the party responsible for the creation of the product. Wheat based products have a range of contributions to the diet of many people. It

is a significant source of calories, as well as vitamins, amino acids, and fiber (Shewry, 2018). Products containing whole wheat are higher in fiber, an essential component to a healthy diet. Wheat production is an absolutely essential sector of the agricultural industry and plays an absolutely vital role in the food industry. Additionally, there is a standard for grain quality that is essential to create high-quality end products with minimal additives.

Hard red winter wheat is the most commonly grown wheat class in the United States. This is a hard endosperm wheat that is planted in mid-autumn, allowed to overwinter, and is harvested during the summer of the following year. It is a hardy crop, and a popular choice in regions that face drought stress. Flour from this wheat is expected to have 10-12% protein concentration and is typically used in bread production, and as the base for all-purpose flour. Breeding programs that focus on the development of hard red winter wheat often have a focus on disease tolerance, insect resistance, and grain quality characteristics. For any trait being researched, having appropriate commercial comparisons is necessary to ensure quality output from the program. In this study, grain quality characteristics are a major focus of the evaluations. Notable commercial lines include Bob Dole (PI 690435), which is utilized in this study as a benchmark for optimal protein concentration and composition in commercial wheat. Another cultivar, Zenda (PI 683512) is used as a benchmark representing the lowest acceptable protein concentration and composition scale for commercial varieties. These benchmarks are based on evaluations of these two cultivars as check cultivars across trials and environments since 2021 (Guttieri, unpublished).

Having satisfactory grain quality is one of the most important factors in determination of the end-product type and quality. Quality is a function of both grain protein concentration and composition. There are two categories of protein in wheat, functional and non-functional. The

majority of non-functional protein is found in the bran and contributes mainly to nutritional quality. The majority of functional proteins are found in the endosperm of the wheat kernel and are constituents of the gluten network, which is responsible for the shape and texture of baked goods. The two proteins responsible for the formation of the gluten network are glutenin and gliadin, which interact to form the support structure during baking. These gluten forming proteins are activated during hydration and are key factors in the balance between the elasticity and extensibility of a dough. Protein concentration and gluten strength of a dough are characteristics of flour that is regularly communicated between the milling and baking industries. Another significant flour constituent is damaged starch; it has a relationship with water absorption of flour. These are characteristics that can be measured by breeders to ensure that the grain quality of their lines is functionally comparable to commercial wheat.

A historically significant focus of the wheat breeding industry has been on increasing yield to feed an increasing world population. There is a known inverse relationship between yield and the functional proteins that form gluten (M. J. Guttieri et al., 2015). This complicates the mission of wheat breeders. The primary purchaser of wheat varieties, the growers, look for options that will provide maximum yield. This leaves grain protein concentration as a secondary selection criterion in most breeding programs, a final characteristic to distinguish superior lines among populations created for other innovative purposes such as disease tolerance or yield increase. Wheat breeders have the uniquely difficult task of ensuring the optimal amount of grain protein concentration without compromising for higher yields.

One of the methods used in research to increase grain protein concentration is through the introgression of wild emmer *(Triticum turgidum* L. subsp. *dicoccoides)* (Nevo, 2014). This is a wild ancestor of wheat and has been proven to pass along favorable grain quality traits. It has a

tetraploid genome with two sets of chromosomes, AABB. However, wild emmer is still a wild relative and has many unfavorable agronomic traits of grass weeds. The seeds are protected by tenacious glumes on the head, and the rachis is brittle in order to easily spread seed (Nevo, 2014). These are two traits that are agronomically unfavorable, presenting complications during milling and having a negative impact on yield. Extensive work was done prior to the experiments reported in this study to eliminate families with these traits. Visual selection was useful in the selection against families that carried undesirable characteristics. The final accessions selected are phenotypically similar to wheat, lacking the traits of brittle rachis and tenacious glumes.

The Hard Winter Wheat Genetics Research Unit of the USDA, located in Manhattan, Kansas, functions primarily as a pre-breeding program, with the intention of creating parent lines of specific value for wheat breeders. One of the focuses of this program is to establish populations that can provide favorable grain protein germplasm, through the introgression of wild emmer. The population in this study was created by the backcrossing of various wild emmer accessions into two Kansas-adapted parents, KanMark (PI 675456) and a Kansas State University breeding line, KS090387K-20 (pedigree = Winterhawk/KS011020-6//Hitch). The wild emmer donor parents were selected to represent the diversity of the collection of wild emmer held by the Wheat Genetics Resource Center (WGRC) at Kansas State University. This study evaluates both agronomic performance and grain quality within this population, with comparison to their bread wheat parents and commercial quality counterparts.

The purpose of this study covers multiple facets of wheat breeding, both the agronomic effects of the wild emmer germplasm integration studied in field experiments, and the grain quality performance as also measured through multiple procedures utilizing both whole meal and milled white flour. One consideration for the types of flour used is that the additional step of

tempering and milling is required to obtain white flour. Whole meal is the most accessible option for many breeding programs, which includes all components of the wheat seed. There are many methods that can provide comparable data between white flour and whole meal, such as solvent retention capacities (M. J. Guttieri et al., 2004). While some methods can utilize either whole meal or white flour, other methods achieve superior results with white flour as a more accurate representation, without the bran inclusion. One of these flour quality assessments, using a new instrument, the GlutoPeak (Brabender, Anton Paar), is also explored in this study as an alternative to the traditional dough testing methods.

Consistent performance from wheat cultivars is an expectation of any line contending for commercialization. While other flour quality parameters such as damaged starch are pertinent information to mills, that is typically affected more by the conditions of the milling process that the endogenous differences among hard wheat cultivars. The protein concentration of wheat is known to be determined by genetics and management and is generally known to be a matter of quantitative protein gene accumulation rather than qualitative genes. The Grain Protein Content gene located on the B1 chromosome (*Gpc-B1*) is a trait previously introgressed from wild emmer that has a significant influence on not only protein content but the content of other nutrients in the grain (Tabbita et al., 2017). Another concern of wheat breeders is the interaction of grain protein concentration and environment. A wheat variety should ideally have only slight variation between environments. Any attempt to increase grain protein concentration, even with wild emmer introgressions, may have a decreasing effect on yield (Tabbita et al., 2017). This requires an approach to breeding for quality that quantifies protein and yield and allows for the selection of lines with a superior relationship.

One method to measure the effect increasing grain protein concentration had on yield is by calculating the grain protein deviation (GPD) for each genotype (Monaghan et al., 2001). This calculation creates a function that is representative of the GPD of the population, ideally from multiple site-years, and is an asset for wheat breeders looking to produce high yielding lines with high protein concentration. In order to select from the population effectively, a minimum yield threshold should first be chosen. Then, the selections should be focused on those with a positive grain protein deviation. Like most quality traits, grain protein deviation has been found to be repeatable (M. J. Guttieri et al., 2015). A genotype that has an acceptable yield and a positive grain protein deviation will likely be a good candidate as a parent breeding line. One consideration while using this method is that the protein gains may not be attributed to functional protein that increases the quality of the grain relevant to the milling and baking industries. The functional proteins are those responsible for the development of the gluten network, and these are vital in the production of high-quality products. Other selection criteria that might indicate lines with higher functional protein are by selecting for test weight and other seed characteristics, which are also measured in this study.

Methods to evaluate functional proteins and damaged starch have evolved, becoming higher throughput and requiring less material. Solvent retention capacity (SRC) testing is one of the methods used to evaluate whole meal and flour quality. Through the years of research, the methods have evolved from the low throughput system of using table shakers and measuring volume to utilizing centrifugation and by pellet weight measurements (Seabourn et al., 2012). These tests allow researchers to rank their genotypes by potential gluten strength and damaged starch content. Each assay has a known solvent that interacts with and binds the chemical constituent of concern, yielding a measurable change in volume or weight that indicates gluten

(Wilson et al., 2020) strength or damaged starch content (M. J. Guttieri et al., 2004). Lactic acid as a solvent in the retention capacity test is useful in the approximation of gluten strength of both a whole meal and flour sample (M. J. Guttieri et al., 2004). Sodium carbonate is the solvent of choice when approximating damaged starch levels of a whole meal or flour sample (M. J. Guttieri et al., 2004). There are specific procedures among this class of grain quality testing that require only a gram of flour or whole meal, making this type of testing accessible to breeders that have limited access to quality testing and small samples in early generations. It can be costly to send away samples for processing, and many dough rheology methods such as the farinograph, extensigraph, and mixograph are costly equipment. Most of the equipment to perform solvent retention capacities may already be possessed by research organizations with a diverse focus, such as a centrifuge and table shaker. These methods can also be performed on a high throughput basis, such as in this study, where tube racks of 24 are simultaneously completed.

Other quality procedures in labs that are commonly performed measure other parameters, such as water absorption and stability via the farinograph. Dough rheology characteristics can be measured such as elasticity and extensibility via the extensograph, which is an indication of a fully formed gluten network. There are a few drawbacks in common among these methods; they require both large sample sizes, have long completion times, and require the full attention of a skilled operator. This puts breeders seeking to perform early generation evaluation between families or genotypes at a disadvantage; these procedures require milling large sample sizes that most breeders do not have in the early stages of selection. In the early generations the seed produced is typically only enough for limited phenotyping and replanting for the next generation. Another complication is that between milling, sample preparation, run time and clean up between samples, there is a significant time requirement per sample. In the past, the mixograph

was an option for early generation testing, but has since faced less manufacturer support (Bock et al., 2024). Amid these drawbacks, there is a clear need for a high-throughput alternative to these traditional methods that has a smaller sample size and time requirement.

The GlutoPeak (Brabender GmbH and Co KG, Duisburg, Germany) is a high shear-based test using a rotating paddle that measures the torque of a stirrer in a slurry of flour and water over a length of time. When the peak torque is reached, it is an indicator that the gluten network has fully formed (Chandi & Seetharaman, 2012). Several measurements of importance throughout the GlutoPeak run time have been investigated, searching for potential correlations. Procedures vary, but most require less than 10 grams of white flour or whole meal and water and take only a few minutes to complete. The GlutoPeak has been a proposed high throughput stand-in for some of the other rheological tests, with correlations being found between farinograph water absorption and maximum torque (Wang et al., 2017), and correlations between peak maximum time and both mixograph and gluten index, indicators of gluten strength (Sissons & Smit, 2018). The GlutoPeak has shown merit in predicting certain flour and dough quality factors, but further investigation is necessary to confirm these and discover any additional quality factors that may be predicted.

In an industry driven by increasing wheat yields, wheat breeding programs have an essential commitment to maintaining and improving grain quality. There are many options to collect grain quality data, although some have extensive requirements and high costs. Wheat breeding programs need to have accessible and reliable procedures for assessing the value of the lines being produced. In order to test the utility of wild emmer as a germplasm source for desirable grain quality traits, this study analyzes data collected on agronomic traits and grain quality traits with the intention of producing parent lines or germplasm resources for wheat

breeders. Two commercial varieties with extensive historical data were selected to perform as checks, Bob Dole and Zenda. The two recurrent parents were also utilized as checks, to illustrate the effect that the wild emmer introgression had on the established lines. Alongside the two original recurrent parents, Bob Dole and Zenda were used as benchmarks during the evaluation of families to determine if the families within the population are comparable to the expectations of typical commercial varieties; Bob Dole is acknowledged as the goal for grain protein concentration and quality, while Zenda was designated as the lower benchmark. Using this set of checks, this study aims to determine the effect of wild emmer introgression on both agronomic and grain quality characteristics. This experiment also contributes to current research being done to improve the utility of the GlutoPeak relative to predicting results of other traditional quality testing methods. The ultimate goal of this study is to contribute to the research on improving grain protein quantity and quality and developing more efficient methods for testing.

Review of Literature

The History and Importance of Modern Wheat

Wheat has been a staple in the human diet since its domestication in the Fertile Crescent nearly 12,000 years ago. Following two suspected hybridization events, domestic wheat as we know it achieved a hexaploid genome, with three sub genomes: AA, BB, and DD (Balfourier et al., 2019). Current theory credits the first hybridization event as contributing the AA and BB genomes, while the DD genome is believed to have been added in a second event (Levy & Feldman, 2022). The AA genome is believed to be contributed by *Triticum urartu*, while the BB genome is believed to be supplied by an extinct member of the Sitopsis section of *Aegilops* genus, with *Aegilops speltoides* as the most closely related existing species. In a later

hybridization event, the AABB tetraploid wheat was believed to have crossed with *Aegilops tauschii,* which contributed the DD genome, completing the hexaploidy of modern wheat (Pont et al., 2019). After these fortuitous hybridizations, wheat spread from its center of origin to the neighboring early civilizations, feeding humanity as societies grew and advanced. As the consumption of wheat spread from its center of origin, it achieved more traits seen in the domestication of cereal crops; it became free threshing, from a dominant gene on the Q locus (Shewry, 2009). It also became less likely to shatter through a mutation at the brittle rachis locus; this is beneficial for minimizing seed loss to pre-harvest shattering (Shewry, 2009).

This hexaploid combination of genomes gave way to the domesticated wheat that many global societies utilize extensively. Commonly grown in regions of both the United States and around the world that have lower average rainfall (Sowell, 2024), wheat is a hardy crop responsible for the flour that consumers can use to make bread, pasta, and other baked goods. The type of product is determined by the classification of wheat, and the protein levels. Made useful by the presence of gluten-forming proteins, even minor modifications of ingredients in recipes can produce vastly different products, with types varying across cultures. For example, hard red winter wheat with an average 12% grain protein concentration, is commonly used as flour to produce bread (Wheat Foods Council, 2024).

In the United States, wheat is typically the crop with the third most planted acres per year, following corn and soybeans. The 2023-2024 growing season for wheat was one of the most productive in wheat production in recent years. According to a World Grains estimate, 18.66 million hectares were planted across the United States, producing an estimated 53.64 million metric tons (Noltemeyer, 2024). Wheat grain production is critical for the manufacturing of many food commodities that feed the entire United States. On top of the need for grain, there

are certain chemical quality factors of the grain that must be met to provide the best quality end-products. There are various factors that affect the progress of wheat breeding, including genetics, industry influences, and technology limitations and advancements.

For winter wheat, a few unique obstacles that slow the progress in wheat breeding are having a massive genome size around 16 Gbp (Alonge et al., 2020), and a roughly eight-month growing season in the field. Unlike other grain crops, winter wheat requires a vernalization period and is planted in the fall and left to overwinter, to be harvested in the summer. It can take much longer to complete line development and selective advancement programs. Alongside an extended breeding timeline, the self-pollinated crop produces seeds that are typically not genetically inferior to the previous generation (unlike some commercial hybrids), allowing farmers to plant saved seed, which is also not profitable for seed companies (Kusunose et al., 2023).

Another issue faced by the producers of wheat cultivars is the inverse relationship between grain yield and grain protein content; in most cases there is a grain quality sacrifice in the name of increasing yields (Vitale et al., 2020). There are many factors that contribute to this relationship, including genotype, temperature, and factors that affect starch synthesis (Dupont & Altenbach, 2003). Wheat is also one of the most prolifically grown crops in the United States that is not produced as a genetically modified organism (GMO); likely because unlike corn and soybeans, most wheat grain goes towards human consumption. In a market that still grapples with GMO acceptance of other crops, traditionally bred wheat varieties are still the only option for growers (Kusunose et al., 2023). Other issues faced by wheat breeders are the limits imposed by breeding for higher yield potential; yield is often a crop grown in and designed for regions with high abiotic stress potential, complicating the selection of elite genotypes based one genetic

by environment impacts (Fischer, 2007). It is necessary for wheat varieties to be more adapted to designated environments, due to the variable GxE varieties can have (Bell et al., 1995). In order to breed for varieties that can be adaptable to more diverse environments, it would be imperative to have a heavy focus on the GxE effects of each genotype (McKendry et al., 1995). Aside from the concerns with marketability, wheat is genetically much closer to its ancestors and wild relatives, as exhibited by the ability to cross with other grass species native to the fertile crescent, such as *Triticum dicoccoides,* commonly known as wild emmer (Shewry, 2009). In the past, there were fewer genetic tools available to wheat breeders compared to those that work with other crops, likely contributing to the slower genetic gain. There are just a few examples that illustrate the complexity of wheat production, and why it fell behind the massive advancements of corn and soybeans in the past.

Regarding nutrition, wheat is consumed by many people around the world every day, playing a much larger role in some cultures than others. In some end-product forms, it can be a great source of fiber, vitamins, and minerals (Shewry, 2018). It can also be further fortified with vitamins and minerals, to address nutritional deficiencies worldwide (World Health Organization, 2023). There is also evidence that suggests that upon development of the protein content of wheat, it can also be a source of protein in human diets (Alomari et al., 2023).

Wheat Flour and Grain Quality

Wheat has many methods of classification in the United States: hardness of endosperm, kernel color, and growing season behavior. Other major wheat producing countries have similar categories and even discriminate further by growing region, such as Canada (*Canadian Wheat Classes*, 2019). All systems of classification serve the same purposes: to identify varieties by

optimum growing environment and season, and the end use of the flour from the grain. Endosperm hardness is broken into two classes: hard and soft wheat; referring to the texture of the endosperm, which is determined by mutations in genes encoding puroindoline proteins (Morris et al., 2001). There are distinctly different uses in the baking industry for each endosperm type, with hard wheat generally being chosen as bread wheats and soft wheats selected for sweet breads and pastries (Wheat Foods Council, 2024). Hard wheats are often selected for use in products with high moisture content, due to the higher flour water absorption capacity compared to soft wheats (Sapirstein et al., 2018). The hardness of the endosperm is one of the most influential factors in the selection of flour based on the desired end product (Morris et al., 2001). Other than hardness of grain, wheat is also differentiated by the growing season type, spring or winter, and color of grain, typically red or white. The categories of wheat include hard red winter wheat (HRW), soft red winter wheat (SRW), hard red spring wheat (HRS), hard white winter (HWW), club wheat, and tetraploid durum wheat. Hard red winter wheat is typically utilized in the production of bread, rolls, all-purpose flour, and cereal (Wheat Foods Council, 2024). This bread flour typically has a higher gluten content compared to flour from the other classes. Hard red spring wheat is used for higher quality bread products, such as bagels, croissants, and pizza crusts; it is also used as an "improver" in flour blends, essentially improving the quality of flour with sub-optimum gluten strength (Wheat Foods Council, 2025b). Soft red winter wheat is most often used in the production of products like cookies, crackers, pretzels in pastries; due to its low-gluten content, products made from these doughs have less "rise" (Wheat Foods Council, 2025b).

White wheat has two recognized categories, hard and soft. Soft white is typically used for cakes, pastries, and flat breads, while hard white is used for Asian noodle types, pan and flat

breads (US Wheat Associates, 2025). The last major category is durum wheat, a tetraploid species, that has the hardest grain; this is used for most of the United States' pasta production. The choice of flour type is generally dependent on the desired commodity; most of the flours are selected based on their chemical qualities, such as protein, gluten, starch, and other quality factors (Wheat Foods Council). If the flour produced by a certain classification of wheat does not meet the criteria needed for the desired end product, alterations can be made by mixing other flours or additives that can help reach the desired quality conditions (Ravi et al., 2000).

Flour for commercial bread production is expected to have a 12% protein concentration; this is also the percentage expected of hard red winter wheat grain. The protein types in wheat fall under two categories: about 75% are prolamins, consisting of glutenins and gliadins. The remaining 25% are non-prolamins referring to globulins and albumins (Khalid et al., 2023). In general, the globulins and albumins function as elements of metabolic functions. Unique to other grains, wheat has a complex protein called gluten, which is derived from the glutenins and gliadins (Biesiekierski, 2017). Gluten is the protein complex responsible for many of the rheological properties of flour, and the quality factor that sets wheat flour above products produced from other grains.

The process of baking bread consists of many chemical interactions, highly dependent on the quality of the grain being used to make flour. Most bread recipes include at least three items: flour, water, and a leavening agent, an agent that chemically creates the rising effect in dough. Typical leavening agents are yeast, baking powder, or baking soda (Bakerpedia, 2024). The expansion of gas bubbles depends on the bread type, and there may be more ingredients, but these are the most basic. The flour provides protein and starches for chemical interaction, the constituents necessary for the bonding of the ingredients. The water acts as a mixing agent,

combining the ingredients into a uniform dough and hydrating the flour molecules. Water also activates leavening agents, such as water-activated yeast. The leavening agent is responsible for carbon dioxide production, the gas bubbles that stretch the dough upwards and/or outwards. This is a basic description of the reactions involved in baking; it can vary based on the desired product and additional ingredients. But the factor that elevates dough made with wheat flour above doughs made with other grains is the structure preserving gluten network.

As a critical factor in breadmaking, breeding for appropriate protein quality is essential for wheat breeders (Thorwarth et al., 2018). The gluten content and strength ranges from high to low between the varieties of wheat. It is one of the functional proportions of protein concerning many baking qualities. One of the rheology characteristics of gluten is the balance between extensibility and elasticity, derived from the physical structure maintained by the gluten network (Anderssen et al., 2004). This refers to the dough's ability to rise or stretch without cracking or breaking. For example, with the optimal gluten elasticity, bread dough will rise to the desired loaf size without over-expanding which can cause the loaf to collapse (Khalid et al., 2023). Gluten content is one of the factors that determines what product can be made from flour. It is the completed network made of glutenin and gliadin after flour is hydrated (Melnyk et al., 2011). There are many significant dough and batter qualities that are influenced by the balance between these two building blocks; both stability and development time (the time at which the gluten network is considered fully formed) are influenced by the glutenin and gliadin contents of a flour (Schuster et al., 2023). Glutenin and gliadin perform very different roles in the mixing, proofing, and baking processes (Wieser et al., 2023). Glutenin contributes to the elastic properties of dough, providing resistance to overworking. Disulfide bonds are one source of the viscoelastic properties of dough (N. Ooms & Delcour, 2019). Inter-chain disulfide bonds contribute to the

high molecular weight (HMW) glutenins, large polymers that are linked to favorable flour quality in regard to baking (Shewry & Tatham, 1997). The addition of just glutenin to a dough can increase the maximum shear viscosity, creating a stronger dough (Uthayakumaran et al., 2000). Gliadin additions have been shown to soften dough, as well as decreasing development time (Barak et al., 2014). Gluten itself is such a valuable component that it is often extracted in the form of 'vital wheat gluten' and can be used in many food and medicine products as an additive that has many different functions (Biesiekierski, 2017).

Flour has classifications based on not only chemical components, but also which parts of the kernel are included in the final flour product. For example, whole wheat flour is made from the entire grain as the name suggests. Bran is the primary source of fiber, minerals, and B-vitamins. The endosperm is the largest portion of the kernel, at around 83% of the dry weight (Wheat Foods Council, 2025a). This is where most of the gluten forming proteins and carbohydrates are stored. The germ has additional protein and is a source of unsaturated fats. Due to the oil content, the germ is removed for most types of flour; it can cause premature rancidity (Wheat Foods Council, 2024). Breads that contain any portion of whole grains often have crumb texture complications; this is partially due to the dilution of gluten content with bran inclusion in the dough, another circumstance in which vital wheat gluten may be beneficial (Ngozi, 2014). White flour consists only of milled endosperm. Before milling for white flour, a process called tempering is performed on the wheat kernels to bring it to an appropriate moisture content, typically 13-15%. This helps loosen the bran, allowing it to be removed cleanly in larger flakes, which can then be used in products that require bran, fibrous health cereals being one example (Kweon et al., 2009). Tempering at a higher moisture can reduce the white flour yield but can increase flour quality (Kweon et al., 2009). For white flour, after the bran and germ is

sieved out, it is likely allocated to produce other commodities and is generally not wasted, having a high fiber content that is beneficial to both human and livestock diets, as well as additional benefits unlocked by the process of fermentation (Ma et al., 2024).

The different classifications of wheat and types of flour have chemical aspects that demonstrate the importance of one particular component: protein. As efforts to increase yield have advanced, grain protein levels can be compromised. This is due to the inverse relationship between yield and protein content, a trait shared by most grain crops (Monaghan et al., 2001). As yield increases, grain protein content decreases (Simmonds, 1995). This is a concern that does not necessarily affect farmers but instead could be a consequence that affects the milling industry's supply and the quality of goods for consumers. This is one reason that grain protein content is typically a secondary concern to wheat breeders, following yield. Yield and factors that contribute to yield such as strong agronomic traits and disease resistances are often primary concerns for growers; whereas a wheat variety that features a higher grain protein content has historically had lower yields (Teng et al., 2022) . However, it is not uncommon for the price paid for grain by the elevators to be dependent on protein concentration; grain with high protein concentration can be bought at a premium price, which provides incentive for growers to be concerned about preserving the quality characteristics of their harvest, even if GPC is secondary to yield (Bekkerman, 2021).

The struggle between improving yield and maintaining grain protein concentration has led wheat breeders to developing methods of raising grain protein content; one of which is the grain protein deviation (GPD), an asset for selecting lines from breeding programs that not only have high yield, but high grain protein content as well (M. J. Guttieri et al., 2015). The grain protein deviation is calculated on a per line basis; the first step is establishing a linear

relationship of yield as a predictor of grain protein content. The residuals of this linear relationship represent the grain protein deviation, the extent to which individual breeding lines either under-perform or over-perform for protein at a given yield (M. J. Guttieri et al., 2015). By selecting the lines that have a positive grain protein deviation on the high end of the yield range, wheat breeders can assure that the lines in their programs have potential to not only have higher yield, but adequate to high protein as well (Monaghan et al., 2001).

Known factors can affect the grain protein content and yield. Environmental effects can have a significant effect on both yield and protein content. Poor environmental conditions in the last month before harvest can lead to poor yields via decreased grain fill, but inversely an increase in protein. For example, heat or drought stress during the grain fill period can lead to a relative increase in protein content, with a decrease in endosperm accumulation (Nuttall et al., 2017). This can explain any interference in data comparing yield and protein content, especially in growing seasons with unfavorable environmental conditions (Oury & Godin, 2007). However, the scale of effect this has on a line varies greatly based on genotype (Ghimire et al., 2021). In order to determine the effectiveness of efforts to increase GPD, researchers and their support staff must have reliable methods of determining the protein content of a line and comparing it to the rest of the population, as well as a consistent set of reliable checks with sufficient historical performance data. Along with protein content data, it is essential to determine the quality of the protein, as the gluten-forming proteins are of particular concern in product-making.

Using Solvent Retention Capacity Procedures to Evaluate Flour and Meal Functionality

There are different methods to analyze the functional chemical components of whole meal and flour, and among these are the solvent retention capacity (SRC) tests. SRCs can give measurable data on the gluten content, damaged starch, and pentosan levels. (Kweon et al., 2011). These are all contributors to overall flour quality and can be quantified by the amount of solvent retained throughout the procedure. There can be variation in results on a lab-to-lab basis, but in general the correlation of genotypes between labs should remain similar, with some of the variation likely attributed to individual storage conditions (Siah et al., 2024). Experiments have been performed that can distinguish the most ideal SRC procedure parameters, which allow for the appropriate methods to be selected based on whether whole meal or white flour is available to the experiment (M. J. Guttieri et al., 2004).

For both whole meal and white flour sample types, SRC methods function by measuring the amount of solvent retained by the flour, through a chemical response based on solvent type (Kweon et al., 2011). As the chemical reaction progresses and bonds are formed, the sample retains solvent as a network is formed, causing the molecules of the semi-solid pellet or sediments to “grow larger” in volume and weight (Kweon et al., 2011). Different solvents test for different chemical factors. Procedures may vary, sedimentation methods utilize volume for measurement, while other methods have been hybridized using a weight-based measurement. The Cereals & Grains Association recognizes four different solutions in their Solvent Retention Capacity Profile that provide a measurement for starch, gluten, and pentosans. These four solutions are water, 5% lactic acid solution, 5% sodium carbonate solution, and 50% sucrose solution (AACC International, 2010f). This method also integrates centrifugation instead of

sedimentation to extract the data (AACC International, 2010f). Two commonly utilized solvents are 5% lactic acid and 5% sodium carbonate, to characterize gluten strength and damaged starch, respectively.

The lactic acid sodium dodecyl sulfate (lactic-acid SDS) sedimentation method provides an approximation of gluten strength, which is a predictor of bread loaf volume and other rheological traits. The AACC 56-70.01 method is one of the original lactic acid-SDS sedimentation procedures and relies on the analysis of volume by the measurement of sedimentation volume, before and after the swelling of the granules (AACC International, 2010e). This process takes around 30 minutes per sample; it is considered to be low throughput, while requiring a slightly larger sample size of six grams (Seabourn et al., 2012).

From these two source methods, a hybrid approach for the lactic acid-SDS SRC was developed that takes a fraction of the time and can run more samples simultaneously (Seabourn et al., 2012). The solutions used were selected from the AACC 56-70 method, while centrifugation in place of traditional sedimentation was modified from the AACC 56-11 method. This new method is considered to be a high throughput alternative to both of the aforementioned methods, requiring only one gram of dry sample and relatively less solvent (Wilson et al., 2020). The hybrid lactic acid-SDS SRC method is measured completely on a weight basis; the weight of the pellet is expressed as a percentage of the weight of the dry sample. Included in the equation is also a moisture adjustment, that accounts for the actual moisture percent of the sample, and adjusts it to 14%, which is the industry standard moisture percent for flour (AACC International, 2010c). This moisture correction allows for an accurate comparison between all samples after eliminating grain moisture content as a source of variability (M. J. Guttieri & Souza, 2003).

This hybrid method allows for the execution of a procedure that takes less than 20 minutes, to more samples at one time than typically allowed by the original methods. Regarding this more efficient method, when comparing the utility between white flour and whole meal, an essentially equivalent correlation to bread loaf volume was found between the SRC results of either whole meal or flour (Seabourn et al., 2012). Meaning, the application of this lactic acid method can yield significantly similar results between either whole grain or white flour. This is a method that can be trusted by labs with limited milling resources or sample sizes insufficient for white flour milling, with sometimes the only option being to produce whole meal (M. J. Guttieri et al., 2004). This makes it an option for early generation protein quality-based selection.

Another SRC method, utilizing a 5% sodium carbonate solution as the solvent, is used as an approximation of damaged starch, another important chemical component of wheat grain quality. While broken starch is thought to have less connection with genetics than it does with conditions of milling, it is still a significant factor to consider when determining flour quality, being a major indicator of water absorption quality of the flour (Jukić et al., 2019). The conditions that affect damaged starch concentration during milling vary as far as tempering conditions to roller diameter, speed, and pressure settings on the mill (Jukić et al., 2019). The genetic effect on damaged starch is a consequence of the hardness of the kernel; for example, hard wheats tend to have more damaged starch, while soft wheats tend to have less (Jukić et al., 2019). This procedure follows the same set up instructions as the lactic acid-SDS SRC 56-11.02 (AACC International, 2010f). This procedure is still considered to be higher throughput than the original alternatives (M. J. Guttieri et al., 2004). Like in the hybrid lactic acid-SDS SRC, the final weight of pellet is recorded and used to calculate solvent retention capacity of the whole

meal, with the same equation containing a moisture adjustment as mentioned before (Seabourn et al., 2012).

There are a few potential sources of variation in SRCs. Conditions of sample handling and storage can affect the quantitative results of SRCs, but there may still be useful qualitative results useful for discriminating between genotypes (Siah et al., 2024). Another potential source of variation is the inclusion of bran and germ in the dry materials, opposed to extracting those materials through milling (M. J. Guttieri & Souza, 2003). These findings demonstrate that whole meal can be a suitable stand in for flour, when considering the limited milling resources most breeders face and the need for rapid early generation grain quality testing.

The GlutoPeak and Methods of Assessing Flour Quality

Wheat breeders and quality technicians utilize a variety of instruments and procedures to determine which of their accessions are best suited for commercial use. Each test provides different information about a flour's quality related to end use. The farinograph, for example, measures stability, development time, and water absorption (Amjid et al., 2013). The water absorption of each flour can be an indicator of the correct proportion of ingredients for future baking purposes. The farinograph generally requires a large sample size, beyond the amount typically available early in the breeding process, as well as taking a significant amount of time to prepare, perform, and clean up after the test (Fu et al., 2017). The extensigraph measures the stretching ability of a dough, otherwise known as the extensibility (ability to stretch) and elasticity (resistance to stretching) (Amjid et al., 2013). The balance between these two extensibility and elasticity can influence final quality factors such as loaf volume. The mixograph measures the mixing qualities such as stability and dough development of a flour

throughout the mixing process (Amjid et al., 2013). This can lead to determination of which end-use product would be most appropriate for flour. While the mixograph was a past option for early generation seed testing, requiring around 10g of sample, there is concern due to lack of manufacturer support (Bock et al., 2024). Other manual procedures such as the solvent retention capacity tests can indicate the gluten strength (Seabourn et al., 2012) or damaged starch (M. J. Guttieri et al., 2004) of flour, both of which affect water absorption and other dough quality factors. The main drawbacks of these procedures are similar; they cost extra time and labor that could be spent elsewhere in a breeding program and often require sample sizes beyond the availability of seed.

The GlutoPeak has been proposed by many quality researchers as a higher throughput replacement for traditional rheology equipment. The GlutoPeak measures the torque of a stirrer in a slurry of flour and water over a length of time. When the peak torque is reached, it is an indicator that the gluten network has fully formed (Chandi & Seetharaman, 2012). Several measurements of importance throughout the GlutoPeak run time have been investigated, searching for associations with other flour characteristics.

Maximum torque has been identified in multiple experiments as a potential predictor of water absorption measured by farinograph (FAB). Wang et al. found that the maximum torque reached throughout the run time had a high correlation coefficient of (r = 0.96, at P < .001) with farinograph measures of water absorption, independent of the gluten strength of the flour (Wang et al., 2017). Similar findings from Fu et al., indicate that farinograph water absorption and maximum torque are related when using either the Buhler Test Mill (r^2 = 0.97) or the Quadromat Junior mill (r^2 =0 .96), demonstrating that there is likely little difference when comparing methods of flour preparation (Fu et al., 2017). This study also found that there was a stronger

relationship between FAB when including wheats of all classifications than when considered within the same classification, and that the maximum torque had no correlation ($r^2 = 0.02$) with the extensigraph dough strength (Fu et al., 2017). Yet more evidence is presented by Wang et al. in 2021, when maximum torque and farinograph water absorption were calculated to have a significant correlation for both whole meal ($r = 0.82$) and "refined flour" ($r = 0.94$); even though the whole meal is less highly correlated, it still has merit as a low-labor stand in for white flour, and is still significant at the $\alpha = 0.001$ confidence level (Wang et al., 2021). Some of other the factors found to influence maximum torque were mixing speed and flour to water ratio; an increase in both caused an increase in maximum torque (Wang et al., 2017).

The peak maximum time (PMT), the time at which the maximum torque is reached, and peak area have also been identified as parameters of interest. There is evidence that both the peak maximum time and peak area measurements are correlated with gluten strength (Wang et al., 2017). Another study similarly found that gluten index (GI) and peak maximum time had a significant correlation coefficient ($r^2 = 0.88$) (Sissons, 2016). Additionally, the peak area and peak maximum time were related to mixograph results (Sissons & Smit, 2018). Additional factors that can affect peak maximum time include salt concentration (via solvent) and type. There is an effect on peak maximum time dependent on solvent type, more evident at high salt concentrations in general, when compared to the same salts at low concentrations (Melnyk et al., 2011).

While there is evidence that measurements of farinograph, mixograph, and other traditional flour quality tests can be predicted by GlutoPeak measurements, further work is necessary to confirm these findings. There is also merit to research using flour made from wheat with a more diverse genome. The GlutoPeak has potential to predict similar characteristics of

these longer, less efficient tests as a reliable predictor of grain quality, which could narrow down the amount of accessions worth investigating further.

The Utilization of Wild Emmer Germplasm to Enhance Wheat Grain Quality

Wheat breeders have many performance factors to incorporate when improving wheat germplasm. Breeding objectives commonly include increased disease resistance and yield, both of which are concerns that often come directly from growers. These two concerns are often the focal points of a breeding program. Quality concerns are often considered to accompany other breeding concerns as a secondary selection criterion, due to the inverse relationship between yield and protein. In wheat breeding's pursuit to increase and maintain yields, protein content and quality are one of the final factors selected for.

Therefore, rapid and effective methods are needed to genetically increase grain quality, in a manner that does not interfere with yield and other desirable traits in new cultivars. Grain protein content in modern wheat is considered to be mostly as a quantitative or additive trait, with most wheat cultivars containing similar genes regarding grain quality. The answer could lie within the germplasm of wild emmer *(Triticum dicoccoides)* (Xie & Nevo, 2008). Wild emmer is a wild relative to domestic wheat and is a diverse germplasm source that features generous desirable grain quality components, such as glutenin (Peng et al., 2021). Given the status of being a native relative, wild emmer is a suitable source of compatible germplasm and has proven to be capable of providing these favorable quality genes to the wheat genome (Nevo, 2014). The similarities in traits such as flowering patterns and height between wild emmer and wheat also speak to the merit of utilizing wild emmer's genetic diversity (Pont et al., 2019).

While most protein content genes are thought to be quantitative, a few genes of large effect have been identified. The 3A and 3B chromosomes in wheat have been identified as associated with grain protein content (Kartseva et al., 2023). The functional allele of *Gpc-B1*, which can be integrated from wheat's wild relatives such as wild emmer, improved grain and flour quality in a population of durum wheat (Kiszonas et al., 2021). Additionally, the *Gpc-B1* loci from wild emmer is known to improve not only grain protein content, but also the micronutrient content of grain including iron, manganese, and zinc concentrations (Peng et al., 2021). The cloning of *Gpc-B1* has contributed greatly to the knowledge of its functionality in modern wheat. Aside from GPC and micronutrients, the presence of *Gpc-B1* can further benefit bread quality through increasing water absorption in hexaploid wheats, and pasta quality increased firmness in tetraploid wheat (durum) (Tabbita et al., 2017). This gene, which has been mostly lost from modern wheat due to any number of deletions or mutations throughout decades of breeding, has been found to successfully be re-integrated into the genome via introgression from native relatives that carry *Gpc-B1* (Tabbita et al., 2017). The *Gpc-B1* allele does not contribute solely to grain quality. It also has been linked to senescence in some wheat varieties (Carter et al., 2012). The *Gpc-B1* allele can accelerate the rate of senescence in bread wheat, in some instances increasing GPC via this mechanism (Uauy et al., 2006).

Another allele of interest to wheat breeding is the *Glu-A1y*, which is historically not active in any modern hexaploid wheats (Margiotta et al., 1996). This allele, found in tetraploid sources, also serves as a potential source of gluten strength improvement (G. Chen et al., 2018). The *Glu-A1y* is also expressed in wild emmer and is another potential source of grain quality improvement. This further increases the merit of using wild emmer germplasm to improve wheat grain quality; not only is grain protein concentration important for breadmaking ability, but also

for the nutritional benefit from increased trace minerals. There are cases in which this allele can be actively expressed in modern wheats (Yu et al., 2019), caution is necessary to ensure the preservation of other traits.

Wild emmer has proven to be effective at improving wheat grain quality following the integration of germplasm. Given the diverse genome found in wild emmer, it could serve as a germplasm source for not only quality traits, but other traits desired by breeders. The use of wild emmer to improve hexaploid wheat grain quality is imperative in the campaign to provide wheat that is both high yielding and high quality. As one of the most important and prevalently consumed crops in the world, it is imperative that the wheat breeding industry work to maintain the grain quality standards set by the milling and baking industry, in order for growers to continue to produce healthy and nutritional wheat.

Chapter 2 - Effects of Wild Emmer Introgression on Hard Winter Wheat Grain Quality

Introduction

There are many considerations wheat breeders must account for when attempting to create lines for commercial production. Modern pests of wheat are evolving beyond the old forms of control, genetic resistance or pesticide application; this prompts the need to breed for genetic resistances to pests. Commercial lines must also continue to grow in yield potential. The requirement of every line producing grain with optimal grain quality further raises the standards for potential commercial varieties. While the grower's primary concern is likely yield, the baking and milling industries are concerned with grain protein and other chemical quality components. These two primary concerns have an inverse relationship, complicating the route to commercialization (Monaghan et al., 2001). Growers are the primary consumers of a newly commercialized variety; yield is the primary concern for most breeding programs. This requires breeders to find quick and effective ways to integrate germplasm that express adequate grain protein content without negatively impacting yield potential (Kiszonas & Morris, 2018).

The introgression of wild emmer germplasm is one strategy to positively affect the grain protein content of wheat (Nevo, 2014). Not only is wild emmer diverse within its own species but also has the potential to share many genes that improve overall grain quality and flour performance (Kiszonas et al., 2021). The goal of this study is to evaluate the utility of wild emmer introgression into hard winter wheat. However, as experienced in this study, wild emmer also can contribute undesirable agronomic traits such as brittle rachis (grain lost to shattering) and tenacious glumes (low threshability). The extremely diverse genome of *T. dicoccoides* can provide not only favorable grain quality genetics, but also the weedy traits of wild native grasses

that ensure the plant can survive and spread offspring. These weedy traits can be easily selected against, as they are typically visible. After selections with undesired traits are removed, choosing the most efficient germplasm evaluation tools is necessary to sort through the remaining breeding selections.

The inverse relationship between yield and grain protein concentration has historically been a challenge for wheat breeders. One strategy to select for both high yielding and high protein content lines is through calculating a grain protein deviation for each accession (Monaghan et al., 2001). Grain protein deviation is the residual of the regression of grain protein concentration vs yield. By setting a minimum yield threshold, accessions that have a positive GPD (residual) can be selected. This is a method to select for increasing grain protein concentration without compromising for yield.

The objectives of this research were to study the agronomic and grain quality influence of wild emmer introgression in bread wheat. This includes measuring and calculating multi-trial best linear unbiased estimates (BLUEs) for every trait to assess the influence of eild emmer genetics on the phenology, physiology, total grain protein concentration, and functional protein concentration of a set of accessions.

Materials and Methods

Population Development

With the goal of advancing grain quality in mind, two Kansas wheat varieties were selected to serve as recurrent parents to be backcrossed to multiple donor parents of the wild emmer family *(Triticum dicoccoides)*. This population was intended to increase grain protein content and quality. The first cross to create this population was performed in the greenhouse in

spring of 2017, crossing one of the chosen recurrent parents with a wild emmer donor parent. In the fall of 2017, the F_1 progeny of the initial cross were backcrossed with each F1's respective recurrent parent. The two recurrent parents were KanMark (PI 675456) and a Kansas State University breeding line, KS090387K-20 (Winterhawk/KS011020-6//Hitch), which were crossed with accessions from a mini-core of 29 wild emmer *(T. dicoccoides)* selections that were chosen to represent the diversity of the WGRC collection. The two recurrent parents were chosen for their adaptation to Kansas climate, short stature, and good straw strength. These traits featured by the two recurrent parents were intended to offset the undesirable traits of the wild emmer donor parents, such as tall stature and poor straw strength as well as poor threshability and high shattering rates. One backcross was made with these recurrent parents, the subsequently produced plants possessing a theoretical DNA percentage that is 25% wild emmer *(T. dicoccoides)* and 75% respective recurrent parent, KanMark or KS090387K-20, on the A and B sub-genomes. From the recurrent and donor parent combinations, 21 families were formed.

Using visual selection during the BC_1F_2 generation, 10 non-brittle plants with acceptable levels of threshability were selected and selfed from each family, from which 5 $BC_1F_{2:3}$ plants were grown. The same visual selection was applied to the F_3 plants in the spring of 2019. In 2020, the remaining derived families of the $BC_1F_{3:4}$ generation were sent to Second Nature Research in Yuma, Arizona for seed increase. In 2020, the germplasm pool had increased to 384 $BC_1F_{3:4}$ families, comprised of 20 populations, and containing genetics from 16 *T. dicoccoides* accessions. Field trials began in 2021 at the Hutchinson field location in which 283 of these families, now in the $BC_1F_{3:6}$ generation, were trialed in the field in a partially replicated augmented design.

High selection pressure was applied the plants that presented with undesirable phenotypic expressions of the wild emmer traits; most of what was removed presented traits such as tenacious glumes and brittle rachis, both of which negatively impact yield. Subsequently, 96 derived lines were selected to be the population of focus. These accessions were chosen based on harvest dockage of less than 8%, a positive Grain Protein Deviation (GPD), short stature (less than or equal to 95 centimeters, and a low shattering score (less than 3 on a 0-9 scale). These were the lines that had the most ideal agronomic traits across the board; not only were they selected for high grain protein deviation, but also for traits that are valuable to growers, that reflect higher yield either based on factors contributing to yield such as good threshability and non-brittle rachis. The final 96 genotypes chosen were comprised of 76 different pedigrees, containing germplasm from 16 *T. dicoccoides* accessions. A range of 1-11 families were included per the 76 pedigrees. These 96 selections were advanced to the 2022 and 2023 growing season field trials, after which advanced grain quality evaluation was completed. All field and laboratory data included in this study was acquired from the two subsequent years of field trials following the 2021 Hutchinson trial. The accession names and pedigrees are listed in Table 2.1.

Table 2.1 Accession Names and Purdy Pedigrees

Accession	Pedigree
BOB_DOLE	BOB_DOLE
KanMark	KanMark
KS090387K-20	KS090387K-20
ZENDA	ZENDA
U8318_U8319_D4.2	KS090387K-20*2/TA 1464
U8323_A4.2	KanMark*2/TA 1108

Accession	Pedigree
U8323_B10.1	KanMark*2/TA 1108
U8323_C2.2	KanMark*2/TA 1108
U8323_C5.2	KanMark*2/TA 1108
U8323_E1.2	KanMark*2/TA 1108
U8323_E3.1	KanMark*2/TA 1108
U8323_E4.1	KanMark*2/TA 1108
U8323_E4.2	KanMark*2/TA 1108
U8330_U8444_U8574_U8346_A10.1	KS090387K-20*2/TA 1005
U8330_U8444_U8574_U8346_C8.1	KS090387K-20*2/TA 1005
U8330_U8444_U8574_U8346_C8.2	KS090387K-20*2/TA 1005
U8382_C8.1	KS090387K-20*2/TA 1077
U8382_C8.2	KS090387K-20*2/TA 1077
U8382_C9.1	KS090387K-20*2/TA 1077
U8382_D6.2	KS090387K-20*2/TA 1077
U8382_E3.1	KS090387K-20*2/TA 1077
U8382_E3.2	KS090387K-20*2/TA 1077
U8386_U8552_E1.2	KanMark*2/TA 1464
U8434_A4.1	KanMark*2/TA 1171
U8438_U8376_C2.1	KS090387K-20*2/TA 123
U8438_U8376_E5.2	KS090387K-20*2/TA 123
U8439_U8554_U8440_A3.2	KS090387K-20*2/TA 1000
U8439_U8554_U8440_C4.2	KS090387K-20*2/TA 1000

Accession	Pedigree
U8439_U8554_U8440_D4.1	KS090387K-20*2/TA 1000
U8439_U8554_U8440_D4.2	KS090387K-20*2/TA 1000
U8439_U8554_U8440_E6.2	KS090387K-20*2/TA 1000
U8439_U8554_U8440_E9.1	KS090387K-20*2/TA 1000
U8441_U8549_A2.2	KanMark*2/TA 1153
U8441_U8549_D8.1	KanMark*2/TA 1153
U8441_U8549_D9.1	KanMark*2/TA 1153
U8441_U8549_E1.1	KanMark*2/TA 1153
U8441_U8549_E1.2	KanMark*2/TA 1153
U8450_D3.2	KS090387K-20*2/TA 1059
U8450_E7.1	KS090387K-20*2/TA 1059
U8450_E7.2	KS090387K-20*2/TA 1059
U8452_U8447_U8539_C1.1	KanMark*2/TA 1191
U8452_U8447_U8539_E8.2	KanMark*2/TA 1191
U8453_A1.1	KanMark*2/TA 1000
U8453_A1.2	KanMark*2/TA 1000
U8453_A6.1	KanMark*2/TA 1000
U8453_C6.1	KanMark*2/TA 1000
U8453_D1.1	KanMark*2/TA 1000
U8453_D1.2	KanMark*2/TA 1000
U8453_D5.1	KanMark*2/TA 1000
U8453_D5.2	KanMark*2/TA 1000

Accession	Pedigree
U8453_E3.1	KanMark*2/TA 1000
U8453_E4.1	KanMark*2/TA 1000
U8453_E4.2	KanMark*2/TA 1000
U8454_U8446_A7.1	KanMark*2/TA 1077
U8454_U8446_B2.2	KanMark*2/TA 1077
U8454_U8446_C1 .1	KanMark*2/TA 1077
U8454_U8446_C1 .2	KanMark*2/TA 1077
U8454_U8446_D2.2	KanMark*2/TA 1077
U8458_U8459_U8460_U8471_A10.1	KanMark*2/TA 54
U8458_U8459_U8460_U8471_A10.2	KanMark*2/TA 54
U8458_U8459_U8460_U8471_C10.2	KanMark*2/TA 54
U8458_U8459_U8460_U8471_C4.2	KanMark*2/TA 54
U8458_U8459_U8460_U8471_C6.1	KanMark*2/TA 54
U8458_U8459_U8460_U8471_C6.2	KanMark*2/TA 54
U8458_U8459_U8460_U8471_D10.1	KanMark*2/TA 54
U8458_U8459_U8460_U8471_D8.1	KanMark*2/TA 54
U8458_U8459_U8460_U8471_D8.2	KanMark*2/TA 54
U8461_D10.2	KanMark*2/TA 1195
U8537_B5.2	KanMark*2/TA 123
U8537_C6.1	KanMark*2/TA 123
U8537_C6.2	KanMark*2/TA 123
U8542_A10.1	KanMark*2/TA 1411

Accession	Pedigree
U8542_A2.2	KanMark*2/TA 1411
U8542_A6.1	KanMark*2/TA 1411
U8542_B3.1	KanMark*2/TA 1411
U8542_C7.1	KanMark*2/TA 1411
U8542_C7.2	KanMark*2/TA 1411
U8542_C8.1	KanMark*2/TA 1411
U8542_D5.1	KanMark*2/TA 1411
U8546_A3.2	KanMark*2/TA74
U8546_A4.1	KanMark*2/TA74
U8546_A4.2	KanMark*2/TA74
U8546_B5.2	KanMark*2/TA74
U8546_C8.2	KanMark*2/TA74
U8546_D2.1	KanMark*2/TA74
U8546_D4.1	KanMark*2/TA74
U8546_D4.2	KanMark*2/TA74
U8546_D6.1	KanMark*2/TA74
U8547_U8433_D2.2	KS090387K-20*2/TA 1020
U8551_A5.4	KanMark*2/TA 144
U8551_B5.2	KanMark*2/TA 144
U8551_C5.1	KanMark*2/TA 144
U8551_D3.1	KanMark*2/TA 144
U8551_E2.2	KanMark*2/TA 144

Field Experiment

This study was conducted in the field over the course of two years, growing seasons of 2022 and 2023. Each year, the same four locations were planted across the state of Kansas: Ashland Bottoms, Hays, Hutchinson, and Colby, making a total of four site trials for each of the two years, the trial abbreviations noted in Table 2.3. The exact dates of planting and harvest were recorded, as well as the coordinates of each field location (Table 2.2). For all site-years, planting was operated entirely with a modified Hege 1000 research plot drill (Zurn Harvesting, Westernhausen, Germany). For both years, the Ashland Bottoms location was harvested using the Hege 140 (Zurn Harvesting, Westernhausen, Germany) All other locations for the 2022 and 2023 seasons were harvested using a Zurn 150 combine (Zurn 150, Zurn Harvesting, Westernhausen, Germany)

Each plot was approximately 6.44 m^2. The seeding rate for all plots over both years was 50 grams of seed per plot. The equates to a 77.64 kg/hectare seeding rate. The row spacing was 21.6 cm (8.5 inch) rows with 6 rows in each plot. Total yield was calculated on a kilogram per hectare basis. At each location there were two complete replications with 100 accessions each. Within each replication there were 10 complete blocks, in an α-lattice experimental design. Within each replication were four checks; one of each of the two recurrent parents, KanMark and KS090387K-20, and two commercial varieties chosen to be references for grain quality; Bob Dole was chosen as a higher grain quality goal, and Zenda was chosen to serve as a lower threshold for grain quality. These were selected based on multiple years of performance data in various HWWGRU trials (M. Guttieri, 2025). Between the two replications, there were 200 samples harvested from each of the four locations, for both years.

Table 2.2 Field Years and Locations

Year	Location	Location	Planting Date	Harvest Date
2022	Ashland Bottoms	39.14380, -96.63173	10/25/2021	6/28/2022
2022	Colby	39.38843, -101.06827	9/29/2021	7/6/2022
2022	Hutchinson	37.93001, -98.02908	10/7/2021	6/17/2022
2022	Hays	38.85361, -99.33464	10/6/2021	6/21/2022
2023	Ashland Bottoms	39.14417, -96.63370	10/17/2022	6/22/2023
2023	Colby	39.38874, -101.06427	9/28/2022	7/18/2023
2023	Hutchinson	37.92945, -98.02911	10/5/2022	6/29/2023
2023	Hays	40.55357, -98.16064	10/6/2022	6/27/2023

Table 2.3 Trial Abbreviations and Field Layout

Year	Location	Trial Abbreviation	Blocks	Ranges Deep	Rows Wide
2022	Ashland Bottoms	22ASM	20	10	20
2022	Colby	22CBM	20	20	10
2022	Hutchinson	22HUM	20	10	20
2022	Hays	22HZM	20	10	20
2023	Ashland Bottoms	23ASM	20	10	20
2023	Colby	23CBM	20	20	10
2023	Hutchinson	23HUM	20	10	20
2023	Hays	23HZM	20	10	20

A number of agronomic traits were recorded throughout both years. During the growing season, plant height excluding awns was recorded in centimeters for all four locations and both

years. Yield per plot was also recorded during harvest and converted to kilograms per hectare. The HarvestMaster Graingage (H3 Classic GrainGage, HarvestMaster, Logan, UT) was added and calibrated prior to harvest in summer 2023, assisting in data collection; it is capable of recording test weight, grain moisture, and grain protein as the samples pass through the combine. Following the addition of the HarvestMaster, grain moisture, and grain protein content were collected during harvest of year 2023 for locations Colby, Hayes, and Hutchinson. Additional data was collected at Ashland Bottoms in both 2022 and 2023, chosen due to close proximity to Manhattan; number of days to reach flowering and days to reach physiological maturity was recorded by Julian date for both years. Flowering and physiological maturity dates were defined as the day that the majority of the plot began flowering and the day the majority of the plot reached Feekes 11.4.

Sample Processing and Whole Grain Analysis

Post-harvest data was collected for both years. In 2022, all test weight data from each of the four locations was measured manually using the 151 Filling Hopper and Stand (Seedburo, Des Plaines, IL). All grain moisture and protein data for samples harvested the year of 2022 for each of the four locations was collected using near infrared spectrometry on the Foss 1241 Grain Analyzer (Foss Infratec 1241 Grain Analyzer, Dresden, Saxony (SN), Germany), which is an AACC approved method for protein concentration measurement (AACC International, 2010d). For samples collected during 2023 harvest season, all samples from Ashland Bottoms were measured for yield, test weight using the Seedburo 151 Filling Hopper and Stand, and grain protein and grain moisture using the Foss 1241 (Foss Infratec 1241 Grain Analyzer, Dresden,

Saxony (SN), Germany) after harvest. Gravimetric test weight was also recorded Additional data was collected on these samples after the completion of processing.

Grain Physical Characteristic Analysis

Additional data was collected on the samples from Ashland Bottoms and Hutchinson in the year of 2023, for a total of 400 samples. 300-500 seeds per sample were analyzed using the Cgrain Value (Cgrain Value, APB, Uppsala, Sweden). Individual pictures were taken of each seed, which are then analyzed and provide a measurement of the physical characteristics of each seed from a sample. These characteristics include the saturation level, the blue, green, and red hue measures of each seed, all of which are measurements of the pigments found in each seed. For each seed, the area, length, width, mean width, and thickness were calculated, from which volume could be calculated by the Cgrain Value. A summary file was created of the Cgrain Value data collected, using an R script that applied the data of each seed of every sample to calculate a representative mean and standard deviation of all physical seed characteristics for each sample.

Whole Meal Preparation

Following the harvest of each trial, each sample was cleaned using an Almaco Air Blast Cleaner (Almaco Air Blast Cleaner, Nevada, IA) blowing out the chaff, broken seed, and foreign materials. Near infrared reflectance (NIR) spectrometry was used to collect the whole grain moisture and protein data for all trials. All locations from the year 2022, and the 2023 Ashland trial were measured with the Foss Infratec 1241 Grain Analyzer (Dresden, Saxony (SN), Germany). The whole grain moisture and protein data for year 2023, locations Hutchinson,

Hayes, and Colby were measured using the H3 Classic Graingage (HarvestMaster, Logan, UT), which was implemented to the Zurn 150 combine (Zurn Harvesting, Westernhausen, Germany). All samples were stored in cold storage to maintain cleanliness while awaiting processing.

Whole meal samples were ground using a Udy Cyclone Sample Mill (2010-030 cyclone sample mill, UDY, Ft. Collins, CO, USA) with a 1 mm stainless steel screen, and stored in cold storage to maintain accurate moisture percentage. Whole meal moisture and protein were recorded for all samples using near infrared spectrometer on the Perten IM 9520 (Perten Instruments North America, Springfield, IL, USA).

Solvent Retention Capacity

Two solvent retention capacity (SRC) procedures were performed on each of the 1,600 whole meal samples. The hybrid lactic acid-sodium dodecyl sulfate (lactic acid-SDS) SRC, as described by Wilson et al. 2020, was completed on each sample in order to differentiate accessions based on respective gluten strength and to compare them to the two industry checks, Bob Dole and Zenda. A sodium carbonate SRC was also performed, as described by Guttieri et al. (2004), to evaluate damaged starch of each sample, which can be an indicator of the water absorption capacity of whole meal.

A hybrid lactic acid-sodium dodecyl sulfate (SDS) solvent retention capacity test (Wilson et al.) was performed on all samples, using parameters selected from Seaborne et al (2012) methodology. This is a hybridized, high throughput approach of the original sedimentation test from the AACC (AACC International, 2010e), and the solvent lactic acid (AACC International, 2010f) in combination with SDS. Using 24-count racks, individual centrifuge tubes were tared on the Mettler Toledo Scale (Mettler Toledo XSE105 Dual Range Scale, Columbus, OH, USA)

then approx. 1 gram of whole meal was added. The tubes were weighed again, and the exact weight of meal was recorded. 5 mL of .47% lactic acid solvent was added to each tube using a bottle top dispenser (Seripettor Pro, BrandTech Scientific, Essex, Connecticut, USA). Each tube was then vortexed for 6 seconds (Wilson et al.), to fully incorporate the meal into solvent. Immediately following, 20 mL of 1.25% (w/v) sodium dodecyl sulfate (SDS) solvent was added to each tube, and vortexed for 6 additional seconds. The rack of tubes was then secured to a table shaker (Orbi-shaker, Benchmark Scientific Inc., Edison, NJ, USA) and shaken for 4 minutes at 300 revolutions per minute. Immediately following that, the tubes were loaded into a balanced centrifuge (Beckman Coulter Avanti J-E, Indianapolis, IN, USA) for 2 minutes at 3200 x g. Each tube was decanted, removing the remaining supernatant, and left upside down for 5 minutes, to drain any lingering liquid into an absorbent lab mat. A "pellet" of material was left at the bottom of the tube. The entire tube was weighed again, and the weight of the pellet was recorded.

A sodium carbonate solvent retention capacity test was also completed on all samples (M. J. Guttieri et al., 2004). Similarly to the lactic acid-SDS method, all centrifuge tubes in a 24-count rack were tared. Approximately 1 gram of whole meal was added, and the tube and contents weighed, with the final weight of whole meal recorded. Using a pipette pump, 10 mL of a 5% sodium carbonate solution was added to each tube. Each tube was inverted once, to loosen the meal from the bottom of the tube. Each tube was vortexed for 15 seconds, the time necessary to fully incorporate the meal into the solvent. For the next 20 minutes, each tube was vortexed for an additional 15 seconds once every 5 minutes. Following the last vortex, the tubes were centrifuged for 15 minutes at 2000 x g (Beckman Coulter Avanti J-E, Indianapolis, IN, USA). The remaining solution was decanted, and the tubes left upside down to drain remaining supernatant for 10 minutes. Again, the remaining pellet weight was recorded.

For both procedures, a measure for solvent retention capacity percentage was calculated using the equation described by Seabourne et al. (2012). This calculation is a percentage utilizing the exact weight of the dry whole meal used and the pellet produced from each method. Included in this equation is a moisture adjustment. Using the whole meal moisture data collected from the Perten NIR, the exact moisture content of each sample was used to correct the SRC measures to a 14% moisture basis, which is considered the industry standard moisture for milled flour. This allowed for a more complete comparison between samples by removing the variation of moisture content.

Combustion Calibration

Randomly selected whole meal samples from 2022 and 2023 were sent to the Hard Winter Wheat Quality Lab in Manhattan, KS for combustion analysis using a LECO FP-428 nitrogen determinator (Leco St. Joseph, MI USA) according to the AACC method 46-30.01 (AACC International, 2010b). A factor of N=5.7 was used for protein determination. A combustion analysis correction was calculated using the protein percentage received from the HWWQL and the Perten NIR. This correction equation was applied to all. Using this combustion adjusted protein for all samples, a moisture correction was also applied. The protein concentration of all samples was adjusted to 14% moisture. To summarize, the final whole meal protein percentages utilized in data analysis were corrected with combustion analysis and adjusted to a 14% moisture basis.

Data Analysis

Data analysis was carried out using R and R Studio (R Core Team, 2024). The *tidyverse* (Wickham et al., 2019) package was utilized throughout, particularly the *dplyr* (Wickham et al.,

2023) and *ggplot2* (Wickham, 2016) packages. All data was imported and exported using *readxl* (Wickham & Bryan, 2023) and *writexl* (J. Ooms, 2024), and supported by the use of *openxlsx* (Schauberger & Walker, 2024) for modification of files. For the agronomic data collected from the field and the two SRC procedures, a mixed model with accession, year, location, and their interactions as fixed effects, and the random effect of block nested within year and location was created using the *lme4* (Bates et al., 2015). This model was used to calculate the Best Linear Unbiased Estimates (BLUEs) of all traits for every accession, experiment, and accession by experiment, using the *emmeans* package (Lenth, 2024).

Data collected from the Cgrain Value was condensed into a summary file. Because the data of only one site-year with two reps was collected, a two-step modeling approach was used. The expected means for each seed characteristic was calculated using the base *stats* package. The variances and heritability of these traits was calculated using a model with accession and block as random effects. The Shapiro-Wilks test of normality was also applied to each trait, listed in Table 2.5.

Grain Protein Deviation (GPD) for each accession was calculated by plotting the grain protein concentration by yield. Correlations between traits were calculated with the *Hmisc* package (Harrell Jr, 2025), using the rcorr function, supplemented with tools from the base stats package. Entry-Mean heritability [$H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_{Error}^2/\text{reps*E} + \sigma_{GxE}^2/\text{E})$], variances, σ_G^2 / σ_{GxE}^2 ratio, and likelihood ratio tests were performed using the packages *lme4* (Bates et al., 2015), *lmtest* (Zeileis & Hothorn, 2002), and *lmeTest* (Kuznetsova et al., 2017).

Results & Discussion

Variances and Heritability of Agronomic and Quality Traits

The variances, heritability, and σ_G^2 / σ_{GxE}^2 ratio for each trait in this study were calculated using R, and listed in Table 2.4. There was a very high genetic variance for yield, which is likely due to the very wide range of low and high yields. This could also be a factor in the relatively high heritability for this trait. Another important calculation made was the σ_G^2 / σ_{GxE}^2 ratio. For every trait, the ratio of genetic influence to genetic by environment effect was > 1, so throughout these results, the multi-trial Best Linear Unbiased Estimates (BLUEs) were used in distributions, plots, and correlations. The features of each trait will be discussed throughout the results, as well as the normality of the distributions.

Table 2.4 Variances, Heritabilities, and Genetic to Genetic by Environment Ratio for Agronomic and Grain Quality Traits.

	Variance				
Trait	σ^2_G	σ^2_{GxE}	σ^2_{Error}	Entry-Mean Heritability	σ^2_G / σ^2_{GxE}
Yield (kg/hectare)	184062***	89742***	135352	0.90	2.05
Grain Protein Concentration (g/kg)	197***	15.5***	34.3	0.98	12.7
Test Weight (g/L)	326***	76.1***	71.5	0.96	4.28
Lactic Acid-SDS SRC	490***	76.8***	87.9	0.97	6.38
Sodium Carbonate SRC	5.03***	1.48***	7.06	0.89	3.40
Protein Quality Score	4.43***	0.634***	1.01	0.97	6.99
Anthesis Date (JD)	1.05***	0.383*	1.68	0.87	2.75
Physiological Maturity Date (JD)	1.34***	0.343 n.s.	1.66	0.90	3.91
Grain Fill Period (days)	1.15***	0.264 n.s.	2.32	0.87	4.36
Height	23.5***	5.44***	14.3	0.94	4.31

*, **, *** indicate significance at $P \leq 0.05, 0.01, 0.001$, respectively. n.s., non-significant

Agronomic Physiological and Phenotypic Data from the Field

Many traits were considered both during and after the growing seasons, to interpret the influence of wild emmer germplasm within the experimental accessions. While comparing the distributions of accessions among the traits to the 4 checks, the Shapiro-Wilks test was used to calculate the normality and *P*-value of all traits. The accessions had a wide range of anthesis date and physiological maturity date (Julian Date) and grain fill period (days); many of the accessions had a shorter grain fill period than the checks, indicating an increased rate of senescence, likely due to the influence of wild emmer germplasm (Uauy et al., 2006). Many of the accessions flowered later than the recurrent parents and commercial checks regarding the estimated date of anthesis, with the latest anthesis date of an experimental accession being 135.7 Julian Days. The check with the latest date was KanMark, with an estimated anthesis date of 131.7. This distribution has a significant deviation from normal distribution ($W = 0.960$, $P = 0.006$**) and is the most visually right skewed of the of the four traits displayed in Figure 2.1. The estimates of the date of physiological maturity had a similarly wide range of distribution, with all of the checks except for the Kansas State University breeding line KS090387K-20 recurrent parent, which reached maturity later in this experiment. This distribution was also slightly skewed to the right ($W = 0.969$, $P = 0.026$*), a significant deviation from normality indicating that many of the experimental accessions may have inherited characteristics that resulted in later maturity dates, based on the distribution of checks. Both anthesis date ($H^2 = 0.87$) and physiological maturity date ($H^2 = 0.90$) were similarly highly heritable. Anthesis and physiological maturity dates both had a σ_G^2 / σ_{GxE}^2 ratio > 1, suggesting that these traits are more dependent on genotype than the effects of environment.

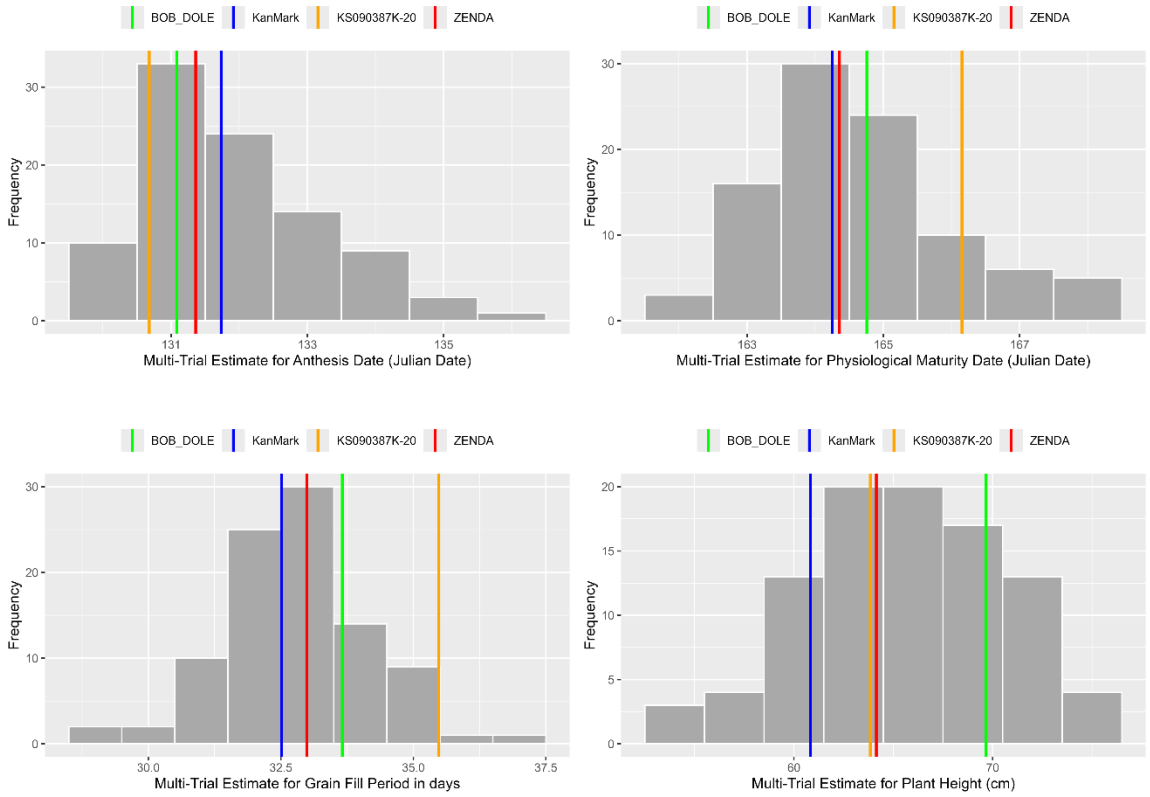

Figure 2.1 Distribution of the Multi-Trial Best Linear Unbiased Estimates of physiological maturity date, anthesis date, grain fill period, and plant height between the experimental accessions and the recurrent parents and commercial checks.

As for Grain Fill Period (GFP), the estimated distribution was found to be more stable (W = 0.968, P = 0.399 n.s.). GFP was also highly heritable among the accessions (H^2 = 0.87). Another agronomic trait, height, was also found to have a significantly normal distribution (W = 0.985, P = 0.354). However, when considering the estimated heights of the recurrent parents (KanMark = 60.8 cm, KS090387K-20 = 63.8 cm) were both less than the mean height of all accessions, 65.7 cm, it can be concluded that the wild emmer donor parents contributed to increasing height of the accessions, similar to the donor parents native form (citation). Further, the estimated height for commercial check Bob Dole was 69.7 cm, meaning that even though many of the accessions exceeded the height of the parents, the heights of the taller accessions were not unreasonably high in the context of commercial wheats. Height was another highly

heritable trait (H^2 = 0.94), and the high genetic to interaction ratio (σ_G^2 / σ_{GxE}^2 = 4.31) suggests that the effect of genetic variance on plant height has more influence than that of the interaction between genotype and experiment. Test weight was another physiological trait measured for all samples (Figure 2.2). Test weight had was highly heritable (H^2 = 0.96).

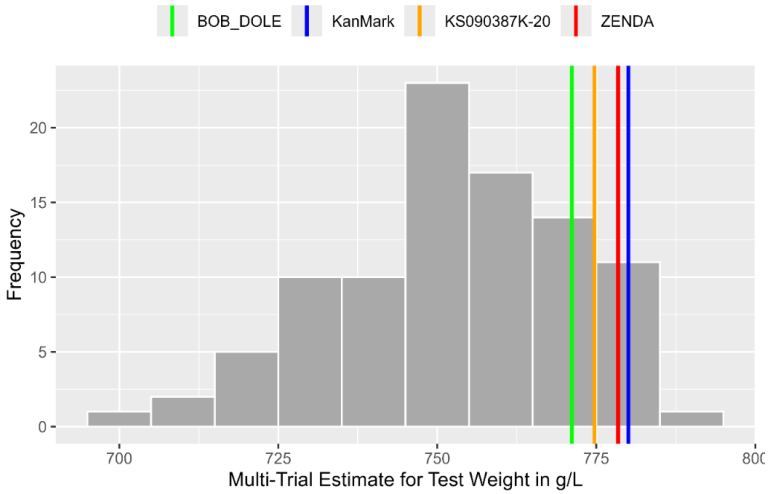

Figure 2.2 Distribution of the Multi-Trial Best Linear Unbiased Estimates of Test Weight.

Table 2.5 Table of Shapiro-Wilks Test of Normality Results (W) and P-Value.

Trait	Shapiro-Wilk Test Statistic (*W*)	P-Value (*P*)
Yield	0.979	0.126
Protein Concentration	0.949	0.001029**
Test Weight	0.976	0.078
Lactic Acid-SDS SRC	0.967	0.018*
Sodium Carbonate SRC	0.965	0.012*
Protein Quality Score	0.953	0.002**
Anthesis Date	0.960	0.006**
Physiological Maturity Date	0.970	0.026*
Grain Fill Period	0.986	0.399
Height	0.985	0.354

*, **, *** indicate significance of normality at $P \leq 0.05, 0.01, 0.001$, respectively. n.s., non-significant

Relationships Between Phenology, Physiological Data, and Yield

There were six physiological and phenological agronomic traits measured in this study. Figure 2.3 is a heatmap of the correlations found between traits. The only notable relationship between the traits collected in the field and the yield of the accessions was height, which was moderately positively correlated with plant height. Yield and GPC were highly negatively correlated (r = -0.73***), as expected. Anthesis date and grain fill period had a moderate negative correlation (r = -0.47***). Physiological maturity date had moderate positive correlations with anthesis date (r = 0.47***) and grain fill period (r = 0.56***), as well as a weak correlation with plant height (r = 0.36***). Test weight was also moderately positively correlated with yield (r = 0.51). GPC was also moderately positively correlated with the Grain Protein Deviation (GPD) of each accession (r = 0.68).

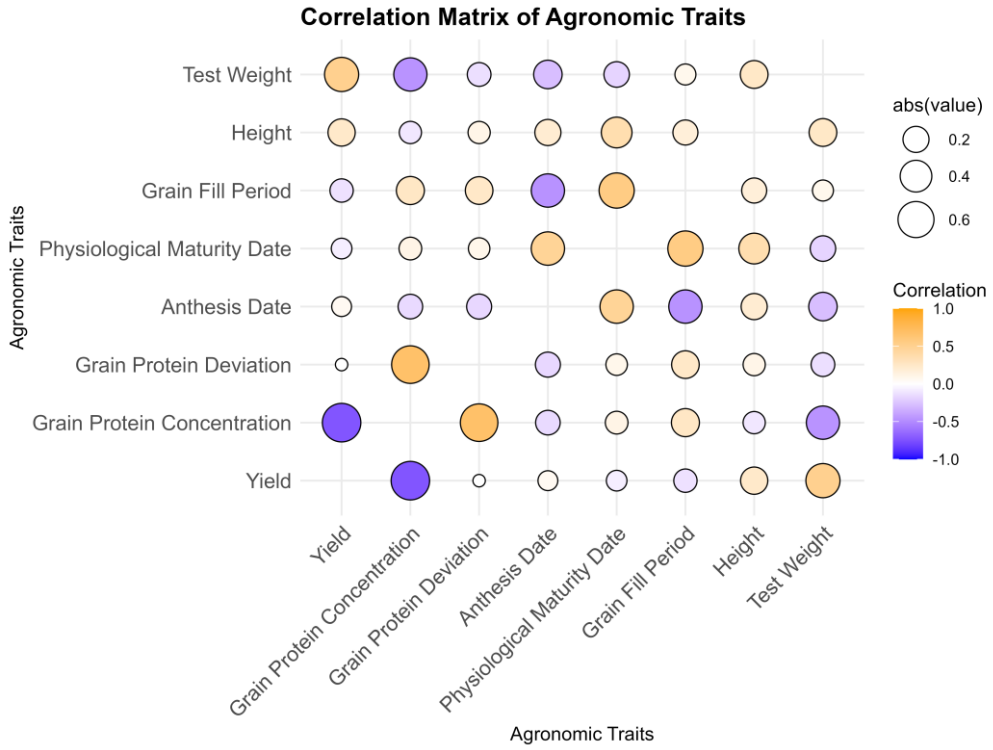

Figure 2.3 Correlations between agronomic physiological traits measured for all samples collected: yield, grain protein deviation, anthesis date, maturity date, grain fill period, and plant height.

Yield and Grain Protein Concentration

Yield and grain protein concentration are the two traits used to calculate grain protein deviation. With these accessions, analyzing them separately first reveals that the impact of the wild emmer introgressions had significant impacts on both of those traits.

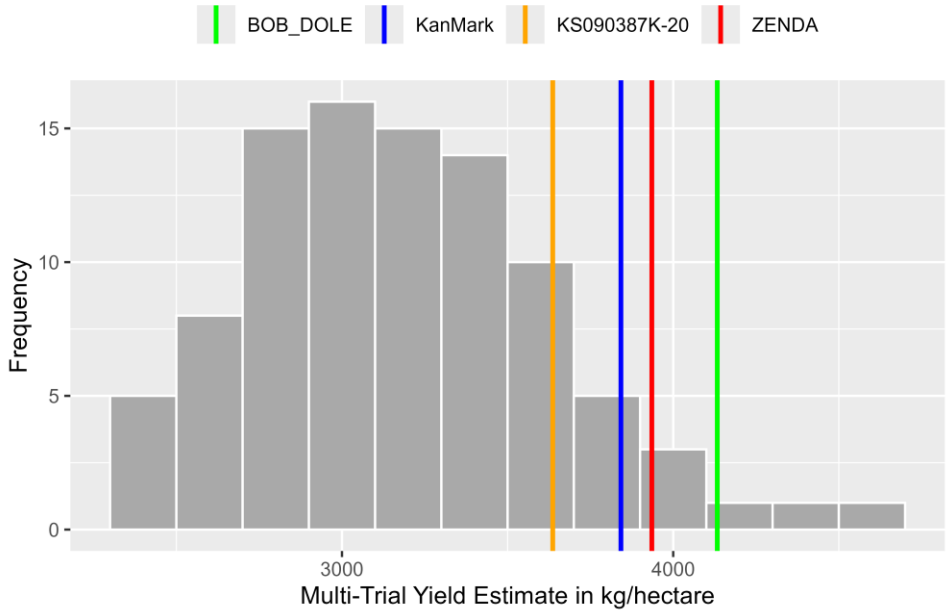

Figure 2.4 Distribution of the Multi-Trial Best Linear Unbiased Estimates of yield (kg/hectare) of each accession to the Recurrent Parents, KanMark and KS090387K-20, and commercial checks Bob Dole and Zenda.

As seen with the distribution (W = 0.979, P = 0.126 n.s.) in Figure 2.4, a large majority of the accessions had lower estimated yields than both the parent line and commercial checks. This is an expected effect, given the documented inverse relationship between yield and grain protein concentration. However, not every accession was beholden to this effect, as seen by small number of accessions on the right tail of this distribution, indicating that a select few had higher estimated yields than all of the checks. Yield was highly heritable (H^2 = 0.90), with σ_G^2 / σ_{GxE}^2 = 2.05, meaning that the effect of genetics was slightly greater than that of the effect imposed by genotype × environment interaction.

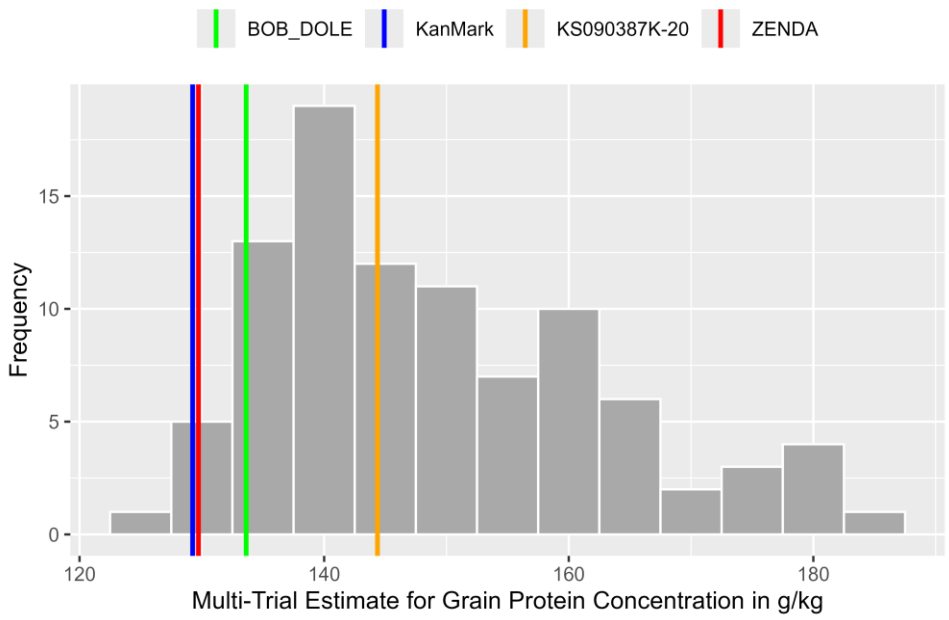

Figure 2.5 Distribution of the Multi-Trial Best Linear Unbiased Estimates of grain protein concentration (g/kg) of each accession to the Recurrent Parents, KanMark and KS090387K-20, and commercial checks Bob Dole and Zenda.

In contrast, grain protein concentration was very highly heritable (H^2 = 0.98) and was determined by genetic effect to a much higher degree (σ_G^2 / σ_{GxE}^2 = 12.7). Also in contrast to the yield distribution, the estimated grain protein content in most accessions was much higher than the checks; this distribution had a significant deviation from normality (W = 0.949, P = 0.001029**) (Figure 2.5). These results suggest that for most accessions, the wild emmer donor parents had a significant influence on a gain of grain protein concentration when compared to the estimates of both recurrent parents (KanMark = 129.2 g/kg, KS090387K-20 = 144.4 k/kg). The mean population estimate for grain protein concentration = 149.1 g/kg, more than estimates for both parents. However, the difference between the population mean estimate and recurrent parent KS090387K-20 estimate is much smaller than the difference between the mean and other recurrent parent estimate, KanMark. This suggests that while many accessions did have increased grain protein concentration, not every accession benefitted from the introgression. Also worth noting is the right tail of the distribution extends past 170 g/kg GPC; this is a much higher

concentration than is necessary for most non-specialty products made of flour and could have a potentially detrimental effect on end-product quality, especially if the grain protein composition is unfavorable (Sharma et al., 2020).

Grain Protein Deviation

Grain Protein Deviation (GPD) is a useful tool for breeders who are attempting to breed for higher GPC. Figure 2.6 is a visual example. The relationship between yield and GPC in this population is inverse, as expected. Yield and GPC were highly negatively correlated (r = -0.73***), making this population a suitable group of accessions to evaluate GPD from. The regression for this relationship is: $y = -24.0x + 6764.7$, $R^2 = 0.54$.

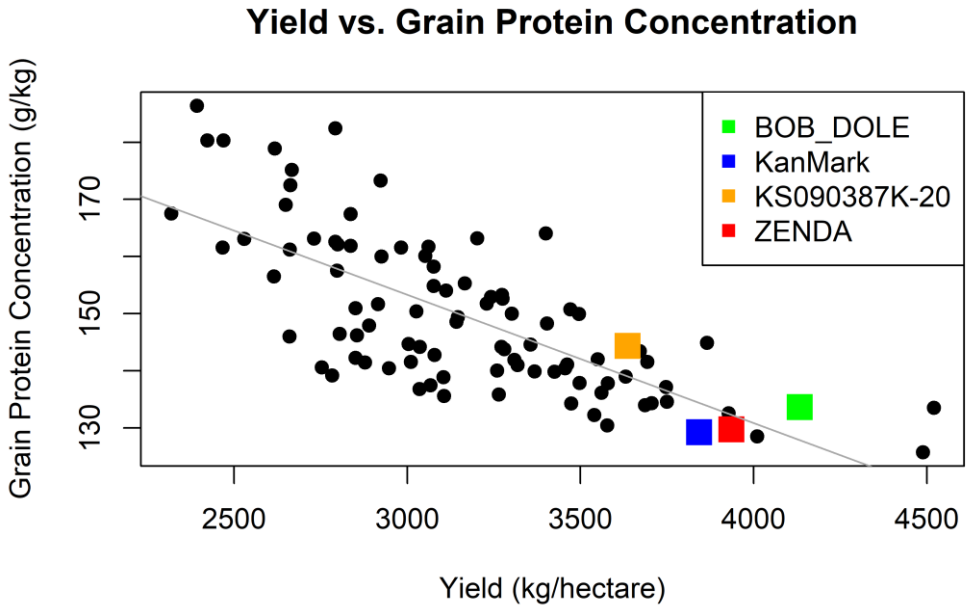

Figure 2.6 Plot of Yield vs Grain Protein Concentration, using best linear unbiased estimates for accessions and checks. An example of how Grain Protein Deviation can be used as a breeding tool using the experimental accessions.

To be used as a tool for selection among a population, a minimum yield threshold can be chosen to ensure the high yielding lines are selected. Then, accessions with positive residuals can be selected as those that have potential for high yield and high GPC. If these accessions were to continue through a breeding program, the accessions that are clustered on the lower right side would be the most viable selections. In this experiment, 43 of the accessions had a positive GPD, including commercial check Bob Dole and recurrent parent KS090387K-20.

Selection of Superior Accessions Based on Agronomic and Quality Characteristics

The selection of superior accessions based on yield, and then positive Grain Protein Deviation (GDP) produced two lines that had higher yield than the commercial check, Bob Dole. One of these checks also had a comparable estimation of GPC, and a very high GPD. These were selected using the multi-trial BLUEs of each trait. These high performing accessions are shown in the table below (Table 2.6).

Table 2.6 Selection of Superior Accessions Based on Yield and Grain Protein Deviation

Accession	Yield (kg/ha)	GPC (g/kg)	GPD
U8546_D4.1	4519.6	133.5	14.4
U8453_E4.1	4487.8	125.7	5.81
Bob Dole	4133.0	133.6	5.75
U8382_C8.2	3865.0	144.9	11.0

Grain Quality Analysis and Solvent Retention Capacities

Grain Protein Concentration (GPC), test weight, lactic acid-SDS SRC, sodium carbonate SRC, and Protein Quality Score (PQS) were the quality traits measures for all samples harvested across all locations for both years. This significant amount of data was used to compare the performance of the accessions, and to allow for the identification of accessions with superior

grain quality for the purpose of selection for breeding to improve grain quality. The distribution of GPC across accessions, discussed above in relation to yield, showed that the wild emmer donor parents had a large effect of the GPC of the experimental accessions.

The left panel in Figure 2.7 displays the distribution of results of the lactic acid-SDS SRC in weight value % form. This SRC given an estimate of gluten strength, which was also later used to create the PQS in this experiment. Bob Dole and the two recurrent parents had similar gluten strength expectations. The commercial check Zenda fell below the mean of these, which indicates that the two commercial checks were correctly chosen as the 'goal' (Bob Dole) and 'floor' (Zenda) for grain protein quality. This distribution for this trait deviates from normality ($W = 0.967$, $P = 0.018$*), with a negative skew (Figure 2.7). This is an indication that even if the wild emmer donor parents contributed to grain protein concentration, it does not ensure improvement of functional grain quality. For the purposes of selection for grain protein quality, accessions with similar or greater lactic acid-SD SRC than the recurrent parents and commercial check Bob Dole would be of great interest. The result of the lactic acid-SDS SRC was very highly heritable ($H^2 = 0.97$), and variances indicate that the genetic effect is much greater than the effect imposed by the environment (σ_G^2 / σ_{GxE}^2 = 6.38).

The right panel of Figure 2.7 displays the Distribution of sodium carbonate SRC results. The results of the sodium carbonate SRC suggest that most of the accessions had lower levels of damaged starch than the checks. This distribution also deviations from normality ($W = 0.965$, $P = 0.012$*) with a negative skew. This could be an indication that the seed of the accessions have traits that negatively impact the hardness of the seed, or other traits that influence the extent of damaged starch accumulation during milling. The thickness of the seed measured by the Cgrain Value was the only measured seed physical characteristic that had a moderately significant

correlation with the sodium carbonate SRC (r = -0.52***). The lower levels of estimated starch damaged in these accessions could be attributed to the effect of seed thickness, but the effect can also be dependent on the conditions of milling (Barrera et al., 2013). This is exhibited by the heritability (H^2 = 0.89) and σ_G^2 / σ_{GxE}^2 ratio (σ_G^2 / σ_{GxE}^2 = 3.40). The estimates of damaged starch was slightly less heritable, and the effect of genetic variance was also lower when compared to the effect imposed by the interaction with environment.

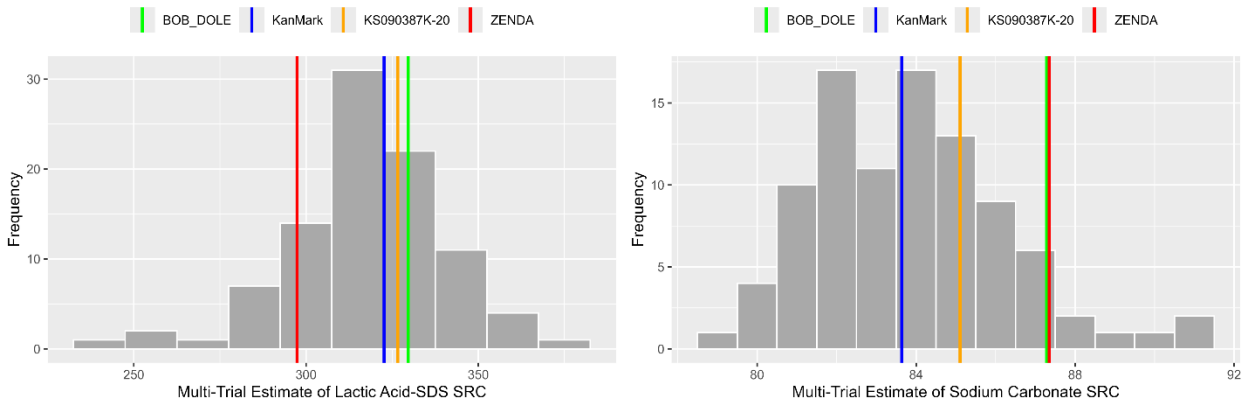

Figure 2.7. Distribution of best linear unbiased estimates of lactic acid-SDS and sodium carbonate SRCs among accessions, recurrent parents (KanMark and KS090387K-20), and commercial checks (Bob Dole and Zenda).

The distribution of the PQS (Figure 2.8) is an informative look into how the grain protein quality was influenced by the wild emmer donor parents. There is a significant deviation from normality for this trait (W = 0.953, P = 0.002**). All four of the checks had PQS higher than the population mean, while the distribution has a negative skew with multiple low scoring outliers. Recall the distribution of GPC, in which all four checks had estimated GPC less than the mean of the population. This, when compared to the estimates of gluten strength for the population, suggests that even if the wild emmer donor parents contributed to the increase of grain protein

concentration, it did not necessarily contribute to protein quality. Like both GPC and the lactic acid-SDS SRC, PQS was very highly heritable (H^2 = 0.97) with genotype having a much larger influence over variance than the interaction with environment (σ_G^2 / σ_{GxE}^2 = 6.99). When breeding to improve grain quality, especially when utilizing ancestral germplasm, the knowledge that the functional proteins can be inherited is invaluable.

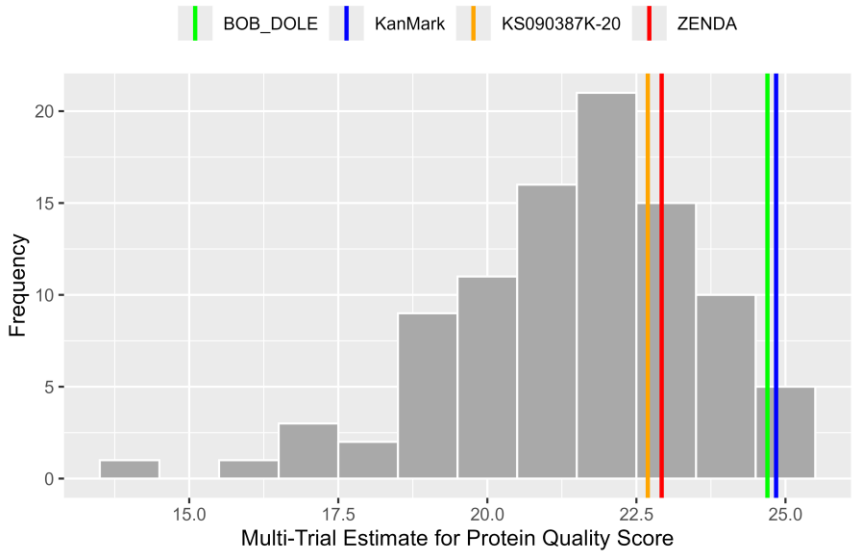

Figure 2.8. Distribution of Best Linear Unbiased Estimates of the Protein Quality Score (ratio of Lactic Acid-SDS SRC result to total GPC) of each accession to the Recurrent Parents, KanMark and KS090387K-20, and commercial checks Bob Dole and Zenda.

Selection of Superior Accessions Based on Quality Characteristics

These accessions were also selected from based on grain quality traits, again using the multi-trial BLUEs (Table 2.7). These were sorted by highest protein quality score (PQS). As seen in the GPC and SRC results of each accession, there can be varying results of each measured trait, and PQS is not necessarily the only factor that needs to be utilized during selection. Other traits such as gluten strength predicted by the lactic acid-SDS SRC (LA-SDS SRC) and the starch damage predicted by the sodium carbonate SRC (NaCarb SRC) are also

valuable information to consider, rather than relying solely on the ratio of functional to total protein provided by the protein quality score. Again, the same accession selected for based on yield and GPD was found to be among the top accessions.

Table 2.7 Selection of Accessions Based on Protein Quality Score.

Accession	PQS	GPC (g/kg)	LA-SDS SRC	NaCarb SRC
U8551_C5.1	24.9	132.6	329.5	85.0
KanMark	24.8	129.2	322.7	83.6
U8551_D3.1	24.7	128.5	318.1	83.9
Bob Dole	24.7	133.6	329.6	87.3
U8546_D4.1	24.6	133.5	326.2	86.7

Relationships between Grain Quality Traits and Agronomic Traits

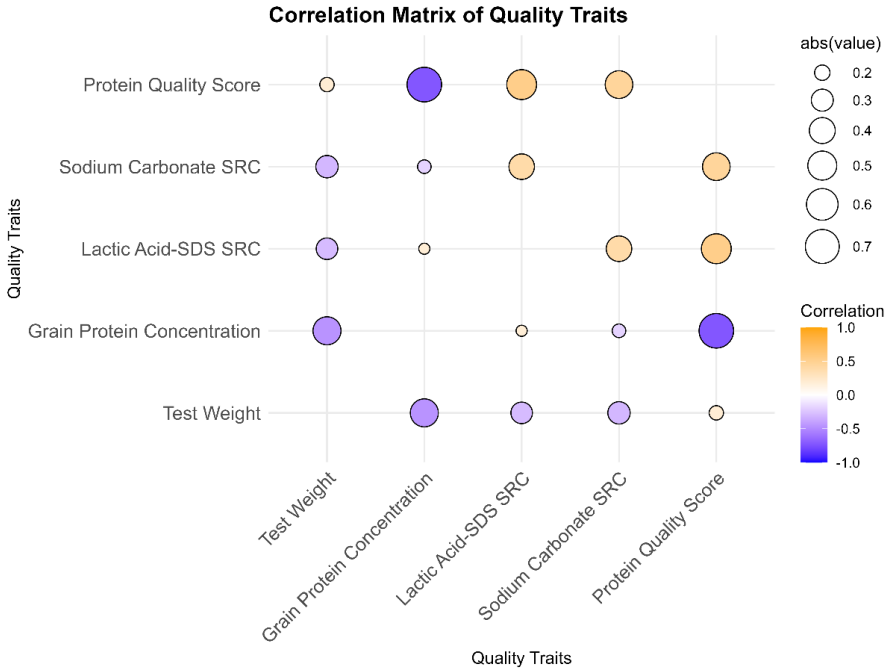

Figure 2.9 Correlations between quality traits measured for all samples collected: GPC, Test Weight, PQS, Lactic Acid-SDS SRC, and Sodium Carbonate SRC.

The relationships between all of the quality traits between themselves and the other agronomic traits have been considered. First, the correlations between the measured quality traits

were calculated (Figure 2.9). GPC had a moderate negative correlation with test weight (r = -0.47***). There were no notable correlations between GPC and either of the SRC methods. There was a highly negative correlation between GPC and PQS (r = -0.73***), which suggests that as GPC increases, the ratio of functional protein to total protein decreases. The results of the lactic acid-SDS SRC were moderately positively correlated with the protein quality score (r = 0.53***). As expected, an increase in predicted gluten strength indicates an increase in the ratio of function protein content to total content; however, given that this is a moderate correlation, there is suggestion of the consistency of this effect.

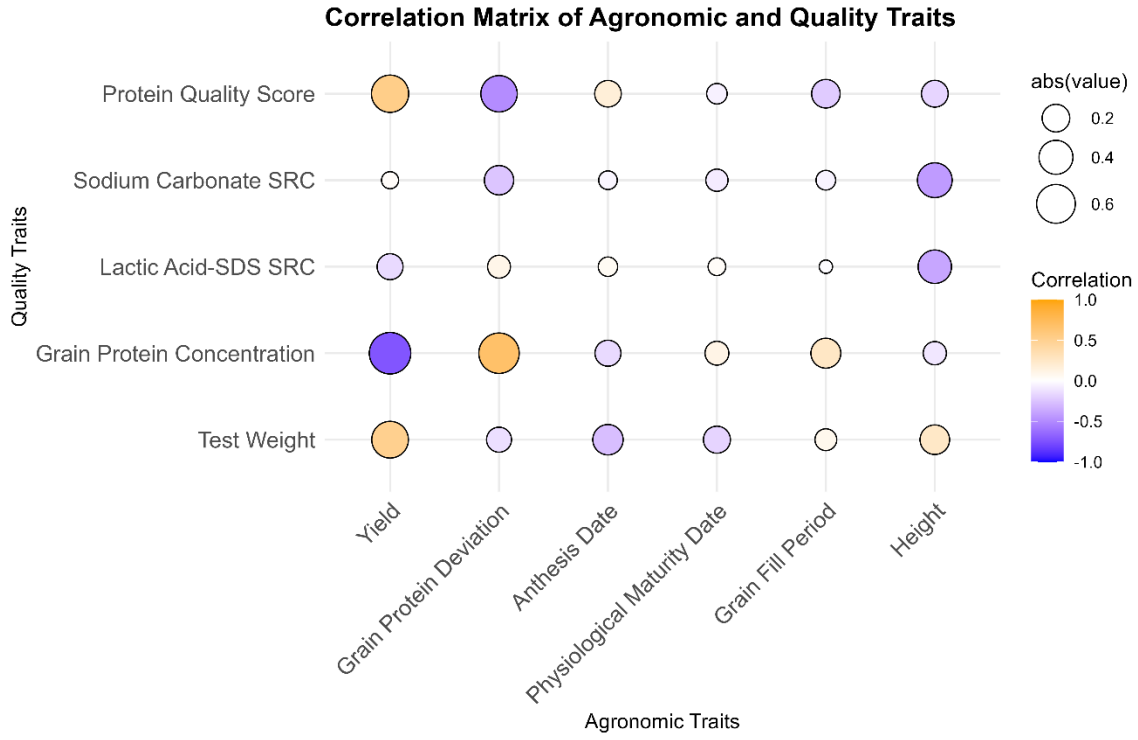

Figure 2.10 Correlations between quality and agronomic traits that were measured for all samples collected.

The correlations between quality and agronomic traits are shown in the heatmap above (Figure 2.10). As discussed in regard to GPD, the correlation between GPC and yield was highly negatively correlated (r = -0.73***). Yield also had notable moderate positive correlations with test weight (r = 0.51***) and PQS (r = 0.54***). The relationship between yield and PQS

suggests that there is a trend of increasing yield as the ratio between function and total protein content increases. Anthesis date, maturity date, and GFP had no notable relationships with any of the quality traits. The last physiological trait, height, had weak to moderate negative correlations with both the lactic acid-SDS SRC (r = -0.38***) and sodium carbonate SRC (r = -0.43***).

Seed Physical Characteristics: Cgrain Value

Whole grain samples from the 2023 Ashland Bottoms and Hutchinson trials were evaluated on the Cgrain Value, which collects measurements of seed size and color. The variance components for genetic, environmental, and genetic by environment were calculated (Table 2.10). Additional data collected on the Ggrain Value was used to compare seed characteristics to the agronomic data collected.

Table 2.8 Correlations between agronomic and quality traits, and seed size characteristics from the Cgrain Value, using data collected from 23ASM and 23HUM.

	Area	Length	Width	Thickness	Volume	Weight
Yield	-0.38***	-0.52***	0.02	0.07	-0.22*	-0.22*
Grain Protein Concentration	0.62***	0.56***	0.42***	0.37***	0.57***	0.57***
Grain Protein Deviation	0.51***	0.26*	0.64***	0.62***	0.60***	0.61***
Test Weight	-0.35***	-0.48***	-0.03	0.07	-0.21*	-0.20*
Lactic Acid-SDS SRC	0.11	0.22*	-0.07	-0.12	0.03	0.03
Sodium Carbonate SRC	-0.27**	-0.07	-0.47***	-0.52***	-0.37***	-0.37***
Protein Quality Score	-0.46***	-0.33**	-0.40***	-0.40***	-0.47***	-0.47***

Anthesis Date	0.08	0.07	0.09	0.05	0.07	0.07
Physiological Maturity Date	0.43***	0.40***	0.24*	0.24*	0.39***	0.39***
Grain Fill Period	0.35***	0.34***	0.15	0.19	0.32**	0.32**
Height	0.40***	0.27**	0.42***	0.46***	0.45***	0.45***

*, **, *** indicate significance at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., non-significant

When comparing the physical characteristics of the seeds and the agronomic and quality measurements, several moderate correlations were found. Seed length had a moderate negative correlation (r = -0.52***) with yield. Several seed characteristics had moderate positive correlations with GPC: area (r = 0.62***), seed length (r = 0.56***), calculated seed volume (r = 0.57***), and estimated weight (r = 0.57***) (Table 2.8). Test weight had a moderate negative correlation to seed length (r = -0.48***). This implies that as seed length increases, the test weight decreases. This could be because the longer seeds do not necessarily pack into the gravimetric weighing cup as well as typical seeds. The estimates of each accession for the lactic acid-SDS SRC had no significant correlations with any of the physical characteristics of the seed, giving the implication that the physical seed characteristics had no relationship with gluten strength (Guzmán et al., 2015). However, the sodium carbonate SRC estimates for each accession had a moderate negative correlation to the seed thickness (r = -0.52***), which indicates that thicker seeds were somewhat associated with lower levels of damaged starch.

As for the Protein Quality Score (PQS) of each accession, seed volume (r = -0.47***) and estimated seed weight (r = -0.47***) both presented moderately negative correlations. Because the PQS was intended to provide a ratio of functional proteins to total protein concentration, this implies that as the volume and weight of the seed increases, the ratio of

functional protein concentration to total protein concentration decreases; this is discussed further in the Grain Quality Analysis section. Physiological maturity date and grain fill period had only moderate to low positive correlations with seed characteristics, while date of anthesis had no relationship with seed size characteristics (Table 2.8). Plant height had moderate positive correlations with seed thickness (r = 0.46***), as well as other moderate-low correlations with seed area, width, volume, and weight (Table 2.8). Grain Protein Deviation had a number of moderate to high positive relationships with seed size characteristics including width (r = 0.64***), thickness (r = 0.62***), volume (r = 0.60***), and weight (r = 0.61***).

Table 2.9 Correlations Between Agronomic Traits and Seed Color, Saturation, Light and Hue Characteristics from the Cgrain Value, using 23ASM and 23HUM data.

	Red	Blue	Green	Hue	Saturation	Light
Yield	0.38***	0.30**	0.35***	0.23*	-0.06	0.38***
Grain Protein Concentration	-0.34***	-0.27**	-0.32**	-0.23*	0.09	-0.34***
Grain Protein Deviation	-0.09	-0.08	-0.09	-0.09	0.06	-0.09
Test Weight	0.01	-0.14	-0.07	-0.18	0.40***	0.01
Lactic Acid-SDS SRC	-0.25*	-0.25*	-0.25*	-0.11	0.19	-0.25*
Sodium Carbonate SRC	-0.06	-0.05	-0.05	0.08	-0.01	-0.06
Protein Quality Score	0.11	0.05	0.09	0.13	0.07	0.11
Anthesis Date	0.08	0.08	0.08	-0.01	-0.05	0.08
Physiological Maturity Date	-0.29**	-0.29**	-0.28**	-0.03	0.25*	-0.29**

Grain Fill Period	-0.37***	-0.36***	-0.35***	-0.02	0.30**	-0.37***
Height	-0.02	-0.01	-0.01	0.04	0.02	-0.02

*, **, *** indicate significance at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., non-significant

The Cgrain Value also provides measurements of seed pigments, saturation, and hues using the HSV color model (Hue, Saturation, and Value) (Table 2.9). In this case, Light is representation of Value. There were fewer correlations between agronomic traits and seed colors of pigment, brightness, and purity. Yield had weak positive correlations with Red (r = 0.38***) and Light (r = 0.38***). Light (r = -0.34**) and Red (r = -0.34**) had a weak negative relationship with GPC. Test weight had a weak positive correlation to Saturation (r = 0.40***). Grain fill period was the only other trait that had notable correlations to seed color characteristics, with weak negative correlations to Red (r = -0.37***), Blue (r = -0.36***), Green (r = -0.35***) and Light (r = -0.37***). Lactic acid-SDS SRC, sodium carbonate SRC, PQS, grain protein deviation, anthesis and maturity dates, and plant height had very weak or no notable correlations to seed color characteristics (Table 2.9).

Table 2.10 below shows the variances, heritabilities, and genetic to genetic by environment ratio calculated for each of these traits. All traits were highly heritable, and each had σ_G^2 / σ_{GxE}^2 ratios greater than 1, implying that the impact of the genetic component had greater influence on the expression of each trait. Genetic variance was highly significant for all traits, while seed volume and weight (both are internal calculations done by Cgrain based on seed dimensions) were less significant across environments and when measuring genetic by environment variance.

Table 2.10 Variances, Heritabilities, and Genetic to Genetic by Environment Variance Ratio for Traits Measured by the Cgrain.

Trait	σ^2_G	σ^2_E	σ^2_{GxE}	σ^2_{Error}	Entry-Mean Heritability	σ^2_G / σ^2_{GxE}
Area	0.651***	0.040***	0.032*	0.141	0.93	20.4
Length	0.069***	0.028***	0.005***	0.012	0.93	13.4
Width	0.009***	0.001***	0.001***	0.002	0.91	10.7
Thickness	0.008***	0.0003***	0.001***	0.002	0.91	10.0
Volume	4.335***	0.00 n.s.	0.293**	0.846	0.92	14.8
Weight	7.721E-06***	0.00 n.s.	5.222E-07**	0.000	0.92	14.8
Red	47.32***	17.22***	2.011***	2.507	0.97	23.5
Blue	19.73***	0.371***	1.433***	1.257	0.95	13.8
Green	32.91***	1.528***	1.789***	1.754	0.96	18.4
Hue	0.134***	0.068***	0.013***	0.016	0.93	10.2
Saturation	0.0002***	0.0005***	0.00002***	0.000	0.91	7.4
Light	0.001***	0.0003***	0.00003***	0.000	0.97	23.5

*, **, *** indicate significance at $P \leq 0.05, 0.01, 0.001$, respectively. n.s., non-significant

Conclusions

There are many conclusions that can be drawn from this data. Wild emmer introgression had an increasing effect on GPC for most accessions, but there was still a trend of decreasing yield. The calculation of GPD will allow for further selection from this population; multiple potentially superior accessions identified. Even though GPC was increased, it did not necessarily mean an increase in functional protein for each accession, which was estimated by the lactic acid-SDS SRC and Protein Quality Score. These quality tests provided more insight based on functional quality of each accession. Wild emmer is an effective source for wheat breeders attempting to breed for increase protein concentration and quality, but the diverse ancestral germplasm can come with complications throughout the breeding process. However, it is important to make an additional distinction between the checks and the accessions in this experiment. There is extensive history of selection for both the recurrent parents and commercial checks, while the wild emmer accessions used as parents only represent the potential of early generation lines that could be further developed. The genetic performance of accessions in this study could be further improved through additional years of pressure.

Chapter 3 - Use of the GlutoPeak to Assess Wheat Quality Characteristics

Introduction

The small-scale methods previously discussed assess grain and whole meal wheat quality generally and are trusted as indicators of quality early in the breeding process. Yet testing on white flour provides the most ideal data for the milling and baking industry. Testing dough and baking quality on white flour is thought to be more accurate, not having the bran or middlings that may interfere with the formation of the gluten network or otherwise alter mixing behavior (Haros et al., 2006). Before the introduction of the GlutoPeak (Brabender Glutopeak, Anton Paar, Duisburg, Germany), most advanced wheat grain quality data was collected in the late stages of breeding due to the requirement of intensive labor and large sample size requirements. The only small-scale early generation testing option was the mixograph, which has an uncertain future of availability and manufacturer support. This data collection has been done using instruments that provide various rheological measurements of dough that are indicators of flour quality, providing valuable information to breeders who are interested in differentiating between breeding selections based on quality.

The farinograph is one of the commonly chosen instruments. The farinograph measures the water absorption capacity of flour, the results of which can indicate the proper proportions of water to flour useful to the food industry (Wrigley et al., 2022). It also provides a measure of stability of the flour, which is the tolerance to mixing (Fowler & P Kovacs, 2004). Another is the extensograph, which measures the resistance to a stretching motion, which is generally a predictor of loaf volume (R. Y. Chen et al., 2009). Another tool, the alveograph, is used to assess the baking quality of a flour by manipulating the dough with inflation (Jødal & Larsen, 2021).

All of these instruments have characteristics in common; they require fairly large white flour sample sizes and take considerably long run times and skilled operators. The mixograph is another historically useful method that also measures water absorption as well as dough development (Okuda et al., 2016). Unlike the others, this requires a smaller sample size but is increasingly difficult to maintain due to lack of manufacturer support (Bock et al., 2024). In the initial stages of breeding, there is very little seed available for quality tests, certainly not enough for protocols such as the farinograph and alveograph which may require hundreds of grams of milled white flour. This leaves breeders with limited options for early generation quality testing. The necessity of white flour requires additional labor throughout the tempering and milling process (Wang et al., 2021). The GlutoPeak provides a potential solution to all three disadvantages of these original instruments.

The GlutoPeak offers a quick run time (2-5 minutes), uses only a small sample size (8-10 grams), and requires less labor through the potential utility of whole wheat meal in place of white flour. Measuring torque over time, the GlutoPeak has previously shown to have correlations between maximum torque and farinograph water absorption capacity (Wang et al., 2017) and correlations between other quality parameters such as gluten strength and GlutoPeak maximum peak time (Sissons, 2016).

The GlutoPeak could prove to be a more efficient alternative to traditional dough testing methods that can be used earlier in a breeding pipeline and save in time, labor, and cost for wheat breeders. This goal of this research was to investigate the relationships between GlutoPeak parameters and grain quality traits, as well as the heritability of the measurements recorded by the GlutoPeak. It is a useful comparison between the created populations containing *T. dicoccoides* germplasm and two established commercial wheat varieties, ‘Bob Dole’ (PI 690435)

and ‘Zenda’ (PI 683512), to observe the utility of wild emmer as a donor parent for breeding programs. The Best Linear Unbiased Estimates for the grain quality traits were calculated and used for correlations.

The samples used for the GlutoPeak were composited from both replicationss of the 22ASM, 22HUM, 23ASM, and 23HUM site-years. A total of 400 samples were run, and 10 from each site-year (40 samples) were randomly selected to be run twice, in an effort to determine the repeatability of the test. The GlutoPeak output provided by Brabender was also tested against a second-by-second (SBS) analysis. The issues raised by the Brabender output of the GlutoPeak data included the added labor of extracting data from multiple different files for one sample, and the concern of incorrect measurement designation. The objective of this experiment was to not only explore relationships between the measurements and quality traits, but to determine whether the initial data output is reliable for this set of accessions, or if the SBS analysis is more appropriate.

Materials and Methods

White Flour Sample Preparation

Both years (2022 and 2023) of samples collected from Ashland Bottoms and Hutchinson were milled into white flour using the junior milling facilities at the HWWQL, for a total of four site-years. Grain from both replications from each site-year was composited to make one representative sample for each accession. The white lfour was prepared follow the approved methods of the AACC for experimental tempering and milling (AACC International, 2010c). The average moisture across samples was found for each site-year and used to temper the grain to 15%; this allows for approximately 1% loss of moisture during milling, with the expected

flour moisture to be 14%, the industry standard. Each sample was milled (AACC International, 2010a) using the Quadromat junior mill (Quadromat Junior, Brabender, Anton Paar TorqueTec, Duisburg, Germany), and sieved for 1 minute at 160 revolutions per minute using a small-scale sample sifter (Great Western Manufacturing Sampl-Sifter, Leavenworth, KS). Two screen sizes were utilized: 405 micron to sift out the bran and 240 micron to remove the middlings, the weights of each recorded for each sample. The 2023 Ashland Bottoms samples were analyzed using the Perten IM 9520 on the white flour setting (Perten Instruments North America, Springfield, IL, USA) to measure the moisture and protein levels to obtain an estimation of the content after junior milling.

GlutoPeak Method and Operation

The GlutoPeak (Brabender, Anton Paar TorqueTec, Duisburg, Germany) was first prepared for running samples by initiating the temperature maintenance system; using the connected Julabo Corio CD system (Seelbach, Germany), the temperature of the receptacle of the measuring bowl is raised to 34 °C and maintained throughout the entire run. The GlutoPeak (Brabender, Anton Paar TorqueTec, Duisburg, Germany) was switched on, and the stirrer secured in the stirrer chuck. This method utilized a 1:1 water to flour ratio. First, 9 grams of deionized water measured into the measuring bowl. The measuring bowl was loaded into the receptable, and then 9 grams of flour was added. The run was initiated on the GlutoPeak MetaBridge Software, starting the stirrer which reached a maximum speed of 2700 revolutions per minute and remained constant for the remainder of the 2:30 minute run time. Torque and mean value were measured throughout the run. Following each run, the measuring bowl and stirrer were removed and cleaned to prepare for the next sample.

The raw GlutoPeak data was retrieved from Brabender's MetaBridge analysis, and a script was written in R to identify the local peaks (maximums) of the torque measured throughout the run time of each sample, as well as the torque of the sample at predetermined time measurements before and after each of the peaks. The two parameters of greatest interest were Maximum Torque (MT), the highest torque registered on the GlutoPeak, and Peak Maximum Time (PMT), the time at which the maximum torque occurred.

Data Analysis

Data analysis was carried out using R and R Studio (R Core Team, 2024). The tidyverse (Wickham et al., 2019) package including dplyr (Wickham et al., 2023) and ggplot2 (Wickham, 2016) packages were used throughout. The packages readxl (Wickham & Bryan, 2023), writexl (Ooms, 2024) and openxlsx (Schauberger & Walker, 2024) were used for management and modification of files.

The GlutoPeak data was assessed with two different approaches. Each sample was run once, except for randomly selected samples run twice to test repeatability. The time and torque measurements for quality characteristics was collected directly from the Brabender software and analyzed. Additionally, an R script was used to assess the torque on a second-by-second basis from the raw data retained by the Brabender system (Price et al., 2025). Using the aforementioned packages, the BLUEs for each measurement were collected for both approaches. The correlations between both approaches were performed using the rcorr function from the *Hmisc* package (Harrell Jr, 2025), as well as the repeatability between duplicated sample runs.

Results and Discussion

Consistency and Repeatability Between Measurement Methods

The GlutoPeak instrument measures torque versus time. Two sets of parameters were analyzed in this study, utilizing the same raw data; the built-in measurements recorded by the Brabender program and exported into Microsoft Excel, and a second-by-second analysis that measures the same features, plus additional parameters, derived using an R script (Price et al., 2025). Both measurement methods have the same objective; provide the most accurate data in order to deliver information about the flour quality of accessions without a significant amount of time, materials, and effort. There are consistencies found between the traits measured by both methods, but also some minor discrepancies.

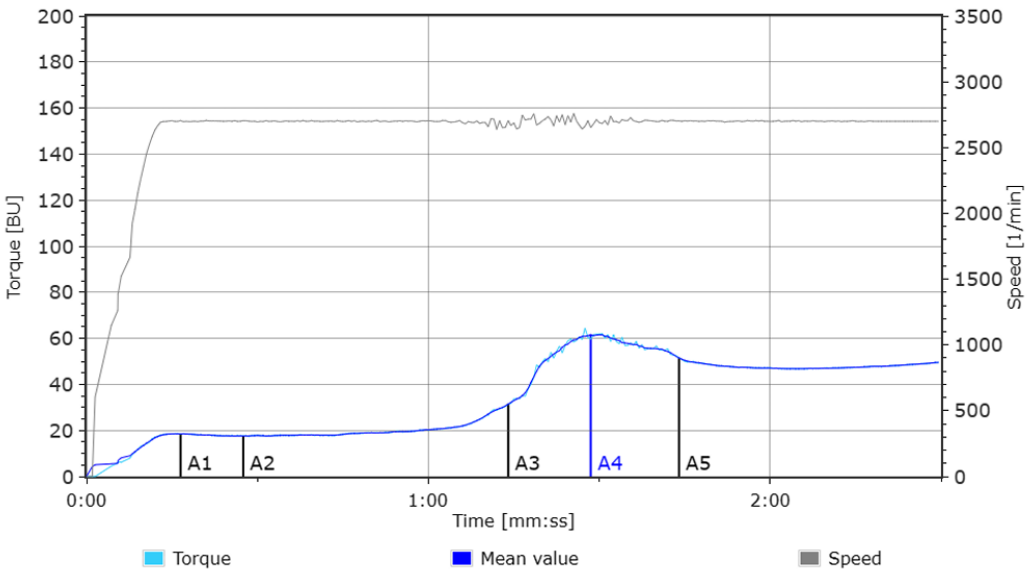

Figure 3.1 Example torque curve of Bob Dole flour using the GlutoPeak and Brabender output.

Figure 3.1 is an example of a typical curve created by the torque over time of a typical commercial flour, in this case, Bob Dole. This example curve illustrates how the Brabender designates the measurements. A1 is the first peak, while A2 is the bottom of the trough following

the first peak. A3 is 15 seconds before the maximum torque, A4 is the peak maximum, and A5 is 15 seconds after the maximum. Figure 3.2 is an example of the curve produced by one of the experimental accessions; it is visually very different, and has a much greater maximum torque, over 160 BU. This is likely due to the influence on gluten strength that wild emmer donor parents had on the experimental accessions. Many of the experimental curves that were very atypical in regard to what was seen with the control flour and the example curve in Figure 3.1 of Bob Dole.

The second-by-second analysis captures the same curve features as the Brabender analysis and calculates additional features using the raw data exported from Brabender. Theoretically, the SBS calculated values should match the Brabender parameters. However, after visual inspection of the measurement curves of the experimental accessions, it was determined that the variability in gluten aggregation curve behavior caused unreliability in the Brabender parameters. While the measurements were designated correctly in this case as seen by the lines labelling the points of interest, it was noticed that among some genotypes from previous tests that if the true peak maximum is reached too early within the timeframe, the torque rises quickly again after the true peak maximum that represents the gluten aggregation of the slurry. The Brabender output seemingly records the absolute maximum torque within the time, even if it is not the true peak maximum. For this reason, a second-by-second (SBS) analysis was also conducted. Tables 3.1 and 3.2 give details regarding the names and relevant measurement types for Brabender output and SBS analysis, respectively. In order to maintain clarity throughout the description of analysis, all measurements exported directly from Brabender will have format "measurement_b".

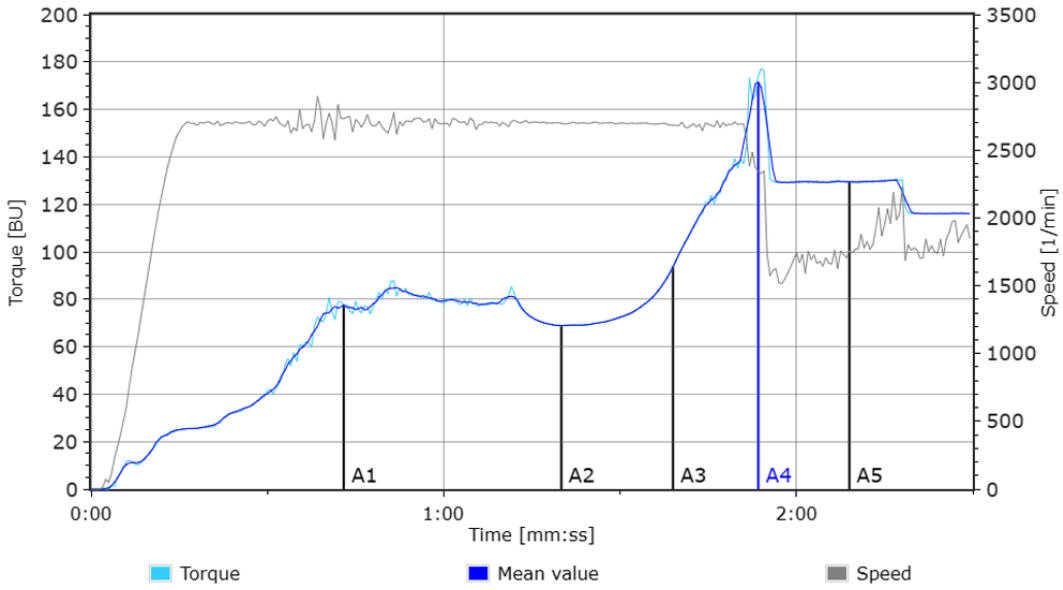

Figure 3.2 Example torque curve of experimental accession flour using the GlutoPeak and Brabender output.

Table 3.1 Abbreviations representing measurements collected by the Brabender GlutoPeak software, unit, and the relevant trait measurement.

Abbreviation	Measurement Name	Unit	Relevant Measurement
PMT_b	Peak Maximum Time	s	Maximum Peak
BEM_b	Maximum Torque	BU	Maximum Peak
AM_b	Torque 15 seconds before Maximum	BU	Maximum Peak
PM_b	Torque 15 seconds after Maximum	BU	Maximum Peak
A01_b	Area from '0' to 'A1'	GPI	Area Between Measurements
A12_b	Area from 'A1' to 'A2'	GPI	Area Between Measurements
A23_b	Area from 'A2' to 'A3'	GPI	Area Between Measurements
A34_b	Area from 'A3' to 'A4'	GPI	Area Between Measurements
A45_b	Area from 'A4' to 'A5'	GPI	Area Between Measurements

Table 3.2 Abbreviations for the Measurements from the Second-by-Second Additional Analysis

Abbreviation	Measurement Name	Relevant Measurement
PMT	Peak Maximum Time	Maximum Peak
MT	Maximum Torque	Maximum Peak
MPArea	Area at 'maximum peak'	Maximum Peak
FPTime	Time at 'first peak'	First Peak
FPTorque	Torque at 'first peak"	First Peak
FPArea	Area at 'first peak'	First Peak
TTime	Time at minimum of trough'	Trough
TTorque	Torque at minimum of trough'	Trough
MTBefore60	Maximum Torque BEFORE 60 seconds	Other Torque Measurements
T150sec	Torque at '150 seconds'	Other Torque Measurements
A12	Area from 'first peak' to 'trough minimum'	Area Between Measurements
A01	Area from 'start' to 'first peak'	Area Between Measurements
PMslope	Slope at '15 seconds after Maximum'	Slope
AMslope	Slope at '15 seconds before Maximum'	Slope
PSlope	Slope at 'maximum torque'	Slope

One of the first considerations between the two analyses types was the repeatability of each type. Forty samples were randomly chosen to be run twice. For each calculation type, the correlation between the first run and the second run was calculated in order to estimate the repeatability. The traits in common that are focused on throughout the results in the context of comparison are highlighted in yellow. The measurements provided by the Brabender data export were for the most part very repeatable, except for the maximum torque (BEM_b, $r = 0.47$***), which is arguably one of the more important measurements provided by the GlutoPeak.

The repeatability correlations for each trait are listed in Table 3.3. Peak maximum time was equally repeatable for both analyses, but the SBS estimation of maximum torque was much more reliable ($r = 0.92$***) from run to run. On the other hand, the A01 and A12 results were

less repeatable when using the SBS method. The additional traits measures in the SBS analysis had mixed repeatability of results, but the calculations for the torque at 150 seconds (r =0.95***) and maximum torque before 60 seconds (r =0.88***) appeared to be reliable. The FPArea and TTime were not repeatable in this experiment, but because each sample was run only twice, there is opportunity for error in the analysis. The analysis of repeatability would undoubtedly benefit from further exploration.

Table 3.3 Table of correlations between GlutoPeak sample and duplicate runs; used to measure the repeatability of both methods of analysis.

Second-by-Second		Brabender	
Measurement	Correlation (r)	**Measurement**	Correlation (r)
PMT	0.97***	**PMT_b**	0.97***
MT	0.92***	**BEM_b**	0.47***
MPArea	0.67***	**AM_b**	0.97***
FPTime	0.42**	**PM_b**	0.78***
FPTorque	0.47**	**A01_b**	0.69***
FPArea	0.31	**A12_b**	0.81***
TTime	0.25	**A23_b**	0.94***
TTorque	0.55***	**A34_b**	0.94***
MTBefore60	0.88***	**A45_b**	0.97***
T150sec	0.95***		
A01	0.43**		
A12	0.45**		
PMslope	0.38*		
AMslope	0.57***		
PSlope	0.70***		

*, **, *** indicate significance at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., non-significant.

Most literature studying the utility of the GlutoPeak focuses heavily on the maximum torque and the time of maximum torque. To take a look at the differences in how each method determined the data, four of the traits that both analysis types measured are compared. The additional two measurements were the area under the curves of A0 to A1, and A1 to A2. These two measurements provide context for the behavior of the first peak seen on almost all curves.

The following sections focus solely on all Brabender and SBS measurements respectively, but in this section, the focus on these four traits is used for comparison of utility. Below are the variances and heritabilities for peak maximum time (PMT/PMT_b), maximum torque (MT/MT_b), the area between A0 and A1 (A01/A01_b), and the area between A1 and A2 (A12/A12_b) (Table 3.4). The full lists of variances and heritabilities for each will be in the corresponding sections.

Table 3.4 Variability and entry-mean heritability of the measurements in common between both analysis methods.

Second-by-Second Analysis				Brabender Analysis			
Trait	σ_G^2	σ_{Error}^2	H^2	**Trait**	σ_G^2	σ_{Error}^2	H^2
PMT	384.9***	759.1	0.70	**PMT_b**	384.3***	759.8	0.66
MT	516.0***	354.7	0.85	**BEM_b**	430.5***	1061	0.62
A01	245592***	576545	0.63	**A01_b**	105553***	108985	0.79
A12	531630***	742712	0.74	**A12_b**	227202***	422530	0.68

*, **, *** indicate significance at $P \leq 0.05, 0.01, 0.001$, respectively. n.s., non-significant

The genetic variances calculated from the data produced by the SBS analysis were all highly significant ($P < 0.001$), while the Brabender variances had both significant and insignificant variances among the traits. Between the two analysis methods, peak maximum time (PMT/PMT_b) appeared to be the most consistent trait, with nearly identical σ_G^2 and σ_{Error}^2. The calculated heritabilities are also the closest among the traits. Maximum torque (MT/BEM_b) is where the methods begin to deviate. The Brabender analysis produced a much higher error variance, which could be the undesirable effect of designating the incorrect maximum torque.

There also was a large difference in calculated heritabilities between the two methods; the largest difference among these four traits. The area under the curves between the start and first peak (A01/A01_b) and the area under the curve from the first peak to the minimum of the trough (A12/A12_b) had similar differences in variance. Both SBS genetic variances were greater and highly significant ($P < 0.001$), while the Brabender genetic variances were lower and

less significant. The heritability of A01 was significantly less than calculated for A01_b, while the heritability of A12 was slightly greater than A12_b.

There were a few significant correlations between the measurements of both analyses. PMT and PMT_b were extremely highly correlated between the two types (r = 1.00***). This means that either method will likely give the same calculation for the time at which peak torque is reached. The calculation of maximum torque was less consistent between the analyses (r = 0.77***); while it was less likely to produce the same outcome, there is still a significant relationship between the two. This called into question which measurement is more accurate. In this case, the second-by-second may be more reliable, due to the high gluten strength of many of these accessions.

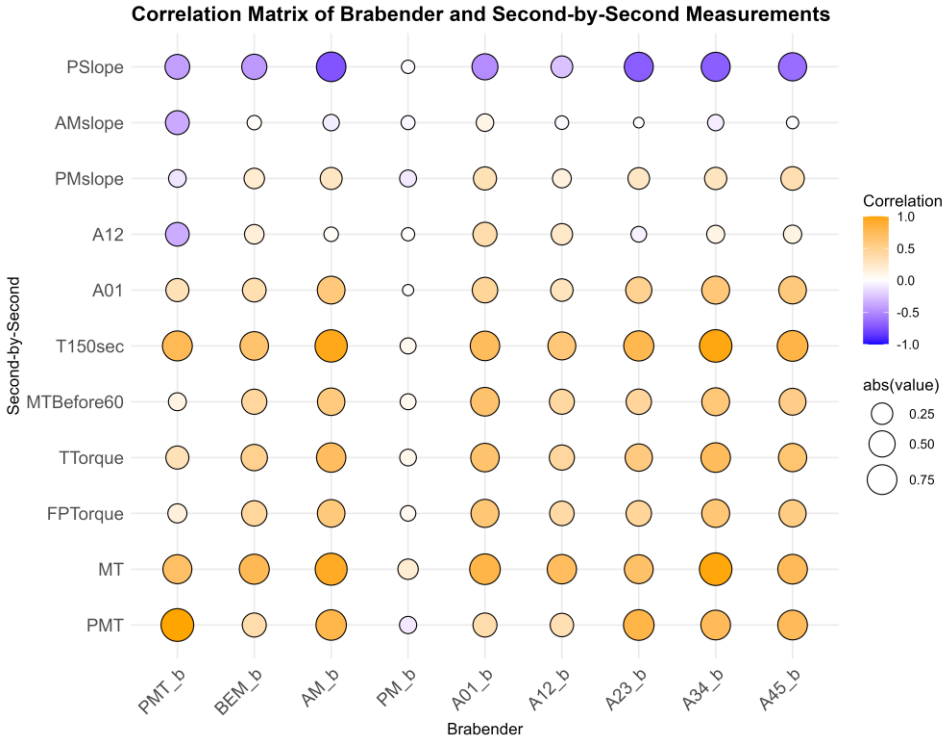

Figure 3.3 Correlation Matrix of Measurements from the Second-by-Second Analysis and Brabender Measurements.

Compared to PMT and MT, the calculation of area under the curves was very inconsistent between the two measurement types. A01 and A01_b were only moderately

correlated (r = 0.46***), while A12 and A12_b had only a somewhat significant relationship (r = 0.25*). Other than those traits measured in common between the two analyses, the Brabender measurement AM_b (torque 15 seconds before the peak maximum) has multiple highly significant positive correlations with SBS measurements: PMT (r = 0.81***), MT (r = 0.94***), T150sec (r = 0.96), and a negative correlation with PSlope (r = -0.74***). Another significant positive relationship was between MT and A34_b (r = 0.99***), as well as T150sec and A45_b (r = 0.83***). Other trends that become visually apparent with Figure 3.3 is that the Brabender measurement PM_b has no significant relationships with SBS measurements. The AMslope measurement from the SBS measurement had only a moderate yet significant negative correlation with Brabender's PMT_b (r = -0.37***).

Table 3.5 Correlations between common measurements made by both analyses and grain protein concentration (GPC), the lactic acid-SDS SRC (LA-SDS), and Protein Quality Score (PQS).

Second-by-Second Analysis				Brabender Analysis			
Trait	GPC	LA-SDS	PQS	**Trait**	GPC	LA-SDS	PQS
PMT	0.38***	0.63***	0.13	**PMT_b**	0.38***	0.64***	0.13
MT	0.76***	0.34***	-0.40***	**BEM_b**	0.55***	0.20	-0.30**
A01	0.58***	0.25*	-0.35***	**A01_b**	0.76***	0.14	-0.53***
A12	0.47***	-0.55***	-0.79***	**A12_b**	0.69***	0.17	-0.43***

*, **, *** indicate significance at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., non-significant.

When comparing the correlations between both analysis types and the quality traits from this study, several differences were found (Table 3.5). For each quality trait (GPC, LA-SDS, PQS), the highest correlation with each measurement type is highlighted in the table above. Both PMT and PMT_b were moderately positively correlated with GPC (r = 0.38***). PMT (r = 0.63***) and PMT_b (r = 0.64***) provided similar correlations for the lactic acid-SDS SRC, which predicts gluten strength. The SBS analysis provided stronger correlations for GPC, LA-

SDS, and PQS than the Brabender analysis. A01_b provided a stronger correlation to GPC (*r* =0.76***) and PQS (*r* = -0.53***) than the SBS counterparts. The measurement A12 from the SBS analysis had a stronger negative relationship between both LA-SDS (*r* = -0.55***) and PQS (*r* = -0.79***). GPC on the other hand, was more highly correlated (*r* = 0.69) with the A12_b calculation.

Brabender Measurements

This section focuses on the output given by Brabender, and the full extent of measurements collected by exporting the data using all three files where the data is located. Table 3.1 contains the abbreviations, units, and descriptions for the measurements provided by Brabender.

Table 3.6 Variances, Entry-Mean Heritability, and σ_G^2/σ_Error^2 of Measurements collected by the Brabender GlutoPeak software.

Trait	σ_G^2	σ_{Error}^2	$\sigma_G^2/\sigma_{Error}^2$	Entry-Mean Heritability
PMT_b	384.3***	759.8	0.51	0.66
BEM_b	430.5***	1061	0.41	0.62
AM_b	332.8***	207.8	1.60	0.86
PM_b	183.1***	869.5	0.21	0.46
A01_b	105553***	108985	0.97	0.79
A12_b	227202***	422530	0.54	0.68
A23_b	1205946***	2793523	0.43	0.63
A34_b	78371***	45811	1.71	0.87
A45_b	1222580***	3148328	0.39	0.61

*, **, *** indicate significance at $P \leq 0.05, 0.01, 0.001$, respectively. n.s., non-significant

The variances for Brabender measurements are listed in the table above (Table 3.6). Two of the measurements that are typically sought after from GlutoPeak are the time at which maximum torque is reached (PMT_b) and the maximum torque (BEM_b). For the output provided by Brabender, the genetic variance of PMT_b was highly significant ($P < 0.001$) and

moderately heritable (H^2 = 0.66). Genetic variance of BEM_b also was significant (P < 0.01) and was similarly moderately heritable (H^2 = 0.62). Genetic variance of AM_b, the torque 15 seconds before the maximum, also was highly significant (P < 0.001) and was among the most highly heritable measurements (H^2 = 0.86), along with A34_b.

Table 3.7 Correlations and Significance Tests for measurements collected by Brabender software.

	PMT_b	BEM_b	AM_b	PM_b	A01_b	A12_b	A23_b	A34_b	A45_b
PMT_b	-	0.38***	0.81***	-0.10	0.38***	0.35***	0.82***	0.75***	0.75***
BEM_b	0.38***	-	0.64***	0.56***	0.78***	0.77***	0.25*	0.75***	0.26*
AM_b	0.81***	0.64***	-	0.10	0.72***	0.58***	0.82***	0.97***	0.80***
PM_b	-0.10	0.56***	0.10	-	0.48***	0.55***	-0.32**	0.19	-0.46***
A01_b	0.38***	0.78***	0.72***	0.48***	-	0.88***	0.28**	0.80***	0.40***
A12_b	0.35***	0.77***	0.58***	0.55***	0.88***	-	0.16	0.69***	0.25*
A23_b	0.82***	0.25*	0.82***	-0.32**	0.28**	0.16	-	0.73***	0.90***
A34_b	0.75***	0.75***	0.97***	0.19	0.80***	0.69***	0.73***	-	0.76***
A45_b	0.75***	0.26*	0.80***	-0.46***	0.40***	0.25*	0.90***	0.76***	-

*, **, *** indicate significance at $P \leq 0.05, 0.01, 0.001$, respectively. n.s., non-significant

Between the measurements themselves, there are a collection of noteworthy relationships (Table 3.7). Of all the measurements, PMT_b and BEM_b had a collection of the most significant relationships with other traits, despite being weakly correlated to each other (r = 0.38***). PMT_b was highly positively correlated with AM_b (r = 0.81***) and A23_b (r = 0.82***). BEM_b was positively correlated with AM_b (r = 0.64***), A01_b (r = 0.78***), and A12_b (r = 0.77***). Along with BEM_b, AM_b was highly positively correlated with A01_b (r = 0.72***), A23_b (r = 0.82***), A34_b (r = 0.97***), and A45_b (r = 0.80***). The measurement A01_b also had highly significant correlations with A12_b (r = 0.88***) and A34_b (r = 0.80***). A23_b and A45_b were highly positively correlated (r = 0.90***). The consistently high correlations between multiple traits suggests that there is consistency within the

gluten aggregation process, while the high variances suggest that the results are highly dependent on genotype. This does not necessarily speak to the predictability of traits but implies that there is an expectation for the behavior of the curve.

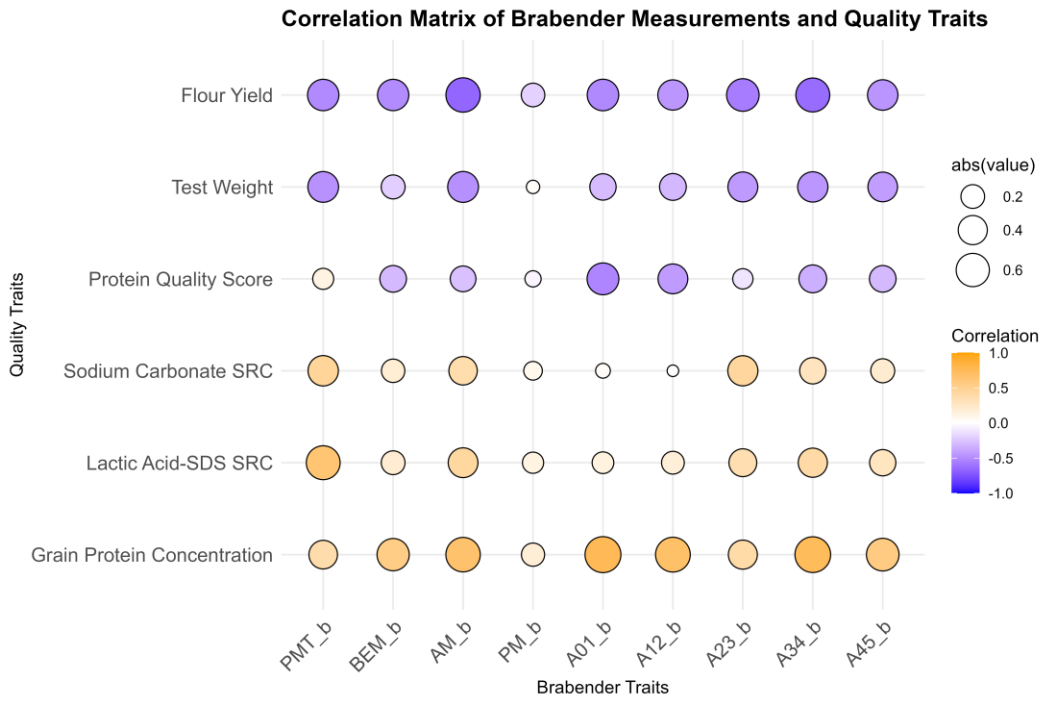

Figure 3.4 Correlation Matrix of Brabender Measurements and Quality Traits.

The correlations between the Brabender measurements and grain quality traits demonstrate moderate relationships between a few of the quality traits collected in this study (Table 3.8 & Figure 3.4). Grain protein concentration (GPC) had several correlations of interest. Protein concentration was moderately positively correlated with maximum torque (BEM_b, r = 0.55***). It was also highly correlated with A01_b (r = 0.76***) and A34_b (r = 0.74***). GPC also had moderate positive correlations with AM_b (r = 0.67***), and A12_b (r = 0.69***). PMT_b had a moderate positive correlation with gluten strength, which was estimated by the lactic acid-SDS SRC (r = 0.64***). Protein Quality Score (PQS) had a moderate negative correlation with A01_b (r = -0.53***). Flour yield had a moderate negative correlation with

AM_b (r = -0.66). There is a general trend for most traits for every measurement; GPC and both SRC types had positive correlations, while PQS, test weight, and flour yield typically had inverse relationships with each Brabender measurement.

Table 3.8 Correlations Between Brabender Measurements and Quality Traits.

	PMT_b	BEM_b	AM_b	PM_b	A01_b	A12_b	A23_b	A34_b	A45_b
Grain Protein Concentration	0.38***	0.55***	0.67***	0.19	0.76***	0.69***	0.40***	0.74***	0.57***
Lactic Acid-SDS SRC	0.64***	0.20	0.43***	0.13	0.14	0.17	0.35***	0.40***	0.29**
Sodium Carbonate SRC	0.46***	0.19	0.37***	0.08	0.03	0.02	0.44***	0.29**	0.21*
Protein Quality Score	00.13	-0.30**	-0.27**	-0.04	-0.53***	-0.43***	-0.11	-0.35***	-0.30**
Test Weight	-0.47***	-0.21*	-0.48***	0.02	-0.29**	-0.30**	-0.44***	-0.45***	-0.42***
Flour Yield	0.51***	-0.50***	-0.66***	-0.20	-0.51***	-0.45***	-0.57***	-0.64***	-0.46***

*, **, *** indicate significance at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., non-significant

Second-by-Second Measurements (SBS)

Alongside the analysis of measurements from Brabender, the second-by-second measurements provided even more valuable information regarding the relationships with traits and GlutoPeak results. The abbreviations and trait descriptions for these measurements can be found in Table 3.2.

Table 3.9 Variances, Entry-Mean Heritability, and σ_G^2/σ_Error^2 for measurements collected on a second-by-second basis.

Trait	σ_G^2	σ_{Error}^2	$\sigma_G^2/\sigma_{Error}^2$	Entry-Mean Heritability
PMT	384.9***	759.1	0.51	0.70
MT	516.0***	354.7	1.45	0.85
MPArea	1914	101284	0.02	0.07
FPTime	125.2***	535.8	0.23	0.48
FPTorque	231.7***	300.3	0.77	0.76
FPArea	37555***	115927	0.32	0.56
TTime	286.4***	1111	0.26	0.51
TTorque	121.2***	140.2	0.86	0.76
MTBefore60	380.0***	168.8	2.25	0.90
T150sec	459.0***	308.5	1.49	0.86
A12	531630***	742712	0.72	0.74
A01	245592***	576545	0.43	0.63
PMslope	0.049***	0.126	0.39	0.61
AMslope	0.238***	0.360	0.66	0.73
PSlope	0.029***	0.028	1.02	0.80

*, **, *** indicate significance at $P \leq 0.05, 0.01, 0.001$, respectively. n.s., non-significant.

Some measurements from the SBS analysis had insignificant genetic variation, and/or only moderate to low heritability (Table 3.9). Others had both significant genetic variability and high heritability, which is an indication that these measurements could have significance regarding the behavior and predictability of other traits. Based on this analysis, maximum torque (MT, $H^2 = 0.85$), peak maximum time (PMT, $H^2 = 0.70$), maximum torque recorded before 60 seconds (MTBefore60, $H^2 = 0.90$), and torque measured at 150 seconds (T150sec, $H^2 = 0.86$)

were highly heritable. Other moderate to highly heritable measurements were the torque of the first peak (FPTorque, H^2 = 0.76), the torque at the minimum of the trough (TTorque, H^2 = 0.76), the area between A0 and A1 (A01, H^2 = 0.63), the area between A1 and A2 (A12, H^2 = 0.74), the slope before the peak maximum (AMslope, H^2 = 0.73), the slope after peak maximum (PMslope, H^2 = 0.61), and the peak slope (PSlope, H^2 = 0.80). The maximum peak area, first peak area, first peak time, and trough minimum time were less heritable. Several of the traits with highly significant genetic variance ($P < 0.01$) and with notable heritability were compared to the quality traits measured in this study.

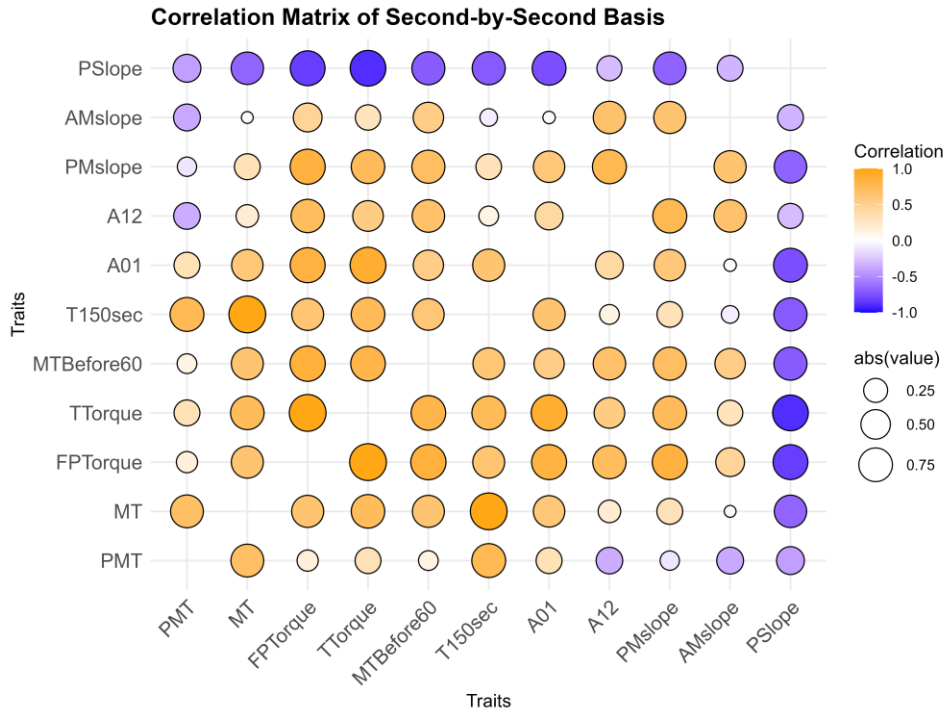

Figure 3.5 Correlation Matrix of Measurements from the Second-by-Second Analysis.

Similar to the findings from the Brabender measurements, there were several significant correlations between the GlutoPeak traits. Peak maximum time (PMT) and maximum torque (MT) were moderately positively correlated (r = 0.70***). PMT was also highly correlated with T150sec (r = 0.77***). MT was very highly correlated with MTBefore60 (r = 0.97***).

MTBefore60 was one of the factors designed to determine whether there was a need for a second-by-second basis and additional parameter measurements. Due to the diversity of this germplasm, there were instances in which the torque measurement increased sharply at the end of the run; this was registered by Brabender as the maximum torque, rather than the true peak maximum that represents gluten aggregation.

Additional measurements collected by the new analysis include that surrounding the "first peak." The time of the first peak (FPTime) was highly correlated with A01 (r = 0.91***). The torque of the first peak (FPTorque) was highly positively correlated to several parameters: MTBefore60 (r = 0.86***) and A01 (r = 0.84***). FPTorque was also highly negatively corelated with PSlope (r = -0.83***). FPArea was highly correlated with TTorque (r = 0.77***) and PMslope (r = 0.78***). TTime was highly positively correlated with A12 (r =0.84***) and moderately positively correlated to PMslope (r = 0.77***). TTorque was also highly positively correlated with MTBefore60 (r = 0.81) and A01 (r = 0.90***), and highly negatively correlated to PSlope (r = -0.89***).

Of these trends, there are two notable parameters that are found in addition to data from Brabender output. The relationship between time of the first peak (FPTime), and time of the minimum of the following trough were moderately correlated (TTime) (r = 0.77***), but neither trait is significantly heritable. Furthermore, the torque of the first peak (FPTorque) and the minimum torque of the following trough (TTorque) were both highly correlated (r = 0.97***), as well as both highly heritable. The inter-measurement trends of correlations can be seen more clearly in the correlation heatmap (Figure 3.5), with a large majority having some level of positive correlation with other traits; the outlying trait in this case would be PSlope, of which all

correlations were inverse. PMT did have some low to moderate negative correlations, particularly to the slope measurements and to A12.

Table 3.10 Correlations between Quality Traits and PMT, MT, FPTorque, and TTorque.

	PMT	MT	FPTorque	TTorque
Grain Protein Concentration	0.38***	0.76***	0.64***	0.66***
Lactic Acid-SDS SRC	0.63***	0.34***	-0.13	0.06
Sodium Carbonate SRC	0.46***	0.24*	-0.03	0.08
Protein Quality Score	0.13	-0.40***	-0.65***	-0.54***
Test Weight	-0.47***	-0.42***	-0.19	-0.25*
Flour Yield	-0.51***	-0.62***	-0.42***	-0.48***

*, **, *** indicate significance at $P \leq 0.05, 0.01, 0.001$, respectively. n.s., non-significant

There were also a few noteworthy correlations between the second-by-second basis analysis measurements and the other quality traits measured in this study (Table 3.10 & Table 3.11). GPC had four moderate-high positive correlations with the measurements: MT (r = 0.76***), FPTorque (r = 0.64***), TTorque (r = 0.66***), and T150sec (r = 0.72***). PMT had a moderate correlation to the lactic acid-SDS SRC (r = 0.63***), suggesting a possible relationship between PMT and gluten strength. The lactic acid-SDS SRC also had a moderately high negative correlation with AMslope (r = -0.72***). FPTorque had a moderately negative correlation with PQS (r = -0.65***), suggesting that as the ratio of function protein to total protein increases, the torque of the first peak had an expectation of decrease. There were only moderate to low correlations between these measurements and the sodium carbonate SRC, test weight, and flour yield.

Table 3.11 Correlations Between Quality Traits and MTBefore60, T150sec, A01, and A12.

	MTBefore60	T150sec	A01	A12
Grain Protein Concentration	0.62***	0.72***	0.58***	0.47***
Lactic Acid-SDS SRC	-0.23*	0.41***	0.25*	-0.55***
Sodium Carbonate SRC	0.01	0.29**	-0.01	-0.45***
Protein Quality Score	-0.70***	-0.32**	-0.35***	-0.79***
Test Weight	-0.16	-0.45***	-0.22*	0.02

*, **, *** indicate significance at $P \leq 0.05$, 0.01, 0.001, respectively. n.s., non-significant

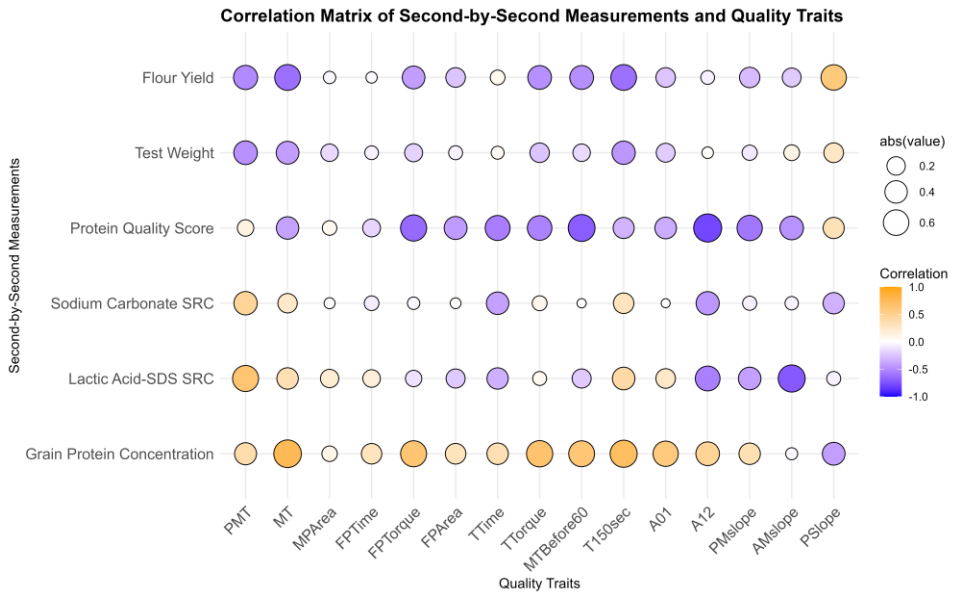

Figure 3.6 Correlation matrix of measurements collected on a second-by-second basis and quality traits.

Figure 3.6 displays all of the relationships discussed between the SBS measurements and the quality traits. The merit of the SBS is seen particularly in the additional data provided, compared to the auto-calculated exported data from Brabender. Based on these findings, it is

possible to utilize the SBS method to calculate reliable data that is not provided by the base software provided by the GlutoPeak supported by Brabender.

Conclusions

There is merit to using both the data export directly from Brabender, and from using the SBS method. Both methods provide relatively similar measurements for heritability, but the lack of accuracy presented by the prediction of maximum torque by the Brabender software is a concern for use, especially considering the nature of this germplasm. Creating the SBS analysis required an initial investment of labor but provided better repeatability of estimations for two of the most important measurements collected by the GlutoPeak, peak maximum time and peak maximum torque. The SBS analysis also simplified the data export procedure, pulling from only one file instead of multiple. Both analyses of PMT were equally correlated with GPC (r = 0.38***) and had very comparable correlations to the lactic acid-SDS SRC, a predictor of gluten strength. However, the SBS analysis was superior in its correlations containing maximum torque with GPC, lactic acid-SDS SRC, and PQS.

On the other hand, the Brabender analysis appeared to have much better repeatability of the areas beneath the curve early in the run. Considering the stronger correlations between quality traits and maximum torque, the SBS analysis appears to be the superior method. Not only did the SBS method possess higher experimental repeatability of maximum torque results but also had the added safeguard of the MTBefore60 measurement, which allows for measurement of consistency on peak maximum time. Many of these accessions in the experiment had significantly higher protein concentrations and predicted gluten strength than both the recurrent parents and the commercial checks, one of the drivers of concern about the Brabender output.

The introduction of the SBS analysis appears to have mitigated these effects, streamlined the data collection process, and provided additional measurements.

Almost all measurements were highly heritable. The GlutoPeak proved to have both positive and negative correlations with multiple of the quality traits collected in this study. Of the measurements provided by the GlutoPeak, peak maximum time and maximum torque are the two found most commonly across relevant literature. In this study, peak maximum time was found to be moderately positively correlated with predicted gluten strength (r = 0.63***). The maximum torque was found to be highly positively correlated with GPC (r = 0.76***). Both methods of measurement were consistent for calculation of peak maximum time. In this study, there was concerningly low repeatability of maximum torque from Brabender output. This could be due to the nature of the accessions used in this study; the intention was to increase functional protein in the progeny, and many of these lines reached maximum torque earlier than expected, introducing error produced by the potential capture of starch gelatinization post-gluten aggregation. Given that the intention of this experimental population was to increase GPC, the second-by-second analysis is likely the more appropriate method to use. However, the Brabender output could still be suitable for testing flour from more stable varieties. Regardless of analysis type, data collected in this experiment attempts to advance the understanding of the relationships between GlutoPeak measurements and flour characteristics that are indicative of end product quality as low cost, time, and material input alternative to traditional rheology methods.

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Appendix A - Best Linear Unbiased Estimates for Agronomic and Grain Quality Traits

Accession	Yield (kg/ha)	Anthesis Date (Julian date)	Physiological Maturity Date (Julian Date)	Grain Fill Period (days)	Height (cm)	Protien (g/kg)	Test Weight (g/L)	Lactic Acid-SDS SRC	Sodium Carbonate SRC	Protein Quality Score
BOB_DOLE	4133.0	131.1	164.8	33.7	69.7	133.6	771.2	329.6	87.3	24.7
KanMark	3842.1	131.7	164.3	32.5	60.8	129.2	780.0	322.7	83.6	24.8
KS090387K-20	3636.3	130.7	166.2	35.5	63.8	144.4	774.7	326.6	85.1	22.7
ZENDA	3935.5	131.4	164.4	33.0	64.1	129.7	778.4	297.4	87.3	22.9
U8318_U8319_D4.2	3061.1	131.2	164.6	33.4	64.8	161.8	725.9	321.0	84.2	20.0
U8323_A4.2	3106.4	133.3	164.2	30.9	63.1	135.6	745.7	329.5	88.0	24.3
U8323_B10.1	2854.6	131.6	164.0	32.4	63.6	146.2	747.3	312.7	84.8	21.5
U8323_C2.2	2662.4	130.2	161.9	31.7	53.0	172.5	743.9	361.9	85.8	21.2
U8323_C5.2	2783.3	130.8	163.5	32.7	61.5	139.2	747.8	308.8	91.1	22.2
U8323_E1.2	2947.3	130.6	161.9	31.3	61.5	140.5	729.2	301.1	91.2	21.5
U8323_E3.1	3355.4	132.8	164.1	31.3	72.5	144.6	758.9	313.7	81.3	21.7
U8323_E4.1	3629.9	130.8	163.7	32.9	65.1	139.0	758.1	295.4	83.9	21.3
U8323_E4.2	3578.5	131.0	163.3	32.4	63.3	137.8	747.5	290.5	83.3	21.1
U8330_U8444_U8574_U8346_A10.1	2797.4	133.6	166.6	33.1	63.5	157.5	749.2	333.9	84.3	21.4
U8330_U8444_U8574_U8346_C8.1	3076.2	131.4	165.2	33.8	72.4	158.3	751.3	320.4	83.7	20.3
U8330_U8444_U8574_U8346_C8.2	2982.1	131.1	167.6	36.5	75.2	161.6	758.8	313.8	80.8	19.5
U8382_C8.1	3051.6	131.1	166.2	35.1	73.4	160.1	747.9	299.9	80.9	18.9
U8382_C8.2	3865.0	131.1	165.3	34.3	65.5	144.9	776.0	332.9	84.6	23.1

Accession	Yield (kg/ha)	Anthesis Date (Julian date)	Physiological Maturity Date (Julian Date)	Grain Fill Period (days)	Height (cm)	Protien (g/kg)	Test Weight (g/L)	Lactic Acid-SDS SRC	Sodium Carbonate SRC	Protein Quality Score
U8382_C9.1	2392.6	131.1	165.3	34.2	59.0	186.4	726.6	307.6	86.1	16.7
U8382_D6.2	2529.3	134.2	166.8	32.7	59.6	163.1	720.9	341.7	86.4	21.0
U8382_E3.1	2922.7	130.7	164.6	33.9	69.9	173.3	750.6	345.5	82.4	20.2
U8382_E3.2	2792.4	130.6	165.1	34.5	70.0	182.5	743.7	349.5	83.5	19.3
U8386_U8552_E1.2	2617.8	132.4	166.2	33.8	58.7	178.9	727.2	336.5	84.6	18.9
U8434_A4.1	3026.2	131.6	163.9	32.3	63.1	150.4	765.3	323.8	81.3	21.6
U8438_U8376_C2.1	2792.0	131.4	166.3	34.9	72.0	162.6	751.3	325.0	82.4	20.2
U8438_U8376_E5.2	3455.5	132.3	163.9	31.6	74.0	140.5	760.2	319.8	84.0	22.9
U8439_U8554_U8440_A3.2	2614.8	131.7	164.4	32.7	70.0	156.5	759.5	286.3	80.6	18.4
U8439_U8554_U8440_C4.2	2925.2	130.8	165.4	34.6	61.2	160.0	738.6	317.1	82.9	20.0
U8439_U8554_U8440_D4.1	3400.0	132.8	162.1	29.3	72.3	164.1	753.5	349.2	82.2	21.4
U8439_U8554_U8440_D4.2	2836.6	132.5	167.1	34.6	70.8	161.9	724.9	337.6	82.3	21.0
U8439_U8554_U8440_E6.2	3202.1	133.7	165.3	31.6	65.2	163.2	734.9	337.7	79.9	20.8
U8439_U8554_U8440_E9.1	3229.5	131.7	164.6	32.9	67.7	151.8	756.7	282.4	82.3	18.7
U8441_U8549_A2.2	2467.1	130.9	165.6	34.7	69.5	161.6	727.9	327.0	81.5	20.4
U8441_U8549_D8.1	2850.3	129.7	163.0	33.4	60.0	150.9	748.3	317.9	84.7	21.1

Accession	Yield (kg/ha)	Anthesis Date (Julian date)	Physiological Maturity Date (Julian Date)	Grain Fill Period (days)	Height (cm)	Protien (g/kg)	Test Weight (g/L)	Lactic Acid-SDS SRC	Sodium Carbonate SRC	Protein Quality Score
U8441_U8549_D9.1	3308.7	132.7	163.2	30.6	63.7	141.9	750.8	311.2	87.5	22.0
U8441_U8549_E1.1	3264.2	130.6	164.5	33.9	68.6	135.8	737.0	314.0	89.4	23.1
U8441_U8549_E1.2	2659.7	132.0	165.5	33.5	67.0	146.0	717.6	321.0	90.3	22.0
U8450_D3.2	2730.3	130.9	167.7	36.8	72.0	163.1	764.8	307.7	82.2	18.9
U8450_E7.1	3165.9	131.2	164.0	32.9	65.7	155.3	751.8	324.0	81.2	20.9
U8450_E7.2	2660.6	131.6	164.4	32.9	65.5	161.2	735.6	329.8	84.4	20.5
U8452_U8447_U8539_C1.1	3425.0	130.9	164.5	33.6	59.8	139.9	754.6	328.6	84.8	23.5
U8452_U8447_U8539_E8.2	2319.0	134.3	167.5	33.3	62.0	167.6	713.2	356.9	86.4	21.4
U8453_A1.1	3111.7	131.8	163.2	31.3	67.2	154.0	749.3	255.6	80.4	16.6
U8453_A1.2	3241.7	130.8	163.8	32.9	69.5	152.9	766.7	254.7	79.4	16.7
U8453_A6.1	3473.2	132.0	165.2	33.2	63.4	134.3	723.1	325.4	84.7	24.2
U8453_C6.1	3549.2	135.3	167.1	31.7	72.8	142.0	722.1	323.2	84.0	22.8
U8453_D1.1	3145.6	133.3	164.1	30.8	68.0	149.4	774.9	317.6	80.2	21.3
U8453_D1.2	2889.6	133.2	165.4	32.2	62.4	147.9	742.2	339.2	84.2	23.0
U8453_D5.1	3274.7	130.4	162.9	32.5	64.9	152.6	761.2	339.9	83.5	22.4
U8453_D5.2	3273.5	132.3	163.1	30.8	69.9	153.3	758.7	288.5	79.6	18.9
U8453_E3.1	3403.4	133.2	162.8	29.5	66.2	148.3	763.2	293.2	82.2	19.8
U8453_E4.1	4487.8	133.0	167.6	34.5	74.9	125.7	775.0	272.4	84.5	21.7
U8453_E4.2	3496.8	134.1	165.0	30.8	66.8	137.9	766.5	296.7	81.7	21.5
U8454_U8446_A7.1	2878.1	131.8	163.7	31.9	60.9	141.4	753.8	316.5	81.7	22.4
U8454_U8446_B2.2	3318.2	133.6	165.8	32.2	65.8	141.0	736.7	314.0	82.8	22.5

Accession	Yield (kg/ha)	Anthesis Date (Julian date)	Physiological Maturity Date (Julian Date)	Grain Fill Period (days)	Height (cm)	Protien (g/kg)	Test Weight (g/L)	Lactic Acid-SDS SRC	Sodium Carbonate SRC	Protein Quality Score
U8454_U8446_C1 .1	3301.5	131.9	164.6	32.7	64.3	150.0	749.3	287.0	81.6	19.1
U8454_U8446_C1 .2	3470.2	129.7	163.4	33.6	68.3	150.8	755.7	314.2	81.8	20.9
U8454_U8446_D2.2	3075.7	130.8	162.9	32.1	57.5	154.9	752.4	299.8	82.1	19.5
U8458_U8459_U8460_U8471_A10.1	3577.0	132.2	164.2	31.9	65.6	130.5	780.7	317.8	84.9	24.4
U8458_U8459_U8460_U8471_A10.2	3539.3	132.4	164.6	32.2	69.7	132.3	786.5	315.8	83.5	23.8
U8458_U8459_U8460_U8471_C10.2	2649.1	131.3	162.9	31.7	53.9	169.1	732.6	368.3	87.7	22.1
U8458_U8459_U8460_U8471_C4.2	3705.1	132.6	164.3	31.6	71.9	134.4	753.0	328.2	85.2	24.5
U8458_U8459_U8460_U8471_C6.1	3104.7	132.8	165.2	32.3	66.3	138.9	739.5	319.6	83.9	23.1
U8458_U8459_U8460_U8471_C6.2	2753.1	133.7	163.3	29.6	66.5	140.6	758.6	310.2	82.6	22.1
U8458_U8459_U8460_U8471_D10.1	3460.9	132.6	165.8	33.2	71.3	141.1	776.5	302.9	82.1	21.5

Accession	Yield (kg/ha)	Anthesis Date (Julian date)	Physiological Maturity Date (Julian Date)	Grain Fill Period (days)	Height (cm)	Protien (g/kg)	Test Weight (g/L)	Lactic Acid-SDS SRC	Sodium Carbonate SRC	Protein Quality Score
U8458_U8459_U8460_U8471_D8.1	3749.5	134.1	167.4	33.3	67.4	134.6	756.8	318.3	83.9	23.7
U8458_U8459_U8460_U8471_D8.2	3560.6	133.8	166.0	32.2	63.6	136.2	759.2	318.8	84.5	23.4
U8461_D10.2	3078.2	134.5	163.8	29.2	60.6	142.8	702.8	343.8	86.0	24.1
U8537_B5.2	2423.2	129.9	163.2	33.3	59.4	180.3	734.0	318.2	85.6	17.9
U8537_C6.1	3141.7	130.5	163.8	33.4	69.0	148.6	776.6	329.8	80.6	22.3
U8537_C6.2	3003.4	131.2	164.0	32.7	71.4	144.7	775.7	330.7	82.4	22.9
U8542_A10.1	3259.4	130.6	162.7	32.0	58.3	140.1	749.7	313.5	85.8	22.4
U8542_A2.2	3271.3	132.6	164.6	32.1	61.2	144.2	731.4	341.2	86.3	23.7
U8542_A6.1	3495.3	131.3	162.9	31.6	69.2	149.9	768.7	295.9	82.5	19.8
U8542_B3.1	3010.7	132.4	163.6	31.2	60.4	141.6	758.4	321.6	83.3	22.8
U8542_C7.1	2836.4	130.2	162.9	32.7	54.1	167.5	745.6	359.3	86.8	21.7
U8542_C7.2	2799.1	130.3	164.4	34.1	56.8	162.1	748.8	362.0	86.7	22.5
U8542_C8.1	3747.1	130.1	164.1	34.0	63.4	137.2	777.7	298.5	81.5	21.7
U8542_D5.1	2469.7	134.6	168.0	33.5	66.2	180.3	732.7	291.2	82.9	16.2
U8546_A3.2	2850.8	132.2	166.1	33.9	69.1	142.3	768.6	313.0	83.5	22.0
U8546_A4.1	3036.4	129.8	164.5	34.7	63.5	144.2	768.9	308.9	82.9	21.5
U8546_A4.2	3367.3	130.6	164.4	33.8	64.1	139.9	778.5	304.2	83.4	21.8
U8546_B5.2	3035.4	132.7	165.8	33.1	66.3	136.8	765.2	309.9	83.0	22.6
U8546_C8.2	2915.3	130.6	163.5	32.9	64.2	151.7	752.0	300.7	82.0	19.9
U8546_D2.1	3685.8	131.6	164.2	32.6	63.1	134.0	765.9	323.6	86.2	24.2
U8546_D4.1	4519.6	131.6	164.6	32.9	72.0	133.5	764.3	326.2	86.7	24.6
U8546_D4.2	3066.7	133.1	165.4	32.3	66.6	137.5	774.8	305.9	83.8	22.3
U8546_D6.1	2804.4	132.2	165.3	33.1	73.3	146.5	771.3	294.5	81.3	20.1

Accession	Yield (kg/ha)	Anthesis Date (Julian date)	Physiological Maturity Date (Julian Date)	Grain Fill Period (days)	Height (cm)	Protien (g/kg)	Test Weight (g/L)	Lactic Acid-SDS SRC	Sodium Carbonate SRC	Protein Quality Score
U8547_U8433_D2.2	2666.5	131.4	164.7	33.3	74.5	175.2	735.1	237.0	80.5	13.6
U8551_A5.4	3693.0	135.8	167.1	31.3	68.2	141.6	711.0	330.6	84.6	23.5
U8551_B5.2	3671.3	132.0	163.7	31.8	70.4	143.4	769.3	292.0	83.8	20.4
U8551_C5.1	3927.5	131.2	164.5	33.4	63.2	132.6	775.8	329.5	85.0	24.9
U8551_D3.1	4009.6	130.6	162.9	32.3	58.4	128.5	764.0	318.1	83.9	24.7
U8551_E2.2	3279.8	132.5	164.3	31.8	66.8	143.8	744.9	340.6	85.4	23.7

Appendix B - Best Linear Unbiased Estimates of Brabender GlutoPeak Measurements

Accession	PMT_b (Peak Maximum Time)	BEM_b (Maximum Torque)	AM_b	PM_b	A01_b	A12_b	A23_b	A34_b	A45_b
BOB_DOLE	92.1	57.9	42.1	38.6	175.9	303.5	1806.4	870.2	2100.8
KanMark	91.0	63.8	34.8	50.5	166.3	210.9	1097.9	814.8	947.1
KS090387K-20	103.9	45.8	36.5	37.8	145.5	233.5	1887.4	847.2	2105.3
ZENDA	96.2	75.6	51.9	23.1	180.5	143.3	2941.3	1028.3	3256.0
U8318_U8319_D4.2	105.3	33.3	54.5	27.5	342.5	91.8	3133.8	1094.0	3563.9
U8323_A4.2	111.5	81.5	54.5	24.8	198.5	427.0	2759.0	1029.7	3210.6
U8323_B10.1	91.1	84.3	48.5	41.0	177.5	329.5	2015.2	1036.6	2329.1
U8323_C2.2	130.5	98.5	73.0	65.3	757.3	1552.3	3403.8	1416.3	3861.9
U8323_C5.2	131.2	107.3	94.0	70.0	625.0	319.3	5513.4	1497.5	2801.3
U8323_E1.2	129.5	105.9	89.2	58.1	605.9	271.5	5510.9	1428.4	3546.4
U8323_E3.1	85.6	60.6	35.6	52.7	157.1	262.6	797.1	761.7	806.6
U8323_E4.1	88.5	61.3	28.5	50.3	157.8	449.3	622.2	734.3	846.2
U8323_E4.2	84.9	60.3	26.2	48.8	147.5	390.5	621.5	701.9	821.4
U8330_U8444_U8574_U8346_A10.1	110.6	86.0	56.4	20.7	526.7	496.8	3006.5	1165.8	3979.0

Accession	PMT_b (Peak Maximum Time)	BEM_b (Maximum Torque)	AM_b	PM_b	A01_b	A12_b	A23_b	A34_b	A45_b
U8330_U8444_U8574_U8346_C8.1	117.8	92.8	71.7	13.5	425.8	690.3	3843.5	1378.6	4680.7
U8330_U8444_U8574_U8346_C8.2	120.3	87.3	69.7	44.0	953.0	1102.0	2872.4	1294.1	3794.6
U8382_C8.1	96.3	106.7	60.6	72.6	863.7	1584.7	1427.7	1243.9	2152.0
U8382_C8.2	91.6	62.5	34.7	53.6	139.5	284.5	882.2	801.5	896.0
U8382_C9.1	127.8	144.3	97.5	122.5	1384.0	2798.3	2724.8	1805.7	1899.7
U8382_D6.2	91.6	50.8	52.2	44.0	289.3	137.5	2093.5	926.9	2108.4
U8382_E3.1	123.1	157.8	96.7	97.8	1650.3	2351.3	2153.4	1881.3	3073.7
U8382_E3.2	124.4	146.3	95.2	97.0	1474.5	2715.8	2242.8	1804.1	3142.0
U8386_U8552_E1.2	130.5	148.0	85.7	65.5	1236.5	2020.3	3083.5	1770.2	4036.0
U8434_A4.1	72.4	62.8	37.2	55.3	196.5	174.0	684.2	833.7	884.9
U8438_U8376_C2.1	105.3	72.3	60.2	29.0	239.0	123.3	3238.0	1233.6	3635.9
U8438_U8376_E5.2	77.3	64.8	36.5	53.3	170.3	319.0	751.5	819.4	883.8

Accession	PMT_b (Peak Maximum Time)	BEM_b (Maximum Torque)	AM_b	PM_b	A01_b	A12_b	A23_b	A34_b	A45_b
U8439_U8554_U8440_A3.2	63.8	55.9	36.0	43.9	597.7	471.3	589.0	838.4	1904.4
U8439_U8554_U8440_C4.2	105.0	33.0	49.5	26.3	178.8	129.8	3098.5	966.7	3386.0
U8439_U8554_U8440_D4.1	127.8	115.0	74.0	48.0	167.5	209.3	4259.7	1456.6	3835.4
U8439_U8554_U8440_D4.2	105.9	80.8	52.2	44.8	158.2	284.5	2297.8	1069.2	2523.0
U8439_U8554_U8440_E6.2	116.6	63.3	55.5	29.0	505.5	937.3	2210.6	1088.2	3565.4
U8439_U8554_U8440_E9.1	49.4	63.3	24.0	50.0	319.0	1390.3	1531.8	693.4	853.7
U8441_U8549_A2.2	145.5	30.5	93.4	1.0	392.9	505.7	5140.5	1555.6	6291.1
U8441_U8549_D8.1	106.8	48.5	49.5	40.3	180.8	259.0	2260.9	886.4	1911.0
U8441_U8549_D9.1	79.3	51.5	49.2	39.8	226.3	172.3	1981.0	987.8	2392.1

Accession	PMT_b (Peak Maximum Time)	BEM_b (Maximum Torque)	AM_b	PM_b	A01_b	A12_b	A23_b	A34_b	A45_b
U8441_U8549_E1.1	121.5	94.3	57.7	24.8	198.8	329.8	3416.3	1176.0	3520.6
U8441_U8549_E1.2	138.1	82.2	85.8	25.7	236.7	283.0	5665.0	1458.6	5406.3
U8450_D3.2	117.5	79.0	73.5	13.8	546.8	617.8	3816.4	1302.5	4643.6
U8450_E7.1	105.9	88.1	60.2	23.0	436.3	587.9	2946.3	1226.0	3899.8
U8450_E7.2	148.0	35.3	75.7	0.0	185.0	122.0	6152.6	1292.7	6012.6
U8452_U8447_U8539_C1.1	81.2	63.3	34.6	52.0	170.9	367.3	635.3	797.1	745.6
U8452_U8447_U8539_E8.2	130.0	44.8	80.2	14.5	638.0	601.8	4318.9	1383.7	5291.8
U8453_A1.1	47.4	64.0	26.7	47.5	350.3	1136.5	1283.1	728.0	848.5
U8453_A1.2	42.6	66.0	28.8	49.1	392.7	8.9	21.7	746.0	856.2
U8453_A6.1	97.4	60.3	38.6	47.3	170.9	303.9	1210.5	802.6	929.6
U8453_C6.1	97.4	62.0	35.2	52.3	160.3	288.3	1045.7	810.9	854.1
U8453_D1.1	78.1	63.6	31.8	55.7	164.1	247.3	766.7	792.0	963.9
U8453_D1.2	90.8	65.3	37.0	56.5	184.3	509.8	795.2	848.3	935.1
U8453_D5.1	133.5	95.3	64.0	14.8	190.5	371.5	3275.0	1201.0	3829.2
U8453_D5.2	84.8	48.0	38.5	39.3	310.8	187.0	1711.2	895.6	2151.4

Accession	PMT_b (Peak Maximum Time)	BEM_b (Maximum Torque)	AM_b	PM_b	A01_b	A12_b	A23_b	A34_b	A45_b
U8453_E3.1	67.8	65.3	32.5	50.0	276.0	137.8	553.7	777.9	875.5
U8453_E4.1	68.5	60.0	24.2	43.3	134.3	162.5	530.9	641.5	776.7
U8453_E4.2	90.8	47.5	40.5	37.3	172.0	147.3	1928.6	839.2	2025.0
U8454_U8446_A7.1	72.8	64.5	34.7	48.3	194.5	250.8	687.7	801.0	844.5
U8454_U8446_B2.2	88.4	62.0	30.5	50.3	167.0	413.8	790.9	737.2	856.0
U8454_U8446_C1 .1	99.6	51.5	48.0	25.0	228.3	137.5	3095.0	891.6	3197.3
U8454_U8446_C1 .2	97.7	57.3	48.8	29.7	192.3	207.5	2646.5	955.0	2992.6
U8454_U8446_D2.2	79.6	51.3	46.5	41.5	755.5	700.0	873.9	937.9	2030.7
U8458_U8459_U8460_U8471_A10.1	90.3	63.5	34.2	48.3	168.2	317.0	990.7	788.7	845.0
U8458_U8459_U8460_U8471_A10.2	97.5	60.8	31.5	48.3	149.8	326.3	995.3	744.9	823.4

Accession	PMT_b (Peak Maximum Time)	BEM_b (Maximum Torque)	AM_b	PM_b	A01_b	A12_b	A23_b	A34_b	A45_b
U8458_U8459_U8460_U8471_C10.2	127.1	74.2	71.0	64.9	470.3	779.3	4056.4	1350.6	3460.0
U8458_U8459_U8460_U8471_C4.2	96.8	61.5	37.7	49.0	157.3	305.5	1099.6	795.7	839.9
U8458_U8459_U8460_U8471_C6.1	93.3	48.3	49.2	38.3	180.5	272.3	1882.4	894.1	2041.6
U8458_U8459_U8460_U8471_C6.2	66.8	64.3	36.7	51.0	165.5	230.0	582.9	817.3	863.5
U8458_U8459_U8460_U8471_D10.1	53.4	66.3	34.6	54.0	253.5	129.1	266.4	814.4	780.4
U8458_U8459_U8460_U8471_D8.1	87.9	63.5	35.2	48.3	166.5	370.5	910.6	800.3	840.7

Accession	PMT_b (Peak Maximum Time)	BEM_b (Maximum Torque)	AM_b	PM_b	A01_b	A12_b	A23_b	A34_b	A45_b
U8458_U8459_ U8460_U8471_ D8.2	97.2	63.4	38.0	50.1	163.3	451.9	1029.6	825.3	938.1
U8461_D10.2	113.9	50.7	45.4	40.7	165.7	530.3	1719.5	856.9	1743.3
U8537_B5.2	135.6	146.2	92.2	46.9	1244.1	2195.5	3288.7	1771.7	4586.5
U8537_C6.1	87.7	62.4	37.4	56.1	151.7	314.0	851.1	808.7	829.8
U8537_C6.2	101.5	65.5	45.2	40.8	162.8	318.8	1841.0	875.2	1955.4
U8542_A10.1	73.5	64.7	35.8	49.7	193.3	232.1	786.1	815.7	991.0
U8542_A2.2	95.1	70.8	44.2	39.8	198.8	365.8	1675.4	924.6	1892.0
U8542_A6.1	52.1	66.0	34.2	51.3	516.1	21.8	135.8	827.6	836.4
U8542_B3.1	94.4	59.0	34.2	51.0	136.3	303.5	917.5	768.4	833.4
U8542_C7.1	117.8	119.8	75.0	81.3	869.3	1309.3	2588.6	1424.7	2762.1
U8542_C7.2	127.0	93.8	73.2	79.3	748.5	714.8	3510.6	1345.6	2627.8
U8542_C8.1	75.5	59.6	27.4	51.3	142.3	288.4	564.0	729.6	785.0
U8542_D5.1	93.8	101.3	69.0	28.3	906.0	1369.0	1570.0	1318.5	3708.7
U8546_A3.2	106.0	31.8	46.5	24.0	180.0	216.3	2736.2	864.9	2998.0
U8546_A4.1	75.1	51.8	40.5	38.8	200.0	126.8	1767.4	880.7	2167.4
U8546_A4.2	73.2	54.0	36.2	41.7	260.5	110.5	1484.7	808.7	1881.7
U8546_B5.2	71.6	61.8	31.7	49.0	165.3	257.3	674.7	764.3	845.8

Accession	PMT_b (Peak Maximum Time)	BEM_b (Maximum Torque)	AM_b	PM_b	A01_b	A12_b	A23_b	A34_b	A45_b
U8546_C8.2	103.9	57.9	49.0	23.0	364.9	96.5	3068.3	906.5	3516.3
U8546_D2.1	81.4	65.5	33.5	52.0	190.8	437.3	666.5	793.6	880.9
U8546_D4.1	110.1	31.8	45.2	23.0	160.3	222.3	2806.3	847.4	3066.8
U8546_D4.2	74.2	58.1	42.6	45.9	172.7	295.1	863.1	805.0	898.5
U8546_D6.1	51.9	67.0	30.2	55.5	303.0	80.0	267.9	797.7	925.2
U8547_U8433_D2.2	56.0	82.5	33.0	38.3	367.8	746.3	793.8	890.0	2185.4
U8551_A5.4	110.0	62.3	34.2	53.3	160.3	412.3	1170.6	799.2	875.9
U8551_B5.2	86.5	48.5	40.7	41.5	180.3	160.3	1868.3	869.6	2065.9
U8551_C5.1	102.8	62.0	38.2	51.5	155.3	348.0	1111.3	820.9	842.5
U8551_D3.1	116.0	57.0	30.0	46.0	133.0	378.8	1112.4	703.7	785.9
U8551_E2.2	82.1	67.0	46.0	57.9	196.7	308.5	1006.9	915.2	1016.2

Appendix C - Best Linear Unbiased Estimates of Second-by-Second GlutoPeak Measurements

Accession	PMT	MT	MPArea	FPTime	FPTorque	FPArea	TTime	TTorque
BOB_DOLE	92.6	70.5	893.7	65.9	59.3	922.3	93.0	45.0
KanMark	91.6	64.4	1114.8	24.3	29.9	495.7	43.9	24.7
KS090387K-20	104.5	72.6	869.9	34.5	29.9	233.1	48.8	26.1
ZENDA	96.5	84.7	713.3	37.5	49.9	625.5	63.3	36.8
U8318_U8319_D4.2	105.5	88.1	867.8	50.8	57.5	669.0	84.5	43.5
U8323_A4.2	111.8	81.6	825.4	61.3	56.0	847.9	84.3	44.7
U8323_B10.1	91.5	84.1	1028.1	44.5	46.3	604.7	68.3	36.3
U8323_C2.2	131.0	116.8	935.7	50.5	63.7	486.5	75.0	51.8
U8323_C5.2	131.8	107.3	945.4	53.0	79.2	896.6	68.3	63.1
U8323_E1.2	130.0	106.6	918.4	49.9	79.7	1033.3	63.2	64.9
U8323_E3.1	86.1	60.5	788.8	75.2	52.3	649.8	111.1	40.8
U8323_E4.1	89.0	61.3	937.9	49.0	40.1	527.0	88.0	30.4
U8323_E4.2	85.5	60.7	807.1	31.3	29.2	404.9	58.8	24.2
U8330_U8444_U8574_U8346_A10.1	111.1	100.3	850.6	60.4	68.9	928.7	111.7	50.5
U8330_U8444_U8574_U8346_C8.1	118.0	113.9	911.8	51.3	72.4	1143.6	87.5	53.7
U8330_U8444_U8574_U8346_C8.2	121.0	106.6	806.6	54.0	71.6	1082.4	93.0	52.8

Accession	PMT	MT	MPArea	FPTime	FPTorque	FPArea	TTime	TTorque
U8382_C8.1	96.7	105.8	917.9	42.0	72.2	693.7	101.3	49.3
U8382_C8.2	91.9	62.4	687.8	35.9	32.9	312.6	65.1	26.0
U8382_C9.1	128.0	145.2	1142.3	40.5	83.1	1335.7	75.5	62.2
U8382_D6.2	92.0	69.4	619.9	34.8	45.3	563.6	58.5	34.8
U8382_E3.1	123.3	159.0	998.3	49.8	79.6	883.8	78.3	62.7
U8382_E3.2	124.8	146.3	1030.1	53.8	79.3	662.0	77.8	63.8
U8386_U8552_E1.2	131.0	148.9	1259.9	51.0	75.6	728.0	81.8	59.0
U8434_A4.1	72.8	62.8	851.4	58.3	51.4	635.3	105.0	38.2
U8438_U8376_C2.1	106.0	101.4	1076.9	68.5	68.4	614.6	99.8	53.4
U8438_U8376_E5.2	77.8	64.7	1115.7	42.3	42.8	705.3	72.3	33.0
U8439_U8554_U8440_A3.2	64.2	71.1	712.6	41.5	69.2	796.8	98.2	46.6
U8439_U8554_U8440_C4.2	105.3	78.5	596.9	50.0	55.3	666.7	99.8	40.0
U8439_U8554_U8440_D4.1	128.5	115.4	1023.4	88.0	68.6	596.1	106.3	57.1
U8439_U8554_U8440_D4.2	106.8	80.6	1220.7	48.5	44.0	393.6	70.8	36.2
U8439_U8554_U8440_E6.2	117.0	82.5	1008.0	70.0	54.7	362.8	93.3	45.4

Accession	PMT	MT	MPArea	FPTime	FPTorque	FPArea	TTime	TTorque
U8439_U8554_U8440_E9.1	49.8	63.5	712.2	49.3	63.0	712.2	102.8	42.7
U8441_U8549_A2.2	145.9	121.9	776.3	64.4	59.6	415.0	82.1	49.2
U8441_U8549_D8.1	106.8	66.4	622.9	26.5	32.6	422.1	44.0	26.5
U8441_U8549_D9.1	79.8	79.0	960.0	58.5	70.3	1173.8	88.8	51.9
U8441_U8549_E1.1	122.0	94.8	1009.6	48.5	45.5	670.2	69.5	36.3
U8441_U8549_E1.2	138.7	120.1	655.5	62.2	75.2	987.0	83.5	59.0
U8450_D3.2	118.0	98.8	861.0	52.3	72.3	804.4	90.0	53.2
U8450_E7.1	106.3	102.1	938.3	55.8	70.5	922.0	95.7	51.5
U8450_E7.2	148.5	104.4	733.7	49.8	57.9	643.8	73.5	45.6
U8452_U8447_U8539_C1.1	81.3	62.3	1187.4	40.4	38.3	605.1	76.9	30.1
U8452_U8447_U8539_E8.2	130.5	108.3	981.1	75.8	74.2	924.9	97.0	60.3
U8453_A1.1	48.0	64.2	831.9	47.0	63.2	831.9	128.3	40.9
U8453_A1.2	43.1	66.2	755.7	42.8	65.5	744.5	120.2	42.1
U8453_A6.1	98.0	61.7	979.6	16.7	20.5	228.0	31.2	18.8
U8453_C6.1	97.8	62.0	909.1	34.3	29.8	394.4	58.8	24.4
U8453_D1.1	78.4	64.0	904.8	67.5	54.0	664.9	109.9	40.2
U8453_D1.2	91.5	65.7	1207.9	70.3	53.9	1039.3	103.5	43.2
U8453_D5.1	134.0	95.4	570.3	52.3	45.5	519.7	72.3	37.7

Accession	PMT	MT	MPArea	FPTime	FPTorque	FPArea	TTime	TTorque
U8453_D5.2	85.5	74.7	1005.8	48.3	54.6	777.7	83.3	40.8
U8453_E3.1	68.0	65.4	999.6	43.0	54.9	825.8	99.5	37.2
U8453_E4.1	69.0	60.0	701.4	51.8	49.5	575.4	111.8	32.5
U8453_E4.2	91.5	67.7	882.8	68.8	64.4	1113.4	117.5	45.5
U8454_U8446_A7.1	73.0	64.5	985.7	73.0	64.2	985.7	121.3	45.1
U8454_U8446_B2.2	88.8	62.0	890.3	52.3	41.5	557.0	79.0	33.1
U8454_U8446_C1 .1	100.5	70.8	588.2	53.0	68.8	985.5	105.0	48.7
U8454_U8446_C1 .2	98.4	75.8	726.9	64.3	67.2	842.4	98.6	49.2
U8454_U8446_D2.2	79.8	70.3	840.9	51.8	69.9	1012.7	96.5	50.0
U8458_U8459_U8460_U8471_A10.1	90.8	63.4	940.4	33.5	32.3	464.4	52.3	27.4
U8458_U8459_U8460_U8471_A10.2	98.0	61.1	962.2	14.8	19.5	199.1	33.3	17.4
U8458_U8459_U8460_U8471_C10.2	127.6	110.1	852.9	45.7	55.9	445.5	65.5	46.3
U8458_U8459_U8460_U8471_C4.2	97.0	61.6	824.3	15.5	20.1	222.3	32.0	18.4
U8458_U8459_U8460_U8471_C6.1	94.3	67.0	1001.2	40.8	42.8	641.3	72.8	32.7
U8458_U8459_U8460_U8471_C6.2	67.3	64.3	1052.5	40.3	42.0	609.9	75.3	30.9

Accession	PMT	MT	MPArea	FPTime	FPTorque	FPArea	TTime	TTorque
U8458_U8459_U8460_U8471_D10.1	53.9	65.3	997.6	51.4	57.1	864.9	86.5	42.6
U8458_U8459_U8460_U8471_D8.1	88.5	63.4	1105.0	32.5	32.0	472.2	58.0	25.9
U8458_U8459_U8460_U8471_D8.2	97.8	63.9	956.9	15.9	21.3	221.0	37.1	18.8
U8461_D10.2	114.4	66.2	947.6	16.9	21.0	234.7	42.2	19.0
U8537_B5.2	136.0	147.6	1087.9	48.3	76.8	971.9	82.3	59.4
U8537_C6.1	88.1	62.0	856.3	62.2	45.6	632.3	90.1	36.9
U8537_C6.2	101.8	65.7	673.7	50.5	42.6	532.3	74.5	34.7
U8542_A10.1	73.6	66.0	855.1	71.9	64.6	845.0	106.4	47.7
U8542_A2.2	96.5	71.1	729.7	36.5	34.1	431.2	50.8	29.6
U8542_A6.1	52.7	65.9	955.0	52.6	66.7	958.5	100.9	46.2
U8542_B3.1	94.8	59.3	754.3	35.8	28.7	275.3	59.3	23.8
U8542_C7.1	118.5	119.5	790.9	54.8	62.3	434.3	78.3	49.6
U8542_C7.2	127.5	111.4	718.0	71.5	72.1	814.1	102.0	56.7
U8542_C8.1	75.7	59.6	906.4	37.0	35.5	502.8	66.3	26.9
U8542_D5.1	94.3	102.1	785.1	48.3	72.1	625.2	101.5	51.8
U8546_A3.2	106.5	65.2	623.3	56.0	53.0	819.7	89.0	40.2
U8546_A4.1	76.0	73.7	881.0	53.5	67.9	801.2	84.5	49.6
U8546_A4.2	74.0	69.5	766.5	54.7	67.2	950.0	102.9	47.3

Accession	PMT	MT	MPArea	FPTime	FPTorque	FPArea	TTime	TTorque
U8546_B5.2	72.5	62.2	865.2	33.3	31.7	196.6	48.8	27.5
U8546_C8.2	104.9	71.5	617.8	57.6	70.3	966.8	102.7	50.6
U8546_D2.1	82.0	65.5	914.2	44.8	43.8	461.0	73.5	33.5
U8546_D4.1	110.5	69.5	475.9	55.8	54.4	740.7	85.0	41.5
U8546_D4.2	73.8	59.1	803.4	28.5	30.7	387.2	43.6	26.9
U8546_D6.1	52.3	67.2	796.0	51.8	66.8	796.0	119.0	46.4
U8547_U8433_D2.2	56.5	83.0	848.3	28.0	74.0	705.5	122.0	43.0
U8551_A5.4	110.3	62.4	946.0	35.3	30.5	437.5	63.3	24.3
U8551_B5.2	87.0	70.8	668.7	59.0	54.5	598.0	85.8	42.5
U8551_C5.1	103.3	62.1	969.0	15.8	19.2	217.5	35.5	17.1
U8551_D3.1	116.5	57.2	881.5	15.3	17.5	186.1	38.5	15.4
U8551_E2.2	82.8	68.0	1194.4	86.3	66.2	897.4	114.9	52.4

Appendix D - Best Linear Unbiased Estimates for Additional Second-by-Second GlutoPeak Measurements

Accession	MTBefore60	T150sec	A01	A12	PMslope	AMslope	PSlope
BOB_DOLE	39.6	64.2	1704.2	1432.5	0.8	1.8	-0.5
KanMark	30.1	50.9	512.7	741.9	0.3	1.4	-0.2
KS090387K-20	23.3	59.9	843.9	470.0	0.2	0.9	-0.2
ZENDA	66.8	72.7	935.3	1276.5	0.6	2.1	-0.4
U8318_U8319_D4.2	58.5	78.8	1520.3	1860.5	0.5	1.6	-0.4
U8323_A4.2	33.4	74.3	1683.4	1171.8	0.7	1.7	-0.5
U8323_B10.1	47.5	73.3	1106.9	1065.9	0.5	1.9	-0.4
U8323_C2.2	64.3	97.0	1905.9	1603.7	0.6	1.2	-0.4
U8323_C5.2	80.5	101.4	1707.0	1051.8	1.0	2.8	-1.2
U8323_E1.2	80.9	90.3	1775.5	1033.5	0.9	2.8	-1.1
U8323_E3.1	20.2	45.2	1792.1	1626.4	0.5	1.3	-0.3
U8323_E4.1	22.4	45.2	1025.3	1480.6	0.4	1.7	-0.2
U8323_E4.2	19.9	43.4	588.2	934.3	0.3	1.4	-0.1
U8330_U8444_U8574_U8346_A10.1	66.2	82.8	1766.6	2805.6	0.6	2.1	-0.4
U8330_U8444_U8574_U8346_C8.1	71.8	104.0	1715.9	2209.7	0.8	2.0	-0.5
U8330_U8444_U8574_U8346_C8.2	71.4	97.7	1716.8	2323.3	0.8	2.1	-0.5
U8382_C8.1	72.7	86.4	1330.2	3215.5	1.0	2.7	-0.4

Accession	MTBefore60	T150sec	A01	A12	PMslope	AMslope	PSlope
U8382_C8.2	26.1	48.2	801.4	977.5	0.3	1.2	-0.2
U8382_C9.1	83.8	122.4	1726.3	2516.3	0.7	2.2	-0.6
U8382_D6.2	48.8	56.1	1031.3	1179.5	0.3	1.4	-0.3
U8382_E3.1	79.8	124.2	1986.4	2080.3	0.6	1.7	-0.6
U8382_E3.2	80.5	118.3	2491.8	1747.8	0.8	0.8	-0.7
U8386_U8552_E1.2	76.5	124.6	2113.8	2088.0	0.7	1.2	-0.5
U8434_A4.1	37.8	49.0	1438.6	2215.5	0.5	1.6	-0.3
U8438_U8376_C2.1	64.7	90.6	2178.8	1864.3	0.7	1.3	-0.5
U8438_U8376_E5.2	35.2	53.8	1111.5	1274.4	0.5	1.4	-0.3
U8439_U8554_U8440_A3.2	68.9	54.1	1235.6	2991.2	0.7	2.7	-0.4
U8439_U8554_U8440_C4.2	60.3	67.8	1295.9	2520.1	0.7	2.1	-0.4
U8439_U8554_U8440_D4.1	39.4	104.1	2996.2	1163.8	0.6	0.5	-0.7
U8439_U8554_U8440_D4.2	31.3	75.2	1412.9	915.7	0.4	1.2	-0.3
U8439_U8554_U8440_E6.2	35.3	76.9	2312.1	1135.8	0.6	0.3	-0.4
U8439_U8554_U8440_E9.1	63.5	47.6	1181.0	2597.0	0.8	2.6	-0.4
U8441_U8549_A2.2	44.0	116.5	2173.1	1022.1	0.6	0.8	-0.6
U8441_U8549_D8.1	33.8	58.1	562.2	652.8	0.4	1.5	-0.3
U8441_U8549_D9.1	69.8	70.3	1824.1	1768.3	1.1	2.1	-0.6
U8441_U8549_E1.1	40.8	84.4	1198.4	896.5	0.6	1.8	-0.4
U8441_U8549_E1.2	61.7	105.5	1989.5	1382.0	1.0	2.4	-0.8
U8450_D3.2	73.2	87.9	1796.2	2254.6	0.9	1.9	-0.5

Accession	MTBefore60	T150sec	A01	A12	PMslope	AMslope	PSlope
U8450_E7.1	69.8	87.1	1712.4	2331.2	0.7	2.3	-0.5
U8450_E7.2	69.4	102.3	1586.6	1409.5	0.5	1.5	-0.4
U8452_U8447_U8539_C1.1	31.5	46.5	950.9	1564.6	0.4	1.3	-0.2
U8452_U8447_U8539_E8.2	56.5	105.8	2809.3	1429.8	0.9	0.7	-0.7
U8453_A1.1	64.2	41.4	1218.6	3757.8	1.0	2.5	-0.3
U8453_A1.2	65.7	46.2	1163.7	3550.4	1.0	2.5	-0.3
U8453_A6.1	22.9	48.4	215.9	274.0	0.1	1.2	-0.1
U8453_C6.1	19.6	46.0	667.4	903.9	0.3	1.4	-0.2
U8453_D1.1	30.1	47.3	1707.6	2052.8	0.5	1.6	-0.3
U8453_D1.2	23.6	62.0	1864.2	1483.2	0.6	1.4	-0.4
U8453_D5.1	24.0	80.6	1498.8	833.9	0.5	1.2	-0.4
U8453_D5.2	56.0	65.6	1283.8	1780.3	0.5	2.0	-0.4
U8453_E3.1	54.9	45.9	1159.1	2661.8	0.8	2.0	-0.3
U8453_E4.1	35.2	39.6	1030.6	2316.0	0.9	2.1	-0.3
U8453_E4.2	43.5	57.0	1783.4	2378.5	0.9	2.2	-0.4
U8454_U8446_A7.1	37.9	46.1	1927.3	2409.7	1.0	2.0	-0.4
U8454_U8446_B2.2	20.9	45.7	1202.5	1129.8	0.4	1.6	-0.3
U8454_U8446_C1 .1	69.0	59.9	1560.4	2764.1	1.0	2.3	-0.4
U8454_U8446_C1 .2	61.4	65.3	1903.0	1914.5	0.8	1.7	-0.5
U8454_U8446_D2.2	69.8	60.1	1624.5	2549.0	0.9	2.3	-0.5
U8458_U8459_U8460_U8471_A10.1	22.3	51.4	708.9	679.1	0.3	1.5	-0.2

Accession	MTBefore60	T150sec	A01	A12	PMslope	AMslope	PSlope
U8458_U8459_U8460_U8471_A10.2	19.8	45.7	147.5	350.0	0.1	1.3	-0.1
U8458_U8459_U8460_U8471_C10.2	63.4	99.1	1530.5	1196.7	0.4	1.2	-0.4
U8458_U8459_U8460_U8471_C4.2	20.4	47.2	162.2	325.3	0.1	1.3	-0.1
U8458_U8459_U8460_U8471_C6.1	42.3	58.1	1049.9	1396.0	0.5	1.5	-0.2
U8458_U8459_U8460_U8471_C6.2	51.4	50.8	948.4	1519.6	0.4	1.4	-0.2
U8458_U8459_U8460_U8471_D10.1	65.5	50.3	1448.4	1890.2	0.5	1.9	-0.4
U8458_U8459_U8460_U8471_D8.1	22.2	50.2	659.6	911.6	0.3	1.6	-0.2
U8458_U8459_U8460_U8471_D8.2	21.9	51.8	190.9	429.2	0.1	1.4	-0.1
U8461_D10.2	20.3	56.1	221.7	487.8	0.1	1.3	-0.1
U8537_B5.2	76.8	126.9	1954.2	2298.2	0.6	1.6	-0.5
U8537_C6.1	21.3	49.8	1512.8	1280.3	0.3	1.1	-0.3
U8537_C6.2	23.2	55.0	1238.9	1107.6	0.4	1.4	-0.3
U8542_A10.1	42.1	56.0	1870.3	1813.2	1.0	1.9	-0.5
U8542_A2.2	27.8	66.0	808.6	530.5	0.3	1.3	-0.3
U8542_A6.1	67.2	49.4	1545.5	2500.6	1.0	2.2	-0.4
U8542_B3.1	18.6	43.3	675.9	816.2	0.2	1.2	-0.2
U8542_C7.1	62.1	91.9	1921.5	1512.5	0.6	1.3	-0.5
U8542_C7.2	58.8	99.0	2272.2	1902.5	0.6	1.0	-0.5
U8542_C8.1	27.4	43.1	732.0	1099.7	0.3	1.7	-0.2
U8542_D5.1	73.0	86.6	1675.8	3019.0	0.8	2.0	-0.4
U8546_A3.2	48.0	56.9	1496.3	1588.4	0.6	1.6	-0.3

Accession	MTBefore60	T150sec	A01	A12	PMslope	AMslope	PSlope
U8546_A4.1	68.7	69.0	1607.1	1777.8	1.0	2.1	-0.6
U8546_A4.2	66.3	59.7	1553.0	2464.0	0.9	2.5	-0.5
U8546_B5.2	38.4	50.2	800.9	594.6	0.2	1.1	-0.2
U8546_C8.2	63.9	63.0	1697.1	2568.2	0.9	2.4	-0.4
U8546_D2.1	33.4	51.1	1073.6	1223.8	0.5	1.7	-0.3
U8546_D4.1	38.6	63.4	1452.5	1487.7	0.6	2.0	-0.4
U8546_D4.2	45.8	52.2	603.7	537.8	0.2	1.4	-0.2
U8546_D6.1	63.6	49.7	1415.2	3448.7	0.7	2.5	-0.3
U8547_U8433_D2.2	75.6	56.8	966.8	4420.8	1.4	3.1	-0.4
U8551_A5.4	20.0	47.4	686.1	869.6	0.3	1.4	-0.2
U8551_B5.2	50.3	58.3	1570.9	1490.4	0.2	1.6	-0.4
U8551_C5.1	19.8	48.3	159.3	366.4	0.1	1.3	-0.1
U8551_D3.1	17.5	43.4	143.6	386.4	0.1	1.1	-0.1
U8551_E2.2	37.3	62.0	2637.2	1703.1	0.7	0.9	-0.5

Appendix E - Multi-Trial Grain Protein Deviations for All Accessions

Accession Name	Grain Protein Deviation
U8546_D4.1	14.35
U8453_E4.1	5.81
BOB_DOLE	5.75
U8551_D3.1	-2.10
ZENDA	-2.58
U8551_C5.1	0.07
U8382_C8.2	11.01
KanMark	-5.16
U8458_U8459_U8460_U8471_D8.1	-1.88
U8542_C8.1	0.63
U8458_U8459_U8460_U8471_C4.2	-3.12
U8551_A5.4	3.82
U8546_D2.1	-3.94
U8551_B5.2	5.21
KS090387K-20	5.34
U8323_E4.1	-0.20
U8323_E4.2	-2.49
U8458_U8459_U8460_U8471_A10.1	-9.90
U8458_U8459_U8460_U8471_D8.2	-4.55

Accession Name	Grain Protein Deviation
U8453_C6.1	1.04
U8458_U8459_U8460_U8471_A10.2	-8.95
U8453_E4.2	-4.30
U8542_A6.1	7.75
U8453_A6.1	-8.41
U8454_U8446_C1 .2	8.02
U8458_U8459_U8460_U8471_D10.1	-1.83
U8438_U8376_E5.2	-2.62
U8452_U8447_U8539_C1.1	-3.91
U8453_E3.1	4.04
U8439_U8554_U8440_D4.1	19.72
U8546_A4.2	-5.15
U8323_E3.1	-0.74
U8454_U8446_B2.2	-5.13
U8441_U8549_D9.1	-4.47
U8454_U8446_C1 .1	3.49
U8551_E2.2	-3.25
U8453_D5.1	5.48
U8453_D5.2	6.13
U8542_A2.2	-3.05
U8441_U8549_E1.1	-11.55

Accession Name	Grain Protein Deviation
U8542_A10.1	-7.43
U8453_A1.2	5.06
U8439_U8554_U8440_E9.1	3.62
U8439_U8554_U8440_E6.2	14.40
U8450_E7.1	5.73
U8453_D1.1	-0.65
U8537_C6.1	-1.53
U8453_A1.1	3.24
U8323_A4.2	-15.34
U8458_U8459_U8460_U8471_C6.1	-12.06
U8461_D10.2	-8.76
U8330_U8444_U8574_U8346_C8.1	6.66
U8454_U8446_D2.2	3.25
U8546_D4.2	-14.33
U8318_U8319_D4.2	9.82
U8382_C8.1	7.96
U8546_A4.1	-8.29
U8546_B5.2	-15.67
U8434_A4.1	-2.33
U8542_B3.1	-11.50
U8537_C6.2	-8.56

Accession Name	Grain Protein Deviation
U8330_U8444_U8574_U8346_C8.2	7.89
U8323_E1.2	-14.04
U8439_U8554_U8440_C4.2	5.05
U8382_E3.1	18.27
U8546_C8.2	-3.55
U8453_D1.2	-7.87
U8454_U8446_A7.1	-14.60
U8323_B10.1	-10.38
U8546_A3.2	-14.41
U8441_U8549_D8.1	-5.72
U8439_U8554_U8440_D4.2	4.91
U8542_C7.1	10.47
U8546_D6.1	-11.21
U8542_C7.2	4.31
U8330_U8444_U8574_U8346_A10.1	-0.34
U8382_E3.2	24.50
U8438_U8376_C2.1	4.63
U8323_C5.2	-19.00
U8458_U8459_U8460_U8471_C6.2	-18.24
U8450_D3.2	3.76
U8547_U8433_D2.2	14.40

Accession Name	Grain Protein Deviation
U8323_C2.2	11.64
U8450_E7.2	0.30
U8441_U8549_E1.2	-14.94
U8458_U8459_U8460_U8471_C10.2	7.88
U8386_U8552_E1.2	17.01
U8439_U8554_U8440_A3.2	-5.44
U8382_D6.2	-0.80
U8542_D5.1	15.09
U8441_U8549_A2.2	-3.67
U8537_B5.2	14.06
U8382_C9.1	19.43
U8452_U8447_U8539_E8.2	-1.04

Appendix F - Correlations Between All Agronomic and Quality Traits from Chapter 2

	YIELD	GPC	GPD	TEST WEIGHT	LACTIC ACID-SDS SRC	SODIUM CARBONATE SRC	PQS	ANTHESIS DATE	MATURITY DATE	GFP	HEIGHT
YIELD	1	-0.73***	-0.16	0.51***	-0.16	0.03	0.54***	0.06	-0.07	-0.13	0.24*
GPC	-0.73***	1	0.22*	-0.47***	0.18	-0.19	-0.73***	-0.16	0.11	0.26*	-0.10
GPD	-0.16	0.22*	1	-0.13	0.13	0.16	-0.10	-0.18	0.15	0.32**	-0.01
TEST WEIGHT	0.51***	-0.47***	-0.13	1	-0.29**	-0.31**	0.19	-0.28**	-0.18	0.08	0.25*
LACTIC ACID-SDS SRC	-0.16	0.18	0.13	-0.29**	1	0.39***	0.53***	0.05	0.03	-0.01	-0.38***
SODIUM CARBONATE SRC	0.03	-0.19	0.16	-0.31**	0.39***	1	0.45***	-0.04	-0.09	-0.05	-0.43***
PQS	0.54***	-0.73***	-0.10	0.19	0.53***	0.45***	1	0.17	-0.06	-0.22*	-0.18
ANTHESIS DATE	0.06	-0.16	-0.18	-0.28**	0.05	-0.04	0.17	1	0.47***	-0.47***	0.2
MATURITY DATE	-0.07	0.11	0.15	-0.18	0.03	-0.09	-0.06	0.47***	1	0.56***	0.36***
GFP	-0.13	0.26*	0.32**	0.08	-0.01	-0.05	-0.22*	-0.47***	0.56***	1	0.17
HEIGHT	0.24*	-0.10	-0.01	0.25*	-0.38***	-0.43***	-0.18	0.2	0.36***	0.17	1

Grain Protein Concentration (GPC), Grain Protein Deviation (GPD), Protein Quality Score (PQS), Grain Fill Period (GFP)

Appendix G - Correlations Between Brabender and Second-by-Second Analysis GlutoPeak Measurements

	PMT_B	BEM_B	AM_B	PM_B	A01_B	A12_B	A23_B	A34_B	A45_B
PMT	1.0***	0.38***	0.81***	-0.10	0.38***	0.34***	0.82***	0.75***	0.75***
MT	0.70***	0.77***	0.94***	0.21*	0.82***	0.72***	0.69***	0.99***	0.74***
MPAREA	0.01	0.37***	0.1	0.38***	0.21*	0.28**	-0.16	0.2	-0.16
FPTIME	0.12	0.1	0.30**	-0.12	0.14	0.01	0.32**	0.29**	0.38***
FPTORQUE	0.16	0.45***	0.59***	0.06	0.62***	0.40***	0.46***	0.62***	0.56***
FPAREA	-0.02	0.24*	0.33**	0	0.38***	0.18	0.27**	0.33***	0.32**
TTIME	-0.29**	0.04	0.02	-0.09	0.17	0.04	0.01	0.06	0.16
TTORQUE	0.33**	0.51***	0.72***	0.09	0.66***	0.44***	0.58***	0.74***	0.64***
MTBEFORE60	0.12	0.44***	0.58***	0.07	0.67***	0.43***	0.45***	0.61***	0.54***
T150SEC	0.77***	0.66***	0.96***	0.07	0.73***	0.62***	0.78***	0.98***	0.83***
A01	0.33**	0.36***	0.60***	-0.01	0.46***	0.30**	0.50***	0.62***	0.60***
A12	-0.36***	0.17	0.04	0.02	0.38***	0.25*	-0.06	0.12	0.13
PMSLOPE	-0.11	0.21*	0.27**	-0.09	0.33**	0.15	0.26*	0.29**	0.35***
AMSLOPE	-0.37***	0.04	-0.08	-0.03	0.11	-0.03	-0.01	-0.08	-0.02
PSLOPE	-0.42***	-0.44***	-0.74***	-0.03	-0.49***	-0.27**	-0.70***	-0.70***	-0.63***

Appendix H - Correlations Between Brabender GlutoPeak Measurements and Quality Traits

	PMT_B	BEM_B	AM_B	PM_B	A01_B	A12_B	A23_B	A34_B	A45_B
GRAIN PROTEIN CONCENTRATION	0.38***	0.55***	0.67***	0.19	0.76***	0.69***	0.40***	0.74***	0.57***
LACTIC ACID-SDS SRC	0.64***	0.2	0.43***	0.13	0.14	0.17	0.35***	0.40***	0.29**
SODIUM CARBONATE SRC	0.46***	0.19	0.37***	0.08	0.03	0.02	0.44***	0.29**	0.21*
PROTEIN QUALITY SCORE	0.13	-0.30**	-0.27**	-0.04	-0.53***	-0.43***	-0.11	-0.35***	-0.30**
TEST WEIGHT	-0.47***	-0.21*	-0.48***	0.02	-0.29**	-0.30**	-0.44***	-0.45***	-0.42***
FLOUR YIELD	-0.51***	-0.50***	-0.66***	-0.20	-0.51***	-0.45***	-0.57***	-0.64***	-0.46***

Appendix I - Correlations Between Second-by-Second GlutoPeak Measurements and Quality Traits

	GRAIN PROTEIN CONCENTRATION	LACTIC ACID-SDS SRC	SODIUM CARBONATE SRC	PROTEIN QUALITY SCORE	TEST WEIGHT	FLOUR YIELD
PMT	0.38***	0.63***	0.46***	0.13	-0.47***	-0.51***
MT	0.76***	0.34***	0.24*	-0.40***	-0.42***	-0.62***
MPAREA	0.1	0.2	0.02	0.07	-0.16	-0.03
FPTIME	0.29**	0.17	-0.08	-0.18	-0.06	-0.02
FPTORQUE	0.64***	-0.13	-0.03	-0.65***	-0.19	-0.42***
FPAREA	0.30**	-0.23*	0.01	-0.43***	-0.06	-0.25*
TTIME	0.34***	-0.34***	-0.40***	-0.56***	0.04	0.08
TTORQUE	0.66***	0.06	0.08	-0.54***	-0.25*	-0.48***
MTBEFORE60	0.62***	-0.23*	0.01	-0.70***	-0.16	-0.48***
T150SEC	0.72***	0.41***	0.29**	-0.32**	-0.45***	-0.61***
A01	0.58***	0.25*	-0.01	-0.35***	-0.22*	-0.25*
A12	0.47***	-0.55***	-0.45***	-0.79***	0.02	-0.06
PMSLOPE	0.34***	-0.41***	-0.07	-0.59***	-0.09	-0.29**
AMSLOPE	-0.03	-0.72***	-0.05	-0.47***	0.11	-0.22*
PSLOPE	-0.42***	-0.06	-0.33***	0.33**	0.26*	0.59***