Genetic Variance, Transgressive Segregation, and Genomic Selection Prediction Accuracy for Fusarium Head Blight Resistance in Advanced Multi-parent Barley Breeding Populations

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Dedication

This thesis is dedicated to my brothers and sisters in M.A.N.R.R.S., who give me hope of a brighter future in agriculture, natural resources, and, of course, related sciences.

Abstract

The contemporary era of molecular breeding includes predicting breeding values based on allelic value estimations with genome-wide markers. The overarching objective of this thesis is to assess the potential use of genomic markers in predicting genetic variance, transgressive segregation, and breeding values within barley breeding populations in the context of Fusarium head blight (FHB) resistance.

Chapter One investigates prediction of genetic variance and transgressive segregation using measures of phenotypic and genotypic parental dissimilarity. To a limited extent, phenotypic dissimilarity could predict transgressive segregation and genetic variance while genetic dissimilarity using a subset of FHB-associated single nucleotide polymorphism markers could predict genetic variance in both populations.

Homogeneity of genomic selection prediction accuracy among families for FHB severity and deoxynivalenol concentration was examined in Chapter Two. Accuracy between predicted and observed values for both traits varied among families. Potential factors for limited ability to predict individual family performance are discussed.

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Abbreviations[a]

AFLP	Amplified fragment length polymorphism
BOPA	Barley oligonucleotide pooled assay
CAP	Coordinated agricultural project
FEG	Fusarium early generation
FHB	Fusarium head blight
GCA	General combining ability
MN	University of Minnesota
MN-ND	Germplasm panel of 30 populations stratified by cross-type from crosses between University of Minnesota and North Dakota State University lines
MNxMN	Cross-type; represents crosses of University of Minnesota lines
MNxND	Cross-type; represents crosses between University of Minnesota and North Dakota State University lines
ND	North Dakota State University
NDxND	Cross-type; represents crosses of North Dakota State University lines
QTL	Quantitative trait loci
RAPD	Random amplified polymorphism DNA
RFLP	Restriction fragment length polymorphism
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
STS	Sequence tagged site
UMN	Germplasm panel of 93 populations from the University of Minnesota

a Chapter One

Chapter One

Predicting Genetic Variance and Transgressive Segregation for Fusarium Head Blight Resistance in Advanced Populations of Barley (*Hordeum vulgare* L.)

INTRODUCTION

Prediction of cross combination merit based on measurable parental characteristics could enable breeders to select the most promising pair-wise combinations from a vast array of crossing possibilities. The number of crosses generated in most breeding programs is generally limited by the practicability of evaluating subsequent progeny, which can quickly rise to a resource-prohibitive level. The number of progeny may be calculated for any breeding program using the combination without repetition formula $\binom{n}{r}$, where *n* equals the number of elite lines selected and *r* equals two (Hall 1967), e.g. a breeding program with an annual goal crossing 100 parents would generate nearly 5000 possible combinations. Making all of these crosses and evaluating subsequent progeny is impractical, emphasizing the advantage in predicting cross combination value prior to making them. Schnell and Utz (1975) defined the value, or usefulness, of a cross as the sum of the population mean for all possible homozygous lines of the cross prior to selection and the expected gain when selection intensity of the breeding program is imposed. Per Jinks and Pooni (1976), cross combination value exists in the currency of phenotypically extreme progeny variants, termed transgressive segregants. Transgressive segregant frequency is statistically related to greater progeny additive genetic variance.

Breeders have historically centered their choice of cross combinations on those projected to maximize the trait mean; consideration of progeny genetic variance has been negligible (Bernardo 2002). Primary rationale for the mean-centered breeding effort is two-fold: 1) an unfavorable association between the variance of genetic standard deviation and the variance of means in progeny populations may lead to an increase of progeny mean variance when maximizing genetic standard deviation (Zhong and Jannink 2007); and 2) accurate prediction of the population mean from parental phenotypic performance evaluations is well-established in single (Bhatt 1973) and

multiple environments (Busch et al. 1974; Utz et al. 2001). Predicting genetic variance, and the associated transgressive segregation, remains a challenge.

Rieseberg et al. (1999) defined transgressive segregants as individual F_2 or later hybrid progeny with breeding values more extreme than either parent. In many breeding programs where cultivar development and plant improvement efforts are centered on autogamous crops, selected homozygous transgressive segregant lines form the foundation of parental germplasm. Generation of transgressive segregants in the desired direction of a phenotypic distribution is associated with the degree of progeny genetic variance, where greater genetic variance widens the potential pool of segregants for selection as promising parents. Based on this relationship, the merit of cross potential for line development is related to the frequency of transgressive segregant occurrence and progeny genetic variance. As seen from Schnell and Utz (1975) and Jinks and Pooni (1976), breeding theory suggests the greatest gains from selection will stem from crosses among parents predicted to have both a favorable progeny population mean and high trait variance. Since the only contribution to genetic variance in a cross will be segregation at loci for which parents carry different alleles, it is then expected that genetic dissimilarity of parents could predict genetic variance of the offspring. Multiple studies have sought to identify parental determinants for use as predictors of transgressive segregant frequency and genetic variance in progeny populations including probability, combining ability, and various measures of parental dissimilarity.

The earliest studies began with a statistical approach based on a formula introduced by Jinks and Pooni (1976) designed to calculate the probability of random sampled transgressive segregants of a normal distribution generated by a cross of two homozygous parents: $[d]/\sqrt{D}$, where *d* equals the sum of additive effects across *k* loci segregating multiplied by the *r* coefficient of distributed genes (*dkr*), and D is additive genetic variance. Assumptions of the probability model include absences of GxE interaction, linkage of trait-associated genes, and epistasis. The requirement of progeny data for the statistical model to generate predictions precludes its use in discerning cross potential prior to making the cross, a limitation also associated with the approach based on combining ability.

General combining ability (GCA), which measures parental potential of a genotype through its mean performance in a series of crosses, has been investigated as an indicator of transgressive segregation frequency and direction. Although many diallel studies have been conducted with barley (*Hordeum vulgare* L.), information relating GCA to transgressive segregant frequency was sparse until Surma (1996) investigated the relationship with doubled haploid barley lines representing parents of the diallel crosses. The studies successfully demonstrated an association between transgressive segregant direction and GCA effects, but trends were inconsistent across traits, exemplified by the directional disparity between grain weight by ear and protein content. Beyond the trait-specific nature of this approach, the key limiting factor in using combining ability to infer cross potential is prior evaluation of parental genotypes, a time-consuming and resource-demanding requisite.

Additional studies revisiting the challenge of predicting progeny genetic variance and transgressive segregation assayed various measures of parental dissimilarity as potential estimators. Schnell (1983) and Cox et al. (1985) were among the first to suggest the use of parental genetic dissimilarity in predicting genetic variance in progeny populations. Plant breeders of the classical era heavily relied on phenotype and coancestry as the primary measures of parental dissimilarity, with the latter as the sole source of genetic variance information from which breeders would select cross combinations. In situations where plausible crosses involved lines with equal phenotypic means, breeders would default to crosses among parents with greater genetic dissimilarity as inferred from lower coancestries, i.e. the less-related parents. Predicting progeny performance from parental measures of dissimilarity included multivariate analyses regressing progeny phenotypic performance on parental phenotypic dissimilarity or parental genetic dissimilarity estimates based on coancestry.

Assessments of prediction accuracy using coancestry-based genetic dissimilarity and phenotypic dissimilarity were conducted on several autogamous crops including oat (*Avena sativa* L.) lines (Cowen and Frey 1987; Souza and Sorrells 1989, 1991; Moser and Lee 1994), winter wheat (*Triticum aestivum* L.) lines (Martin et al. 1991; Burkhamer et al. 1998; Bohn et al. 1999), soybean [*Glycine max* (L.) Merrill] lines (Kisha et al. 1997; Manjarrez-Sandoval et al. 1997), and barley lines (Kuczyńska et al. 2007). The majority

of these studies focused primarily on F_1 progeny, doubled haploids, and other early generation hybrids. By contrast, Burkhamer et al. (1998) and Bohn et al. (1999) examined the predictive ability of coancestry in more advanced generations of winter wheat lines, an experimental design better representative of germplasm in small grain breeding programs. Results of the advanced generation winter wheat studies corroborated those using doubled haploid and early hybrid lines. Coancestry continually emerged as the best predictor of progeny genetic variance and transgressive segregant frequency, but performance was trait-specific and associations were weak.

With the shift from classical to molecular breeding, molecular markers were proffered as an alternate means of estimating parental genetic dissimilarity with more accuracy than coancestry. Van Berloo and Stam (1998) advocated the use of marker information to select parents with different alleles at each quantitative trait locus (QTL). Expectations included an increase of phenotypically extreme progeny lines attributable to increased segregation at more loci. Subsequent studies using small grains compared the prediction accuracy of marker-based genetic dissimilarity estimates with those using coancestry. Marker systems of choice for these studies included sequence tagged site (STS; Martin et al. 1995; Burkhamer et al. 1998), amplified fragment length polymorphism (AFLP; Burkhamer et al. 1998; Bohn et al. 1999), restriction fragment length polymorphism (RFLP; Moser and Lee 1994; Barbosa-Neto et al. 1996; Kisha et al. 1997; Bohn et al. 1999; Corbellini et al. 2002; Parker et al. 2002), simple sequence repeat (SSR; Bohn et al. 1999; Parker et al. 2002; Xu et al. 2002), and random amplified polymorphism DNA (RAPD; Marić et al. 2004; Kuczyńska et al. 2007). In general, marker-based genetic dissimilarity of parental lines did not strongly predict progeny genetic variance or transgressive segregant frequency. In cases where weak associations were found, marker-based genetic dissimilarity did not predict genetic variance better than coancestry.

The more commonly cited explanations for the poor prediction accuracy of marker-based genetic dissimilarity included factors related to the marker system and genetic architecture of the trait of interest. Acquisition of the best genetic dissimilarity estimates requires markers to be highly polymorphic, abundant, and well-distributed throughout the genome. Failure to meet these criteria may introduce sampling error related to

insufficient genome coverage, increasing standard error of estimates. RFLPs are limited by the lack of polymorphisms found in autogamous crops, resulting in limited genome coverage (Bohn et al. 1999). Although markers such as AFLPs and SSRs are considered better able to capture more polymorphisms than RFLPs (Russell et al. 1997), AFLP loci have been shown to cluster in low recombination regions (Hayes et al. 1997). This may explain the low correlations observed by Burkhamer et al. (1998) using AFLP and STS markers. Beyond factors associated with marker systems, trait genetic architecture and phenotypic diversity appear to significantly influence predictive ability. Using doubled haploid barley lines, Kuczyńska et al. (2007) demonstrated plasticity of the relationship between parental genetic dissimilarity and both progeny genetic variance and transgressive segregation. The most significant factor related to transgressive segregant frequency was parental gene distribution, which can vary among parents and differ for various traits.

Grain yield has been a primary trait of interest in the majority of genetic variance and transgressive segregation prediction studies. While yield remains an important trait for improvement in barley, a prominent trait for barley varieties destined for Midwest cultivation is resistance to Fusarium head blight (FHB). FHB, or “scab,” is a grain disease primarily associated in North America with isolates of *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch]. Significant economic repercussions concomitant with the disease have revitalized breeding efforts centered on identification and introgression of FHB resistance genes in barley. Bi-parental mapping studies have identified several putative QTL, although QTL validation has been limited (de la Peña et al. 1999; Zhu et al. 1999; Ma et al. 2000; Dahleen et al. 2003; Mesfin et al. 2003; Canci et al. 2004; Hori et al. 2005, 2006; Horsley et al. 2006; Sato et al. 2008; Lamb et al. 2009). Contemporary association mapping studies define FHB resistance as a quantitative trait controlled by several genes with small effect, and multiple QTL associated with reduced FHB severity have been identified using single nucleotide polymorphism (SNP) markers (Massman et al. 2011; Vikram et al. In preparation).

SNPs represent the smallest detectable unit of genetic variation and are the most abundant type of polymorphism in the genome (Rafalski 2002). The ease and low cost of

genotyping with SNP assays as compared with other marker systems and phenotyping are the primary reasons many breeding programs are transitioning to SNPs as the marker system of choice. Further, these bi-allelic markers have been shown to follow the infinite sites model (Kimura 1969) in many organisms, including barley (Morrell et al. 2006). Since the infinite sites model indicates each mutation occurs at a new location in the genome, each SNP generally occurs only once for the genomic segment where it has been identified. For this reason, SNPs have a high probability of being both identical by state and identical by descent, which overcomes bias potentially associated with other marker systems. Microsatellites, among many other marker systems used in related past studies, are identical by state markers with a high probability of homoplasy, where similar marker states appear the same but do not share common ancestry. Estimates of genetic dissimilarity based on markers with a low probability of being identical by descent may be an underlying cause of the poor association of genetic dissimilarity relative to coancestry-based estimates.

Incorporating SNPs, where identity by state infers identity by descent, may strengthen correlations between parental genetic dissimilarity and genetic variance and transgressive segregation. Identification of SNPs presumably linked with FHB resistance QTL suggests another method of strengthening correlations. Hayes et al. (1997) posits that improvement of prediction accuracy using genetic dissimilarity necessitates integration of markers linked to QTL of desired traits. Genetic dissimilarity estimates based on a subset of FHB-specific SNPs may strengthen the correlation between parental genetic dissimilarity with genetic variance in progeny populations. To augment the correlation of parental genetic dissimilarity with transgressive segregant frequency, the genetic architecture of FHB resistance is a significant factor to consider as suggested by Kuczyńska et al. (2007).

Genetic architecture of the FHB resistance trait in barley differs from previously studied traits related to grain yield in terms of QTL number, genomic distribution, and frequency of QTL segregation among Midwest barley breeding programs (Massman et al. 2011; Vikram et al. In preparation). Further, introgression history of FHB QTL is distinct from that of yield since most breeding programs do not generally make wide crosses to introgress grain yield gene sources. In the case of FHB resistance, barley breeding

history at the University of Minnesota includes systematic identification of resistant sources and introgression into the breeding program. Results include lines with relatively similar disease levels and diverse genetic backgrounds since FHB resistance genes trace to multiple sources (Huang et al. 2012). Crossing lines with different genetic backgrounds would presumably lead to increased genetic variance and increased probability of transgressive segregation in progeny populations.

Empirical evidence suggests there is little to no association of parental dissimilarity for progeny genetic variance, and correlation with transgressive segregation is too weak to be used as a reliable predictor for selecting cross combinations with maximized potential. However, the distinct introgression history of FHB resistance genes, the genetic architecture of the FHB resistance trait, and availability of trait-specific markers with a genome-wide marker system where identity by descent may be inferred create a unique framework within which to revisit the classical breeding goal in a contemporary context: to predict progeny genetic variance and transgressive segregation from measures of parental dissimilarity. Two panels of barley breeding lines were utilized for this study, including a larger set which comprised many populations from a single breeding program and a smaller set of populations with greater genetic diversity representative of two Midwest breeding programs. Four predictors based on various measures of parental dissimilarity were generated for each cross combination. Two responses in terms of genetic variance and transgressive segregant frequency were estimated from evaluated progeny populations. The strength of associations was investigated with regression and correlation analyses.

MATERIALS AND METHODS

Germplasm

Two data sets were used to investigate the relationship between parental dissimilarity and genetic variance of six-rowed, spring barley progeny populations for FHB resistance: 1) UMN, an historical set of 93 populations derived from crosses among 112 parent lines from the University of Minnesota for which 20-101 lines (average = 66 lines per population) from each cross were evaluated in replicated FHB disease trials; and 2) MN-ND, a contemporary set of 30 populations derived from crosses between 15 parent lines in the barley breeding programs of both the University of Minnesota (MN) and

North Dakota State University (ND) divided into three subsets (n = 10) based on cross-type (MNxMN, MNxND, NDxND). Parental lines of the UMN panel were selected for either suitable malting qualities (M-lines) and/or demonstration of early generation FHB resistance (FEG-lines). MN-ND parents were chosen for FHB resistance, among other agronomic characteristics, and stratification of diversity among breeding programs. Investigation of the relationship between parental dissimilarity and transgressive segregant frequency was conducted with the UMN panel due to its relatively large population sample sizes.

Experimental Design

Progeny lines were advanced to the $F_{4:5}$ generation for phenotypic evaluation. No prior selection was imposed on progeny lines. From 2003 to 2010, UMN populations were evaluated for FHB severity in disease nurseries located at two of three Minnesota locations each year: Crookston, St. Paul, and Morris. Concatenation of location and year formed a 19-level trial factor for regression analysis. Parent lines were evaluated in all trials with progeny lines. Each UMN line was replicated twice per location in randomized complete block design with three checks: MNBrite or Quest, Robust, and Stander. MN-ND populations were evaluated in 2011 and 2012 at two disease nurseries in Minnesota (Crookston and St. Paul) and two disease nurseries in North Dakota (Langdon and Osnabrock) for a total of eight trials. Parent lines were evaluated in 2010 and 2011 at the Minnesota disease nurseries. All MN-ND lines were replicated twice per location in randomized incomplete block design using six incomplete blocks with three checks: ND20493, Quest, and Tradition. All entries were represented in each replicate and all checks represented in each block. Minnesota plots were single 1.5-m rows with 0.3-m row-spacing. North Dakota plots were single hill plots spaced 0.3-m apart.

Fusarium Head Blight Evaluation

St. Paul disease nursery plots were inoculated with conidial spray consisting of approximately 30 *Fusarium graminearum* isolates at a concentration of 100,000 spores per liter of water (Steffenson 2003). To promote spore adherence to leaf surfaces, 20.0-mL of Tween-20 surfactant was added to the macroconidial solution immediately prior to inoculation. Application of the inoculum was conducted using backpack sprayers with ss80015 TeeJet sprayer tips dispensing the solution at a rate of 10.0-mL per second

with a pressure of 40-psi. Each plot was evenly sprayed for six seconds. Inoculum was applied twice per trial, once at heading and again two to three days after the first inoculation. Overhead mist irrigation began at plant heading with DAN 8000 spray sprinklers (Netafim, Fresno, CA) spaced 4.0-m apart and mounted 120-cm above the ground surface. Plants were misted daily at 10-minute timed intervals every hour from 18:00 to 6:00 until phenotypic evaluation. Disease severity was scored approximately 14 days after heading.

Inoculation of plants at Crookston and Morris locations followed the grain spawn method (Prom et al. 1996). At the Crookston disease nursery in 2011, two applications of *F. graminearum*-inoculated corn kernels were dispersed between rows at the five-leaf growth stage (jointing) and again at the flag leaf (boot) stage. In 2012, application of inoculum was conducted once at jointing due to inoculum shortage. For all corn inoculum applications, kernels were spread at a rate of six grams per square meter. Mist irrigation began with the first inoculation and followed the St. Paul location protocol. FHB severity was scored approximately 18 days after inoculum was spread.

Langdon and Osnabrock disease nursery plots were inoculated using the grain spawn method. Application of corn inoculum occurred two weeks prior to anthesis. Rate of inoculum dispersal was 50 grams per square meter. Plots were overhead mist irrigated using XS-360 F xeri-spray sprinklers (Rain Bird, Glendora, CA) spaced 4.0-m apart and mounted 120-cm above the ground surface. Plants were misted at a rate of 1.2-L per hour for 30 seconds every 30 minutes daily between the hours of 04:00 to 08:00 and 18:00 to 20:00. Disease was scored approximately 14 to 18 days from first inoculation.

Phenotypic Evaluation

For all disease nurseries, each line was scored for FHB severity by arbitrary selection of ten spikes from main tillers per row. At the St. Paul disease nurseries, where disease pressure was low, FHB severity was scored by counting infected kernels of each spike and later converted to percentage. At disease nurseries with higher disease pressure, as observed at the Crookston, Morris, and North Dakota disease nurseries, FHB severity was scored by visual estimation of infection percentage per spike using the 0, 1, 3, 5, 10, 15, 25, 35, 50, 75, 100% rating scale.

Genotypic Evaluation

UMN and MN-ND parent lines were sent to the Fargo USDA genotyping facilities and genotyped with the Illumina GoldenGate barley oligonucleotide pooled assays (BOPA; Close et al. 2009) and 9K array. Although BOPA SNP assays included 1536 BOPA I SNPs and 3072 BOPA I and II SNPs, the genotypic data used for calculating genetic dissimilarity for any pair-wise combination on average was 1387 BOPA I SNPs for the UMN parent lines and 2794 BOPA I and II SNPs for the MN-ND parents. Genotypic data for all lines analyzed are available through the Triticeae Toolbox (http://triticeaetoolbox.org/).

FHB-associated SNPs were identified by Vikram et al. (In preparation) through a genome-wide association mapping study involving all MN and ND barley lines genotyped with BOPA I and II SNPs and evaluated for FHB resistance. The phenotypic and genotypic data used were derived from the Barley Coordinated Agricultural Project (Barley CAP; http://www.barleycap.org). SNPs identified using a kinship alone model with a negative log (p) value between 2.01 to 5.38 were considered to have a significant association with FHB resistance and were included in the subset of FHB SNP markers used to calculate parental genetic dissimilarity. Twenty-three unique BOPA I FHB SNPs were used for the UMN panel and 56 unique BOPA I and II FHB SNPs were used for the MN-ND panel.

Data Analysis

Four measures of parental dissimilarity were estimated for each parent combination based on phenotypic and genotypic data. Phenotypic dissimilarity was calculated from FHB severity scores. Genetic dissimilarity was estimated based on polymorphism proportion using all SNPs, FHB SNPs, and an inverse relationship of the kinship coefficient. Pearson's product moment correlations between each measure of parental dissimilarity and genetic variance or transgressive segregant frequency of progeny populations were calculated in SAS v. 9.2 (SAS Institute 2008) with PROC GLM. Correlations were tested for significance at alpha = 0.05 using PROC CORR for each population in the UMN and MN-ND data sets.

Of the eight MN-ND FHB trials, five were removed from analyses due to lodging and use of disparate disease rating methodology, resulting in three trials for use in data analysis. Confounding effects attributable to lodging resulted in non-detectable measures of phenotypic variation in three trials (CR2011, LA2012, OS2012). The 1-5 scale of FHB scoring for two of the trials (LA2011, OS2011) could not be standardized to the method used for additional trials outlined in Phenotypic Evaluation. Data of the remaining three trials (CR2012, SP2011, SP2012) demonstrated homogeneity of error variance based on Levene's HOV test implemented in PROC GLM and were subsequently combined and adjusted to LS-Means calculated with PROC MIXED.

Parental Phenotypic Dissimilarity

UMN parents were evaluated for FHB severity in the same trials as progeny populations. Parents of the MN-ND panel were evaluated in four trials: CR2010, CR2011, SP2010, and SP2011. Phenotypic data were standardized prior to analysis using PROC MIXED to adjust for effects relative to each panel. Of the UMN panel, replication and trial effects were resolved while the MN-ND panel required resolution of replication, block, and trial effects. Replication, block, and trial were used as fixed effects. Genotype was the random effect in the mixed model equation. Measures of phenotypic dissimilarity were calculated from FHB severity differences between parents for each cross combination based on adjusted LS-Means.

Parental Genotypic Dissimilarity

Estimates of kinship-based genetic dissimilarity were calculated as $(2 - \Theta)$, where Θ equals the coefficient of kinship for each pair-wise parental line combination generated in kinship matrices through TASSEL v. 2.1 (Bradbury et al. 2007). SNP data used were downloaded in TASSEL V3 format from the Triticeae Toolbox. Estimates of genetic dissimilarity with all SNPs and FHB SNPs were calculated as genetic distance between parents of each population using the formula given by Nei and Li (1979): $GD = 1 - 2N_{ij} / (N_i + N_j)$ where N_{ij} is the number of shared SNPs between parent i and parent j, N_i is the number of parent i alleles, and N_j is the number of alleles of parent j. SNPs missing from either or both parents were removed from analysis.

Progeny Genetic Variance Estimates and Transgressive Segregant Frequency

Additive genetic variance of each population was estimated using PROC MIXED with genotype as the random effect. Significant differences between cross-types in the MN-ND panel were identified using Fisher's Least Significant Difference (LSD) test. Each UMN parent was designated as a high or low FHB-scoring parent per cross based on adjusted LS-Means. Favorable transgressive segregant individuals were defined as lines in a population with a lower FHB severity score than the lowest scoring parent. Favorable transgressive segregant frequency was calculated as a proportion by dividing the number of low transgressive segregants by the total number of lines for each population. Total transgressive segregant frequency was similarly calculated using the sum of favorable and high transgressive segregants divided by the total number of lines per population.

RESULTS

Parental Phenotypic Dissimilarity

A wide range of phenotypic differences among parents was observed for both germplasm panels (Table 1). As expected, FEG parent lines of the UMN panel consistently contributed lower FHB scores than those of the M-lines (data not shown). The range of phenotypic dissimilarity values in the MN-ND panel was slightly greater than that of the UMN panel (Table 1). Dissection of the distribution by cross-type demonstrated a wider range of phenotypic dissimilarity within the MNxND cross-type, although phenotypic dissimilarity means were not significantly different (Figure 1a). Mean FHB severity scores of the MN-ND progeny populations varied significantly by cross-type (Figure 1b).

Parental Genotypic Dissimilarity

Of the three parental genetic dissimilarity measurements in the UMN panel, genetic dissimilarity based on all SNPs had the smallest range of values. FHB SNP genetic dissimilarity had a slightly greater range than the kinship-based genetic dissimilarity (Table 1). Twenty-six of the 93 UMN populations had an FHB genetic dissimilarity value of zero. Estimates of kinship genetic dissimilarity for the UMN cross combinations ranged from 2.00 (least related) to 0.13 (most related). Correlation between the TASSEL-generated kinship genetic dissimilarity and Nei-based genetic dissimilarity

using all SNPs was significant (r = 0.91). A significant association between measures of phenotypic dissimilarity and genetic dissimilarity in both panels was absent (data not shown).

The greatest range of genetic dissimilarity measurements in the MN-ND panel was associated with kinship, and the smallest with all SNPs (Table 1). MN-ND kinship genetic dissimilarity estimates were significantly correlated with those based on all SNPs (r = 0.99). Significant differences by cross-type were observed for kinship and all SNPs based on LSD mean comparisons (Figure 2). Two groups were identified for FHB genetic dissimilarity, one comprising MNxMN and NDxND populations, and a separate group of MNxND populations. In general, populations of the MNxMN cross-type were most related, MNxND populations were least related, and NDxND populations were observed at both extremes of the relatedness distribution.

Correlations between Parental Dissimilarity, Progeny Genetic Variance Estimates, and Transgressive Segregant Frequency

Estimates of progeny genetic variance in the UMN populations were significantly different among populations (Levene HOV test: $p < 0.001$) and ranged from zero to 31.50 (Figure 3). A significant positive association (r = 0.38) was identified between the phenotypic dissimilarity predictor and progeny genetic variance in the UMN panel (Table 2; Figure 3). FHB genetic dissimilarity was the only genetic-based predictor to demonstrate significant correlation (r = 0.32) with progeny genetic variance in the UMN populations (Table 2; Figure 4). Significant correlations were absent when using any of the genetic dissimilarity measures to predict favorable or total transgressive segregant frequency. A significant negative association was observed for both favorable transgressive segregant frequency (r = - 0.55) and total transgressive segregant frequency (r = - 0.83) with the UMN phenotypic dissimilarity predictor (Table 2; Figure 5).

In the MN-ND panel, progeny genetic variance estimates were significantly different among cross-types (Levene HOV test: p = 0.04). An LSD mean separation test on genetic variance by cross-type resulted in two groups, one comprising NDxND and MNxMN populations and the other composed of MNxMN and MNxND populations (data not shown). These identified groups were the same as those identified in the LSD tests

on parental genetic dissimilarity based on FHB SNPs (Figure 2 and 6). In terms of predictors, FHB genetic dissimilarity was the sole measure of parental dissimilarity to demonstrate a significant correlation ($r = 0.53$) with progeny genetic variance (Table 2; Figure 6). The majority of significance for the association between FHB SNP genetic dissimilarity and genetic variance could be traced to the MNxND populations when dissected by cross-type with PROC CORR.

DISCUSSION

The use of genetic dissimilarity between parents as an indicator of genetic variance in progeny populations was first put forth by Schnell (1983) and Cox et al. (1985). Their rationale centered on the contribution of heterozygous QTL to genetic variance, with the proportion of such QTL ideally estimated from the degree of genetic dissimilarity. Historically, genetic dissimilarity has been measured either through the pedigree-based coefficient of coancestry or molecular marker polymorphisms. The coefficient of coancestry relies on the assumptions that: 1) ancestors are unrelated; 2) each parent equally contributes alleles to the offspring; and 3) selection and genetic drift are both absent. These assumptions are not upheld in the breeding methodology of many autogamous crops. In particular, pedigree-based breeding methods, as is commonly used in barley, can impose relatively high selection intensity, which introduces bias into genetic dissimilarity estimates, accumulating throughout generations. Genetic dissimilarity estimated with molecular markers is expected to overcome such bias.

Assuming SNPs follow Kimura's (1969) infinite sites model in barley, which appears to be true (Morrell et al. 2006; Toleno et al. 2007), then mutations occur only once at a specific site in the genome for the population's history. A SNP identical in state is then far more likely to be identical by descent than other types of molecular markers. For this reason, it was reasonable to expect genetic dissimilarity estimates based on SNPs would emerge as the best predictor of progeny genetic variance and transgressive segregation. Trait-specific genetic dissimilarity estimates were expected to demonstrate even stronger associations. Although FHB genetic dissimilarity was a significant predictor in the UMN set and for the MNxND cross-type, phenotypic dissimilarity emerged as the most effective predictor in the UMN panel. This would indicate the best method to select cross combinations is to first identify those with the best projected

phenotypic mean and then choose from that subset those with the greatest phenotypic dissimilarity. This method requires knowledge of parental line phenotypes. In terms of contemporary breeding, genotyping continues to advance as the more cost-efficient and reliable option. Continuity of the breeding community along the path of genomic-based selection, where emphasis is on genotypic data, outlines a future where phenotypic data may not be available until years after selection is made. Application of trait-associated markers in estimating parental dissimilarity will be increasingly useful for predicting genetic variance.

Genetic Variance and Transgressive Segregation in UMN Germplasm

It was observed that an increase of phenotypic dissimilarity between parent lines was positively associated with an increase in genetic variance in the UMN panel. Such an association was expected as a reflection of allelic segregation at QTL related to the trait. Since genetic dissimilarity based on FHB SNPs would be a more direct measure of this genetic phenomenon in context of the trait studied, an increase of FHB genetic dissimilarity in parent lines was expected to significantly correlate with an increase in genetic variance, which was also observed. In terms of transgressive segregants, none of the genetic dissimilarity predictors were significant, indicating transgressive segregants may not be predicted from measures of parental genetic dissimilarity. Phenotypic dissimilarity was the sole predictor significantly associated with transgressive segregation. For both favorable and total transgressive segregants, an increase in phenotypic dissimilarity was associated with a decrease in segregants observed. Mathematical expectation dictates that an increase in phenotypic dissimilarity between parent lines will require individual progeny to be at the most extreme positions of the distribution tails to be defined as transgressive segregants. The observed negative association then matches expectation.

Genetic Variance in MN-ND Germplasm

Although progeny population means for FHB severity were significantly different by cross-type, means of phenotypic dissimilarity for parent lines were not significantly different (Figure 1). The lack of phenotypic relationship between parents and progeny is underscored by the limited ability of the mid-parent means to predict progeny population means ($r = 0.57$; Figure 7) as compared with the UMN panel ($r = 0.93$; Figure 8). Genetic

dissimilarity based on FHB SNPs was the only predictor significantly associated with genetic variance in the MN-ND panel. Dissection of the correlation by cross-type demonstrated the majority of the significance was contributed by the MNxND cross-type. Greater measures of genetic dissimilarity between parents was expected for this cross-type based on previous research which identified differences in FHB QTL segregation frequency between the MN and ND barley breeding programs (Massman et al. 2011). This presumably translated into the significant association with progeny genetic variance in this cross-type.

Poor Prediction Performance of Phenotypic Dissimilarity in MN-ND Germplasm

Unexpected observations included the absence of significant associations between phenotypic dissimilarity and genetic variance for the MN-ND panel. Two potential explanations that may address this observation are related to sample size bias and the absence of phenotypic dissimilarity. It is possible phenotypic dissimilarity does not exist to a sufficient degree to be a significant predictor in this panel. It is also possible the phenotypic dissimilarity is present, but significance is occluded by the small sample size of the populations. The MN-ND panel was not expected to have a great degree of phenotypic dissimilarity as parent lines were chosen for demonstration of partial resistance to FHB. However, the MN-ND panel demonstrated a greater range of values for phenotypic dissimilarity between parents, surpassing the UMN panel where such dissimilarity was expected (Table 1).

Presence of such dissimilarity may be attributable to the nature of the partial FHB resistance, where many lines are considered resistant but can vary in the degree of disease severity. Based on the presence of the dissimilarity, it would then be possible that correlation coefficients may be significant between phenotypic dissimilarity and genetic variance for the MN-ND panel. Pearson's correlation coefficients must be at least 0.423 for the two-tailed t-test associated with the population size of the entire panel to be considered significant. However, the observed correlation coefficient is far below this value ($r = -0.18$). Additionally, the LSD mean separation test demonstrated that means of phenotypic dissimilarity by cross-type were not significantly different. For these reasons, it is likely that the poor predictive ability is due to absence of significant phenotypic dissimilarity between parents.

Poor Prediction Performance of Genetic Dissimilarity Using All SNPs and Kinship in UMN and MN-ND Germplasm

Genetic dissimilarity estimates based on a genome-wide set of markers, such as with the BOPA SNPs and kinship, were expected to be more predictive than phenotypic dissimilarity and to have greater associations than those of past studies using other marker systems. All SNP and kinship measures of genetic dissimilarity were not significant in either panel, corroborating the general findings of past studies. The primary limitation often implicated for weak associations has been related to the marker system, e.g. the clustering of markers in regions of low recombination and the low probability of identity by state markers being identical by descent. The SNP markers used in this study are abundant, well-distributed throughout the genome, and follow the infinite sites model. These factors contribute to overcoming bias related to genome coverage and should yield more accurate genetic distance estimates since identity by state infers identity by descent. Previously cited shortcomings of marker systems are then likely not a factor influencing this study. Bias inherent to the SNP marker system is the most probable factor responsible for the poor predictive ability of the kinship and all SNP predictors.

Ascertainment bias associated with use of the SNP marker system can include multiple factors, two of which are related to the choice of lines and site frequency spectra of the discovery panel. SNP assay design involves the process of generating a discovery panel based on EST databases where bioinformatic efforts result in putative gene alignment. These data derive from a number of lines sequenced for multiple purposes, thus the discovery panel is optimized to differentiate between lines of that particular panel. Application of those SNPs to assay germplasm groups other than those on the discovery panel generates bias inherent to that assay affecting estimates of genetic diversity, known as ascertainment bias. In context of the BOPA I and II SNP assays, the majority of genotypes used in the discovery panel include parental lines commonly used for mapping, many elite lines, some landraces, and one wild barley line (Waugh et al. 2009). Morex, an elite malting line common to all pedigrees of the UMN panel, is included on the discovery panel. As such, line-related ascertainment bias is likely not a confounding factor responsible for the poor predictive performance of the SNP and kinship determinants. In this case, the most influencing factor is ascertainment bias related to site frequency spectra, which is the number of times a SNP is observed in a sample. The

site frequency spectrum for the BOPA SNPs is very different from the site frequency spectrum for the cause of FHB resistance. The frequency spectrum of the BOPA SNP assays is pushed towards more common alleles based on the assay's designed application in generating data for estimating breeding values for multiple traits simultaneously. With the BOPA SNPs selected to be common consensus, there is a heavy skewness towards common SNPs. However, the causative variance for FHB resistance is, by definition, rare variance.

The amount of variation for the trait which can be explained by any one SNP is very small, and the marker allele frequency for FHB SNPs is extremely small (Vikram et al. In preparation). For this reason, it is possible to observe high levels of polymorphisms between pairs of parents without an increase in progeny genetic variance or transgressive segregation frequency for FHB resistance. There is little correlation between the frequencies of these two types of SNPs, resulting in poor prediction ability using all the BOPA SNPs or kinship, which is also based on all SNPs. For SNPs to predict a relatively rare trait, the SNP panel must include many rare SNPs. This is the primary reason genetic dissimilarity calculated from the subset of FHB-associated SNPs had better predictive capacity.

A Trait Difficult to Phenotype

The measures of genetic dissimilarity may be stronger predictors than revealed in this study. The majority of genetic variance components of populations in both panels were much smaller than the error variance components in ANOVA analyses. Potential confounding of estimates with sources of error is not unexpected with an environmentally-sensitive trait difficult to consistently phenotype. Screening for FHB resistance is notoriously challenging and fraught with innumerable elements with capacity for error introduction. The most common sources of error stem from divergent inoculation methodology between locations. The grain spawn method of Crookston, Morris, and North Dakota encourages higher disease pressure attributable to uncontrolled inoculum timing and concentration. Other uncontrollable components include location-specific differences in soil type, temperature, and precipitation, which may amplify effects of GxE interactions. Inconsistent phenotypic data will lower the strength of correlations between parental genetic dissimilarity and progeny genetic

variance and transgressive segregation. This may be a reason phenotypic dissimilarity emerged as the stronger predictor in the UMN panel. Increasing repeatability by testing more lines representative of each population, or cross-type, in more trials could reduce standard error and subsequently increase the correlation between parental genetic dissimilarity, progeny genetic variance, and transgressive segregation.

Conclusion

The challenge of evaluating complex traits such as FHB resistance has remained throughout breeding history, although breeding methodology has transitioned from classical to molecular. As new marker systems are developed and breeding contexts change, the foundational question of whether we can predict genetic variance and transgressive segregation endures. This study defined cross potential in terms of both genetic variance and transgressive segregant frequency. In terms of barley breeding in the Midwest, transgressive segregation related to FHB resistance is a significant trait. Parental dissimilarity based on phenotype could predict favorable and total transgressive segregant frequency to a certain extent. Results from using measures of genetic dissimilarity were consistent with past studies showing weak, if any, associations with transgressive segregation and genetic variance. Prior knowledge of FHB QTL genetic architecture, although limited, contributed to the capture of more trait variation, as indicated by the significant association between the FHB-related SNP predictor and genetic variance in both germplasm panels.

Although parental dissimilarity based on phenotype was the more powerful predictor, the ability of the FHB SNPs to predict genetic variance is more relevant to the contemporary era of breeding. Depending on the breeding program, phenotypic data for many lines may not be available for several years since the primary basis for selection are breeding values predicted from genotypic data. Breeding value prediction models which incorporate genotypic information could be modified to generate additional predictions related to genetic variance without additional cost to the breeder. The ability of a model to identify the best parental pairs from thousands of cross combinations would contribute towards improvement of the trait. Even if the improvement is small, as in this study, small improvement is still improvement in the nature of quantitative traits.

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Table 1. Measures of pair-wise dissimilarity based on parental combinations representing all populations per germplasm panel including the smallest measure of dissimilarity (min value) and the largest (max value)

	UMN Panel		MN-ND Panel[a]	
Parental Dissimilarity	**Min Value**	**Max Value**	**Min Value**	**Max Value**
Phenotypic	0.04	24.32	0.23	27.89
All SNPs[b]	0.03	0.50	0.05	0.19
FHB SNPs[b]	0.00	0.92	0.11	0.50
Kinship[c]	0.13	2.00	0.49	1.84

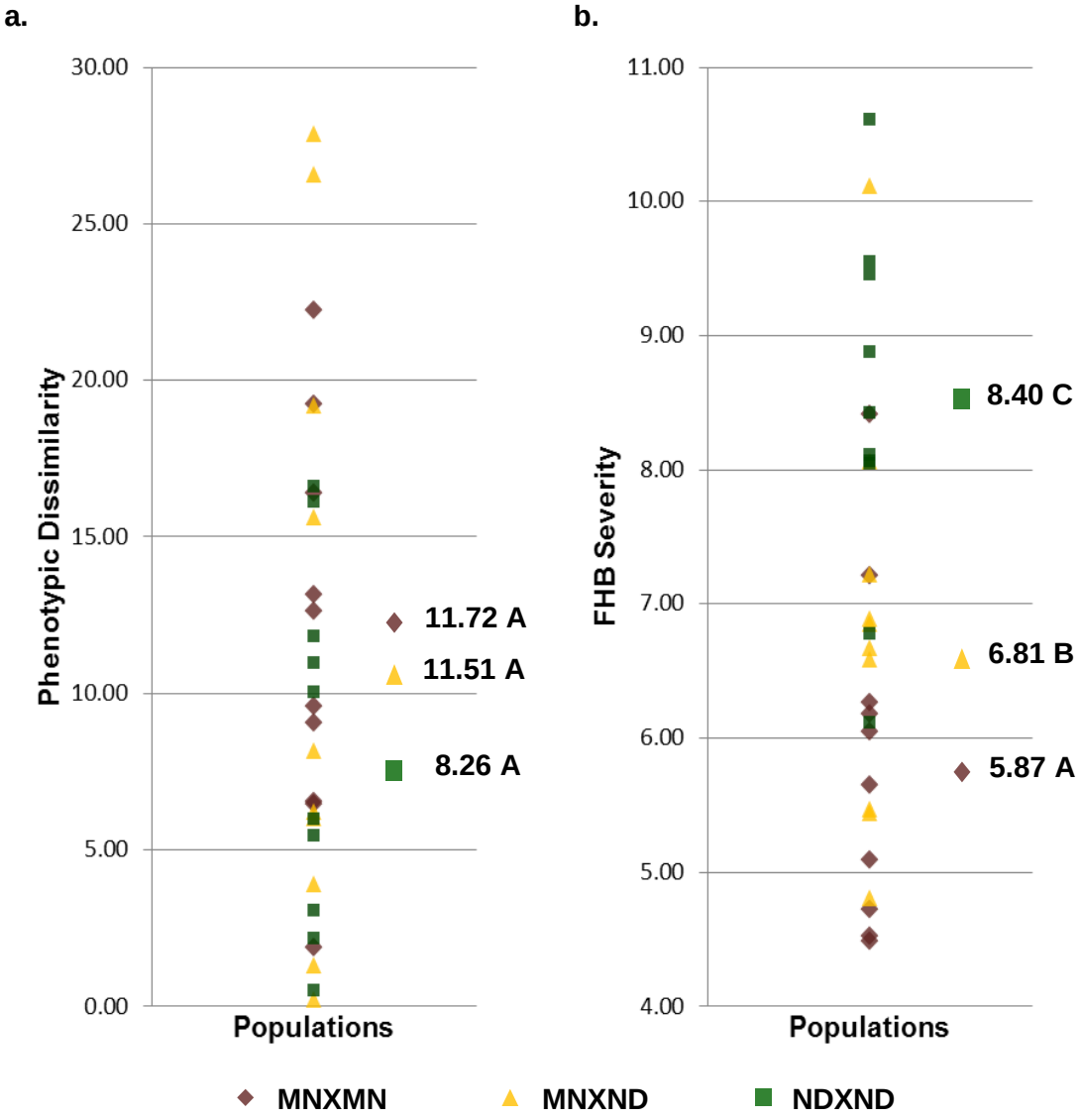
a Includes populations from all three breeding program cross-types
b Possible range for genetic dissimilarity based on all SNPs and FHB SNPs is zero (least dissimilar) to one (most dissimilar)
c Possible range for genetic dissimilarity based on kinship is zero (most related) to two (least related)

Table 2. Significant correlation coefficients for parental dissimilarity and progeny genetic variance in UMN and MN-ND populations and for parental dissimilarity and transgressive segregant frequency in UMN populations

	UMN Panel			MN-ND Panel
Parental Dissimilarity	**Genetic Variance[a]**	**Low Transgressive Segregant Frequency**	**Total Transgressive Segregant Frequency**	**Genetic Variance[b]**
Phenotypic	0.38	-0.55	-0.83	NS
All SNPs	NS	NS	NS	NS
FHB SNPs	0.32	NS	NS	0.53
Kinship	NS	NS	NS	NS

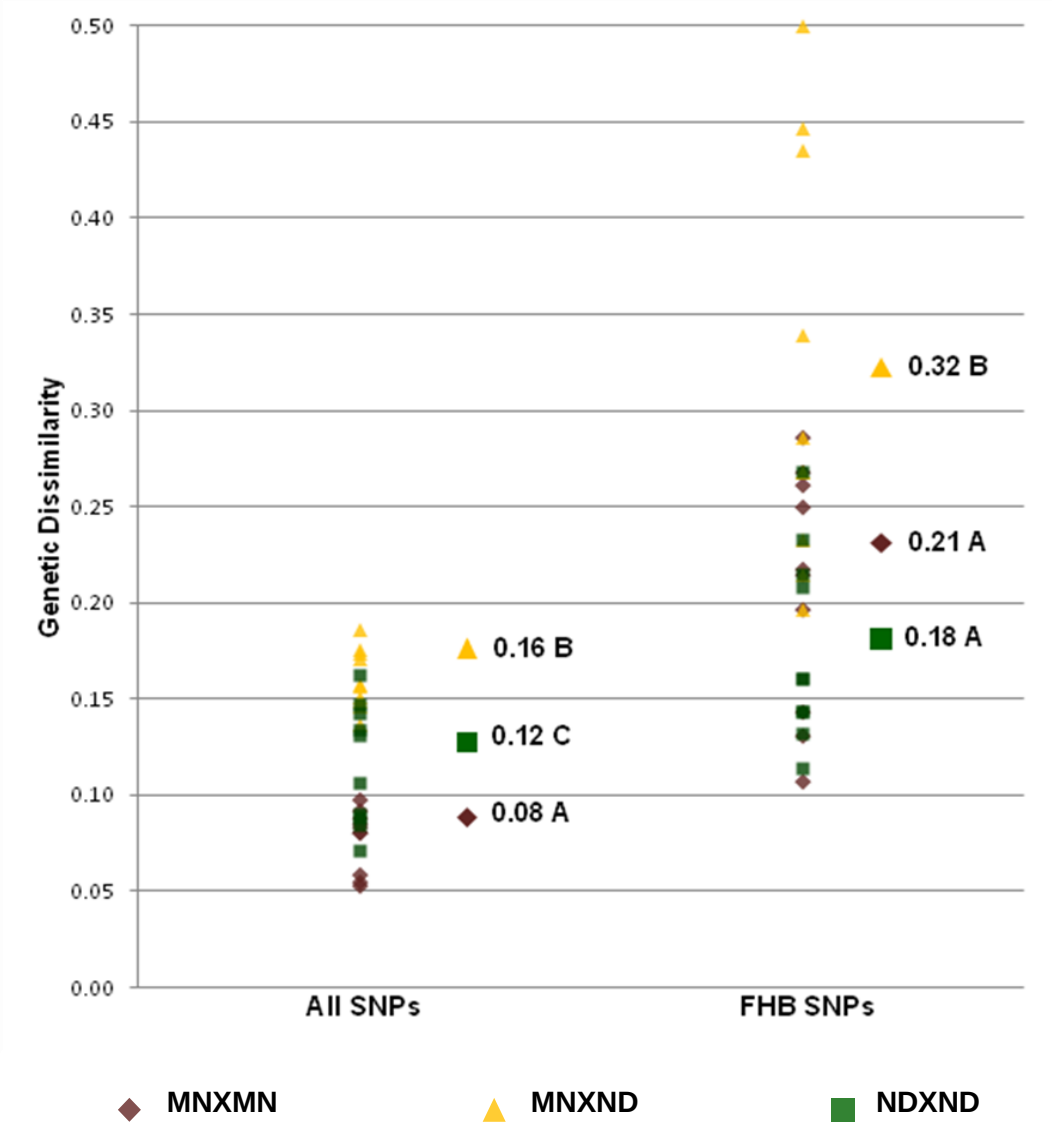
a Correlation coefficients with significance $p \leq 0.05$ listed; NS = non-significant values indicated
b Includes populations from all three breeding program cross-types

Figure 1. Phenotypic dissimilarity means by cross-type of each population (a.) and progeny population Fusarium head blight (FHB) severity means by cross-type (b.) for the MN-ND panel



Values followed by the same letter per figure are not significantly different
Based on Fisher's least significant difference (LSD) mean separation tests

Figure 2. Means of genetic dissimilarity based on all single nucleotide polymorphism (SNP) markers and Fusarium head blight (FHB)-associated markers in the MN-ND panel by cross-type



Values followed by the same letter per figure are not significantly different
Based on Fisher's least significant difference (LSD) mean separation tests

Figure 3. Genetic variance of UMN progeny populations plotted against phenotypic dissimilarity based on Fusarium head blight (FHB) severity differences per cross combination

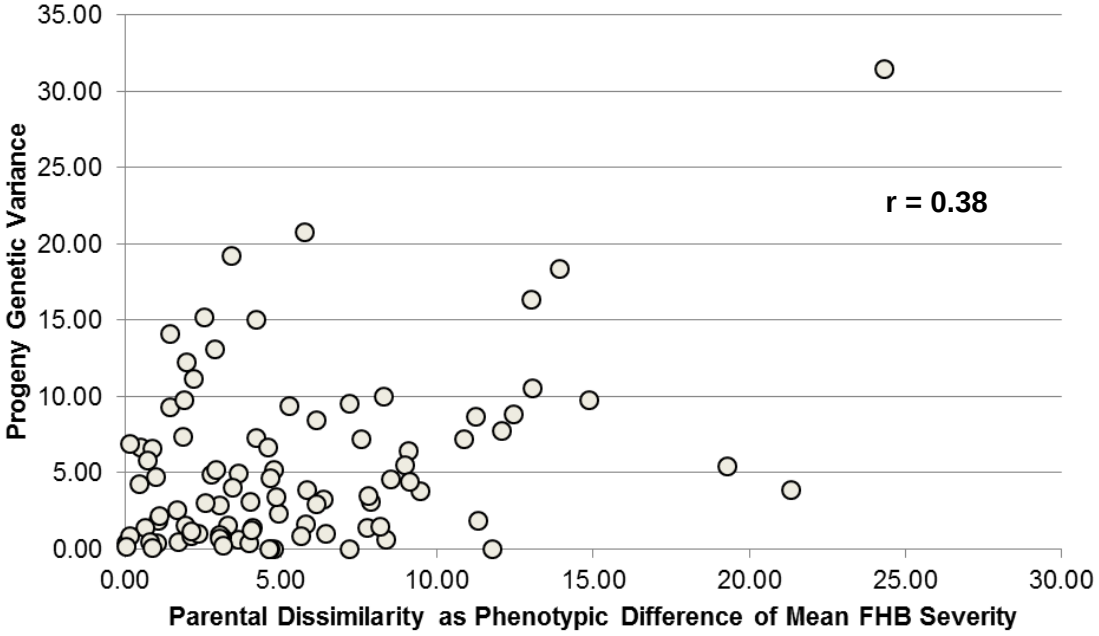


Figure 4. Genetic variance of UMN progeny populations plotted against genetic dissimilarity based on calculated genetic distance using a subset of Fusarium head blight (FHB)-associated single nucleotide polymorphism (SNP) markers

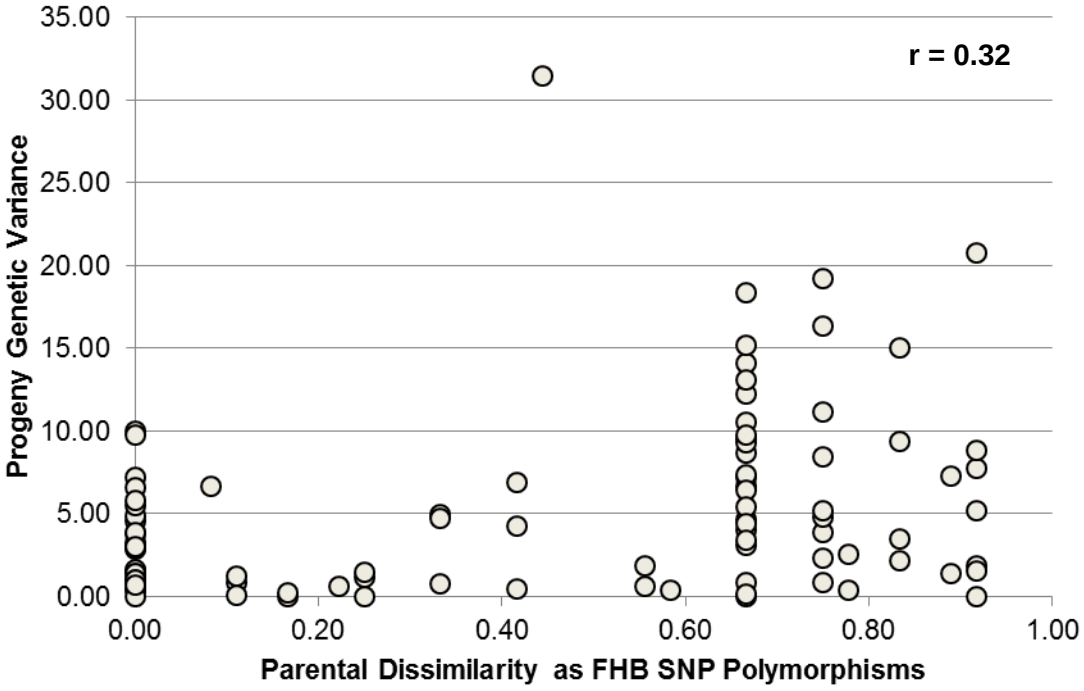


Figure 5. Low FHB severity transgressive segregant proportion and combined low and high FHB severity transgressive segregant proportion per UMN population plotted against phenotypic dissimilarity based on Fusarium head blight severity scores per cross combination

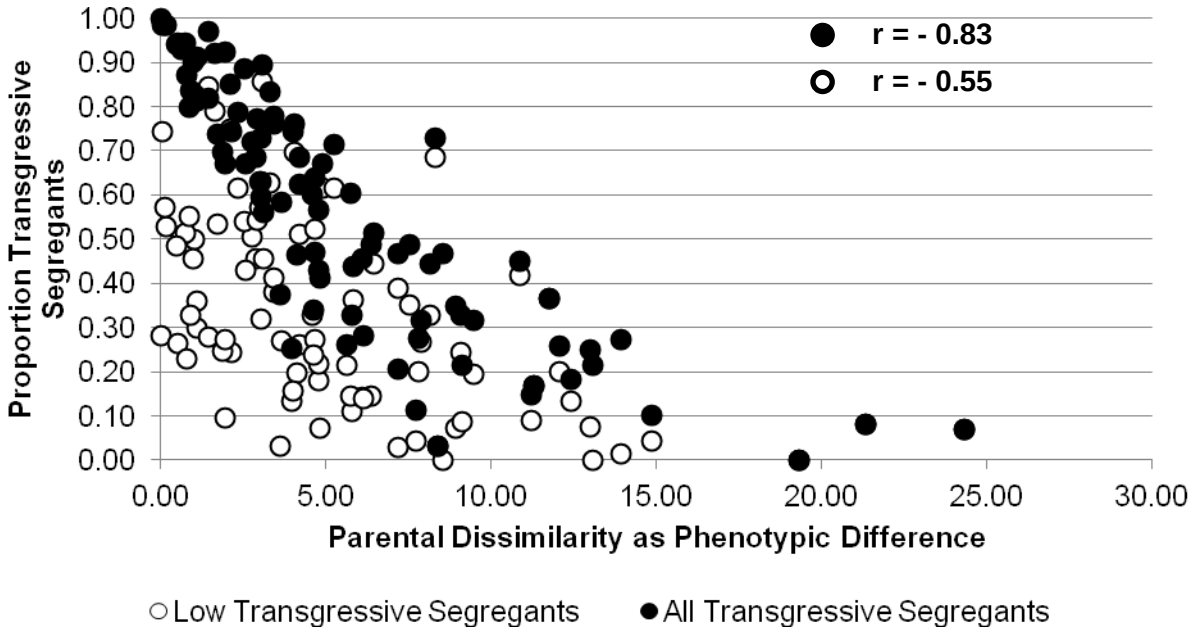


Figure 6. Genetic variance of MN-ND progeny populations plotted against genetic dissimilarity based on calculated genetic distance using a subset of Fusarium head blight (FHB)-associated single nucleotide polymorphism (SNP) markers

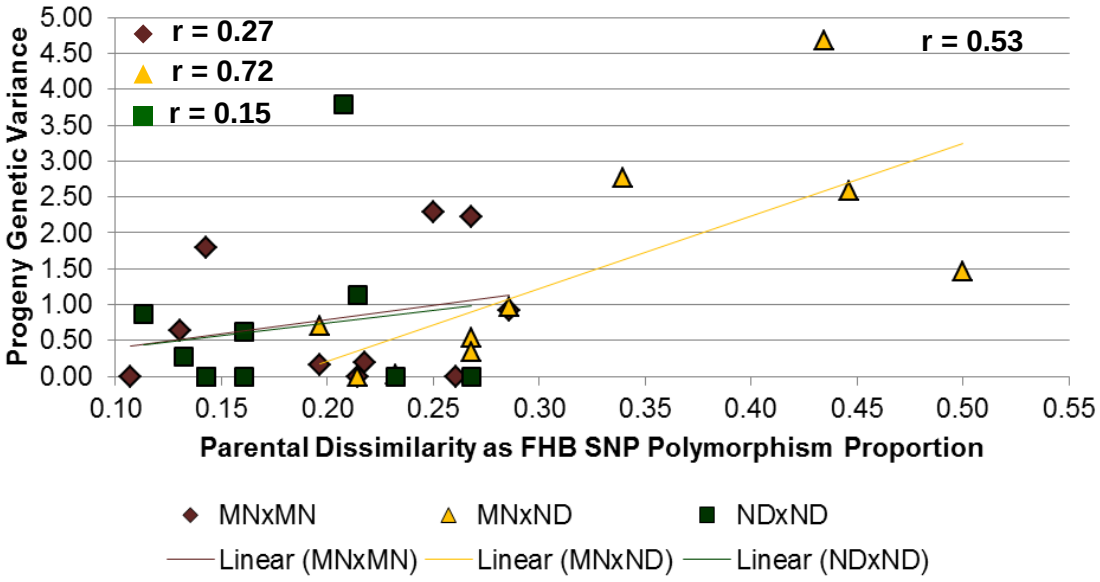


Figure 7. MN-ND progeny population means plotted against mid-parent means for Fusarium head blight (FHB) severity

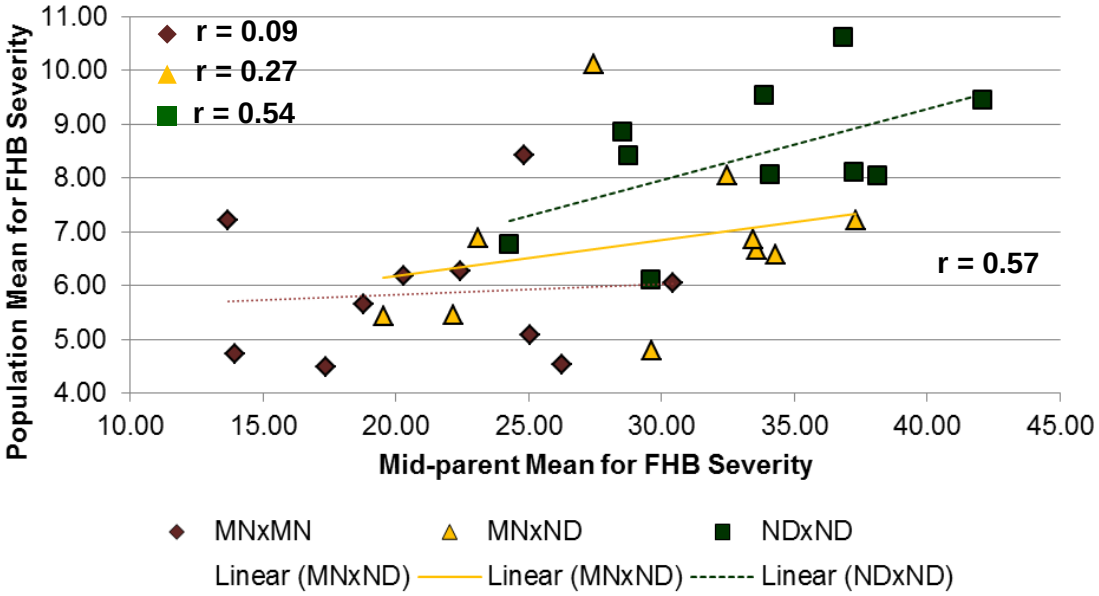
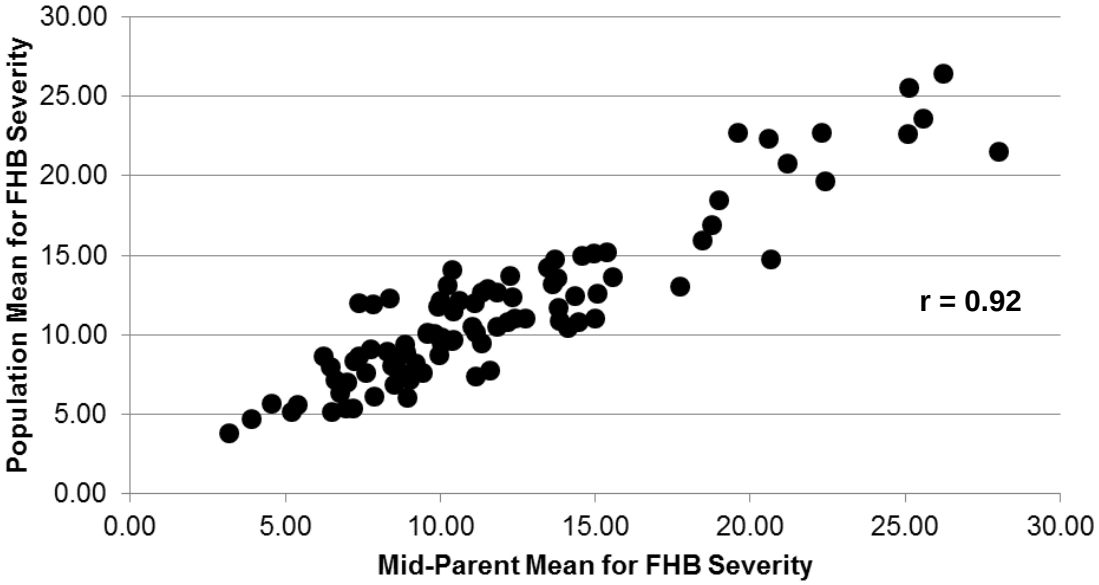


Figure 8. UMN progeny population means plotted against mid-parent means for Fusarium head blight (FHB) severity



Chapter Two

Assessing Homogeneity of Genomic Selection Prediction Accuracy for Fusarium Head Blight Resistance among Families in Multi-parent Barley Breeding Populations

INTRODUCTION

One of the most significant factors leading to the reduction of barley acreage in the Midwest is Fusarium head blight (FHB), a toxin-associated grain disease. The current era of FHB research has dawned molecular, characterized by comprehensive screenings of germplasm collections throughout the world and identification of quantitative trait loci (QTL) associated with FHB resistance. Realization of FHB resistance QTL introgression into elite malting barley germplasm is still rife with challenges. Phenotypic evaluation of the trait is sensitive to an array of error-introducing variables with potential to confound putative influence of small-effect FHB resistance genes. Of the FHB QTL identified through bi-parental mapping efforts, validation remains elusive for most. Association between the few validated QTL and unfavorable agronomic/morphologic characters has further frustrated improvement efforts.

Such complications have challenged breeders to pursue alternate approaches in breeding for FHB resistance, including the use of a genome-wide marker-based selection method. The ability to predict breeding values from allelic effect estimates using genomic markers in linkage disequilibrium with QTL of interest overcomes many obstacles innate to quantitative trait breeding. Related studies suggest prediction accuracy in terms of FHB resistance is adequate for genetic gain in six-row malting barley germplasm. It remains unknown whether prediction accuracy observed across breeding populations is maintained across families, the subsets of lines derived from bi-parental crosses within barley breeding populations. This study investigates the potential homogeneity of genomic selection (GS) prediction accuracy across families of two Midwest barley breeding populations in the context of FHB severity and concentration of an FHB-associated mycotoxin, deoxynivalenol (DON).

Renowned globally to growers of gramineous crops, FHB, or “scab,” is considered one of the primary limiting factors of grain production. Though associated with several

Fusarium species, FHB epidemics in the Midwest United States are primarily attributable to isolates of *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch]. Symptoms of the disease on barley include darkened, shrunken kernels with reduced germination rates and presence of trichothecene mycotoxins. Nivalenol, deoxynivalenol (DON), and associated acetylated derivatives produced by the *Fusarium graminearum* species complex are prominent factors in grain quality reduction and some cases of human and animal mycotoxicoses (Binder et al. 2007; Alexander et al. 2011; Zain 2011). DON is most commonly detected and is considered responsible for the majority of economic losses related to FHB (McMullen et al. 1997; Pestka 2007). Although the United States Food and Drug Administration (USFDA) recommends no more than 1 ppm DON in finished grain products for human consumption, the malting industry may depreciate or reject grain containing DON levels at 0.5 ppm or less (Windels 2000; USFDA 2010). Concomitant with regional establishment of the disease is the sharp decline in barley production in the Upper Midwest by approximately 70% from 1990 to 2010 (http://quickstats.nass.usda.gov; verified 17 March 2013).

Contemporary attempts at FHB management include an integrated approach involving regional blight risk forecasting (Prandini et al. 2009), application of systemic fungicides, inoculum-reducing cultural practices, and development of FHB-resistant barley cultivars. Resistant cultivars represent the most effective and cost-efficient component of the integrated approach, spurring multiple germplasm screenings in pursuit of resistance sources. FHB immunity remains elusive; however, results of screening efforts proffer an array of genetically diverse resistance sources with moderate effects of FHB resistance relative to initial infection (Type I resistance; Mesterházy 1995) in cultivated barley, land races, and wild barley (Takeda and Heta 1989; Chen et al. 1991; Zhou et al. 1991; Buerstmayr et al. 2004; Choo et al. 2004; McCallum et al. 2004; Ma et al. 2009; Huang et al. 2012). Subsequent mapping studies identified multiple QTL associated with Type I FHB resistance and DON concentration (de la Peña et al. 1999; Zhu et al. 1999; Ma et al. 2000; Dahleen et al. 2003; Mesfin et al. 2003; Canci et al. 2004; Hori et al. 2005, 2006; Horsley et al. 2006; Lamb et al. 2009; Sato et al. 2008; Massman et al. 2011), a requisite for application in marker-assisted selection (MAS).

Despite discovery of major QTL through mapping endeavors, transition from QTL identification to introgression remains unrealized due to challenges related to mapping methodology, genetic architecture of the trait, and phenotyping difficulties. Many QTL identified are bi-parental cross-specific, thus limiting allelic diversity and putative QTL to the genetic background of the mapping population or, as more recently hypothesized, associated with specific haplotypes (Massman et al. 2011; Huang et al. 2012; Navara and Smith In review). QTL discovered via bi-parental mapping are difficult to validate and explain little genetic variation of the trait. Further, several QTL for FHB resistance and decreased DON concentration map to the same genomic regions as heading date, plant height, and spike morphology QTL (Zhu et al. 1999; Canci et al. 2004; Lamb et al. 2009). Such unfavorable associations with resistance may be explained by tight linkage with heading date QTL (Nduulu et al. 2007) and pleiotropic effects of the *vrs1* locus with row-type (Sato et al. 2008). The polygenic nature of FHB resistance, as indicated by the association mapping study of elite breeding germplasm by Massman et al. (2011), affirms the challenge of implementing MAS on a complex trait influenced by several small-effect loci where additive effect direction varies across environments (Lamb et al. 2009).

Phenotypic evaluation of the environmentally-sensitive trait is resource-intensive, and consistent results are difficult to achieve (Van Sanford et al. 2001). The high degree of GxE interaction characteristic of FHB resistance necessitates multiple replications and environments. Mist-irrigated disease nurseries with location-specific inoculation methodologies are assembled and maintained throughout planting and inoculation to disease evaluation and seed harvest for DON assays (Steffenson 2003). Measurements of DON concentration represent a significant component of the total phenotypic cost but are an essential expense. Just as presence of orange sporodochia does not necessarily indicate the presence of DON-producing *Fusarium* species, absence of FHB symptoms is not a reliable indicator of DON levels, as discovered by barley growers at grain elevators. The partial correlation between DON concentration and FHB severity indicate separate genetic control of the traits (Lamb et al. 2009). Evidence for the independent genetic mechanisms affecting disease symptoms and DON levels further supports the need for phenotyping DON concentration (Smith et al. 2004)

The degrees to which complex trait architecture, high GxE interaction, and resource-demanding phenotyping limit MAS extend beyond FHB resistance breeding. Such challenges are inherent to quantitative trait breeding in general, and serve as inspiration to pursue alternate breeding approaches. One alternative is genomic selection (GS), a marker-based selection method using high-density markers distributed throughout the genome to estimate allelic contributions to a trait of interest for generation of breeding value predictions (Meuwissen et al. 2001). As compared with the step-wise regression approach of Lande and Thompson (1990), where markers are first qualitatively assessed for phenotype association before estimation of effects, GS estimates all marker/locus/haplotype/allele effects in one step. Simultaneous effect estimation circumvents the step-wise regression problem of arbitrary statistical thresholds for marker significance declaration, resulting in decreased ability to capture genetic variation and inflation of marker effects concomitant with using marker subsets (Beavis 1998; Xu 2003; Goddard and Hayes 2007).

Summation of marker effects yields predicted breeding values for each individual, termed “genomic estimated breeding values” (GEBVs; Heffner et al. 2009; Jannink 2010). The GS framework may be summarized in four steps: 1) train a statistical model with genotypic and phenotypic data of individuals, defined as a training population; 2) estimate marker effects in the training population; 3) calculate GEBVs for a new set of individuals related to the training population, defined as a validation population, based on genotypic data; and 4) select desirable individuals based on GEBVs (Heffner et al. 2009; Jannink 2010). Application of this method towards parental selection at early stages in the breeding process significantly shortens the breeding cycle, increasing gain per unit time.

Several statistical models are capable of accommodating simultaneous estimation of marker effects, when the number of predictive markers, *p,* exceeds the number of observations, *n,* a concept known as “the large *p*, small *n* problem” (Heffner et al. 2009; Lorenz et al. 2011). Ridge-regression best linear unbiased prediction (RR-BLUP), first advocated for GS by Whittaker et al. (2000), is the most parsimonious model, assigning marker effects as random, assuming equal variance, and equally shrinking marker effects towards zero. Such simple assumptions do not meet the realistic expectations of

QTL distribution that some Bayesian estimation models incorporate, e.g. BayesB (Meuwissen et al. 2001), where marker effects may equal zero to reflect absence of QTL in some genomic regions. Accuracy of GEBV predictions is defined as the Pearson correlation between the GEBV and the true breeding value (TBV; Heffner et al. 2009), though TBVs are generally phenotypically estimated (PEBVs). Empirical studies contrasting estimation methods demonstrate RR-BLUP performance is at least comparable in terms of accuracy to that of Bayesian estimations as observed with maize (*Zea mays* L.) bi-parental populations (Lorenzana and Bernardo 2009), winter wheat (*Triticum aestivum* L.) advanced cycle breeding lines (Heffner et al. 2011), and oat (*Avena sativa* L.) elite breeding lines (Asoro et al. 2011).

Results of a cross-validation study using phenotypic and genotypic data derived from the Barley Coordinated Agricultural Project (Barley CAP; http://www.barleycap.org) for training and validation populations corroborate those of referenced empirical studies, indicating RR-BLUP prediction accuracy is sufficient to realize genetic gain in context of FHB-related traits (Lorenz et al. 2012). Such results infer the RR-BLUP method of GS captures a sufficient degree of FHB-related QTL genetic variance to adequately predict associated breeding values in barley breeding germplasm. Cross-validation efforts have recently translated into empirical study within the framework of FHB resistance and DON concentration using Barley CAP data to first train an RR-BLUP GS model and then validate prediction accuracy with three cross-type-stratified progeny sub-populations representing crosses within and between two Midwest barley breeding programs (Vikram et al. In preparation). Although the magnitude of the correlation between GEBVs and PEBVs [r(GEBV:PEBV)] varies by cross-type, initial results indicate prediction accuracy for the FHB-associated traits approaches that of the Lorenz et al. (2012) cross-validation study. This accuracy is sufficient to realize genetic gain across breeding programs based on cross-type (Vikram et al. In preparation), but it is unknown whether prediction accuracy is retained among families within barley breeding populations.

To investigate the homogeneity of GS prediction accuracy among families for FHB resistance and DON concentration, two sets of barley breeding germplasm were used, including a larger panel comprising three sub-populations stratified by cross-type that were generated for the Vikram et al. (In preparation) study and a smaller panel of elite

breeding lines specific to one breeding program. In this study, a population is defined as a panel encompassing progeny of multiple bi-parental crosses. A family is defined as a set of progeny lines derived from a single bi-parental cross. Prediction accuracy was assessed through regression and correlation analyses between FHB severity and DON concentration GEBVs and validation population PEBVs.

MATERIALS AND METHODS

Germplasm

The following two germplasm panels were used as validation populations: 1) MN-ND, a set of lines (n = 300) composed of three sub-populations (i.e. cross-types) with 10 families per sub-population (MNxMN, MNxND, NDxND) and ten lines per family representing crosses among University of Minnesota (MN) breeding lines, North Dakota State University (ND) breeding lines, and crosses between MN and ND lines (Table 1); and 2) MN-RS, a contemporary set (n = 130) of six MN families which comprised two sub-populations (RxS, RxR) representing crosses between lines demonstrating partial FHB resistance (R) and susceptible malting standard lines (S) with three families per sub-population and 19-24 lines per family (Table 2). Most parental lines of both germplasm panels were included in the training population.

The training population was a third panel of six-row barley breeding lines advanced to or beyond the F_4 generation (n = 691). Each line was genotyped and evaluated for FHB resistance and DON concentration in Barley CAP I, II, and III, representing years 2006, 2007, and 2008 respectively. Lines were selected based on representation of contemporary advanced breeding lines in the MN, ND, and Busch Agricultural Resources six-row programs (Massman et al. 2011).

Experimental Design

The training population was evaluated in FHB nurseries at locations in Minnesota (Crookston and St. Paul) and North Dakota (Fargo, Langdon, and Osnabrock). CAP I lines were phenotyped in 2006 at Crookston, Fargo, and Langdon, as well as in 2007 at Crookston. CAP II lines were evaluated in 2007 at Crookston, Fargo, Langdon, and St. Paul. CAP III entries were evaluated in 2008 at Crookston, Fargo, Langdon, and

Osnabrock. All lines were replicated twice with three checks: MNBrite, Robust, and Stander (Lorenz et al. 2012; Massman et al. 2011).

MN-RS and MN-ND progeny populations were advanced to the $F_{4:5}$ generation and were evaluated in 2011 and 2012 at two FHB disease nurseries located in Crookston and St. Paul, Minnesota. Each MN-RS line was replicated twice per trial in randomized complete block design with four checks: Quest, Robust, Stander, and Tradition. The two locations and years resulted in a total of four trials.

MN-ND populations were further evaluated in both years at two trials located in Langdon and Osnabrock, North Dakota, for a total of eight trials. All MN-ND lines were replicated twice per trial in randomized incomplete block design using six incomplete blocks with three checks: ND20493, Quest, and Tradition. All entries were represented in each replicate and all checks represented in each block. Minnesota plots were single 1.5-m rows with 0.3-m row-spacing. North Dakota plots were single hill plots spaced 0.3-m apart.

Fusarium Head Blight Evaluation

Plots at the St. Paul disease nursery were inoculated using conidial spray and backpack sprayers (Steffenson 2003). Inoculum was applied twice per trial, at heading and two to three days after the first inoculation. Overhead mist irrigation was maintained daily at timed intervals of ten minutes every hour from 18:00 to 6:00 from heading until disease evaluation, approximately 14 days after heading.

Inoculation of plants at Crookston followed the grain spawn method (Prom et al. 1996). At the Crookston disease nursery in 2011, two applications of inoculum were dispersed between rows at the five-leaf growth stage and at the flag leaf stage. A shortage of inoculum in 2012 resulted in a single application at the jointing stage. For all applications, inoculated corn kernels were spread at a rate of six grams per square meter. Mist irrigation began at inoculation and followed the St. Paul location protocol. FHB severity was scored approximately 18 days after heading.

Langdon and Osnabrock disease nursery plots were inoculated using the grain spawn method where inoculated corn was applied two weeks prior to anthesis. Rate of dispersal was 50 grams per square meter. Plots were overhead mist-irrigated at a rate of 1.2 L per hour for 30 seconds every 30 minutes daily between the hours of 04:00 to 08:00 and 18:00 to 20:00. Disease was scored approximately 14 to 18 days after heading.

Phenotypic Evaluation

FHB severity was measured at each location by arbitrarily selecting ten main tiller spikes per row per sample. At the St. Paul disease nurseries, where disease pressure was low, FHB severity was scored by counting infected kernels of each spike and conversion to percentage. In disease nurseries with higher disease pressure, as observed at the Crookston and North Dakota disease nurseries, FHB severity was scored by visual estimation of infection percentage per spike using the 0, 1, 3, 5, 10, 15, 25, 35, 50, 75, 100% rating scale.

To prepare samples for DON concentration assays, harvested seed from the entire row or hill was bulk-threshed, cleaned using a sieve with 0.2 x 1.9 cm slotted openings, and ground into flour using a Cyclotec Sample Mill with a 1 mm mesh screen (Foss North America, Eden Prairie, MN). DON concentrations for both populations were measured at the University of Minnesota Mycotoxin Laboratory (Dr. Yanhong Dong, Director) per sample with 20 g of barley flour using gas chromatography and mass spectrometry (Fuentes et al. 2005). DON assays were conducted on four MN-RS trials and four MN-ND trials. Grain samples of MN-RS parents, progeny, and checks were bulked by line in 2011. In 2012, only MN-RS progeny samples were bulked, resulting in two DON measurement replicates per parent and check. Of the MN-ND populations, each check and progeny line evaluated in the four 2011 trials was represented by two DON concentration replicates.

Genotypic Evaluation

Genotyping of the training population, including several parental lines of the families included in the MN-RS and MN-ND populations, was conducted with Illumina GoldenGate barley oligonucleotide pooled assays I and II (BOPA I and II; Close et al.

2009) scoring 3072 single nucleotide polymorphism (SNP) markers. Data for all lines analyzed, and for all CAP years, are available through the Triticeae Toolbox (http://triticeaetoolbox.org/). SNPs with a minor allele frequency less than 0.05, or with a missing data frequency greater than 0.10, were removed and imputed by Lorenz et al. (2012). Genotyping of MN-RS and MN-ND progeny lines inbred to the F_3 generation was conducted at the Fargo USDA genotyping facilities using a custom-made 384-SNP assay derived from a subset of BOPA I and II SNPs.

Genomic Estimated Breeding Value and Prediction Accuracy

GEBV predictions were generated using the RR-BLUP prediction model trained with the CAP I-III population as described by Lorenz et al. (2012). RR-BLUP mixed-model equations were resolved for year and marker effects. FHB-related GEBVs for the MN-RS and MN-ND validation populations were calculated by summation of estimated marker effects.

Data Analysis

Phenotypic data were standardized prior to analysis using PROC MIXED (SAS Institute 2008) to adjust for effects relative to each panel. Replication and trial effects were resolved as fixed effects in the MN-RS panel and genotypes were considered random effects. The MN-ND panel required resolution of replication, block, and trial effects, which were treated as fixed effects while genotypes were the random effects in the mixed models.

Prediction accuracy was determined with Pearson's product moment correlations between GEBVs and PROC MIXED-adjusted LS-Means for FHB severity and DON concentrations per family. Although accuracy relative to phenotypic selection may be calculated through division of correlations by the square root of their respective entry mean-based heritabilities (Lorenz et al. 2012), this step was excluded in the present study. Correlations were tested for significance at alpha = 0.05 using PROC CORR for each family in the MN-RS and MN-ND data sets.

Of the eight MN-ND FHB trials, five were removed from analyses due to lodging (CR2011, LA2012, OS2012) and use of disparate disease rating methodology (LA2011,

OS2011), resulting in three trials for use in FHB data analysis. Confounding effects attributable to lodging resulted in non-detectable measures of phenotypic variation. The 1-5 scale of FHB scoring for two of the trials could not be standardized to the method used for additional trials outlined in Phenotypic Evaluation. Data of the remaining three trials (CR2012, SP2011, SP2012) demonstrated homogeneity of error variance based on Levene's HOV test implemented in PROC GLM and were subsequently combined and adjusted to LS-Means calculated with PROC MIXED. All data from the four DON trials were retained for correlation analyses. Unresolved heterogeneity of error variance of DON data among the trials required separate adjustments and analyses by trial.

All trials of the MN-RS families evaluated for FHB and DON were retained for analyses. Homogeneity of error variance for FHB severity in the four trials allowed data to be standardized to LS-Means through PROC MIXED then pooled and analyzed. Heterogeneity of DON error variance among trials could not be resolved with standardization, necessitating separate analyses by trials after adjusting for within-trial effects.

RESULTS

For both FHB-related traits, prediction accuracy varied across families in the MN-RS (Table 3) and the MN-ND populations (Table 4; Figure 1 and 2). Significant correlation coefficients were associated with four of the six MN-RS families for at least one trial. Two families (MN-RS03, MN-RS04) demonstrated significant associations between GEBVs and PEBVs for both FHB severity and DON concentration. One MN-RS population (MN-RS04) could consistently be predicted for DON concentration for three of the four trials, and the correlation between predicted and observed FHB scores was also significant for this family.

There was a wide range in prediction accuracy across families for the MN-ND population both within and across FHB and DON trials (Figure 1 and 2). Correlation coefficients for FHB ranged from -0.48 and 0.86. Within DON trials, the range of accuracies was relatively consistent: -0.39 to 0.85 (CR2011), -0.42 to 0.71 (LA2011), -0.61 to 0.82 (OS2011), and -0.41 to 0.78 (SP2011). Four families (MN-ND05, MN-ND17, MN-ND25, MN-ND27) consistently had negative correlations for three of the four DON trials.

Significant correlations were absent for the majority of MN-ND families for FHB severity scores (Figure 1) and for DON concentration (Figure 2), regardless of sub-population. By trait, correlation coefficients were significant for 11 of the 30 MN-ND families (Table 4), of which all three sub-populations were nearly equally represented. None of the MN-ND families demonstrated significant association between GEBVs and PEBVs for both traits, although two families (MN-ND09, MN-ND18) had significant correlation coefficients for DON concentrations at two trials each, which did not overlap.

DISCUSSION

The relatively high prediction accuracy for FHB resistance and DON concentration across populations and sub-populations of Vikram et al. (In preparation) may be attributable to a GS model accurately predicting the means of individual populations. However, heterogeneity of prediction accuracy observed within both MN-RS and MN-ND populations suggests the model is limited in ability to predict individual family performance. It is feasible to have a composite of families poorly predicted without decrease in accuracy of the population as a whole, provided the mean of the whole matches the predicted mean for that population, which appears to be true in this study (Vikram et al. In preparation). Weak prediction performance among families despite relatively strong GS predictive performance at the population level is not without precedent in empirical and simulation investigations, the reasons for which may be related to phenotypic variance and relatedness of lines in the validation population relative to lines in the training population (Bernardo and Yu 2007; Albrecht et al. 2011; Zhao et al. 2011; Heslot et al. 2012; Schulz-Streeck et al. 2012).

Effect of Phenotypic Variance

Phenotypic variance differed among families in both germplasm panels, which influenced genetic variance estimates for each family. Families with lower phenotypic variance would have lower calculations of genetic variance, resulting in lower prediction accuracy. To determine whether this factor potentially influenced association between GEBVs and PEBVs, the relationship between genetic variance estimates and correlation coefficients for each family per trait was tested with PROC CORR at the significance level alpha = 0.05. Significant associations were absent (results not shown).

Effect of Genetic Dissimilarity

Prediction accuracy is expected to decrease with an increase in genetic dissimilarity, thus a decrease in prediction accuracy may be observed if relatedness between lines of the training and validation populations is low. Parents of each family studied were present in the training population, with the exception of one MN-RS family (MN-RS06) and four MN-ND families (MN-ND02, MN-ND04, MN-ND05, MN-ND13). It would then be expected the families with a parent excluded from the training population would demonstrate lower prediction accuracies. None of these five families had significant associations; however, many other families with both parents included in the training population also lacked significant prediction accuracy.

Relatedness between each line in the validation populations with those of the training population was not tested in this study. Such an investigation may be conducted using the F statistic measuring allelic frequency dissimilarity between individuals in subpopulations or families, *Fst*. Regression and correlation analyses using *Fst* values and the GEBV:PEBV correlation coefficients could test the hypothesis that line relatedness influences GS prediction accuracy. Expected results supporting such a hypothesis would include a significant correlation between higher average *Fst* values of validation population families and a decrease in prediction accuracy (Heslot et al. 2012).

Conclusion

The premise of GS is that a set of markers distributed throughout the genome are in linkage disequilibrium with the polymorphisms influencing variation in the trait of interest. High prediction accuracy observed in multiple cross-validation and empirical studies suggests the RR-BLUP method of GS captures enough of a quantitative trait's genetic variation to adequately predict breeding values and make selections accordingly. This trend extends to FHB-related traits in barley breeding germplasm (Vikram et al. In preparation). Although GS application is adequate across populations to garner genetic gain, prediction accuracy is heterogeneous within populations. Further research is needed to understand the inconsistent accuracy across families within multi-parent breeding populations. Whether adjusting current methods to more consistently predict accuracy over a broad set of families or focusing predictions on specific families to acquire better accuracy with a given prediction model, GS utility can improve.

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Table 1. Pedigree and number of individuals per family in the MN-ND population

Family	Pedigree	Family Size
MN-ND01	QUEST / FEG154-47	9
MN-ND02[a]	FEG141-20 / FEG153-25	11
MN-ND03	FEG141-20 / FEG154-47	10
MN-ND04[a]	FEG153-25 / FEG175-57	10
MN-ND05[a]	FEG153-25 / FEG183-52	10
MN-ND06	FEG153-58 / FEG183-52	10
MN-ND07	FEG154-47 / FEG175-57	10
MN-ND08	FEG154-47 / FEG183-52	10
MN-ND09	FEG183-52 / FEG141-20	10
MN-ND10	QUEST / FEG183-52	10
MN-ND11	QUEST / ND25728	10
MN-ND12	FEG141-20 / ND24906	10
MN-ND13[a]	FEG153-25 / ND25728	10
MN-ND14	FEG153-58 / ND25160	10
MN-ND15	FEG175-57 / ND25986	10
MN-ND16	FEG175-57 / ND26104	10
MN-ND17	ND24906 / FEG183-52	10
MN-ND18	ND25160 / FEG154-47	10
MN-ND19	ND26036 / FEG154-47	10
MN-ND20	ND26104 / QUEST	10
MN-ND21	ND20448 / ND25160	10
MN-ND22	ND20448 / ND25986	10
MN-ND23	ND24906 / ND20448	10
MN-ND24	ND24906 / ND25652	10
MN-ND25	ND25160 / ND24906	10
MN-ND26	ND25652 / ND25160	10
MN-ND27	ND25652 / ND25986	10
MN-ND28	ND25986 / ND26104	10
MN-ND29	ND26036 / ND24906	10
MN-ND30	ND26104 / ND24906	10

a Parent not in training population

Table 2. Pedigree and number of individuals per family in the MN-RS population

Family	Pedigree	Family Size
MN-RS01	FEG175-57 / RASMUSSON	24 or 22[b]
MN-RS02	FEG181-42 / LACEY	21
MN-RS03	FEG189-05 / M05-08	22
MN-RS04	FEG212-09 / FEG195-33	23
MN-RS05	FEG214-32 / FEG141-20	21
MN-RS06[a]	FEG232-21 / QUEST	19

a Parent not in training population
b Family size = 24 for CR2011 and SP2011; family size = 22 for CR2012 and SP2012

Table 3. Correlation coefficients between predicted and observed means by trait for the MN-RS population

Family	Trial[a]	Trait	r(GEBV,PEBV)[b]
MN-RS01	CR2011	DON	-0.03
MN-RS01	CR2012	DON	0.19
MN-RS01	SP2011	DON	0.23
MN-RS01	SP2012	DON	0.34
MN-RS02	CR2011	DON	0.21
MN-RS02	CR2012	DON	0.37
MN-RS02	SP2011	DON	0.35
MN-RS02	SP2012	DON	0.37
MN-RS03	CR2011	DON	0.28
MN-RS03	CR2012	DON	0.23
MN-RS03	SP2011	DON	0.40
MN-RS03	SP2012	DON	**0.47
MN-RS04	CR2011	DON	**0.65
MN-RS04	CR2012	DON	0.35
MN-RS04	SP2011	DON	**0.47
MN-RS04	SP2012	DON	**0.60
MN-RS05	CR2011	DON	0.05
MN-RS05	CR2012	DON	0.21
MN-RS05	SP2011	DON	-0.16
MN-RS05	SP2012	DON	0.07
MN-RS06	CR2011	DON	0.09
MN-RS06	CR2012	DON	0.18
MN-RS06	SP2011	DON	-0.07
MN-RS06	SP2012	DON	0.20
MN-RS01	4LM	FHB	0.37
MN-RS02	4LM	FHB	**0.58
MN-RS03	4LM	FHB	**0.51
MN-RS04	4LM	FHB	**0.48
MN-RS05	4LM	FHB	**0.44
MN-RS06	4LM	FHB	0.68

a 4LM = Four location mean of CR2011, CR2012, SP2011, and SP2012

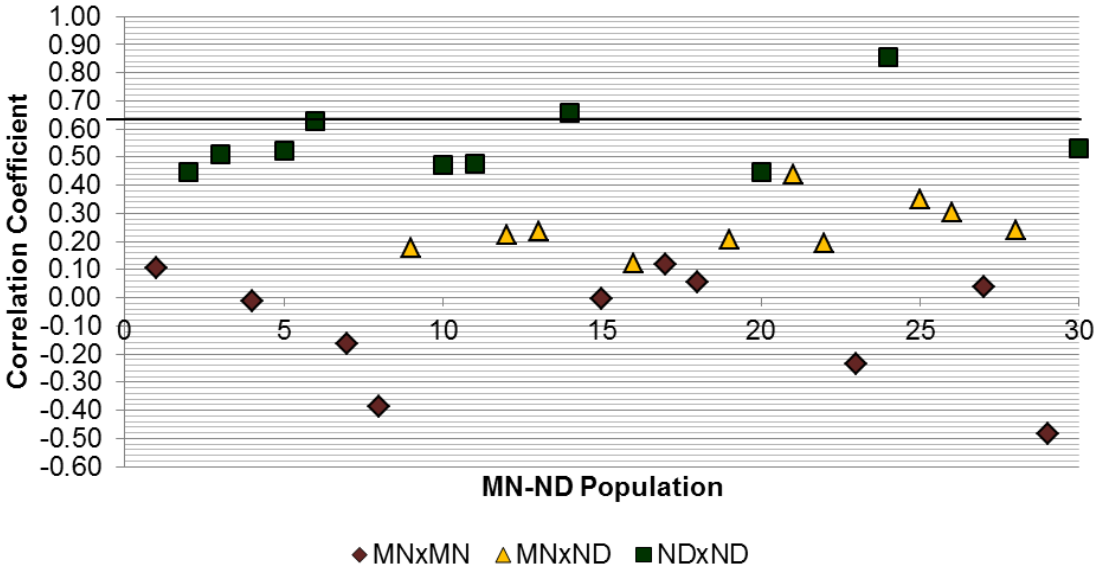
b **Correlation coefficients significant at $p \leq 0.05$

Table 4. Correlation coefficients for the 11 families with significant associations between predicted and observed means in the MN-ND population listed by trait

Family	Trial[a]	Trait	r(GEBV,PEBV)[b]
MN-ND06	3LM	FHB	0.63
MN-ND07	CR2011	DON	0.64
MN-ND09	LA2011	DON	0.71
MN-ND09	OS2011	DON	0.75
MN-ND14	3LM	FHB	0.66
MN-ND16	OS2011	DON	0.74
MN-ND18	CR2011	DON	0.85
MN-ND18	SP2011	DON	0.64
MN-ND19	SP2011	DON	0.78
MN-ND20	OS2011	DON	0.82
MN-ND22	SP2011	DON	0.68
MN-ND23	CR2011	DON	0.69
MN-ND24	3LM	FHB	0.86

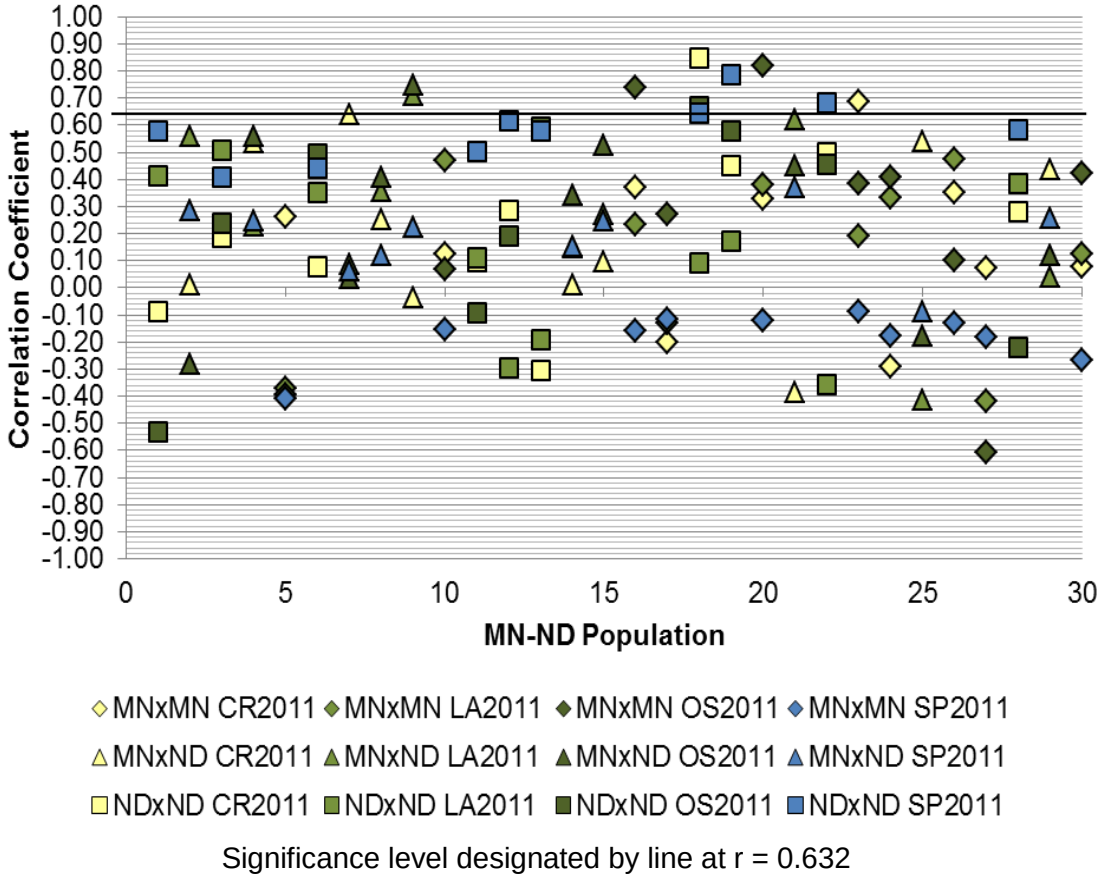
a 3LM = Three location mean of CR2012, SP2011, and SP2012
b Correlation coefficients significant at $p \leq 0.05$

Figure 1. MN-ND correlation coefficients between predicted and observed mean Fusarium head blight (FHB) severity within families based on performance across trials in Crookston 2012, St. Paul 2011, and St. Paul 2012



Significance level designated by line at r = 0.632

Figure 2. MN-ND correlation coefficients between predicted and observed mean deoxynivalenol concentration within families



Significance level designated by line at r = 0.632

Chapter Three

Future Directions

The conclusions of Chapter One suggest there is some merit to predicting genetic variance of a trait with trait-associated SNP markers. Translation of results from the study into breeding application would be facilitated by strengthening the prediction ability of genetic dissimilarity. Further, to be generally useful for a breeding program, the promising results must make the transition from relevance at the level of a single trait to that of multiple traits.

Strengthening Trait-specific Associations between Genetic Variance and Genetic Dissimilarity

The ability to predict genetic variance for Fusarium head blight resistance based on genetic dissimilarity of parental lines has been shown to increase when the marker data set is biased towards the specific trait. Using genetic dissimilarity based on all of the SNPs of the BOPA assays did not have enough power to capture variance related to FHB resistance since these SNPs are likely in low to medium frequency in the assays, which are skewed to represent common variants. A potential solution to overcoming such ascertainment bias, which would presumably lead to increased association between dissimilarity using the SNPs and genetic variance of that trait, would be to use a method that does not require a discovery panel. Genotype by sequencing (GBS) could provide such a method.

The objective of this study would be to determine if associations between SNP-based genetic dissimilarity and genetic variance may be strengthened using a method bypassing the ascertainment bias inherent with SNP assays. The association between parental dissimilarity calculated based on GBS could be compared with the strength of associations in Chapter One using BOPA SNPs and FHB-specific BOPA SNPs. The UMN panel comprises 112 parent lines and 93 populations. Eight more populations could be added with genotypic data acquired on seven additional parent lines. Phenotypic data for Fusarium head blight severity scores are available for UMN parent and progeny lines evaluated from 2003 to 2010. Genotypic data could be acquired by

first generating a GBS library and then genotyping the lines by next generation sequencing.

Since the restriction enzyme-cut fragments form such a reduced representation of the genome, 30 to 40 samples could be multiplexed. With the genotypic data of the parent lines, Nei's genetic distance could be calculated for each pair-wise parent combination reflecting each progeny population. The GBS-based genetic dissimilarity could then be regressed on estimates of progeny genetic variance. By comparing the strength of the association between the GBS genetic dissimilarity and genetic variance with the associations seen in the Chapter One study, the merit of using a method bypassing ascertainment bias of SNP assays to predict genetic variance could be assessed.

Investigate Accuracy among Multiple Traits

Trait-specific genetic dissimilarity of parent lines can predict genetic variance to a certain extent. Although marginally effective for the FHB resistance trait, in order for this finding to effectively translate into breeding program application, such an association should extend to the additional traits for which breeders must simultaneously select. Development and improvement of cultivars require the balancing of both directional selection and stabilizing selection. For example, traits such as yield and disease resistance would benefit from maximized genetic variance. For other traits, such as those related to malting quality or protein content, it is more desirable to stabilize the variance. As such, the ability to select cross-combinations in context of genetic variance for multiple traits simultaneously would most benefit breeders.

The objective of this study would be to determine how effectively genetic dissimilarity can be used to predict genetic variance in context of multiple traits. This would involve the creation of an index estimating measures of genetic variance for several traits and then validating the accuracy of the predictions. Such an index would encompass measures of genetic dissimilarity based on trait-specific SNP marker subsets derived from association mapping studies. Multiple association mapping studies using BOPA SNPs have been conducted in barley centered on several malting quality traits. The index would comprise traits for which both directional selection and stabilizing selection are relevant. Based on the measures of genetic dissimilarity of the traits, cross-

combinations would be identified having projected maximization of genetic variance for a directionally selected trait, e.g. FHB resistance, and concomitantly projected to minimize genetic variance for traits that are generally the focus of stabilized selection, e.g. malt extract, protein, or enzyme levels. Although the predictor, genetic dissimilarity, will have specific values, projected estimates of genetic variance will not have clearly defined values. To address this, the predictions could be assigned rankings reflecting the degree of expected genetic variance. Progeny populations could then be generated by crossing the lines representing the cross-combinations identified by the index. The degree to which results match expected genetic variance-related trends, i.e. the strength of correlations between expected and observed rankings of genetic variance for the traits, would assess the merit of using such an index incorporating information from association mapping studies to predict genetic variance for multiple traits.

Potential Challenges

Potential limitations which could be associated with the proposed studies involve low estimates of genetic variance and the relationship between genetic variance and the variance of trait means. Low or undetectable genetic variance for a trait could confound correlations validating accuracy of genetic variance predictions, more so for traits where directional selection is the general rule. Increasing replication of trait evaluation trials could help reduce the error component to a limited extent, but if genetic variance is too low to detect, it will be difficult to correlate to expectations.

A second concern worth addressing is whether there exists an inflection point where increasing genetic variance is no longer useful. If increasing genetic variance comes at the cost of gains made from progeny means for the trait, where the variance of the trait means is also greatly increased, then this would be a scenario exemplifying redundancy of genetic variance increase. However, there is merit to investigating where this point is for traits of interest such that it could be predicted and breeding efforts could be conserved. Estimation of this breaking point could be done using the t-ratio of Zhong and Jannink (2007), which is a ratio looking at the standard deviation of progeny means to the standard deviation of genetic variance.

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Appendix A. UMN population parameters and markers used in parental dissimilarity estimates

Population	No. Lines	Pedigree	No. SNPs[a]	No. FHB SNPs[b]	FHB Trials[c]
UMN01	78	M97-31 / FEG27-96	1235	17	CR2003 SP2003
UMN02	77	FEG16-30 / M97-57	1235	17	CR2003 SP2003
UMN03	79	FEG18-27 / M97-16	1418	22	CR2003 SP2003
UMN04	64	FEG32-107 / M97-54	1235	17	CR2003 SP2003
UMN05	99	FEG33-89 / M97-54	1235	17	CR2003 SP2003
UMN06	61	FEG39-66 / M97-16	1235	17	CR2003 SP2003
UMN07	86	FEG20-18 / FEG39-66	1235	17	CR2003 SP2003
UMN08	88	FEG26-50 / FEG18-27	1419	22	CR2003 SP2003
UMN09	38	FEG27-96 / FEG31-68	1236	17	CR2003 SP2003
UMN10	77	FEG43-46 / M110	1235	17	CR2003 SP2003
UMN11	67	FEG55-14 / M116	1387	22	CR2003 MO2003
UMN12	66	FEG59-09 / M110	1408	22	CR2003 SP2003
UMN13	59	FEG59-21 / M115	1403	22	CR2003 SP2003
UMN14	76	M112 / FEG31-68	1418	22	CR2004 SP2004
UMN15	51	M112 / FEG59-36	1417	22	MO2004 SP2004
UMN16	78	M96-203 / FEG55-14	1386	22	MO2004 SP2004
UMN17	85	M115 / FEG59-21	1403	22	CR2004 SP2004
UMN18	90	M116 / FEG55-04	1418	22	CR2004 SP2004
UMN19	72	FEG31-68 / FEG43-47	1416	22	CR2004 SP2004
UMN20	52	FEG43-47 / FEG55-14	1385	22	CR2004 SP2004
UMN21	52	FEG43-47 / FEG59-36	1415	22	CR2004 SP2004
UMN22	56	FEG59-21 / FEG55-14	1385	22	CR2004 SP2004
UMN23	61	FEG59-21 / FEG56-43	1235	17	CR2004 SP2004

UMN24	88	M98-34 / FEG66-08	1410	22	CR2004 SP2004
UMN25	101	FEG69-38 / M114	1386	22	CR2004 SP2004
UMN26	56	FEG73-49 / M115	1449	23	CR2004 SP2004
UMN27	82	FEG52-01 / FEG69-38	1240	18	CR2004 SP2004
UMN28	89	FEG73-49 / FEG31-68	1406	22	CR2004 SP2004
UMN29	70	M99-52 / FEG66-08	1410	22	CR2004 SP2004
UMN30	66	FEG93-36 / M00-60	1399	23	CR2005 SP2005
UMN31	69	FEG80-74 / FEG67-12	1428	23	CR2005 SP2005
UMN32	71	FEG82-19 / M116	1402	22	CR2005 SP2005
UMN33	67	FEG90-22 / RASMUSSON	1401	22	CR2005 SP2005
UMN34	70	FEG97-44 / M118	1431	20	CR2005 SP2005
UMN35	65	FEG96-22 / M123	1410	22	CR2005 SP2005
UMN36	69	FEG90-31 / M118	1405	23	CR2005 SP2005
UMN37	70	FEG96-22 / RASMUSSON	1401	22	CR2005 SP2005
UMN38	67	ND20407 / M118	1396	22	CR2005 SP2005
UMN39	70	M00-26 / FEG80-06	1445	23	CR2006 SP2006
UMN40	70	M00-33 / QUEST	1421	23	CR2006 SP2006
UMN41	70	M00-33 / FEG66-08	1434	23	CR2006 SP2006
UMN42	70	M119 / FEG96-10	1401	22	CR2006 SP2006
UMN43	70	FEG80-06 / M00-35	1445	23	CR2006 SP2006
UMN44	61	M127 / M00-24	1442	23	CR2006 SP2006
UMN45	40	FEG97-23 / M00-33	1451	23	CR2006 SP2006
UMN46	60	FEG97-56 / RASMUSSON	1400	22	CR2006 SP2006
UMN47	64	FEG117-24 / M00-26	1453	23	CR2006 SP2006
UMN48	61	FEG116-48 / FEG122-60	1418	22	CR2006 SP2006
UMN49	29	CIHO3942 / RASMUSSON	1256	18	CR2006 SP2006

UMN50	40	FEG66-08 / M99-106	1407	22	CR2006 SP2006
UMN51	30	FEG97-05 / M00-26	1448	23	CR2006 SP2006
UMN52	23	COMP351 / M98-102 // RASMUSSON	1399	22	CR2006 SP2006
UMN53	57	M00-51 / M123	1433	23	CR2006 SP2006
UMN54	48	M123 / FEG97-56	1409	22	CR2006 SP2006
UMN55	62	M120 / M123	1160	16	CR2006 SP2006
UMN56	63	QUEST / M123	1476	23	CR2006 SP2006
UMN57	69	FEG118-69 / RASMUSSON	1438	23	CR2007 SP2007
UMN58	69	FEG86-03 / M130	1440	22	CR2007 SP2007
UMN59	70	M129 / FEG100-41	1445	23	CR2007 SP2007
UMN60	61	FEG99-10 / QUEST	1418	23	CR2007 SP2007
UMN61	70	FEG109-44 / FEG100-41	1444	23	CR2007 SP2007
UMN62	69	FEG117-24 / FEG109-44	1446	23	CR2007 SP2007
UMN63	70	FEG148-56 / RASMUSSON	1444	23	CR2007 SP2007
UMN64	70	FEG144-17 / QUEST	1373	23	CR2007 SP2007
UMN65	68	FEG144-17 / FEG89-73	1385	23	CR2007 SP2007
UMN66	70	FEG148-56 / FEG105-33	1435	23	CR2007 SP2007
UMN67	70	FEG150-49 / QUEST	1433	23	CR2007 SP2007
UMN68	70	SEP8-04 / QUEST	1400	22	CR2007 SP2007
UMN69	56	ND23657 / M132	1448	22	CR2008 SP2008
UMN70	68	M133 / QUEST	1421	23	CR2008 MO2008
UMN71	50	M133 / M132	1450	22	CR2008 MO2008
UMN72	59	M03-12 / FEG168-19	1449	23	CR2008 MO2008
UMN73	70	RASMUSSON / FEG154-46	1256	18	CR2009 SP2009
UMN74	70	FEG154-46 / M04-09	1261	18	CR2009 SP2009
UMN75	70	M04-11 / M140	1452	23	CR2009 SP2009

UMN76	70	M04-40 / QUEST	1412	23	CR2009 SP2009
UMN77	70	FEG153-31 / FEG141-18	1449	23	CR2009 SP2009
UMN78	70	FEG153-35 / M137	1271	18	CR2009 SP2009
UMN79	70	FEG160-03 / M137	1451	23	CR2009 SP2009
UMN80	69	FEG167-25 / M141	1261	18	CR2009 SP2009
UMN81	70	FEG169-04 / M136	1327	18	CR2009 SP2009
UMN82	70	FEG182-61 / M115	1406	23	CR2009 SP2009
UMN83	70	FEG183-25 / M115	1408	23	CR2009 SP2009
UMN84	68	M04-40 / FEG192-69	1403	23	CR2009 SP2009
UMN85	66	M143 / M137	1416	23	CR2009 SP2009
UMN86	70	FEG192-69 / RASMUSSON	1405	23	CR2009 SP2009
UMN87	20	NEG2-48 / M137	1445	23	CR2009 SP2009
UMN88	68	M133 / M135	1451	23	CR2009 SP2009
UMN89	70	M137 / SEP10-51	1450	23	CR2009 SP2009
UMN90	46	M135 / NEG5-102	1268	18	CR2009 SP2009
UMN91	42	M132 / CELEBRATION	1417	22	CR2010 SP2010
UMN92	70	M130 / FEG183-52	1406	23	CR2010 SP2010
UMN93	70	FEG204-45 / M129	1404	23	CR2010 SP2010

a Total BOPA I SNPs = 1536

b Total unique FHB-associated SNPs = 23

c CR = Crookston, MO = Morris, SP = St. Paul

Appendix B. MN-ND population parameters and markers used in parental dissimilarity estimates

Population[a]	Cross-type	Pedigree	No. SNPs[b]	No. FHB SNPs[c]
MN-ND01	MNxMN	QUEST / M140	2830	19
MN-ND02	MNxMN	M135 / M138	2498	15
MN-ND03	MNxMN	M135 / M140	2884	19
MN-ND04	MNxMN	M138 / M141	2489	15
MN-ND05	MNxMN	M138 / FEG183-52	2470	15
MN-ND06	MNxMN	M139 / FEG183-52	2851	19
MN-ND07	MNxMN	M140 / M141	2888	19
MN-ND08	MNxMN	M140 / FEG183-52	2852	19
MN-ND09	MNxMN	FEG183-52 / M135	2850	19
MN-ND10	MNxMN	QUEST / FEG183-52	2812	19
MN-ND11	MNxND	QUEST / ND25728	2827	27
MN-ND12	MNxND	M135 / ND24906	2843	27
MN-ND13	MNxND	M138 / ND25728	2487	21
MN-ND14	MNxND	M139 / ND25160	2841	27
MN-ND15	MNxND	M141 / ND25986	2849	27
MN-ND16	MNxND	M141 / ND26104	2841	27
MN-ND17	MNxND	ND24906 / FEG183-52	2847	27
MN-ND18	MNxND	ND25160 / M140	2842	27
MN-ND19	MNxND	ND26036 / M140	2851	27
MN-ND20	MNxND	ND26104 / QUEST	2802	27
MN-ND21	NDXND	ND20448 / ND25160	2811	29
MN-ND22	NDXND	ND20448 / ND25986	2819	29
MN-ND23	NDXND	ND24906 / ND20448	2819	29
MN-ND24	NDXND	ND24906 / ND25652	2845	30
MN-ND25	NDXND	ND25160 / ND24906	2841	30
MN-ND26	NDXND	ND25652 / ND25160	2838	30
MN-ND27	NDXND	ND25652 / ND25986	2847	30
MN-ND28	NDXND	ND25986 / ND26104	2846	30
MN-ND29	NDXND	ND26036 / ND24906	2851	30
MN-ND30	NDXND	ND26104 / ND24906	2843	30

a Ten lines per population with exception of MN-ND01 (nine lines) and MN-ND02 (11 lines)

b Total BOPA I and II SNPs = 3072

c Total unique FHB-associated SNPs = 56

Appendix C. Fusarium head blight marker subset parameters

Mapping Panel	Marker	Chromosome	Gene Position	- log (p)	Panel Applied
MNxMN	11_10132	4	24.59	2.38	UMN, MN-ND
MNxMN	11_11509	1	135.56	2.36	UMN, MN-ND
MNxMN	11_20109	4	26.19	3.18	UMN, MN-ND
MNxMN	11_20119	4	99.28	3.09	UMN, MN-ND
MNxMN	11_20302	4	26.19	4.28	UMN, MN-ND
MNxMN	11_20422	4	24.59	4.54	UMN, MN-ND
MNxMN	11_20603	1	135.56	2.36	UMN, MN-ND
MNxMN	11_20680	4	26.19	3.43	UMN, MN-ND
MNxMN	11_20777	4	26.66	4.62	UMN, MN-ND
MNxMN	11_21070	4	26.19	4.54	UMN, MN-ND
MNxMN	11_21201	7	98.50	2.23	UMN, MN-ND
MNxMN	11_21418	4	26.19	3.44	UMN, MN-ND
MNxMN	12_10507	5	110.26	2.03	MN-ND
MNxMN	12_10752	10	0.00	3.11	MN-ND
MNxMN	12_30390	4	84.30	2.01	MN-ND
MNxMN	12_30705	5	110.26	2.02	MN-ND
MNxMN	12_30793	10	0.00	4.42	MN-ND
MNxMN	12_30823	2	150.67	2.11	MN-ND
MNxMN	12_31428	3	0.00	2.93	MN-ND
MNxND	11_10132	4	24.59	3.60	UMN, MN-ND
MNxND	11_10565	3	19.15	2.83	UMN, MN-ND
MNxND	11_11042	4	51.30	2.07	UMN, MN-ND
MNxND	11_20076	7	0.00	2.26	UMN, MN-ND
MNxND	11_20134	5	106.16	2.38	UMN, MN-ND
MNxND	11_20302	4	26.19	4.69	UMN, MN-ND
MNxND	11_20367	5	75.40	3.19	UMN, MN-ND
MNxND	11_20422	4	24.59	4.73	UMN, MN-ND
MNxND	11_20475	1	92.80	2.03	UMN, MN-ND
MNxND	11_20777	4	26.66	3.11	UMN, MN-ND
MNxND	11_20960	2	74.37	2.02	UMN, MN-ND
MNxND	11_21001	5	75.40	3.01	UMN, MN-ND
MNxND	11_21070	4	26.19	4.72	UMN, MN-ND
MNxND	11_21073	4	48.50	2.04	UMN, MN-ND
MNxND	11_21239	5	69.34	2.57	UMN, MN-ND
MNxND	11_21309	5	76.30	2.49	UMN, MN-ND
MNxND	12_10507	5	110.26	2.35	MN-ND
MNxND	12_10542	10	0.00	3.91	MN-ND

MNxND	12_10752	10	0.00	2.20	MN-ND
MNxND	12_30592	1	52.46	2.31	MN-ND
MNxND	12_30705	5	110.26	2.54	MN-ND
MNxND	12_30793	10	0.00	5.06	MN-ND
MNxND	12_31240	10	0.00	2.22	MN-ND
MNxND	12_31252	2	64.24	2.11	MN-ND
MNxND	12_31394	2	74.37	2.39	MN-ND
MNxND	12_31395	7	99.67	2.06	MN-ND
MNxND	12_31473	10	0.00	4.20	MN-ND
NDxND	11_10132	4	24.59	3.04	MN-ND
NDxND	11_10565	3	19.15	2.38	MN-ND
NDxND	11_10577	4	48.50	2.24	MN-ND
NDxND	11_10756	4	48.50	2.24	MN-ND
NDxND	11_20053	6	72.54	2.46	MN-ND
NDxND	11_20210	4	24.59	2.05	MN-ND
NDxND	11_20302	4	26.19	2.15	MN-ND
NDxND	11_20367	5	75.40	2.17	MN-ND
NDxND	11_20422	4	24.59	2.04	MN-ND
NDxND	11_20488	6	72.54	2.11	MN-ND
NDxND	11_20495	7	25.70	2.13	MN-ND
NDxND	11_20746	6	76.55	2.22	MN-ND
NDxND	11_20889	6	75.21	2.11	MN-ND
NDxND	11_21001	5	75.40	2.06	MN-ND
NDxND	11_21032	6	9.06	2.18	MN-ND
NDxND	11_21070	4	26.19	2.04	MN-ND
NDxND	11_21073	4	48.50	2.32	MN-ND
NDxND	11_21224	6	77.89	2.17	MN-ND
NDxND	12_10395	4	24.59	3.09	MN-ND
NDxND	12_10542	10	0.00	3.94	MN-ND
NDxND	12_10960	1	66.70	2.04	MN-ND
NDxND	12_21386	3	145.89	5.38	MN-ND
NDxND	12_30331	4	48.50	2.10	MN-ND
NDxND	12_30573	6	76.55	2.70	MN-ND
NDxND	12_30777	4	49.50	2.10	MN-ND
NDxND	12_30793	10	0.00	2.20	MN-ND
NDxND	12_30940	6	72.54	2.31	MN-ND
NDxND	12_31382	4	48.50	2.10	MN-ND
NDxND	12_31458	4	12.02	4.45	MN-ND
NDxND	12_31473	10	0.00	4.17	MN-ND