DISSERTATION

IMPROVING END-USE QUALITY IN HARD WINTER WHEAT THROUGH GLUTENIN

ALLELE COMBINATIONS AND GENOMIC SELECTION

Submitted by

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ABSTRACT

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Wheat (*Triticum aestivum* L.) has unique properties that allow for a variety of end products, such as pan bread, steamed bread, cookies, cakes, and tortillas. Most wheat-breeding programs focus on increasing yield and yield-related traits as primary objectives. However, end-use quality is also crucial as quality characteristics influence grain sale price and market success of a variety. Large-effect quantitative trait loci (QTL) have been identified for quality related traits. The *Glu-1* loci encoding high molecular weight glutenin subunits (HMWGS) have a major effect on dough mixing properties. However, many quality traits are too complex to be controlled by only a small number of loci. These traits may benefit from genomic selection (GS), which utilizes all effective loci regardless of effect size. Genomic selection can accelerate genetic progress especially for traits that are costly or time consuming to phenotype, like quality-related traits. This research focused on the genetic improvement of end-use quality in hard winter wheat by targeting specific loci with known effects or by using all loci in a GS approach. The objectives of this study were to: i) evaluate agronomic and quality effects associated with different combinations of HMW-GS at the *Glu-B1* and *Glu-D1* loci among a set of near isogenic lines (NILs); ii) use a genome-wide association approach to identify QTL and develop predictive models for pre-harvest sprouting tolerance (PHST) and iii) assess GS models for milling and baking traits in hard winter wheat lines representative of west-central U.S. Great Plains germplasm.

A set of NILs that varied for alleles at the *Glu-B1* and *Glu-D1* loci were evaluated for dough mixing properties, kernel characteristics, and agronomic effects. Results confirmed the Bx7^{OE} + By8 HMW-GS (*Glu-B1a1* allele) at *Glu-B1* contributed to greater dough strength compared to the common Bx7 + By8 HMW-GS (*Glu-B1b* allele); however, the effect was not as significant as that conferred by Dx5 + Dy10 subunits (*Glu-D1d* allele). Near isogenic lines with the combination of both favorable alleles at *Glu-B1* and *Glu-D1* had the largest mixograph mixing time. However, a decrease in yield was observed for groups containing the Bx7^{OE} + By8 subunits. These results suggest glutenin allele combinations are useful for improving bread-making characteristics in winter wheat but some combinations may be associated with negative effects on yield.

Pre-harvest sprouting (PHS) is a major problem in wheat that results in decreased yield and quality. Genomic selection was evaluated as a potential breeding method for PHST given the complex inheritance and phenotyping difficulty of this trait. In this study, genotyping-by-sequencing (GBS) markers were used to identify QTL associated with PHST among a panel of hard red and white winter wheat lines. Genomic selection models were developed with the GBS data and phenotype data collected across seven growing seasons. The effect of including identified QTL and kernel color as fixed effects in the model was assessed, as kernel color has been generally associated with sprouting tolerance. Optimum marker number was also determined as accuracy can vary with different numbers of markers. Results showed model accuracy did not improve with kernel color information but weighting major QTL increased predictive performance. Optimum marker number was 4,000 with no improvement in accuracy above this threshold. Overall, model accuracies were promising and confirmed wheat breeding programs would benefit from incorporating GS models for PHST.

Lastly, the accuracy of GS models for 11 end-use quality traits in a panel of hard red and white winter wheat breeding lines phenotyped across multiple years and locations was assessed. Trait heritability, marker number, and marker imputation method were evaluated for their effect on model accuracy. Traits measured included flour yield, single kernel characteristics, protein concentration, mixograph mixing time and tolerance, bake absorption, bake mixing time, crumb grain score, and loaf volume. Genotyping-by-sequencing marker data varied for marker density and imputation method used for missing data. Across traits, model accuracies ranged from 0.30 to 0.63 and trait heritability ranged from 0.03 to 0.61. Imputation method and marker density had little to no effect on model accuracy. Heritability appeared to have the greatest effect on accuracy as GS models for traits with higher heritability had higher accuracies. Additionally, GS models for moderate to high heritability traits performed better than expected when predicting a set of genotypes separate from the training panel. Results showed model accuracies for end-use quality traits were sufficient for increasing genetic gain in a wheat breeding program.

In summary, genetic improvement in end-use quality can be made by utilizing both large effect and small effect loci in the wheat genome for such traits and will reduce phenotyping costs while increasing efficiency in a breeding program. In many winter wheat breeding programs, particularly those at higher latitudes, phenotypic quality evaluations from one season cannot be used for planting decisions of the next season due to the short turn-around time from harvest to planting. Genomic selection potentially solves this problem as selection decisions based on genotypic data can be implemented before the next season of planting. Thus, results from this study support the implementation of GS to reduce phenotyping costs and increase the rate of genetic gain for end-use quality in wheat.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Cereal crops are an importantfood source worldwide due to their high nutritional value, good storing capacity, and easy transporting ability (Feuillet et al., 2008). One cereal crop in particular, wheat (*Triticum aestivum* L.), has become one of the world's most significant food crops with a global production of over 660 million tons in 2013 (FAO, 2014). Wheat is unique from other cereal crops in its distinctive proteins that allow for various end products depending on the protein make-up of the flour, as well as other characteristics. An important objective in wheat breeding programs is to improve or maintain end-use quality to produce cultivars with good milling and baking characteristics while retaining high yields.

GLUTENIN PROTEINS

A key factor in the bread-making process is the formation of gluten, which is a cohesive mass that has the ability to deform, stretch, recover and trap gas molecules. These are key components in determining dough structure and strength. Gluten gives dough its viscoelastic properties allowing for elasticity and extensibility (Cauvain, 2003).

Rheological characteristics that positively affect bread baking properties are due in part to glutenin subunits (Payne et al., 1988b). Glutenins and gliadins make up the storage protein groups found in the kernel endosperm (Kreis et al., 1985). Glutenins are defined as the proteins extractable in dilute acids or bases but not in 70 percent ethanol (Payne, 1987). They are divided into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS; Payne and Holt, 1981; Jackson et al., 1983). These subunits are linked

together by disulfide bonds between cysteine residues forming polymers that contribute to elasticity and strength (Branlard and Dardevet, 1985).

Studies have focused on improving the end-use quality of wheat through analyzing effects of glutenin genes (Lawrence and Shepherd, 1980; Payne et al., 1988a; Rogers et al., 1991; Peña et al., 1995; Obreht et al., 2007; Zheng et al., 2009; Gao et al., 2012). Glutenin genes have been identified at the *Glu-A1, Glu-B1* and *Glu-D1* loci. Payne et al. (1987) confirmed the relationship between glutenin genes and bread baking quality by identifying a subunit that was significantly correlated (r=0.72) with high loaf volume and demonstrated that the difference in bread baking quality was due to relative amounts of glutenin. Allelic variation at the loci for HMW-GS has given rise to over 500 million possible combinations of these genes (Payne, 1987). Partial additive effects on bread making were inferred from combining desired alleles at different loci (Payne et al., 1984). Drawing from this, some breeders are approaching the task of increasing end use quality in wheat by choosing parents with complimentary alleles to produce better progeny. Thus, the influence of glutenin allele combinations on quality is of value.

Rogers et al. (1991) found that allelic variation at the *Glu-D1* locus had a greater effect on baking quality than the *Glu-B1* locus. HMW-GS are encoded by "x" and "y" type genes that are tightly linked (Payne et al., 1987). In a study of near isogenic lines (NILs) with either an "x" or "y" type subunit missing at *Glu-B1* or *Glu-D1*, the lines with null alleles at *Glu-D1* suffered more loss of dough strength than lines with a null allele at *Glu-B1* (Rogers et al., 1991). However, further analysis is needed to confirm this claim.

Studies have characterized alleles at the *Glu-D1* locus (Bekes et al., 2001; Zheng et al., 2009). In a study of 96 cultivars and lines by Zheng et al. (2009), the most common subunits were Dx5 + Dy10 (*Glu-D1d*), which was shown to be beneficial over the 2+12 subunits (*Glu-*

D1a) according to mixograph peak time, a common indicator of dough strength. Other studies have also shown *Glu-D1d* to be associated with enhanced dough strength and bread-baking characteristics (Payne et al., 1987; Butow et al., 2003; He et al., 2005; Huang et al., 2006).

Subunits $Bx7^{OE}$ + By8 (*Glu-B1a1* allele) at the *Glu-B1* locus have been shown to enhance dough strength over the common Bx7 + By8 subunits (Butow et al., 2003). $Bx7^{OE}$ is an overexpression of Bx7 (Gao et al., 2012), and several studies have discussed this allele (Butow et al., 2003, 2004; Obreht et al., 2007; Ragupathy et al., 2008; Jin et al., 2011; Gao et al., 2012). Butow et al. (2003) differentiated alleles at the *Glu-B1* locus to define at the molecular level the $Bx7^{OE}$ + By8 subunits and characterize its association with dough quality parameters. Butow et al. (2004) identified the source of this allele to be from a Uruguayan landrace, Americano 44D. Ma et al. (2003) developed a marker to discriminate Bx17 from Bx7 which was later used by Obreht et al. (2007) to discriminate Bx7* and $Bx7^{OE}$. A recent survey of 718 cultivars from across the world showed $Bx7^{OE}$ + By8 to be present in 3.1 percent (Jin et al., 2011). In another survey of 316 hexaploid wheat cultivars, 40 were found to carry the allele for $Bx7^{OE}$ + By8 (Ragupathy et al., 2008). Ragupathy et al. (2008) concluded that the overexpression of Bx7 was likely due to a single duplication event at the locus facilitated by a retroelement insertion.

The $Bx7^{OE}$ + By8 subunit is found in the Colorado variety, 'Snowmass,' and has become a focal point of the breeding program for hard white wheat at Colorado State University (Haley et al., 2011). Despite having been identified as sources of dough strength, there is little known about the influence of variation at *Glu-B1* in combination with variation at *Glu-D1* among Great Plains hard winter wheat germplasm.

PRE-HARVEST SPROUTING

Pre-harvest sprouting (PHS) is the mature germination of a wheat kernel in a physiologically mature spike before harvest, usually occuring after periods of prolonged rainfall and high humidity. Besides causing a reduction in grain yield, PHS can be very detrimental to end-use quality (Gale and Lenton, 1987), causing economic losses for the farmer and processor.

Previous studies have shown pre-harvest sprouting tolerance (PHST) to be a complex trait controlled by many QTL across the genome (Tyagi and Gupta, 2012). QTL studies have identified many loci associated with PHST but the most important loci are thought to belong to homoeologous group 3 (3A, 3B, 3D; Kulwal et al., 2005; Liu et al., 2008)) and chromosome 4A (Mares et al., 2005). On the long arms of group 3 are vivipary genes (*TaVp1*) which encode a transcription factor that represses genes involved with germination. However the group 3 chromosomes also carry genes for red grain color (R-A1, R-B1, R-D1; Sears, 1944). Breeding efforts have been made to develop PHS tolerant white-grained varieties as these are usually preferred for whole grain products (Liu et al., 2008).

Improving tolerance to sprouting is a challenge to breeders because of its quantitative inheritance and laborious procedures required for phenotyping. Additionally, winter wheat breeding often occurs with a short window of time between harvest and planting, preventing phenotypic evaluations from one season from being applied for planting decisions for the next season. Using molecular markers to screen for tolerant lines would be helpful in addressing this problem. However, due to the polygenic nature of PHS using only one or a few markers may not be effective. In an association study, as many as 30 markers were identified for associations with PHST (Jaiswal et al., 2012). Utilizing markers across the genome could be a better option when breeding for PHST. Also, the genetic architecture of pre-harvest sprouting has not been

thoroughly studied. Genomic data could help provide answers to this issue and shed light on how to breed more efficiently for this trait.

QUALITY TESTING PROCEDURES

Measuring quality and predicting bread making characteristics of wheat varieties are important to produce varieties that meet quality standards. Unfortunately, there is no universal test to measure all the components of end-use quality. This is not surprising given the many processes and products involved with wheat flour. Thus, the following mentions some but not all testing procedures and traits measured for end-use quality of wheat.

Mixograph

A mixograph records a time curve of dough resistance during mixing. This test measures dough strength and is used in many end-use quality studies. The mixograph measures four properties of flour: water absorption (the amount of water needed to produce a representative curve), dough strength (related to peak time), extensibility (related to curve bandwidth), and tolerance to over-mixing (related to length, thickness, and slope of the curve; Bekes et al., 2001; Chung et al., 2001). The mixograph measures the resistance of dough against the continual mixing movements of pins. A mixograph of high quality hard winter wheat flour will have a longer peak time and greater width indicating greater tolerance to over-mixing.

Single Kernel Characterization System

The single kernel characterization system (SKCS) evaluates texture characteristics of the wheat kernels, which are related to milling properties of the grain (Wheat Marketing Center, 2008). In this process, a sample of kernels are poured into the access hopper of the SKCS instrument, which analyzes 300 individual kernels and outputs means and standard deviations. Kernels are tested for weight, diameter, moisture content and hardness.

Sedimentation Test

The sedimentation test indicates the protein quality of ground wheat or flour. Sedimentation volume is highly correlated with gluten strength and loaf volume. To conduct this test, the wheat sample is emersed in a lactic acid and sodium dodecyl sulphate (SDS) solution. Glutenin proteins will swell and precipitate as sediment under such conditions. Volume of the sediment is recorded with a higher value indicative of a wheat with strong gluten. Correction for differences in protein concentration among samples may be done by reporting SDS sedimentation volume per unit of protein concentration.

NIRS, Protein, Moisture and Ash Content

Near-infrared reflectance spectroscopy (NIRS) measures the moisture, protein concentration, and ash concentration of the flour and grain without destroying the sample. NIRS is a commonly used tool due to its accuracy and precision, ease-of-use, consistency, and relatively low operating cost (Ross and Bettge, 2009). The near-infrared region of the electromagnetic spectrum, about 500-800 nm, is used to determine the concentration of physical or chemical constituents in materials (Pasquini, 2003). A typical sample will be approximately 12 percent moisture. It is desirable for a flour to have low moisture for more stability during storage. High grain moisture content will lead to deterioration problems due to mold, bacteria, and insects. There are also some downsides to a moisture content that is too low as special equipment or processes may be needed before milling to reach standard moisture levels. Ash concentration is typically measured with NIRS but can be measured by high temperature incineration of a flour sample in an electric muffle furnace. The previously weighed sample is heated overnight at 585 °C until its weight is stable. The process of incinerating the sample drives out the moisture and burns away organic materials such as the starch, protein, and oil.

Remaining inorganic minerals are concentrated in the bran layer. After cooling and weighing the residue, the ash concentration is recorded as the percentage of the initial sample weight. Ash concentration also indirectly reveals the amount of bran contamination and can affect color, leading to darker color of end products.

Protein is considered to be the most important functional property of wheat grain and flour. Therefore, it is of interest in hard wheat breeding programs to select lines that have an above average value for protein. This may be difficult due to the negative relationship that exists between protein concentration and grain yield. There is also a positive relationship between water absorption and protein. Both of these traits are desirable to improve through breeding while still maintaining high grain yield. The goal of developing a predictive model for end-use quality is to select candidates that possess high values for yield, water absorption and protein concentration.

Solvent Retention Capacity

Solvent retention capacity (SRC) is determined by the weight of a solvent held by flour after centrifuging for 15 minutes. It is expressed as a percent of flour weight on a 14 percent moisture basis. Four different solvents are prepared and used one at a time to determine different characteristics. Water as a solvent is used to determine absorption components, sodium carbonate for damaged starch, sucrose for pentosan components, and lactic acid is related to gluten protein characteristics. A functionality profile combining all four results is developed from this test and useful for predicting baking performance of the flour.

PPO

Polyphenol oxidase (PPO) is mostly a concern with noodle making but can also lead to discoloration in other end-products (Glen Weaver, personal communication, 2010). It is

desirable to have low levels of PPO for products with color specification as excess PPO enzyme can lead to darkening and shorter shelf-life (Ross and Bettge, 2009).

Extensibility and Dough Strength

An extensograph produces a curve which records maximum dough resistance to stretching as Rmax and the amount the cylindrical shaped dough piece can be stretched before breaking. Nash et al. (2006) found extensibility to be negatively correlated with mixograph tolerance (r= -0.45) and loaf volume (r = -0.26). Thus it may be difficult to select for both dough strength and extensibility. The aim is to have high dough strength with moderate extensibility.

GENOMIC SELECTION

To improve end-use quality of wheat, breeders have utilized recent advancements in genetic technologies. Marker-assisted selection (MAS) is one breeding tool that has led to many genetic improvements in breeding programs (Graybosch et al., 2013; Kumar et al., 2010; Liu et al., 2014; Varshney et al., 2009). However, MAS is most successful with large effect QTL for traits with high heritability. A new technology that has entered the molecular breeding world is genomic selection (GS). Genomic selection was first proposed in animal breeding by Meuwissen et al. (2001) as a method of statistically estimating the breeding value of individuals where dense genome-wide molecular markers are available. The genomic estimated breeding value (GEBV) may be used to initiate another cycle of mating and selection. Genomic selection has been used and discussed in many areas of plant and animal breeding (Heffner et al., 2009; Iwata and Jannink, 2011; Kumar et al., 2011; Zhao et al., 2011). Application of GS is described in two steps; First, estimating the marker effects in a reference population, usually referred to as the 'training population' and second, predicting GEBVs of new individuals not necessarily in

the training population (Hayes, 2007). These new individuals are referred to as the selection

candidates and may be used as parents of new crosses in the breeding program. During GS,

candidate lines are selected based on their genotype with little or no phenotypic evaluation.

Figure 1.1 depicts depicts the application of GS into a breeding program (Heffner et al., 2009).
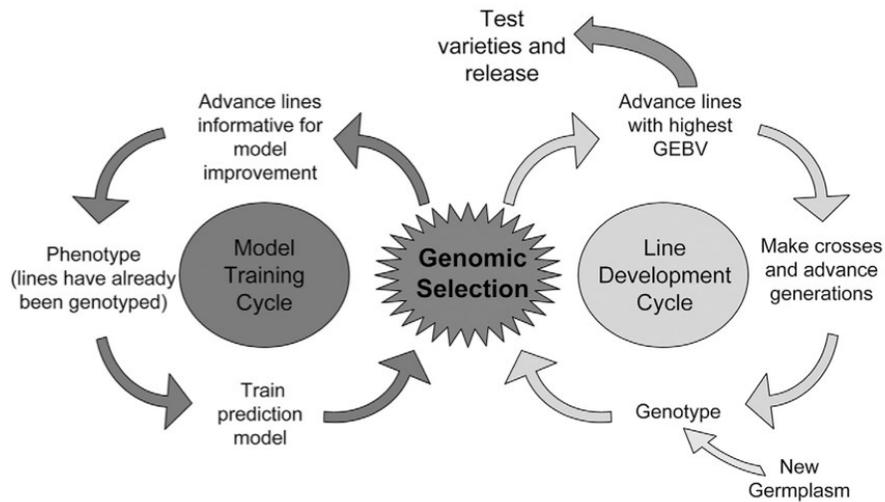
Figure 1.1 Flow diagram of genomic selection in a breeding program (Heffner et al., 2009).

GEBVs are predicted according to genotype information by summing marker effects of

the individual:

$$GEBV = \sum_i^n X_i g_i$$

where n is the number of chromosome segments across the genome, X_i is a design matrix

assigning individuals to the marker effects at segment i, and g_i is the vector of marker effects

effects (Hayes, 2007).

To estimate the breeding values, various statistical approaches have been used to develop

the prediction model. Least squares is not a valid option as all haplotype effects cannot be

estimated simultaneously and only including the largest effects gives a low accuracy (Meuwissen et al., 2001). Accuracy is described as the correlation between true and estimated breeding value. Other models and methods have been evaluated for their influence on model accuracy. De los Campos et al. (2009) described methods available for the use of GS, such as linear models, penalized estimation methods, Bayesian methods, and semi-parametric models.

In a traditional breeding program, elite parents are crossed and their progeny evaluated. Depending on the number of parents, it is usually impossible to evaluate all possible crosses from a set of elite lines. Thus, a model that predicts the value of the crosses before resources are used in creating and phenotyping them would be advantageous. Potentially, GS will perform in this way, producing superior inbreds in an efficient and feasible manner (Zhong and Jannink, 2007). Even for polygenic traits with low heritability, simulation studies have shown sufficient accuracies for breeding values (Heffner et al., 2009). Thus, GS would aid in saving time, money, field space, and fieldwork while still producing desired phenotypes.

Selection gain per unit time is critical in the comparison of genomic to phenotypic selection. Meuwissen et al. (2001) concluded that using GS could increase the rate of genetic gain especially if combined with reproductive techniques that shorten the generation cycle. Genetic gain was particularly increased when GS was applied to traits difficult to measure or with low heritability. Simulated data for polygenic traits in maize (*Zea mays* L.) showed GS to have 43% greater genetic gain than MAS (Bernardo and Yu, 2007). Schaeffer (2006) found genetic gain per year increased two-fold with GS in dairy cattle (*Bos Taurus* L.) breeding. Studies have also shown benefits of GS in a wheat breeding program (Heffner et al., 2010; Poland et al., 2012b).

Considerations for Implementing Genomic Selection

Several factors are important to consider when implementing GS into a breeding program. These include model choice, composition and size of training panel, marker density, marker platform, linkage disequilibrium, and trait heritability. Resources such as money, field space, time and work force available to the breeder play a major role in making decisions for implementing GS.

Next generation sequencing (NGS) methods allow for massively parallel analysis, are high throughput, and are much lower in cost than previous marker platforms (reviewed in Liu et al., 2012). Liu et al. (2012) compared various sequencing platforms side by side and found advantages and drawbacks to all, depending on the goals of the user. Illumina Hi-seq provided the biggest output of short reads at the lowest cost compared to the Roche 454 and SOLiD system.

During the last few years array-based marker platforms have become increasingly popular in genotyping plants (Gupta et al., 2013). The basic procedure of an array-based platform is the hybridization of DNA with targeted mRNA labeled with a fluorescent dye on an array (Gibson and Spencer, 2009). Arrays may be used for detection of single nucleotide polymorphisms (SNP) like Illumina's SNP chip, which is based on Bead Array Technology. SNP Chips are useful in generating a lot of marker data, which can be used for GS in a breeding program. Diversity arrays are a platform for producing diversity array technology (DArT) markers. These arrays are crop-specific and contain a large amount of diverse anonymous clones. Diversity arrays may be used to develop polymorphic markers for marker assisted selection in a breeding program (Gupta et al., 2013). Array-based marker platforms are useful for detecting QTL or genes through association mapping and for improving efficiency of

breeding programs through GS (Gupta et al., 2013). The limitations in array-based platforms are the ascertainment bias due to the reference population used to develop the array and the higher costs relative to recent alternatives.

Genotyping by sequencing (GBS) uses restriction enzymes to capture a reduced set of the target genome (Poland and Rife, 2012). These DNA samples are barcoded, which allows for multiplexing of the samples via NGS platforms. Genotyping by sequencing markers are useful in a breeding program for GS and genome-wide association studies. Genotyping by sequencing markers are beneficial because they produce a vast amount of marker data on a large amount of samples with a low cost. Another major benefit of GBS markers is the lack of ascertainment bias seen in array-based platforms. In one study, the commonly used DArT marker platform was compared to a GBS platform for a panel of wheat lines (Heslot et al., 2013). Results showed the DArT markers to have more clustering of markers and ascertainment bias than GBS markers, however this in itself did not lead to a reduction in GS accuracy. The GBS platform produced over 38,000 SNPs, whereas the DArT platform produced about 1,500 markers. In comparing the two platforms with an equal number of markers (i.e., reducing GBS to 1,500), there was no difference in GS accuracy except for the pre-harvest sprouting trait (Heslot et al., 2013). Therefore the better prediction was due to an increase in available markers and not due to a lack of ascertainment bias. The drawback of the GBS platform is the high amount of missing data but this can be overcome with imputation methods (Poland et al., 2012).

With GS, there are many more marker estimates than phenotypic entries. This leads to a problem referred to as "large-p-small-n" and must be addressed through shrinkage of the estimates (de los Campos et al., 2013a). Accuracy of estimators in a model can be measured with mean square errors (MSE) which are the distance between estimated and true values of a

parameter. With ordinary least squares and maximum likelihood methods, the mean square error estimates will be very high and problematic but shrinking the marker estimates towards zero will reduce the marker variance. Ridge regression methods perform shrinkage that is homogenous across all markers, thus addressing the "large-p-small-n" problem (de los Campos et al., 2009).

There are various methods for estimating marker effects for GS. The ridge regression best linear unbiased prediction (RR-BLUP) is a common method used with the assumption that marker variances are equal. An alternative to this approach is to estimate variances for each marker individually. Bayesian methods have been produced to achieve this and the R package BLR (de los Campos et al., 2013b) contains several Bayesian regression models for GS. In Bayesian methods, a prior distribution for the variance associated with marker effects must be assumed. Fernando et al. (2007) observed that accuracy with a Bayes B model didn't decline with an increase in markers compared to the decline while using RR-BLUP and suggested Bayesian methods might be better suited to handle situations with marker colinearity due to large marker sets and limited phenotypic data. In another study using dairy cattle data, Bayesian modeling had superior accuracies (r=.75) to traditional BLUP (r=.51; de Roos et al., 2007). Although Bayesian methods make a more correct assumption, the RR-BLUP method is computationally more convenient. In more recent studies, Bayesian models have shown little to no gain over RR-BLUP (Asoro et al., 2011; Heffner et al., 2011; Heslot et al., 2012), and in some cases RR-BLUP has outperformed Bayesian methods (Schulz-Streeck et al., 2012). Some studies have shown Bayesian methods to result in a slight increase in accuracy for traits involving large-effect QTL but generally there is little difference between models (de los Campos et al., 2013a).

If optimal genome coverage is achieved, the next limiting factor to genomic prediction accuracy is the reference population used to train the model (Clark et al., 2011). Increasing the size of the training panel has been shown to improve accuracy (Heffner et al. 2011b). Riedelsheimer et al (2012) found that reducing the training panel to a core set by removing highly related lines did not have an effect on prediction accuracy for hybrid maize testcrosses. Thus, removing redundancy from the training panel did not improve the model. Perhaps more important than size is the composition of the training panel. Clark et al. (2011) showed that genetic relatedness between the training and validation panel has a strong effect on GS accuracy. Thus, it is important to consider this when choosing an effective population panel.

Another consideration is to decide when to implement GS into the breeding program. GS involves selection for two components in the pipeline; i.) selecting for advancement to the next breeding stage, and ii.) selecting for individuals to return back to the crossing block. It is best to make the latter decision as early as possible to see the benefits of reduction in cycle time and optimization of genetic gain.

RESEARCH OBJECTIVES

Use of molecular tools has led to improvements in plant breeding and will continue to enhance genetic trend. Benefits of MAS and GS have been clearly described in the literature (Heffner et al., 2009, 2010; Heslot et al., 2012; Varshney et al., 2009). The focus of this research was to evaluate potential methods of marker-assisted and genome-wide selection for end-use quality of hard winter wheat. The objectives of this study were to i) evaluate agronomic and quality effects associated with different combinations of HMW-GS at the *Glu*-B1 and *Glu-D1* loci; ii) identify significant QTL for PHST and develop predictive models for this trait; and iii) evaluate GS models for milling and baking traits in hard winter wheat.

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CHAPTER 2

QUALITY AND AGRONOMIC EFFECTS OF HIGH-MOLECULAR-WEIGHT GLUTENIN

SUBUNIT COMBINATIONS IN HARD WINTER WHEAT

SUMMARY

Glutenin proteins play an important role in determining end-use properties of wheat (*Triticum aestivum* L.). In particular, high molecular weight glutenin subunits $Bx7^{OE}$ + By8 have been targeted for selection in hard winter wheat breeding programs to increase dough strength. In this study, agronomic and quality effects of different allele combinations at the *Glu-B1* and *Glu-D1* loci encoding high molecular weight glutenin subunits (HMW-GS) were evaluated. Four groups of near-isogenic lines (NILs) were developed with variation at the *Glu-B1* and *Glu-D1* loci. Kernel characteristics and composition, dough mixing properties, and agronomic traits were measured from multiple Colorado locations across two growing seasons. Results confirmed the $Bx7^{OE}$ + By8 subunits contributed to greater dough strength over the common Bx7 + By8 subunits. However, the effect was not as significant as that conferred by the Dx5 + Dy10 subunits. NILs with the combination of $Bx7^{OE}$ + By8 and Dx5 + Dy10 at the two loci had the longest mixograph mixing time. However, a decrease in yield was observed for groups containing the $Bx7^{OE}$ + By8 subunits in some environments. These results suggest combinations of glutenin alleles are useful for improving bread-making characteristics in hard winter wheat germplasm but may be associated with negative effects on yield.

INTRODUCTION

A hard wheat cultivar with good dough rheology and therefore good bread-making characteristics will have high values for gluten strength. Studies have focused on improving the end-use quality of wheat through understanding the genetic basis underlying gluten strength, specifically focusing on glutenin genes (Lawrence and Shepherd, 1980; Payne et al., 1988; Rogers et al., 1991; Peña et al., 1995; Obreht et al., 2007; Zheng et al., 2009; Gao et al., 2012). These genes have been identified at the *Glu-A1, Glu-B1* and *Glu-D1* loci, with the greatest influence at the B and D loci. Allelic variation at the glutenin loci allow for various combinations of glutenin genes resulting in different effects on the phenotype. Therefore, the influence of different glutenin allele combinations on mixing and baking traits is an important consideration in breeding for end-use quality in wheat. Alleles at these loci are defined by their x and y subunits. The Dx5 + Dy10 subunits (*Glu-D1d* allele), at the *Glu-D1* locus, tend to be superior to the Dx2 + Dy12 subunits (*Glu-D1a* allele) with regard to dough strength for bread making (Payne et al., 1987; Butow et al., 2003; Zheng et al., 2009). Subunits $Bx7^{OE}$ + By8 (*Glu-B1a1* allele) at the *Glu-B1* locus, have been shown to enhance dough strength over the common Bx7 + By8 subunits (*Glu-B1b* allele; Butow et al., 2003). Increased gluten strength associated with $Bx7^{OE}$ + By8 is due to the increased amount of x-type subunit as inferred by Radovanovic et al. (2002) and confirmed by Butow et al. (2003). Thus, these data suggest that $Bx7^{OE}$ + By8 may be useful as a selection target when breeding for cultivars with enhanced dough strength.

To determine the effects of glutenin genes, it is necessary to test for quality among lines that differ for these genes. A common small-scale test for assessing dough quality is the mixograph, which records a time curve of dough resistance during mixing (reviewed in Chung et al., 2001). The mixograph works by measuring the resistance of dough against the continual

mixing movements of pins. Three main properties of flour can be obtained from a mixograph: water absorption (the amount of water needed to produce a representative curve), dough strength (related to peak time), and tolerance to over-mixing (related to length and thickness of the curve) (Gras et al., 2000).

The environment also plays a role in determining the end-use quality of wheat. Studies have shown that environmental factors like temperature, moisture, and nutrient availability can affect end-use quality of wheat (Dupont and Altenbach, 2003). Cornish et al. (2001) found properties related to dough extensibility to be more influenced by the environment than those related to dough strength. Some studies have assessed stability of quality traits across environments. Guttieri et al. (2000) observed some bread-making properties to be stable across different irrigation levels. Zheng et al. (2010) found that effects of glutenin alleles on dough mixing did not differ greatly between irrigated and non-irrigated environments. As studies have found varying results, environmental effects should be considered when analyzing genotypic effects on quality.

Although glutenin alleles have been associated with dough strength and bread-making quality, there is little known about the influence of variation for alleles at *Glu-B1* in combination with variation at *Glu-D1* among U.S. Hard Winter Wheat germplasm. While the Dx5 + Dy10 subunits are quite common in Great Plains hard winter wheats (Graybosch, 1992; Zheng et al., 2009), the Bx7[OE] + By8 subunits are especially rare, only being found in two percent of regional nursery entries since 1995 (B. Seabourn, personal communication). In this study, we estimated the effects of glutenin combinations on quality and yield in environments typical of the west central U.S. Great Plains winter wheat-growing region. Previous studies have investigated the effects of allele combinations at these two loci but they included germplasm from other countries

and trials in different environments (Cornish et al., 2001; Butow et al., 2003; Vawser and Cornish, 2004). An overall evaluation of how glutenin subunit composition affects dough rheological properties and yield in U.S. Great Plains regions has not been conducted. Therefore, the objective of this study was to determine the effects of different combinations of alleles at the *Glu-B1* and *Glu-D1* loci among Great Plains environments on agronomic and quality characteristics. The specific objectives were to i) characterize the influence of different allelic combinations at the *Glu-B1* and *Glu-D1* loci in a set of NILs on end-use quality traits, specifically dough mixing properties; and ii) determine if these allelic combinations are associated with any agronomic advantage or disadvantage. In addition, observations were made about environmental interactions with glutenin alleles.

Abbreviations

BC, backcross; CSU, Colorado State University; HMW-GS, high molecular weight glutenin subunits; MPT, mixograph midline peak time; MPH, midline peak height; MPW, midline peak width; MRS, mixograph right slope; MRW, mixograph right width; NIL, near isogenic line; STS, sequenced-tagged sites

MATERIALS AND METHODS

Germplasm

BC$_3$F$_{2:4}$ and BC$_4$F$_{2:4}$ near isogenic lines (NILS) were developed from a backcross population of 'Glenlea' and 'Ripper', which differ for alleles at the *Glu-B1* and *Glu-D1* loci. The recurrent parent Ripper is a hard red winter wheat cultivar released by Colorado State University in 2006 (Haley et al., 2007). Ripper carries the Bx7 + By8 subunits at *Glu-B1* (*Glu-B1b*) and the Dx2 + Dy12 subunits at *Glu-D1* (*Glu-D1a*). The donor parent Glenlea is a Canadian hard red spring wheat, released in 1972, that carries the Bx7^{OE} + By8 subunits at *Glu-*

B1 (*Glu-B1a1*) and the Dx5 + Dy10 subunits at *Glu-D1* (*Glu-D1d*), which confers high dough strength (Evans et al., 1972). The NILs were derived using marker-assisted selection to identify heterozygotes for the *Glu-B1a1* and *Glu-D1d* alleles after each cycle of backcrossing and then selecting lines homozygous for the respective alleles in selfed progeny. Allele combinations were denoted by the allele at *Glu-B1* locus followed by the allele at *Glu-D1* locus (ie. *Glu-B1a1/Glu-D1a* refers to lines with the subunits 7^{OE} + 8 at loci *Glu-B1* and subunits 2 + 12 at loci *Glu-D1*). For the 2012 harvested trials, there were 19 lines containing the *Glu-B1b/ Glu-D1a* combination, 17 lines containing the *Glu-B1b/ Glu-D1d* combination, 7 lines containing the *Glu-B1a1/ Glu-D1a* combination, and 21 lines containing the *Glu-B1a1/ Glu-D1d* combination. For the 2013 harvest trials, the number of lines for each group was 20 for *Glu-B1b/ Glu-D1a*, 18 for *Glu-B1b/ Glu-D1d*, 13 for *Glu-B1a1/ Glu-D1a*, and 21 for *Glu-B1a1/ Glu-D1d*.

Locations and Field Design

The NILs were planted in replicated field trials in a latinized row-column trial design at five locations in Colorado during the 2011 – 2012 season and four locations during the 2012 – 2013 season. Henceforth, trials will be referred to by the harvest year. The 2012 trial locations were Akron (40.15˚N, 103.14˚W, elevation 1383 m), Burlington (39.27˚N, 102.11˚W, elevation 1271 m), Dailey (40.6˚N, 102.74˚W, elevation 1230 m), Julesburg (40.59˚N, 102.1˚W, elevation 1060 m), and Sheridan Lake (38.54˚N, 102.5˚W, elevation 1208 m) and the 2013 locations were Burlington, Dailey, Julesburg, and Fort Collins (40.65˚N, 105˚W, elevation 1558 m). In 2012, the two trials planted at Akron and Fort Collins included a high and low nitrogen treatment at each location as part of a separate study. At Akron trials, 112 kg NO3 N ha-1 and 22 kg NO3 N ha-1 were applied for high and low nitrogen treatments, respectively. At Fort Collins, 112 kg NO3 N ha-1 and 56 kg NO3 N ha-1 were applied for high and low nitrogen treatments,

respectively. A total of 11 environments were used and labeled according to location, nitrogen treatment if applicable, and harvest year (ie. AkHi12 refers to Akron,CO with a high nitrogen treatment during the 2012 season). At each location, plots consisted of six rows, 3.5 m long with 23 cm between rows except for Akron where plot length was 1.8 m. Irrigation was applied as needed to maximize yields at Fort Collins by a linear overhead sprinkler irrigation system while all other environments were rainfed.

Quality Data

All quality evaluations were done in the wheat quality lab at CSU in Fort Collins, Colorado. Traits were measured using approved methods of the American Association of Cereal Chemists (AACC, 2000). Single kernel hardness, diameter, and weight were determined using the single kernel characterization (SKCS4100; Perten Instruments, Springfield, IL). Protein concentration was measured using near-infrared spectroscopy (NIRS) on whole grain samples with a Foss NIRSystems model 6500 (Foss North America Inc., Eden Prairie, MN). Samples of 50 g of grain from each plot were tempered to 155 g kg^{-1} (15.5%) moisture and milled using a modified Brabender Quadrumat Junior milling system (C.W. Brabender Instruments, Inc. South Hackensack, NJ; Method AACC26-50 for milling). Mixing properties were obtained with a 10-gram mixograph according to AACC Method 54-40A and included optimizing for water absorption based on protein output from the NIRS according to the equation:

$$predicted\ absorption = 42.7 + 1.69*sampleprotein14$$

where sampleprotein14 is the protein concentration at 140 g kg^{-1} moisture basis. The prediction equation was calibrated based on the variety 'Cheyenne' (PI 553248; B. Seabourn, personnel communication).

Mixograph parameters were reported with MIXSMART software for computer-analyzed parameters (v. 1.0.484 for Windows, National Manufacturing, Lincoln, NE). The following key parameters were measured: mixograph peak time (MPT), midline peak height (MPH), midline peak width (MPW), midline right slope (MRS), and curve width at two minutes after peak time (MRW). Previous studies have used MPT to estimate dough strength (Butow et al., 2003; Zheng, 2011). MRS and MRW have been used to estimate mixing tolerance as these parameters were highly correlated with visual tolerance scores in a previous study (Chung et al., 2001).

Field Data

The following data were collected from the field trials: grain yield, test weight, height, and heading date. Plots were combine harvested at maturity and grain yield (120 g kg^{-1} moisture basis) was recorded with an on-combine HarvestMaster GrainGage (Juniper Systems, Logan, UT). Test weight (grain volume weight) was measured by hand with a 151 filling hopper (Seedburo, Des Plains, IL). Plant height was recorded as the distance from the soil surface to the tip of the spikes, excluding the awns. Heading date was determined as the number of days from the first of January when half of the spikes in a plot were fully visible above the flag leaf collar. Yield was measured in all environments, test weight in 10 environments, plant height in nine environments, and heading date in five environments.

Genotyping

Sequence-tagged site (STS) markers were used for marker analysis in development of the NILs. Sequence-tagged site markers were first introduced by Olson et al. (1989) as markers developed with sequence-specific primers, usually for a particular genome region. Leaf tissues from 11 seedlings of each line were bulked and placed into a single well in 96 deep-well plates. Tissues were freeze-dried for approximately 48 h and ground to a fine powder using a 4.5 mm

stainless steel bead for two to five min of agitation at 25 cycles per sec. A 10 µL reaction mix

was used for the polymerase chain reactions consisting of 10 to 100 ng DNA template, 25 mM

$MgCl_2$, 0.2 nM deoxyribonucleotide triphosphates (dNTPs; Bioline, Boston, MA), 10 x $(NH4)_2$

SO4 buffer, 1 U *Taq* DNA polymerase (New England BioLabs, Ipswich, MA), 0.1 µM reverse

primer, and 0.05 µM each of M13-tailed forward primer and M13 universal primer labeled with

either FAM (blue) or VIC (green) fluorescent tags.

Fragments were amplified using a PTC-200 Thermo Cycler with a 384-well block (MJ

Research, Inc., Waltham, MA). The PCR products were multiplexed for detection by pooling

two markers with different fluorescent labels to a final volume of 12 µL with 0.06 µL GeneScan-

500 LIZ size standard (Applied Biosystems, Carlsbad, CA) and 9.94 µL Hi-Di Formamide

(Applied Biosystems, Carlsbad, CA). Pooled marker fragments were analyzed on an ABI 3730

Genetic Analyzer (Applied Biosystems, Carlsbad, CA) at the USDA Central Small Grain

Genotyping Laboratory (Manhattan, KS). Fragments were visualized and scored using

GeneMarker v1.6 software (SoftGenetics, 2007). A codominant marker, Bx MAR, was used to

detect alleles at *Glu-B1* (Butow et al., 2004), while the co-dominant marker UMN26 was used to

target alleles at *Glu-D1* (Liu et al., 2008). For *Glu-B1* alleles, the reference cultivar Ripper

(contains *Glu-B1b*), has a band at 530 bp, while the reference cultivars 'Snowmass' (Haley et al.,

2011) and Glenlea (both contain *Glu-B1a1*), have a bands at 573 bp. For *Glu-D1*, the reference

cultivar Ripper (contains *Glu-D1a*) has a band at 311 bp, while the reference cultivars Snowmass

and Glenlea (both contain *Glu-D1d*) have bands at 293 bp. Marker analysis was performed twice

to confirm homogeneity of each NIL for the respective alleles at the *Glu-B1* and *Glu-D1* loci.

Statistical Analysis

All quality data were analyzed using the statistical software R (R Development Core Team, 2011). Within each environment, main and interaction effects of *Glu-B1* and *Glu-D1* were modeled as fixed effects with the lme4 package (Bates et al., 2014). Least square means were calculated for each allele combination by quality trait using the lsmeans package (Lenth, 2014). For agronomic data, least square means for each allele combination by trait were calculated with PROC MIXED in SAS software 9.2 (SAS Institute, 2008). Because of potential spatial variation within each environment, six different spatially adjusted models were run to account for the following spatial patterns: row-column, spherical, exponential, power, anisotropic power, and Matern (Littell et al., 2006). The model with the lowest Akaike information criterion value was chosen for each environment by agronomic trait. Because spatial patterns varied among environments, the environments could not be combined for data analysis. Effects of alleles at the *Glu-B1* and *Glu-D1* loci, and their interaction, were treated as fixed effects and spatial patterns as random effects. Agronomic means for allele combinations were compared with the 'lsmean/pdiff' statement of PROC MIXED.

RESULTS AND DISCUSSION

In this study, effects of allele combinations at the *Glu-B1* and *Glu-D1* loci on agronomic and end-use quality characteristics in winter wheat production environments typical of the west-central Great Plains region of the U.S. were examined. Little is known about the agronomic effects associated with HMW-GS (Graybosch et al., 2011). Butow et al. (2003) studied dough properties of these same combinations in Australia but no associations with field traits were determined. Also, this previous study included various genetic backgrounds, which could impact

results. Thus, our study included a population of NILs to control for genetic background variation.

End-Use Quality

Endosperm texture is an important characteristic as it affects both milling and bread baking. Hard wheat will have starch that is tightly fixed to the protein matrix. Harder wheat is more difficult to fracture and results in more starch damage leading to broken granules that absorb more water. In this study, NILS with the *Glu-B1a1/Glu-D1a* allele combination had significantly lower ($P < 0.05$) values for grain ash and hardness than other NIL groups (Table 2.1). The desired range for hardness index is 60-80 for pan bread (B. Seabourn, personal communication, 2006). It is unclear if this glutenin allele combination would be beneficial for bread-making because results across environments were inconsistent within this range. However, the decrease in grain ash associated with this group is desirable for bread-making. Reasons for this decrease in grain ash and hardness are unclear but this could be useful in selection for lower ash and hardness to meet quality standards.

Mixograph testing has been used in many studies to assess dough properties. In this study, MPT was used to assess dough strength while MRS and MRW were used to assess mixing tolerance as these parameters are correlated to visual tolerance scores (Chung et al., 2001). Midline peak height and MPW involve a combination of factors that contribute to mixing properties as they are affected by protein concentration, dough strength, and water absorption. These factors can be informational in understanding mixing properties of wheat flour.

Dough strength is an important quality criterion for end-use quality. In this study, the *Glu-D1d* allele contributed the most to dough strength ($P < 0.001$ in all environments) but *Glu-B1a1* also had a significant contribution ($P < 0.05$ in 6 out of 9 environments). Across

environments, there was a clear pattern seen for MPT with NIL groups ranking *Glu-B1a1/Glu-D1d > Glu-B1b/Glu-D1d > Glu-B1a1/Glu-D1a > Glu-B1b/Glu-D1a* (Table 2.2). Similar results were reported by Butow et al. (2003) using Australian spring wheats. The NIL group with the *Glu-B1a1/Glu-D1d* allele combination had the highest values for MPT, MRS, MRW, and intermediate values for MPH compared to other groups. Lines with glutenin allele combination *Glu-B1b/Glu-D1d* had the second highest MPT with low values for MPH and MPW and high values for MRS and MRW. The NIL group with *Glu-B1a1/Glu-D1a* combination had lower values for MPT and MRS but the highest values for MPH and MPW compared to other groups. Lastly, the NILs with *Glu-B1b/Glu-D1a* had the lowest values for MPT, MRW, MRS and intermediate MPH.

NILs with the *Glu-B1a1/Glu-D1d* combination had the greatest dough strength with the greatest contribution due to *Glu-D1d*. However, there was a significant contribution from *Glu-B1a1* even though the increase was not great enough to make up for the decrease in NIL groups with *Glu-D1a*. In this study, interaction between alleles at the two loci was significant for mixograph mixing time ($P < 0.05$) but no other mixograph traits. It appears that the interaction between *Glu-B1* and *Glu-D1* is important for dough strength but less important for other mixograph properties. Radovanovic et al. (2012) observed no interaction between alleles at the *Glu-B1* and *Glu-D1* loci for MPT, MPH, and MPW. Butow et al. (2003) found significant interaction for mixing time in one out of two locations. Zheng et al. (2010) found significant interaction effects for MPH but not for MPT, MPW, MRW, or MRS. Differing results from previous studies could be due to differences in genetic backgrounds and environments.

Agronomic Characteristics

Across environments NIL groups were not consistent in yield or test weight rankings. There appears to be a year by allele effect as consistencies can be found across locations within years. In 2013, NIL groups ranking the highest and lowest for yield (*Glu-B1b/Glu-D1a* and *Glu-B1a1/Glu-D1d*, respectively) ranked the opposite for dough strength, suggesting a negative correlation between dough strength and yield. In this study, a significant negative correlation was observed between yield and mixograph mix time ($R^2 = -0.44$, $P < 0.05$). In 2013 trials, the NILs with the *Glu-B1a1/Glu-D1d* combination were consistently among the lowest yielding groups. However in 2012, NILS with the *Glu-B1a1/Glu-D1d* combination were not significantly different from other groups except in 12AkHi where they were among the top yielding (Table 2.3). Thus, I cannot conclude there is a consistent negative association with yield for this group. In all environments, NILs with the *Glu-B1b/Glu-D1d* combination, which ranked second in dough strength, had yield higher than or equal to NILs with the *Glu-B1a1/Glu-D1d* combination.

NILS with *Glu-B1b/Glu-D1a* had the lowest test weight in 2012 but NILs with *Glu-B1a1/Glu-D1a* had the lowest test weight in 2013 (Table 2.3). The NIL groups with *Glu-D1a* had the lowest test weight across environments, and NILs with *Glu-D1d* were the highest group for test weight in all but one environment. For plant height and heading date, lines with *Glu-B1a1* were taller than those with *Glu-B1b* and the NIL group with the combination *Glu-B1a1/Glu-D1*a had earlier heading dates than other groups.

Environments

Field environments utilized for this study represented target environments for winter wheat production in the west-central region of the U.S. Great Plains. Because the data from each

trial were analyzed with spatial models, environments could not be combined for data analysis. Meteorological data were obtained from the Colorado Agricultural Meteorological Network (Colorado State University and USDA, 2010) for environments where trials were planted and data were available. Maximum temperatures during the 2012 season (41 to 43 °C) were higher than for the 2013 season (35 to 39 °C; Figure 2.1). All sites, except the irrigated site at Fort Collins, were characterized by little to no precipitation from September to March (Figure 2.2). The 2013 season was characterized by a few late freezes in April and severe drought resulting from inadequate precipitation throughout the season. The average heading date for 2012 trials was 16 days earlier than the average heading date for 2013. This difference in heading date is most likely due to inadequate moisture and high temperatures in the 2011–2012 season, causing heat stress and leading to an earlier harvest than the 2012-2013 season. The 2012 harvest began early on June 18 and lasted only two and half weeks, while harvesting in 2013 began later on June 25 and lasted through the end of July.

Across environments, similar results were seen for mixograph parameters by glutenin allele combinations (Table 2.2). This consistency suggests a strong genetic influence of these HMW-GS on dough mixing properties and less environmental influence. For kernel and compositional traits, there was less of a pattern observed although discrimination of these traits was not observed in most environments (Table 2.1). Yield and test weight were more affected by environmental conditions as rankings were not consistent across environments (Table 2.3).

The negative effect on yield associated with groups containing the *Glu-B1a1* allele (Bx7^{OE} + By8) was more prevalent in the 2013 trials. In 2012 environments, there was a negative correlation between heading date and yield (R^2= -0.55, P < 0.05). Thus, there was a yield advantage for earlier maturing lines in 2012, possibly to avoid heat stress from high

temperatures observed later in the season. Lines from the NIL group with the *Glu-B1a1/Glu-D1a* allele combination had significantly earlier heading dates than other groups (Table 2.3), which could explain why a yield decrease wasn't observed in most of the 2012 trials for this NIL group. However, 2013 trials showed no yield advantage in earlier heading lines ($R^2 = 0.12$, $P > 0.05$) thus the association with heading did not improve yield for NILs with the *Glu-B1a1/Glu-D1a* allele combination.

CONCLUSIONS

Both *Glu-B1* and *Glu-D1* are important loci influencing dough strength. This study confirmed the superior dough strength due to the glutenin allele combination of *Glu-B1a1* with *Glu-D1d,* but showed the *Glu-D1d* allele to contribute more so than *Glu-B1a1*. The environment played a major role in determining yield and had less of an effect on dough properties. Caution should be taken when utilizing this allele combination, as there may be an effect on yield that negates the quality advantage of *Glu-B1a1*. However, more research is needed to determine if this negative effect on yield occurs in other genetic backgrounds and why this occurs in order to determine effective breeding strategies that mitigate yield reductions while increasing dough strength.
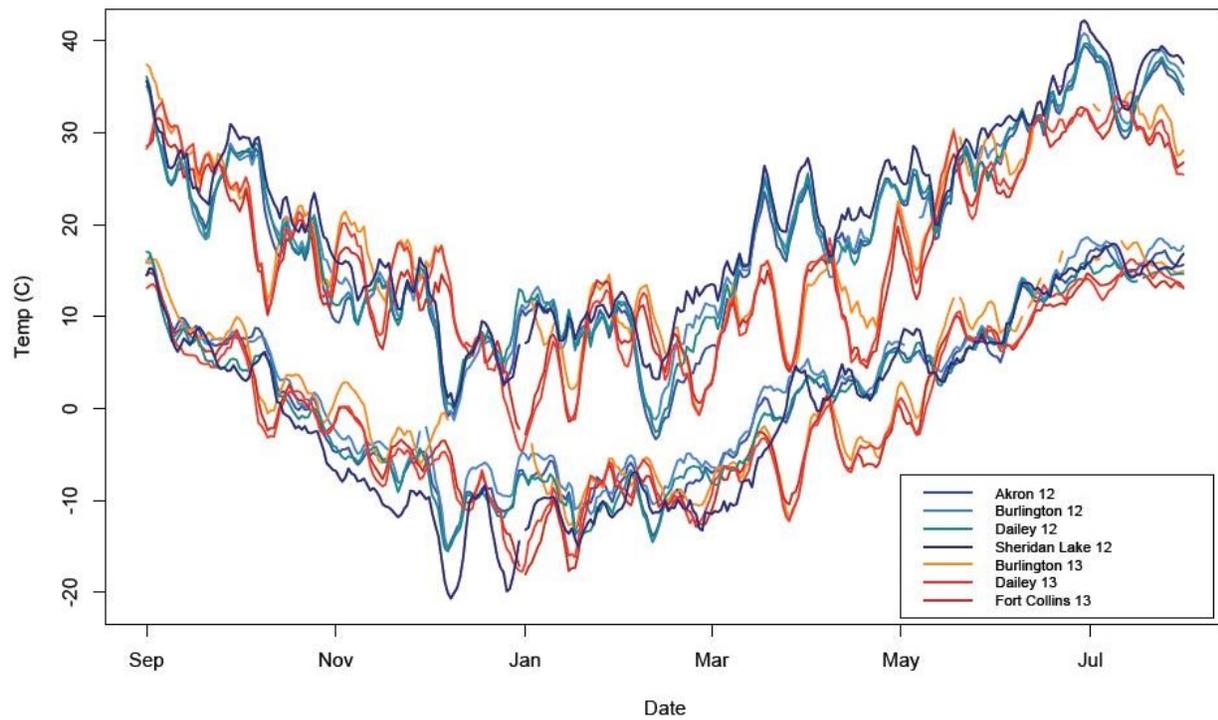
Figure 2.1 Rolling seven day average of maximum and minimum temperatures observed at field trial locations.
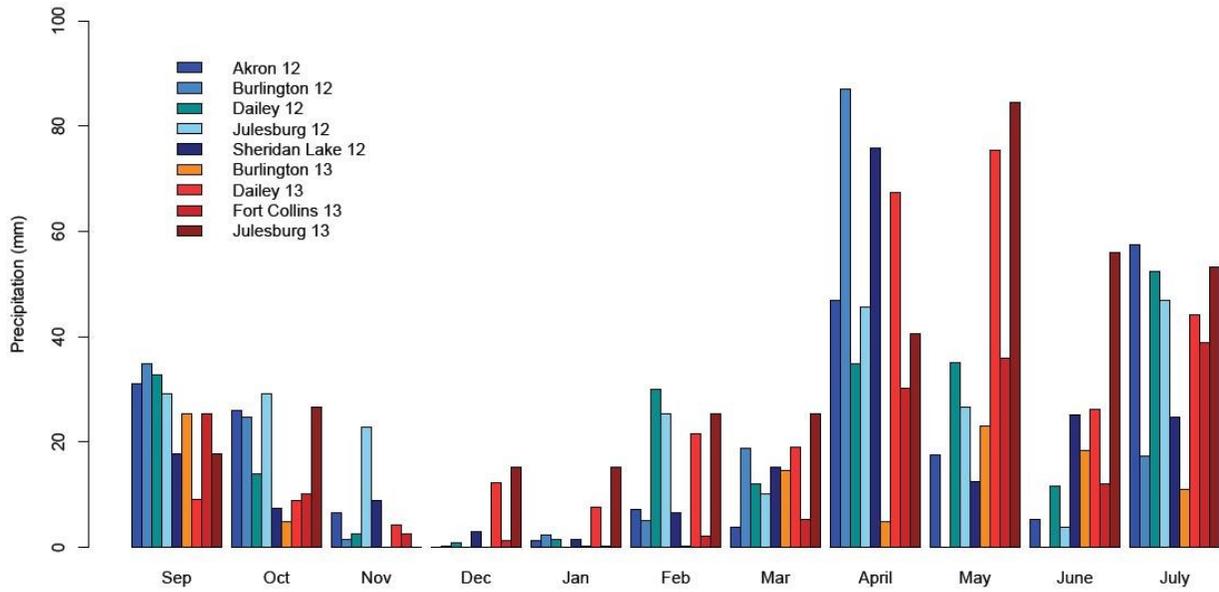
Figure 2.2 Monthly precipitation observed at field trial locations.

Table 2.1 Least square means of kernel characteristics and composition of glutenin allele combinations across Colorado environments

		Environment																
Glu-B1	*Glu-D1*	12Akhi		12AkLo		12Bu		12Ju		13Bu		13Da		13FoHi		13FoLo		13Ju	
Grain Protein (g kg^{-1})																		
b (7+8)	*a (2+12)*	157.0	a[†]	130.0	a	121.5	a	112.1	ab	170.1	a	133.6	a	118.4	a	117.8	a	148.3	a
	d (5+10)	155.5	a	132.0	a	124.1	a	113.4	a	168.5	a	131.4	a	121.3	a	118.0	a	148.0	a
a1 (7^{OE}+8)	*a (2+12)*	157.5	a	133.8	a	126.5	a	110.4	ab	165.2	a	131.5	a	118.5	a	118.4	a	150.4	a
	d (5+10)	155.8	a	130.3	a	124.8	a	109.3	b	167.9	a	134.5	a	120.1	a	116.6	a	150.0	a
Grain Ash (g kg^{-1})																		
b (7+8)	*a (2+12)*	14.3	ab	13.7	a	13.9	a	13.5	a	15.9	bc	14.3	bc	13.3	ab	13.6	b	15.1	b
	d (5+10)	14.4	a	13.9	a	14.1	a	13.6	a	16.2	a	14.7	a	13.6	a	14.0	a	15.4	a
a1 (7^{OE}+8)	*a (2+12)*	13.9	b	13.4	a	13.1	b	13.0	b	15.8	c	14.2	c	13.0	b	13.2	c	14.9	b
	d (5+10)	14.3	a	13.9	a	13.8	a	13.4	a	16.2	ab	14.6	ab	13.4	a	13.6	b	15.4	a
Kernel Weight (mg kernel^{-1})																		
b (7+8)	*a (2+12)*	26.4	a	28.7	a	30.0	a	29.6	a	26.3	a	29.3	a	40.6	a	38.9	a	28.1	a
	d (5+10)	26.6	a	28.9	a	30.0	a	30.2	a	26.0	a	30.2	a	40.1	ab	39.0	a	28.1	a
a1 (7^{OE}+8)	*a (2+12)*	27.0	a	28.6	a	30.2	a	30.7	a	25.8	a	29.5	a	39.1	b	38.2	a	27.2	b
	d (5+10)	26.4	a	28.6	a	29.9	a	30.1	a	26.4	a	29.2	a	39.2	b	38.2	a	27.4	ab
Kernel Diameter (mm)																		
b (7+8)	*a (2+12)*	2.50	a	2.58	a	2.56	a	2.60	a	2.45	a	2.57	b	2.94	a	2.90	a	2.55	a
	d (5+10)	2.52	a	2.59	a	2.57	a	2.64	a	2.45	a	2.61	a	2.92	ab	2.91	a	2.55	a
a1 (7^{OE}+8)	*a (2+12)*	2.52	a	2.55	a	2.58	a	2.64	a	2.45	a	2.59	ab	2.91	ab	2.90	a	2.53	a
	d (5+10)	2.51	a	2.57	a	2.58	a	2.63	a	2.47	a	2.57	ab	2.90	b	2.88	a	2.53	a
Hardness (score)																		
b (7+8)	*a (2+12)*	83.1	a	83.2	a	76.3	a	76.7	a	69.8	a	52.1	a	60.7	a	59.5	a	65.4	a
	d (5+10)	83.4	a	82.3	ab	76.8	a	76.0	b	70.2	a	51.0	a	61.1	a	59.1	a	66.0	a
a1 (7^{OE}+8)	*a (2+12)*	81.0	a	80.3	b	68.9	c	72.2	c	65.7	b	47.2	b	54.7	b	53.7	b	62.1	b
	d (5+10)	82.4	a	81.9	ab	72.8	b	74.8	b	68.5	a	49.9	ab	58.3	ab	56.3	ab	65.4	a

†Within environment and trait, means followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 2.2 Least square means of mixograph properties for glutenin allele combinations across Colorado environments

Glu-B1	Glu-D1	Environment																	
		12Akhi		12AkLo		12Bu		12Ju		13Bu		13Da		13FoHi		13FoLo		13Ju	
MPT[†]																			
b(7+8)	*a(2+12)*	3.19	d[‡]	3.36	c	3.55	c	3.60	c	3.72	d	3.48	d	2.89	d	3.02	c	4.09	d
	d(5+10)	5.58	b	4.92	b	5.14	b	5.20	b	7.36	b	5.14	b	3.80	b	4.13	b	7.93	b
a1(7^{OE}+8)	*a(2+12)*	4.09	c	3.67	c	4.08	c	3.84	c	5.22	c	4.09	c	3.22	c	3.41	c	5.62	c
	d(5+10)	7.52	a	6.17	a	6.81	a	6.33	a	9.17	a	6.44	a	4.54	a	5.03	a	10.16	a
MPH																			
b(7+8)	*a(2+12)*	63.66	b	55.95	b	54.65	bc	49.60	ab	64.57	b	52.54	a	49.93	ab	48.30	ab	55.17	b
	d(5+10)	59.56	c	53.51	c	52.62	c	47.85	c	58.33	c	49.37	b	48.39	b	45.93	c	50.38	c
a1(7^{OE}+8)	*a(2+12)*	67.28	a	59.92	a	57.91	a	50.97	a	67.55	a	54.36	a	52.08	a	50.52	a	60.01	a
	d(5+10)	63.23	b	56.58	b	55.01	b	48.52	bc	63.47	b	52.25	a	49.50	b	47.53	bc	55.90	b
MPW																			
b(7+8)	*a(2+12)*	28.79	b	23.18	b	24.55	b	20.96	b	30.40	b	25.40	b	22.28	b	22.39	b	28.26	b
	d(5+10)	27.10	b	23.82	b	25.32	b	20.09	b	27.18	c	24.38	b	22.74	b	23.27	b	27.66	b
a1(7^{OE}+8)	*a(2+12)*	31.94	a	29.31	a	31.42	a	24.02	a	33.52	a	29.01	a	25.93	a	26.85	a	33.69	a
	d(5+10)	31.05	a	28.07	a	28.53	a	23.28	a	30.50	b	29.97	a	26.97	a	26.79	a	31.57	a
MRS																			
b(7+8)	*a(2+12)*	-3.22	c	-2.40	b	-1.72	b	-1.57	b	-2.97	b	-2.47	b	-2.41	c	-2.36	b	-2.57	b
	d(5+10)	-2.00	a	-1.63	a	-1.18	a	-0.94	a	-1.63	a	-1.49	a	-1.95	b	-1.44	a	-1.25	a
a1 (7^{OE}+8)	*a(2+12)*	-3.81	c	-2.96	b	-1.88	b	-1.68	b	-2.87	b	-2.47	b	-2.71	c	-2.11	b	-2.75	b
	d(5+10)	-2.51	b	-1.57	a	-1.01	a	-0.97	a	-2.11	a	-1.43	a	-1.30	a	-1.10	a	-2.02	ab
MRW																			
b (7+8)	*a(2+12)*	15.05	b	16.28	b	17.28	b	14.76	c	19.85	b	17.45	b	13.49	c	15.14	c	19.63	b
	d(5+10)	19.63	a	17.38	b	18.67	b	15.23	bc	20.59	a	17.09	b	15.95	b	16.86	b	20.48	b
a1 (7^{OE}+8)	*a(2+12)*	12.59	b	16.21	b	21.23	a	16.29	ab	22.50	b	18.79	b	16.54	b	17.46	b	22.95	a
	d(5+10)	20.61	a	19.66	a	21.30	a	16.81	a	23.11	a	20.89	a	18.87	a	19.51	a	23.61	a

[†]MPT, mixograph peak time (min); MPH, mixograph peak height; MPW; mixograph peak width; MRS, mixograph right slope; MRW, mixograph right width.
[‡]Within environment and traits means followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 2.3 Least square means for agronomic traits of wheat NILs by glutenin allele combinations across Colorado environments

Glu-B1	Glu-D1	12AkHi	12AkLo	12Bu	12Da	12Ju	12SL	13Bu	13Da	13FoHi	13FoLo	13Ju
Yield (Mg ha^{-1})												
b (7+8)	a (2+12)	2.27 bc†	3.26 a	4.19 a	3.75 a	3.96 a	0.96 a	1.02 a	4.56 a	5.96 a	5.43 a	2.00 a
	d (5+10)	2.41 a	3.27 a	4.25 a	3.77 a	3.97 a	0.97 a	0.99 ab	4.44 ab	5.64 b	5.20 bc	1.97 a
a1 (7^{OE}+8)	a (2+12)	2.21 c	3.09 a	4.34 a	3.80 a	4.10 a	0.91 a	0.94 bc	4.38 b	5.69 b	5.32 ab	1.75 b
	d (5+10)	2.37 ab	3.21 a	4.19 a	3.75 a	3.96 a	1.01 a	0.94 c	4.19 c	5.58 b	5.09 c	1.72 b
Test Weight (kg hL^{-1})												
b (7+8)	a (2+12)	96.7 b	99.4 b	96.9 a	98.1 b	98.6 c	89.2 a		92.3 c	99.6 a	98.5 a	86.0 b
	d (5+10)	97.7 a	100.0 a	97.4 a	99.2 a	99.7 b	89.1 a		94.1 a	99.6 a	98.7 a	87.6 a
a1 (7^{OE}+8)	a (2+12)	98.2 a	100.5 a	97.6 a	99.4 a	100.4 a	89.6 a		92.9 bc	99.1 b	98.1 b	84.8 c
	d (5+10)	97.8 a	100.0 a	97.2 a	99.4 a	100.1 ab	89.3 a		93.8 ab	99.5 a	98.6 a	85.3 bc
Height (cm)												
b (7+8)	a (2+12)	56.1 b	66.3 b	75.2 c	72.4 b	73.7 a	51.8 b		67.1 a	74.9 ab	71.9 b	
	d (5+10)	56.6 b	67.3 b	75.2 c	73.7 b	73.2 a	52.1 ab		68.3 a	73.4 b	72.9 ab	
a1 (7^{OE}+8)	a (2+12)	58.4 a	68.1 ab	79.8 a	75.4 a	75.4 a	51.3 b		67.8 a	75.2 ab	73.9 a	
	d (5+10)	57.9 a	69.6 a	77.2 b	76.2 a	74.7 a	53.3 a		67.6 a	75.2 a	73.4 ab	
Heading date (days)												
b (7+8)	a (2+12)		136.7 a		133.9 a	130.4 a				150.8 a	149.0 a	
	d (5+10)		136.1 b		133.1 b	129.9 ab				150.6 a	148.2 bc	
a1 (7^{OE}+8)	a (2+12)		133.2 c		131.7 c	128.4 c				149.5 b	147.6 c	
	d (5+10)		136.3 ab		133.2 b	129.5 b				150.6 a	148.8 ab	

†Means followed by different letters indicate a difference significant at $P < 0.05$

Empty cells indicate no data available for that environment.

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CHAPTER 3

GENOMIC SELECTION MODELS FOR PRE-HARVEST SPROUTING TOLERANCE ARE

IMPROVED BY WEIGHTING QTL IDENTIFIED VIA GENOME-WIDE ASSOCIATION

SUMMARY

Pre-harvest sprouting (PHS) is a major problem in wheat (*Triticum aestivum* L.) that occurs when grains in a mature spike germinate before harvest, resulting in reduced yield, quality and grain sale price. Improving PHS tolerance (PHST) is a challenge to wheat breeders because it is quantitatively inherited and tedious to score. Genomic selection (GS) is particularly useful for predicting phenotypes that are costly and time-consuming to assess. In this study single nucleotide polymorphism (SNP) markers obtained by genotyping-by-sequencing (GBS) were used to identify significant marker trait associations and develop predictive models for PHST. A panel of 1118 breeding lines representative of U.S. Great Plains hard winter wheat germplasm was scored for PHST over multiple years. A genome-wide association approach was used to identify quantitative trait loci (QTL) among the individuals. Two primary factors were examined for their influence on model accuracy: the effect of including identified QTL and kernel color as fixed effects in the model, and increasing marker number. Model accuracy did not improve with kernel color information but weighting QTL increased predictive performance. Optimum marker number was about 4,000 with no significant improvement in accuracy above this threshold. Overall, model accuracies were promising and confirm effectiveness of GS for predicting PHST in wheat.

INTRODUCTION

Pre-harvest sprouting (PHS) is the germination of a wheat kernel in a physiologically mature spike before harvest. It usually occurs after periods of prolonged rainfall and high humidity. Besides causing a reduction in grain yield, PHS can be detrimental to end-use quality leading to smaller bread loaf volumes or sticky crumb grain (Gale and Lenton, 1987). This reduction in yield and quality leads to economic losses for the farmer as well as those in the milling and baking industry. Worldwide, it was estimated about one billion dollars are lost per year as a result of pre-harvest sprouting (Black et al., 2006), and as such, reducing PHS carries a significant economic benefit.

Improving tolerance to pre-harvest sprouting (PHST) has been a challenge for breeders because of its quantitative inheritance and laborious procedures required for phenotyping. It is also difficult to breed for PHST in winter wheat as turnaround time is much shorter than the time required to phenotype and make selections decisions for PHST. Using molecular markers to screen for tolerant lines would be helpful in addressing this problem. Although the genetic architecture is still unknown, quantitative trait loci (QTL) studies have identified several chromosomal regions associated with PHST. The most important loci are thought to belong to

homoeologous group 3 (3A, 3B, 3D; Kulwal et al., 2005; Liu et al., 2008), chromosome 4A (Mares et al., 2005) and chromosome 2B (Munkvold et al., 2009). Also located on the homoeologous group 3 are genes for red grain color, *R-A1, R-B1, R-D1* (Sears, 1944). Several studies have shown a general association between red kernel color and tolerance to PHS, possibly due to linkage between these genes (Groos et al., 2002; Gao et al., 2013). Because white grain wheat is generally preferred for whole-grain products, efforts have been made to develop PHS tolerant white-grained varieties (Kottearachchi et al., 2006; Liu et al., 2008; Graybosch et al., 2013). Despite this general association between red wheat and sprouting tolerance, genetic diversity exists among both red and white wheat for PHST (Wu and Carver, 1999). Marker assisted selection techniques have shown some promise in this area, with several QTL identified for PHS (Kumar et al., 2010; Liu et al., 2008; Mares et al., 2005). For example, a major QTL for PHST was identified in the hard white wheat cultivar 'Rio Blanco' (Liu et al., 2008), and the underlying gene, designated as *TaPHS1,* has been cloned and characterized (Liu et al., 2013). Even so, some studies have shown PHS to be affected by many small effect loci throughout the genome (Tyagi and Gupta, 2012). For this reason, using many markers across the genome could be a more effective approach when breeding for tolerance to PHS. Genomic selection (GS) could enable more rapid gains for PHST as all underlying loci would be exploited, regardless of prior identification for significant effects.

Meuwissen et al. (2001) first proposed the fundamentals of GS with the basic concept of regressing phenotypes on all available markers using a linear model. Since this first proposal, advancements in statistical models have led to greater gains in prediction accuracies. However, there are factors to consider when implementing GS into a breeding program, including model choice, size and composition of the training panel, and marker density. Other factors, such as

linkage disequilibrium (LD) and genetic architecture of the trait, may influence the accuracy of the models but these are outside the scope of factors controlled by the breeder.

Ridge regression best linear unbiased prediction (RR-BLUP) is a common method used for estimating marker effects for GS (Whittaker et al., 2000; Meuwissen et al., 2001; Endelman, 2011). However, RR-BLUP makes the assumption that marker variances are equal, which is unlikely in practice. An alternative to this approach involves estimating variances for each marker individually with Bayesian methods (de los Campos et al., 2013). Although Bayesian models make a more realistic assumption, the RR-BLUP method is computationally more convenient. In addition, Bayesian models have shown little to no gain over RR-BLUP (Asoro et al., 2011; Heffner et al., 2011; Heslot et al., 2012), and in some cases RR-BLUP can outperform Bayesian models (Lorenzana and Bernardo, 2009; Schulz-Streeck et al., 2012). De los Campos et al. (2013) found that Bayesian methods resulted in a slight increase in accuracy for traits involving large-effect QTL but generally there has been little difference between the two approaches, and as such, there does not appear to be a universally preferred model. If the trait follows the infinitesimal model, then RR-BLUP should be sufficient (Clark et al., 2011). In the infinitesimal model, many small effect loci across the genome contribute to the phenotype and therefore a model that includes all of the markers with equal marker variances would suffice. As such, RR-BLUP may serve as a more computationally efficient means for implementing GS models in breeding programs without reducing accuracy of predictions.

Some studies have shown an improvement in prediction accuracy when including functional markers in the model. Zhao et al. (2014) used a weighted RR-BLUP prediction approach (W-BLUP) to include effects of known functional markers for heading date and plant height in a hybrid wheat population. In this study, the accuracy of models for heading date and

plant height improved with the weighted model relative to the base model. The W-BLUP approach improved prediction accuracy by utilizing both marker-assisted and genomic selection approaches (Zhao et al., 2014). Bernardo (2014) demonstrated the effectiveness of using major genes in a genomic selection model with simulated data. Conclusions from this study showed that selection for a trait with high heritability (H^2 = .80) and large effect QTL (R^2 = .50) modeled as fixed effects increased the relative efficiency compared to a model that did not weight the QTL. In another study on rust (*Puccinia graminis, P. triticina, and P. striiformis*) resistance in wheat, model accuracy improved with the inclusion of known rust resistance genes (Daetwyler et al., 2014). Combining MAS with GS in this way is worth exploring as others have seen improvements by weighting markers known to be associated with the target trait.

In addition to QTL, it may be advantageous to include kernel color in the model as this is often associated with PHS (Groos et al., 2002; Himi et al., 2002; Morris and Paulsen, 1992; Nilsson-Ehle, 1914). Previous studies on using GS for PHS have not included kernel color in the model or weighted specific markers to make genomic predictions (Heffner et al., 2011; Heslot et al., 2013). Therefore, these two approaches were implemented in this study to determine their effects on model accuracy. The specific objectives were to i) use genome-wide markers to identify QTL for pre-harvest sprouting tolerance in an association panel of hard winter wheat lines; ii) develop a GS model to predict PHST in winter wheat; iii) determine if modeling kernel coat color or PHST-QTL as fixed effects could improve prediction accuracy of the GS model, and iv) determine the effect of marker number on model accuracy.

MATERIALS AND METHODS

Germplasm and Phenotyping

This study included a panel of 1118 hard winter wheat breeding lines and released

cultivars representative of the Colorado State University Wheat Breeding program. Entries were

from various nursery trials harvested in 2006 – 2013 from the Colorado State University

Agricultural Research, Development, and Education Center (ARDEC; 40.65 N, 105 W,

Elevation 1560 m) in Fort Collins, Colorado. Trials included the following: CSU Elite Trials,

Variety Performance Trials, Advanced and Preliminary Yield Nurseries, and Single-Seed

Descent-Derived Preliminary Nurseries. Of the 1118 breeding lines, 30 percent were hard red

winter wheat (HRW), 66 percent were hard white wheat (HWW), and 4 percent were mixed for

kernel color. On average 75 genotypes were evaluated in each year from trials harvested from

2006 to 2011. The number of genotypes evaluated in 2012 and 2013 was 411 and 670,

respectively.

Samples of wheat spikes were collected from plots at physiological maturity, as

determined by the lack of green color in the peduncles. Samples were dried at room temperature,

threshed, and stored in a freezer at -20 °C until germination tests were performed. For

germination tests, approximately 50 kernels were placed in petri dishes with filter paper and

moistened with distilled water. Samples were then placed in a lighted growth chamber at 20 °C

for 12-h days. After one to two days, entries were scored by counting the number of germinated

kernels each day for seven days. To break dormancy, samples were placed in a cold room at 4

°C for four d and then moved back to the growth chamber for three to four d. The remaining

germinated kernels were counted and any non-germinated kernels were removed from the

analysis for the total grain count. A germination index was calculated to give greater weight to

kernels that germinated earlier in the seven day period (Walker-Simmons, 1988). The

germination index (GI) was calculated from the following formula:

$$\text{GI} = \frac{(7x_{n1} + 6x_{n2} + 5x_{n3} + 4x_{n4} + 5x_{n3} + 6x_{n2} + 1x_{n7})}{\text{total days} * \text{total kernels}}$$

where x_{n1}, x_{n2}…x_{n7} refer to the number of germinated kernels on day 1, day 2 …day 7.

Across environments, a mixed model was used to calculate best linear unbiased

predictors (BLUPs) for each line using the lme4 package in R (Bates et al., 2014; R

Development Core Team, 2011).

Genotyping

Breeding lines were genotyped using a two-enzyme genotyping-by-sequencing approach

(Poland et al., 2012a). Libraries were prepared according to protocols reported previously

(Elshire et al., 2011) and modified by using the restriction enzymes *PstI* and *MspI* (Poland et al.,

2012a). Single nucleotide polymorphisms (SNPs) were called from GBS tags using a

population-based filtering approach as described previously (Poland et al., 2012b).

Markers were binned to nearest reference sequences using a combination of wheat survey

sequences from the International Wheat Genome Sequencing Consortium (IWGSC;

http://www.wheatgenome.org) and the 90 K SNP array developed at Kansas State University

(Wang et al., 2014) as a reference map (Manmathan et al., unpublished). Markers were filtered

for LD values below 0.9 and minor allele frequencies above 0.027, as such frequencies indicated

that at least 30 lines carried the allele (R. Bernardo, personal communication, 2014). Marker

subsets were created by setting the maximum percentage of missing values to various thresholds

followed by mean imputations of remaining missing values with the A.mat function in the R

package rrBLUP (Endelman, 2011). Table 3.1 summarizes the marker subset information. For

the analysis of trait associations the marker file with a maximum missing value of 70 percent was used (M7; Table 3.1).

Genome-Wide Association

Principal component analysis (PCA) was performed with GBS markers to estimate and visualize population structure. Association analysis was conducted with a compressed mixed linear model (Zhang et al., 2010) implemented in the R package GAPIT (Lipka et al., 2012). GAPIT uses the efficient mixed model association (EMMA) algorithm developed by Kang et al. (2010). The mixed-linear model (MLM) can account for both population structure and marker-based kinship. The "Model.selection" parameter was called to determine the optimized number of principal components (PCs) to include based on Bayesian information criterion (BIC) values (Schwarz, 1978). It was determined that the best fit was the model with only the kinship matrix and no PCs. The detection threshold for significant SNPs corresponded to the Bonferroni corrected value of $-\log_{10} (0.05/ 17158) = 5.53$ at an alpha level of 0.05 with the M7 subset of 17,158 markers. Sequences of significant markers were blasted to the IWGSC draft sequence via Ensembl Plants (http://plants.ensembl.org).

Genomic Selection Models

Genomic estimated breeding values (GEBVs) were calculated using ridge regression BLUP (Meuwissen et al., 2001). RR-BLUP assumes equal variances for marker effects. The general form of the model was:

$$y = \mu 1_n + Zu + \varepsilon$$

where y is a vector of phenotypic means for individuals; μ is an intercept term, 1_n is a vector of ones; Z is a design matrix (*n x m)* allocating marker values to individuals; u is a vector (*n x m*) of marker effects; and ε is a vector of error terms with a variance of σ_ε.

Fixed effects were added to the model according to the equation:

$$y = X\beta + Zu + \varepsilon$$

where all symbols are the same as the previous equation with the addition of X as the design matrix ($n \times p$) allocating fixed effect values to individuals and β is a vector ($p \times 1$) of fixed effects.

Accuracy of the models was determined with cross-validation (CV) to assign individuals to training or validation sets. A random 5-fold CV involved randomly assigning lines to one of five folds and using four folds to train the model and one to validate. This was repeated until all folds were used as validation sets. Accuracy was calculated as the correlation between GEBVs and BLUPs from the validation set, and reported as the mean across folds. This was repeated 100 times for each marker set and model combination.

Calculating MAS Accuracy

To determine accuracy values for a hypothetical MAS approach, a model was developed that included only the top five most significant markers as fixed effects. Similar to GS, accuracies were determined with a 5-fold CV and calculated as correlation between estimated breeding values (EBVs) and BLUPs from the validation set. This was repeated 100 times and averaged across iterations.

Least significant differences were calculated between models for the same marker subset and between marker subsets for the same model. This was done with LSD.test for multiple comparisons under the agricolae package in R (de Mendiburu, 2014). Heritability was calculated on an entry-mean basis (Fehr, 1987, p. 98) with ASREML-R (Butler et al., 2009). Row and column values from field plots were included as random effects in the model for each site.

RESULTS AND DISCUSSION

Population Structure

PCA analysis was performed to gain insight into population structure that may affect association analysis (Figure 3.1). The first two principal components explained only 10.5% of the total variation, reflecting a lack of population structure. However, the visual of the PC biplot shows a slight clustering of two groups, especially defined by PC1. Reasons for this clustering are unclear and no apparent pattern was observed for kernel color. Thus, red wheat and white wheat were not distinct genetic groups in this breeding population but instead share many common alleles as seen by the mixture of red and white genotypes in the biplot.

Association Analysis

Results from the mixed model showed that five SNPs were significantly associated with PHST (Table 3.2). The quantile-quantile plot can be used to assess how well the model accounted for population structure or relatedness among the lines (Figure 3.2). The observed negative logarithm of p-values will fall along the dotted line if they fit the null hypothesis of no trait association. It is expected that most points will fall along the dotted line but those furthest to the right will deviate as they are most likely associated with PHST. This was the case in this study as the p-values for the highly significant SNPs deviated while most of the remaining ones did not. Significant marker trait associations were all located on chromosome 3AS according to blast results, and had R^2 values above 30 percent (Table 3.2). The short arm of chromosome 3A is well established in the literature as being associated with PHST (Liu et al., 2008). Currently efforts are underway to determine more precise locations of QTL identified in this study, especially in relation to the *TaPHS1* gene.

Although QTL have been identified on several chromosomes in wheat, there has been inconsistent detection across genetic backgrounds, thus it is necessary to identify markers that allow for selection across a range of genetic backgrounds (DePauw et al., 2012). Some chromosomal regions reported in the literature as being involved in PHST were not identified in our study, including the QTL on 2B (Somyong et al., 2014) or 4AL (Chen et al. 2008). Although these QTL could have gone undetected in this breeding population for various reasons, one possibility could be because this breeding population lacks these alleles. If this is true, then there is potential to introduce these alleles into the germplasm for greater improvement of PHST.

Genomic Selection Models

Genomic selection accuracies in the base model, without fixed effects of kernel color or QTL, ranged from 0.49 to 0.58 for various marker subsets (Table 3.3). Accuracies were comparable to other studies of genomic selection for PHST in wheat (Heffner et al., 2011; Heslot et al., 2013). An estimate of MAS accuracy was calculated by using only the highly significant SNPs reported in Table 3.2. The average accuracy for MAS was 0.40 and even with the lowest marker set of 500 markers there was significant improvement in accuracy when using GS over MAS. The highest accuracy for the base GS model (.59) was 49 percent higher than with MAS. Therefore the GS approach, which captured all marker effects, performed significantly better than the approach of identifying and using only QTL. These results strongly support the use of GS in breeding for PHST in wheat.

Marker Density

Ascertainment bias can occur in marker assays when markers are not obtained from a random sample of polymorphisms in the population of interest, and can lead to false estimates of allele frequencies and LD (Albrechtsen et al., 2010; Nielsen and Signorovitch, 2003). In one

study, the commonly used DArT marker platform was compared to a genotyping-by-sequencing (GBS) platform for a panel of wheat lines (Heslot et al., 2013). Results showed the DArT markers were more clustered and led to more ascertainment bias than GBS markers, although this in itself did not lead to a reduction in GS accuracy. The GBS platform produced over 38,000 SNPs, whereas the DArT platform produced only 1,500. In comparing the two platforms with an equal number of markers (i.e., reducing GBS markers to 1,500), there was no difference in accuracy for most traits. Therefore the better prediction was due to an increase in available markers and not due to a lack of ascertainment bias.

In this study, we observed an increase in model accuracy with the inclusion of more markers (Figure 3.3, Table 3.3). However, after a threshold of about 4,000 markers (M3 subset) there was no significant increase in accuracy. Other studies have also identified an optimum marker size with diminishing return above a certain threshold (Heffner et al., 2011a; Heslot et al., 2013b; Lorenzana and Bernardo 2009). For GS, it is desirable to obtain adequate genome coverage so that all contributing loci are in LD with at least one marker. Studies have shown this optimum marker size to be trait and population specific (Erbe et al.,2013; Poland et al., 2012b) . Heffner et al. (2011a) identified the optimum marker number to be 384 in a panel of 288 soft winter wheat breeding lines. Lorenzana and Bernardo (2009) also found diminishing returns from increasing marker size, while Heslot et al. (2013b) observed optimum marker densities varied depending on the target trait. In a simulated study, Muir (2007) found increasing marker density without also increasing training panel size would not increase accuracy and in some cases could actually decrease accuracy. In the present study, a slight but statistically insignificant decrease in accuracy was observed when increasing the marker density from 17,000 to 22,000 in all GS models (Table 3.3). Resend et al. (2012) observed a decrease in accuracy

with greater marker densities for disease resistance traits. This decrease was due to model over parameterization, which can occur if a large number of markers are used in the model for a trait controlled by a few major loci. This over parameterization could explain the slight decrease observed in our data but should not be a major concern as the decrease was insignificant. Muir (2007) suggested increasing marker density would only increase accuracy if it scaled with an increase in the training panel size. Implementing GS into a breeding program would likely involve increasing training panel size over time as more phenotypic data are generated. Thus, as more individuals are added to the model, there may be a need to recalculate the optimum marker size as it has the potential to vary with changes in the data set.

Inclusion of Fixed Effects in the Model

Studies have found that phenotypic information correlated to the target trait can be useful in increasing prediction accuracy. For example, Rutkoski et al. (2012) found that including correlated trait information, such as incidence, severity, and kernel quality, improved prediction accuracy for Fusarium head blight (*Gibberella zeae*) resistance in wheat. However, including such information involves the added step of phenotyping for these traits which can be costly and time consuming, thereby mitigating one of the key benefits of GS (i.e., minimizing resources spent on phenotyping). In this study, including kernel color as a fixed effect in the model had no effect on the prediction accuracy (Table 3.3). The coefficient of determination for the model with kernel color explaining sprouting tolerance was very low ($R^2 = 0.01$). Thus, in this breeding population kernel color was not a reliable indicator of PHST.

Although reports have suggested that white wheats generally have less tolerance than red wheats (Morris and Paulsen, 1992), it is not uncommon to find a red wheat with poor tolerance to PHS or a white wheat with good tolerance. Among Great Plains hard white winter wheats,

'Rio Blanco' has been identified as a good source of PHST (Wu and Carver, 1999) with the major QTL on 3AS identified in this cultivar (Liu et al., 2008). Liu et al.'s (2013) results suggested major differences in PHST were independent of grain color; however color may contribute in modifying PHST regulated by the TaPHS1 gene. In this study, genotypes with red kernel color had on average a slightly lower sprouting rate (GI = .33) compared to the white (GI = .36) but the difference was not significant. Thus, kernel color genes could modify the level of tolerance but do not have a large enough effect to lead to an increase in accuracy when included as fixed effects in GS models.

In a simulated analysis, Bernardo (2014) demonstrated the effectiveness of adding major genes as fixed effects in a GS model. Conclusions from this study showed that selection for a trait with high heritability (H^2 = .80) and large effect QTL (R^2 = 50%) would benefit from a GS model that weighted the QTL as fixed effects. There was no disadvantage to specifying a fixed effect unless the R^2 was below 10%. Results from this study confirmed findings from Bernardo's simulated study as including fixed effects of major PHST-QTL identified via GWAS increased model accuracy (Table 3.3). However, including color as a fixed effect did not decrease accuracy despite the low R^2 value. The average heritability for pre-harvest sprouting tolerance was 0.42 with a standard deviation of 0.22. Even with moderate heritability and R^2 values, an improvement in accuracy was observed when weighting large-effect QTL. Accuracies for models with both color and QTL as fixed effects were not significantly different from the model with only QTL (Table 3.3). This confirms the significant improvement in accuracy was due to weighting QTL and not to including color as a fixed effect.

Differing from our results, Rutkoski et al. (2012) observed no increase in accuracy when adding targeted QTL to the base model for traits which responded better to GS over MAS. It is

unclear if low trait heritability or low R^2 values for targeted QTL contributed to these results, as these parameters were not reported. Similar to our results, Daetwyler et al. (2014) also observed a slight increase in GS accuracy by including markers for known rust (*Puccinia* sp.) resistance genes into the model as fixed effects. However, a greater improvement was observed with diagnostic markers than linked markers. This comparison could not be made in our study as it was unclear where in relation to the known gene the markers were located. However, knowledge of positions of these QTL may allow for this comparison in future work.

CONCLUSION

Unlike with spring wheat breeding, winter wheat breeding programs ahve a much shorter turnaround time from harvest of one season to planting of the next. Thus, selections for tolerance to PHS cannot be made in one year to plant for the following season, and the rate of genetic gain under phenotypic selection is less than for traits like yield because cycle time is increased. This issue of short turnaround time makes GS especially valuable as genotypic information would be readily available to make selections prior to planting the next year's crop.

This study used data from a wheat breeding program to confirm a previous simulated study concerning major genes as fixed effects in a GS model (Bernardo, 2014). Accuracies from these models endorse implementing GS in a wheat breeding program for traits that are controlled by both large-effect and small-effect loci throughout the genome. Genome-wide association analysis shed light on the underlying loci that control for PHST, with results showing that this trait is controlled by both large effect and many small effect loci. If only the large effect loci were contributing to the trait, then MAS accuracy would be comparable to GS accuracy. However, the model with the highest predictive accuracy included all small effect loci in addition to weighted large effect loci, suggesting that MAS may fail to capture useful loci

affecting PHST. In addition, the large effect loci identified here will be strong candidates for

future research. Further work may include a candidate gene study to validate if any of the

identified SNPs reported here may be used as markers in a marker-assisted pre-screening before

collecting genome-wide data on new breeding material.

Table 3.1 Marker subset information for genotype-by-sequencing markers

Subset Name	Max Missing[†] (%)	n[‡]
M1	0.1	507
M2	0.2	2,083
M3	0.3	4,340
M5	0.5	9,816
M7	0.7	17,158
M8	0.8	21,951

[†]Maximum percentage of missing data allowed per marker

[‡]Number of markers in the subset

Table 3.2 Quantitative trait loci for pre-harvest sprouting tolerance identified through genome-wide association in 1118 inbred wheat lines

QTL[†]	Chr[‡]	Score[§]	Blast hit[¶]	Present (%)[#]	MAF[††]	R^2 (%)
Phst-qtl-3AS-gbs2177	3AS	6.601	IWGSC_CSS_3AS_scaff_3406427	51	0.15	30.5
Phst-qtl-3AS-gbs24267	3AS	5.434	IWGSC_CSS_3AS_scaff_3444297	59	0.36	30.1
Phst-qtl-3AS-gbs30209	3AS	7.990	IWGSC_CSS_3AS_scaff_3340678	89	0.33	30.9
Phst-qtl-3AS-gbs392	3AS	7.813	IWGSC_CSS_3AS_scaff_3406679	89	0.31	31.0
Phst-qtl-3AS-gbs25739	3AS	5.629	IWGSC_CSS_3AS_scaff_3343774	73	0.33	30.7

† QTL were named according to four parts: first: *Phst* indicates pre-harvest sprouting tolerance; second: *qtl* indicates a quantitative trait locus;
  third: the chromosome number and arm; and fourth: the name of the genotyping-by-sequencing marker showing significant association with phst
‡ Chromosome position of QTL
§ –log(p) with the K model
¶ According to IWGCS draft sesquences (http://plants.ensemble.org/Triticum_aestivum/)
# Percent of 1118 wheat lines with available data for marker
†† MAF, Minor allele frequency

Table 3.3 Mean accuracies of genomic selection models for pre-harvest sprouting tolerance in wheat

Marker Subset	n[†]	Mean Accuracies of Model[‡]							
		Base Model[§]		Color Effect[¶]		QTL Effect[#]		Both Effect[††]	
M1	507	0.49	b[‡‡]	0.50	b	0.54	a	0.55	a
M2	2,083	0.54	b	0.54	b	0.58	a	0.58	a
M3	4,340	0.57	b	0.57	b	0.59	a	0.60	a
M5	9,816	0.58	b	0.59	ab	0.61	a	0.60	a
M7	17,158	0.59	b	0.60	ab	0.61	a	0.62	a
M8	21,951	0.58	b	0.58	b	0.60	a	0.61	a

† Size of marker subset (refer to Table 3.1)
‡ Accuracies were calculated from 5-fold cross validation repeated 50 times.
§ Model includes all markers as random effects with no fixed effects.
¶ Model includes all markers as random effects and kernel color as a fixed effect.
# Model includes all markers as random effects and five QTL as fixed effects.
†† Model includes all markers as random effects and kernel color and five QTL as fixed effects.
‡‡ Within each row means with different lower case letters are significantly different (LSD t test; $\alpha = 0.05$).
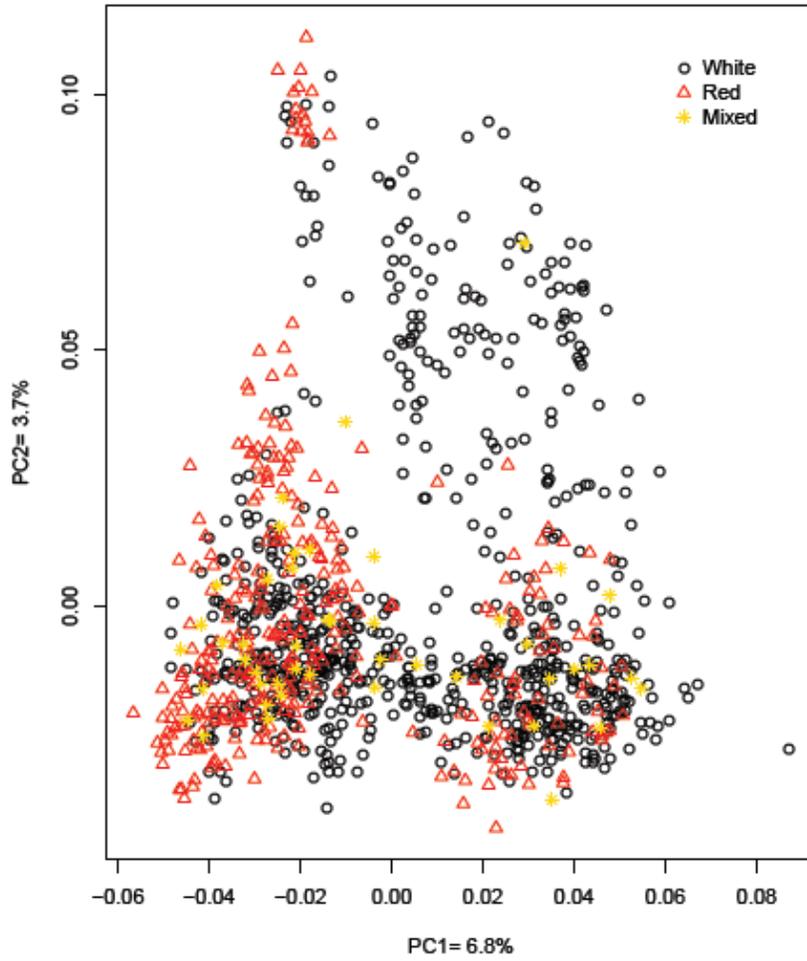
Figure 3.1 Principal component analysis of 1118 inbred wheat genotypes with genotyping-by-sequencing marker data, labeled according to kernel color.
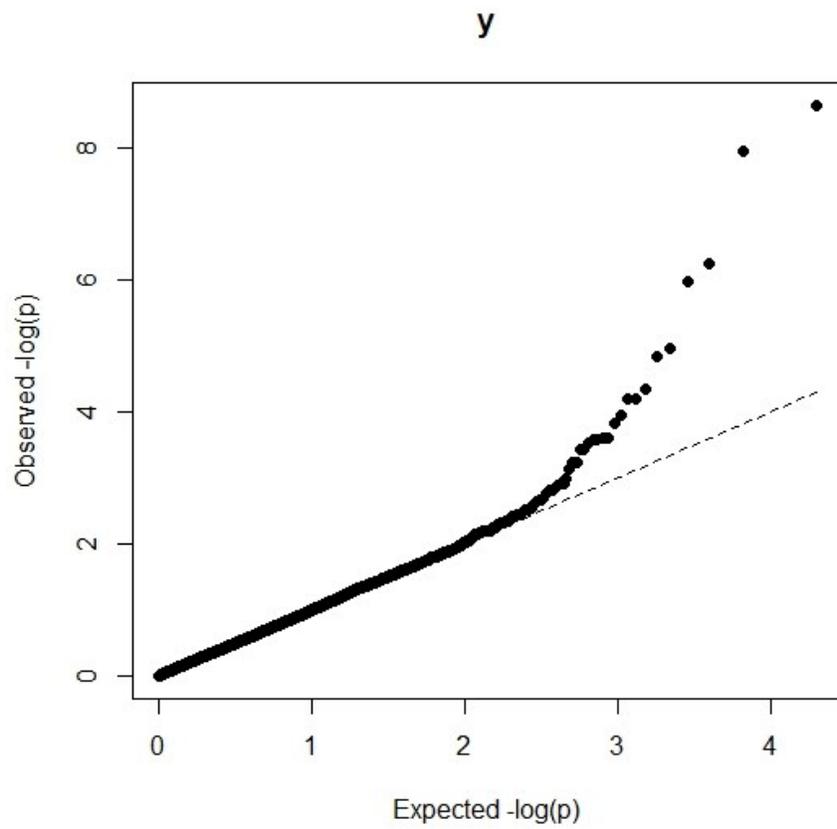
Figure 3.2 Quantile-Quantile plot of negative base 10 logarithm of p-values for each genotyping-by-sequencing marker.
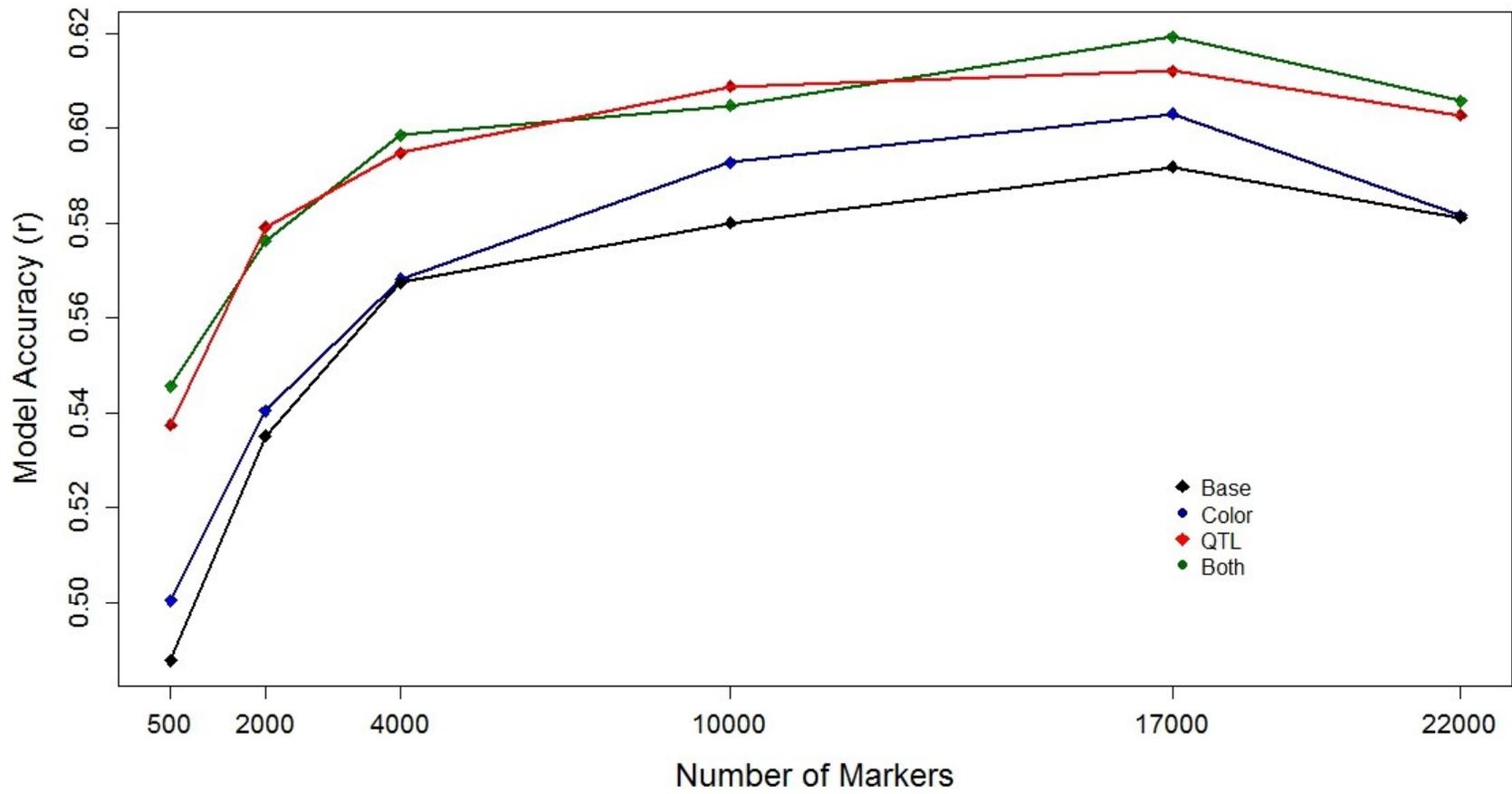
Figure 3.3 Model accuracy versus number of markers used in the model.

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CHAPTER 4

GENOMIC PREDICTION FOR END-USE QUALITY IN HARD WINTER WHEAT

SUMMARY

End-use quality is an important breeding objective for wheat (*Triticum aestivum* L.) as quality characteristics influence grain sale price and market success of a variety. However, breeding for quality traits can be difficult due to high costs, sample size requirements, and short turn around time for winter wheat. Genomic selection (GS) can accelerate genetic progress especially for traits that are costly to phenotype and can't be implemented in selection decisions for the following year's crop. In this study, we assessed the accuracy of GS models for 11 end-use quality traits in a panel of hard winter wheat breeding lines phenotyped across multiple years and locations in Colorado. Traits measured were flour yield, single kernel characteristics, protein concentration, mixing time and tolerance, bake absorption, bake mixing time, crumb grain, and loaf volume. The panel included experimental lines and cultivars of hard red and white winter wheat. Single nucleotide polymorphism (SNP) markers obtained by genotyping-by-sequencing (GBS) were filtered for varying thresholds of maximum missing data and remaining missing markers were subject to imputation via the mean or a random forest (RF) algorithm. Random 5-fold cross-validation accuracies ranged from 0.30 to 0.63 for quality traits with heritabilities ranging from 0.03 to 0.61. No difference in model accuracy was observed between mean and RF imputations. Slight improvement of accuracy was seen with increased marker number but this was trait dependent. Traits with higher heritability had greater model accuracy than traits with low heritability. GS models for moderate to high heritability traits performed better than expected when predicting most recent genotypes in the breeding program.

Results suggest GS models for end-use quality traits with low heritatiblity may require more resources in developing training panels to increase predictive performance for these traits.

Abbreviations

BLUP, best linear unbiased predictor; CV, cross-validation; GBS, genotyping-by-sequencing; G x E, genotype by environment interaction; GS, genomic selection; MAS, marker-assisted selection; QTL, quantitative trait locus; RF, random forest; RR-BLUP, ridge-regression best linear unbiased predictor; SNP, single nucleotide polymorphism.

INTRODUCTION

Breeding for end-use quality in wheat (*Triticum aestivum* L.) presents numerous challenges, in part because many traits related to quality are quantitatively inherited and costly to phenotype. Quantitative trait loci (QTL) for end-use quality traits have been identified for the use in marker-assisted selection (MAS; Chen et al., 2008; Liu et al., 2008; Zhang et al., 2009) and have been used as an effective tool in making breeding decisions. However, MAS carries a number of limitations, such as the time and cost required for identifying and implementing selection for significant QTL. Also, many traits are too complex to be captured by a few QTL. An alternative to utilizing specific loci for selection decisions is to use loci across the entire genome. Whereas MAS assesses a limited number of target loci, genomic selection (GS) examines the influence of the entire genome on phenotypes. The GS approach involves regressing phenotype on genotype to develop a model that predicts new phenotypes based solely on genome-wide markers (Meuwissen et al., 2001). With GS, the focus is not on the underlying genetic architecture or QTL associated with the target trait but rather on the sum of the additive effects, or the breeding value of the individual. Benefits of GS for plant breeding have been discussed in the literature (Heffner et al., 2009), especially for complex genomes like wheat

(Heffner et al., 2011b; Poland et al., 2012b; Lado et al., 2013). Simulated and empirical studies have demonstrated advantages of GS over other methods of selection in terms of maximizing the efficiency of breeding programs (Mayor and Bernardo, 2009; Rutkoski et al., 2012).

Yield and yield-related traits have been primary targets for GS models for wheat. However, quality is also a crucial breeding objective given the direct influence of quality characteristics on grain sale price and the market success of the end product. A successful wheat variety will have high values for yield while maintaining desired values for quality. Therefore, it is desirable that a breeding program utilizing GS will implement models that simultaneously promote selection for both yield and quality characteristics.

A primary benefit of GS is the ability to select for traits in fewer years than is possible using phenotypic selection. Some quality parameters like protein concentration can be measured in early generations with rapid high-throughput tests like near infrared reflectance spectroscopy (NIRS). However, this does not fully explain the end-use quality of the samples evaluated. Traits related to dough viscoelasticity, mixing properties, and baking performance must be delayed until later generations due to high costs and limited grain supply. As a result, lines may be advanced through the breeding pipeline only to be discarded in subsequent seasons due to inferior quality. Genomic selection can allow for milling and baking traits to be considered in selection earlier in the breeding pipeline, increasing the efficiency of selection decisions.

In many winter wheat breeding programs, particularly those at higher latitudes, selections for end-use quality in one year cannot be used for planting decisions in the following year due to the short turn-around time from harvest to planting. By the time the quality evaluation has been performed the next year's crop is already planted. This issue makes GS even more valuable as it can allow for genotypic information to be readily available in enough time to make selections for

quality traits before planting the subsequent year's crop. Overall, GS can reduce the years to select for specific characteristics by at least half.

When implementing GS into a breeding program, a prerequisite is to decide on a marker platform. Genotyping-by-sequencing (GBS) is an alternative to fixed-array marker platforms used for generating genome-wide markers (Poland et al., 2012a). Genotyping-by-sequencing can be used for de novo genotyping of polyploid organisms with large, complex genomes, making it an ideal platform for wheat. It reduces the genome complexity by targeting gene-rich areas and is more cost effective than other array-based platforms (Poland et al., 2012b). Problems associated with ascertainment bias are not a concern with the GBS platform because there is no fixed assay. However, one drawback of this platform is the high percentage of missing data that can occur as genomic fragments are sequenced at a low depth leading to very low coverage in some individuals. Even so, this shortcoming can be addressed through various marker imputation methods. One method involves using the average marker effect across all individuals to impute missing values. Mean imputation is considered to be sufficient in cases where there are few missing values or low marker density but may be insufficient for GS under conditions of very high marker density with a high percentage of missing values. Random forest (RF) is another imputation method that involves creating "trees" of data and choosing the largest cluster of trees (Breiman, 2001). Random forest is considered to be a sufficient imputation method for GBS data but has the major constraint of being more computationally intensive and thus may be impractical for large data sets. Previous studies have compared imputation methods for genome-wide markers (Poland et al., 2012b; Rutkoski et al., 2013). Although differences were found in accuracy of marker imputations, imputation method did not reliably affect accuracy of the GS

models. The influence of imputation method on GS accuracy for quality traits in wheat is not yet clearly established.

GS for end-use quality traits such as mixograph parameters or pup-loaf baking parameters in wheat is limited. Heffner et al. (2011a) compared GS to MAS and phenotypic selection for end-use quality traits in two bi-parental wheat populations. The authors noted that a multi-family approach would capture more effects in a breeding population and be more effective than a bi-parental model. Phenotyping individuals across many environments would also be beneficial when implementing GS, as marker estimates would be more robust under these conditions. In another study, Heffner et al. (2011b) followed through with this idea of multi-family and multi-environment data to develop GS models for agronomic and quality traits using a population of wheat breeding lines phenotyped across two years. Their results indicated that GS was superior to MAS and phenotypic selection; however, the effects of marker imputation method and trait heritability when implementing GS for wheat quality were not investigated.

The objective of this study was to develop and assess the accuracy of GS models for 11 end-use quality traits in hard winter wheat utilizing GBS markers and phenotypic data from multiple years and locations. To meet this objective, cross validation was performed by randomly assigning individuals phenotyped across multiple locations from 2006 – 2012 to training or testing populations. Effects of trait heritability, marker imputation, and marker number on GS accuracy were investigated. Additionally, implications of validation methods to determine model accuracy were investigated.

MATERIALS AND METHODS

Source Materials

The germplasm used in this study consisted of experimental lines and released cultivars representative of the Colorado State University (CSU) Winter Wheat Breeding Program. Entries were from various trials harvested from 2006 to 2013. Trials included the following: CSU Elite Trials, Variety Performance Trials, Advanced and Preliminary Yield Nurseries, and Single-Seed Descent-Derived Preliminary Nurseries. Locations were in Colorado and included the following: Akron, Arapahoe, Burlington, Dailey, Fort Collins, Genoa, Haxtun, Julesburg, Lamar, Orchard, Rocky Ford, Roggen, Sheridan Lake, Walsh, and Yuma. For all traits, the most data available was used to develop models. Because every individual was not subject to all quality testing in the breeding pipeline, training panel sizes varied among traits (see Table 4.1).

Phenotyping

Evaluation of end-use quality in wheat involves measuring traits that are related to overall milling and baking characteristics. Milling involves physical characteristics relating to the kernel structure and make up, while baking involves characteristics related to dough rheology, or how the dough will form, stretch, and deform. To assess milling and baking characteristics, eleven traits were measured: kernel hardness, kernel diameter, kernel weight, protein concentration (grain protein), flour yield, mixograph peak time (mixo mix time), mixograph tolerance (mixo tolerance), bread dough water absorption (bake abs), bread dough mixing time (bake mix time), crumb grain score, and loaf volume.

All quality evaluations were done in the wheat quality lab at Colorado State University in Fort Collins, Colorado. Traits were measured using approved methods of the American Association of Cereal Chemists (AACC, 2000). Single kernel hardness, diameter, and weight

were determined using the single kernel characterization system (SKCS4100; Perten Instruments, Springfield, IL). Protein concentration was determined using NIRS on whole grain samples using a Foss NIRSystems model 6500 (Foss North America Inc., Eden Prairie, MN) and reported on a 120 g kg^{-1} (12%) moisture basis. Samples of 300 g of grain were tempered to 155 g kg^{-1} (15.5 %) moisture and milled using a modified Brabender Quad Senior milling system (C.W. Brabender, South Hackensack, NJ; Method AACC 26-10A for milling). Flour yield was determined as the percentage of total grain weight accounted for by all flour fractions. Mixograph parameters of dough peak time and mixing tolerance were obtained with a 10-gram mixograph and MIXSMART software for computer-analyzed parameters (v. 1.0.484 for Windows, National Manufacturing, Lincoln, NE). Mixograph tolerance was scored visually on a 7-point scale of 0–6, where zero is unacceptable and six is outstanding. To measure bread-making parameters of bake water absorption, bake mix time, and loaf volume, the pup loaf (100 g of flour) straight-dough procedures were used (method 10-10B; AACC 2000). After cooling, loaves were sliced in half and evaluated by a trained panelist for crumb grain on a 7-point scale of 0-6, where zero is unsatisfactory and six is outstanding.

All phenotypic data were collected from 14 Colorado locations over a seven-year period from 2006 to 2012. An imbalanced set of year-by-location combinations generated a total of 39 environments. Across all environments a mixed model was used to calculate best linear unbiased predictors (BLUPs) for each genotype by trait using the lme4 package (Bates et al., 2014) in R (R Development Core Team, 2011). Environments were treated as random effects in the model. Heritability for each trait was calculated on an entry-mean basis (Fehr, 1987, p. 98) with ASREML-R (Butler et al., 2009).

Additionaly, phenotypic data was collected from nine Colorado locations harvested in 2013 and used in the forward prediction approach described later. Any common genotypes between the two data sets were removed from the 2013 data. BLUPs for each genotype by trait were calculated with the same procedures used for the 2006 – 2012 dataset.

Genotyping

Materials were genotyped using a two-enzyme GBS approach as described by Poland et al. (2012a). Libraries were prepared according to protocols reported previously (Elshire et al., 2011). A total of 45,816 single nucleotide polymorphisms (SNPs) were detected from GBS tags using a population-based filtering approach (Poland et al., 2012b). Markers were filtered for minor allele frequencies above 0.01 and missing data below various thresholds (>0.2, >0.3, >0.5, and >0.7). Remaining missing markers were imputed with the mean or by a random forest algorithm using the R packages rrBLUP (Endelman, 2011) and randomForest (Breiman et al., 2012), respectively. Random forest regression is an ensemble approach that involves the creation of decision trees followed by selection of the most common output from the trees (Breiman, 2001). Marker sets ranged in size from 5,085 to 35,159 SNPs. Due to computational demand, random forest was used on marker sets containing up to 50 percent missing data per marker, while mean imputation data sets included marker sets including up to 70 percent missing data.

GS Models and Prediction Accuracy

Genomic estimated breeding values were calculated using ridge regression best linear unbiased prediction (RR-BLUP; Meuwissen et al., 2001). RR-BLUP makes the incorrect assumption of equal marker effect variances but is computationally more convenient than Bayesian methods, which allow for unequal variances of marker effects and epistasis. RR-

BLUP marker estimates were calculated using the R package rrBLUP (Endelman, 2011) according to the following model:

$$y = \mu 1_n + Zu + \varepsilon$$

where y is a vector of phenotypic means for individuals; μ is an intercept term, 1_n is a vector of ones; Z is a design matrix (*n x m)* allocating marker values to individuals; u is a vector (*n x m*) of marker effects; and ε is a vector of error terms with a variance of σ_ε.

Cross-Validation Accuracy

Cross-validation (CV) is a common technique in determining accuracy of genomic predictions and has been used in previous GS studies (Crossa et al., 2010; Hayashi and Iwata, 2013; Heffner et al., 2011a; Heslot et al., 2013a; Lorenzana and Bernardo, 2009). A random five-fold cross-validation method was used to estimate accuracy on models utilizing data from 2006 - 2012. This involved randomly assigning genotypes to one of five folds, using four folds to train the model, and one fold to validate the model. This was repeated until all folds were used to validate the model, following the procedure used by Lorenzana and Bernardo (2009). Accuracy was calculated as the correlation coefficient between GEBVs and BLUPs from the validation set, and reported as the mean across folds. This was repeated 100 times for each trait and marker set combination. Least significant differences (LSD) of accuracies were calculated between marker sets for each trait with the LSD test for multiple comparisons in the R package agricolae (de Mendiburu, 2014).

Forward Prediction Accuracy

An additional validation method was used to estimate model accuracy that involved predicting the most recent breeding lines phenotyped in 2013. This involved using all available

data from 2006 – 2012 as the training set and individuals from 2013 data as the validation set, with no common genotypes between training and validating sets. Accuracies were calculated as the correlation between GEBVs and BLUPs from 2013 individuals and averaged across mean marker subsets. Across traits, percentage of validation lines out of total number of lines ranged from 10 to 20 percent so that no trait had an unusual ratio of training to testing populations and size of validation sets were comparable to CV testing sets.

RESULTS AND DISCUSSION

Genomic Prediction for Quality Traits

Although the cross-validation approach used in this study did not partition years or environments between training and testing populations, phenotypic values used to train the model came from BLUPs calculated across 39 environments. Using multi-environment data should reduce error due to genotype by environment interaction (G x E) and provide a greater sample of the target environmental conditions for the breeding program. Previous studies have shown the benefit of phenotyping across multiple years and locations to establish robust models with high predictive performance (Heffner et al., 2011b; Wang et al., 2014). Environmental factors like temperature, moisture, and nutrient availability have been shown to affect end-use quality of wheat (Cornish et al., 2001; Dupont and Altenbach, 2003). Thus, it was necessary in this study to address G x E interaction by phenotyping across several years and locations and including environment as an effect in the estimation of BLUP phenotypic values. This is the most common method of addressing G x E in GS but another approach is to incorporate G x E effects directly into the model. Dawson et al. (2013) explored modeling G x E in prediction models for a large historical dataset of international wheat genotypes but found no increase in prediction accuracy compared to the global random cross-validation accuracies. The wheat

breeding program at CSU does not breed for quality specific to target environments because of the difficulty and expense of separating grain based on quality. For these reasons, the method employed in the current study was likely sufficient in addressing G x E effects.

Average cross-validation accuracies for quality characteristics ranged from 0.30 for bake absorption to 0.63 for bake mix time (Table 4.1). Accuracies for flour yield and grain protein were similar to previous studies in wheat (Heffner et al., 2011a, 2011b), while GS accuracies for other quality traits in wheat have not been reported. In a simulated study for winter wheat, a GS accuracy of 0.3 was suggested as a threshold for genetic gain via GS to exceed that of MAS (Heffner et al., 2010). Thus, in this study, even GS for the trait with the lowest accuracy would likely lead to greater rates of genetic gain.

The value of obtaining an estimate for a quality parameter in wheat is high relative to many other traits because of the time and cost involved in phenotyping (e.g., measurements from a pup loaf baking test). In addition, the short turnaround time between harvest and planting for winter wheat makes it very difficult to apply phenotypic data from the present year on planting decisions for the following year. This delay in phenotypic evaluations also delays the process of returning a line to the crossing block to be used as a parent, also known as the cycle time. The benefit of GS lies in the reduction of cycle time in a breeding program. Target traits that are time consuming to phenotype will result in a longer cycle time under phenotypic selection, and thus benefit more from GS. Therefore, models for such traits may allow for lower GS accuracies as the primary benefit is in the reduction of cycle time even with a low predictive ability. Results from this study show moderate to high accuracies, giving more support for implementing a GS approach for breeding wheat varieties with acceptable end-use quality characteristics. Among quality traits measured, two traits commonly used when assessing dough rheology and baking

performance are mixograph mix time and loaf volume. In this study, predictive accuracies for

these two traits were among the highest reported (0.62 and 0.55, Table 4.1). As such, these traits

may be useful to emphasize when implementing GS into a wheat breeding program with a goal

of maintaining end-use quality. Additionally, mix time was positively correlated with loaf

volume ($R^2 = 0.54$, $P < 0.05$). Thus, GS models for predicting loaf volume may benefit from

phenotypic information on mix time as studies have shown correlated trait information could

improve GS accuracy (Rutkoski et al., 2012). More phenotypic data on mix time would be

available as this test is performed on more individuals in the breeding program than loaf volume.

Trait Heritability and CV Accuracies

Across traits, a positive linear relationship was observed between trait heritability and CV

accuracy (Figure 4.1). This is expected as heritability is a measure of the proportion of

phenotypic variation that can be explained by genotypic variation. The data used to train the

model was poor for traits with low heritability, which is why the model accuracy was low

relative to models for other traits. Heffner et al. (2009) observed an increase in prediction

accuracy as heritability increased. Other studies have observed a positive correlation between

trait heritability and model accuracy (Combs and Bernardo, 2013; Daetwyler et al., 2014). Bake

absorption followed this linear trend as heritability (0.07) and accuracy (0.30) were both low

relative to other traits in this study (Table 4.1). However, GS accuracy for grain protein was

moderate (0.47) despite its low heritability (0.03). Previous studies have also observed

exceptions to this trend in some traits (Combs and Bernardo, 2013; Heffner et al. 2011a).

Reasons for these exceptions could be due to other factors contributing to model accuracy such

as number of individuals included in the training panel. In this study there was a larger panel of

individuals (1095) for grain protein compared to other traits measured. Other studies have

observed an increase in accuracy due to an increase in training panel size (Heffner et al., 2011b). Thus, the high GS accuracy for protein may simply reflect the large training panel available for the trait in this study. However, studies have shown the effective training panel size to be dependent on the targeted trait and population (Combs and Bernardo, 2013). This could explain why some traits, e.g., bake mix time, had a smaller training panel but greater prediction accuracies. To determine the effective training panel size, one must look at each trait separately and test for accuracies among different sizes of the training panel, which was beyond the scope of this study.

Effect of Marker Imputation and Density

Choosing a marker platform is an important step in incorporating GS into a breeding program. An advantage of the GBS platform is the ability to generate an abundance of marker information at a relatively lower cost compared to other platforms (Poland et al., 2012b). Prior work has shown that GBS markers are superior to array-based markers because of this increase in marker density (Heslot et al., 2013b). However, the limitation to GBS markers is the high degree of missing data per marker. To address this, it is important to have a marker imputation method that is successful at making genomic predictions and easy to implement during the marker data filtering process. As such, two common methods for marker imputation were compared with regard to their impact on GS accuracy: mean imputation and Random Forest imputation.

Across marker sets, differences in GS accuracies among marker imputation methods were minimal (< 0.02; Table 4.2). When comparing imputation methods for each trait and marker density, only two out of 33 scenarios showed a significant difference, with the mean imputation method superior in one (flour yield, 50% missing) and RF superior in the other (mixo mix time,

20% missing). For all other scenarios, differences in accuracies were not statistically significant ($P > 0.05$). Poland et al. (2012b) demonstrated that RF had less imputation error than the mean imputation method, but similar to this study, this didn't affect the predictive accuracy of their model. Rutkoski et al. (2013) found some cases where mean imputations led to a decrease in GS accuracy when using marker sets with 70 percent or more missing data. In this study, we were unable to compare the two imputation methods for the largest marker set with 70 percent missing data due to the computational demands of RF imputation. However, no significant improvement was observed from the RF markers with 50 percent missing data compared to the mean markers with 50 percent missing data (Table 4.2). Results suggest that the simpler mean imputation method should be sufficient given its computational ease and similar resultant model accuracy.

Another consideration when implementing GS concerns the number of markers needed to obtain optimal prediction accuracy. The goal of this step is to obtain genome coverage so that all contributing loci are in complete linkage disequilibrium with at least one marker. Because the GBS platform produces an abundance of markers at a relatively low cost, this goal is attainable for most breeding programs. In this study, reducing number of markers had little impact on model accuracy across all traits for both imputation methods tested. For all traits, reducing the marker sets from 35,000 (for mean imputation) or 21,000 (for RF imputation) to 5,000 reduced the model accuracy by less than 0.03 (Table 4.2). Despite these small differences, significant differences were found among marker sets as accuracy calculations were repeated 100 times for each scenario, reducing the standard errors. In this case, results showed that optimum marker number varied depending on the trait. Only one trait, grain protein, increased in accuracy when the largest marker set of 35,000 was used (Table 4.2). Because differences were small in this study, it was concluded that accuracy was generally not influenced by marker number.

Heffner et al. (2011b) observed a benefit in increasing number of markers up to 384 but found no significant increase in model accuracy after this threshold, with the largest marker set used in the study containing 1158 markers. Lorenzana and Bernardo (2009) also found diminishing returns from increasing number of markers, indicating that increases in marker density do not always result in greater accuracy. Similar to these results, Heslot et al. (2013b), found that optimum marker densities varied from 4,787 to 38,120 depending on the trait. It has been suggested that the advantage of increasing marker density will only be realized if it scales with an increase in training panel size (Muir, 2007). This may explain why the trait with the largest training panel (grain protein) was the only trait to show a significant increase in model accuracy when the largest number of markers were used. However, the single kernel characteristics had almost as many individuals in the training panel (1060) but varied in the optimum marker density (hardness: 21K; diameter: 10K; and weight: 5K). Thus, other factors such as the number of effective loci contributing to the trait may affect the optimum marker size, as traits controlled by fewer underlying loci would require fewer markers.

Forward Prediction Accuracy

Random CV accuracies were compared to 'forward prediction' accuracies to investigate if the cross-validation method used was a good estimate of how the model would perform when predicting new material in the breeding program. This forward prediction approach represents implementation of GS in a breeding program as past individuals will likely be used to predict phenotypes of new lines. Similar to CV estimates, a positive linear relationship was observed for forward prediction accuracy and trait heritability (Figure 4.2), demonstrating the strong influence of heritability on GS accuracies. A significant positive correlation existed between CV and forward prediction accuracy ($R^2 = 0.35$; $P < 0.05$), although forward prediction accuracies of

traits varied in their deviation from CV estimates (Table 4.3). It was expected that CV accuracies would be higher than forward prediction accuracies as environmental effects are shared between training and testing sets in the former method. However, this did not occur for all traits in our study. With the exception of bake absorption for traits with heritability above 0.3, the forward prediction accuracy was greater than the CV accuracy. For traits with heritability below 0.3, the forward prediction accuracy was less than the CV accuracy (Table 4.1, Table 4.3). Thus, in this scenario, if the heritability was high enough, the model would perform better than expected when predicting new lines.

Reasons for this increase in accuracy are unclear but could be due to the difference in training panel size as forward prediction included all 2006-2012 individuals while CV used 80 percent of the individuals to train the model. A decrease in accuracy for low heritability traits could be due to poor estimates of phenotypic data. To increase accuracy in low heritability traits, more resources could be put into obtaining accurate phenotypic data for model training. This could involve more replications or better experimental designs to reduce error in the phenotypic calculations but could also negate the cost reduction benefit of GS.

The forward prediction method only involved prediction for one year and more data are needed to draw clear conclusions about validation methods as forward prediction estimates can vary from year to year (Dawson et al., 2013). It has been suggested that random cross validation is a sufficient assessment of accuracy for a breeding program (Daetwyler et al., 2013). From a breeding perspective, the goal in calculating GEBVs is to predict a breeding value for an individual representative of the individual's worth over the next several years. Therefore, prediction of performance for one year would be insufficient compared to a cross-validation prediction using data from several years and locations.

Table 4.1 Heritability, population size, and mean genomic selection accuracy for quality traits in wheat

Trait	Heritability	N[†]	Cross-Validation Accuracy[‡]
Flour Yield	0.18	428	.56
Kernel Hardness	0.21	1060	.50
Kernel Diameter	0.18	1060	.50
Kernel Weight	0.19	1060	.49
Grain Protein	0.03	1095	.47
Mixograph Mix Time	0.45	908	.62
Mixograph Tolerance	0.39	458	.48
Bake Absorption	0.07	458	.30
Bake Mix Time	0.61	448	.63
Crumb Grain	0.44	454	.54
Loaf Volume	0.32	420	.55

[†]Number of unique individuals genotyped and phenotyped.
[‡] Mean 5-fold cross-validation accuracy.

Table 4.2 Genomic selection cross-validation accuracies† for end-use quality traits with different marker sets based on imputation and density

Marker Set‡ (% Missing)	n	Flour Yield	Kernel Hardness	Kernel Diameter	Kernel Weight	Grain Protein	Mixo Mix Time	Mixo Tolerance	Bake Abs	Bake Mix Time	Crumb Grain	Loaf Volume
Mean (20)	5K	0.57 ab§	0.49 cd	0.48 b	0.49 abc	0.47 b	0.60 b	0.47 c	0.30 ab	0.61 cd	0.52 c	0.54 b
Mean (30)	10K	0.57 ab	0.50 bc	0.49 ab	0.49 bc	0.47 b	0.62 a	0.48 abc	0.32 a	0.64 ab	0.55 a	0.56 ab
Mean (50)	21K	0.57 a	0.51 ab	0.50 a	0.50 a	0.47 b	0.62 a	0.48 bc	0.31 ab	0.64 ab	0.54 a	0.56 ab
Mean (70)	35K	0.57 ab	0.51 a	0.50 a	0.50 abc	0.49 a	0.63 a	0.47 bc	0.31 ab	0.62 bc	0.54 a	0.57 a
RF (20)	5K	0.55 b	0.48 d	0.50 ab	0.48 c	0.47 b	0.62 a	0.47 bc	0.31 ab	0.60 d	0.52 bc	0.54 b
RF (30)	10K	0.55 ab	0.49 cd	0.50 a	0.49 abc	0.47 b	0.62 a	0.50 a	0.30 ab	0.64 a	0.55 a	0.54 b
RF (50)	21K	0.55 b	0.50 ab	0.50 a	0.50 ab	0.47 b	0.62 a	0.49 ab	0.29 b	0.64 ab	0.54 ab	0.57 a
Average		0.56	0.50	0.50	0.49	0.47	0.62	0.48	0.30	0.63	0.54	0.55

†Mean 5-fold cross-validation repeated 100 times for each marker set and trait combination.

‡ Marker sets labeled by imputation method used for missing data and threshold of maximum percent missing per marker allowed in parenthesis.

§Within traits, means with the same letter are not significantly different ($\alpha = 0.05$).

Table 4.3 Genomic selection accuracies for end-use quality traits in winter wheat

Trait	Cross-Validation 2006-2012 accuracy[†]	Forward Prediction of 2013 accuracy [‡]	Difference[§]
Flour Yield	0.57	0.34	-0.23
Kernel Hardness	0.50	0.45	-0.05
Kernel Diameter	0.49	0.24	-0.25
Kernel Weight	0.49	0.13	-0.36
Grain Protein	0.47	0.24	-0.23
Mixograph Mix Time	0.62	0.67	0.05
Mixograph Tolerance	0.48	0.66	0.18
Bake Absorption	0.32	0.50	0.18
Bake Mix Time	0.64	0.73	0.09
Crumb Grain	0.55	0.70	0.15
Loaf Volume	0.56	0.77	0.21

[†]Random 5-fold cross validation accuracies from 2006-2012 data set.
[‡] Accuracies from models trained with 2006-2012 data to predict 2013 data.
[§] Difference between accuracy methods calculated by subtracting cross-validation from forward prediction accuracy.
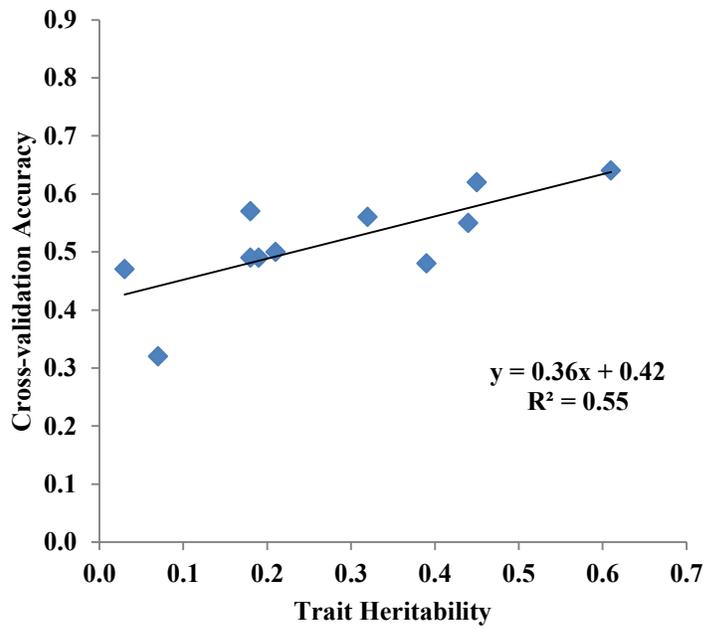
Figure 4.1 Regression of genomic selection cross-validation accuracies on heritability for 11 quality traits in wheat
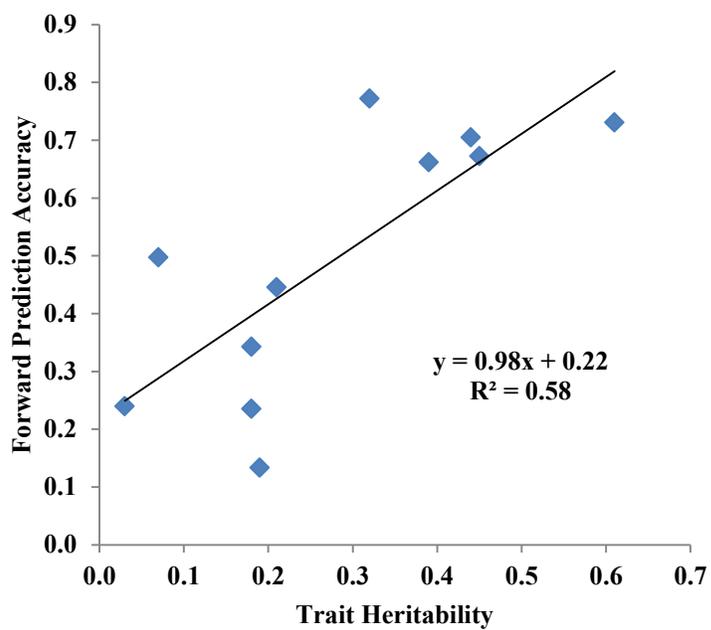
Figure 4.2 Regression of genomic selection forward prediction accuracy on heritability for 11 quality traits in wheat

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