Characterization of Hulled and Hulless Winter Barley, *Hordeum vulgare* L., Through Traditional Breeding and Molecular Techniques

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ABSTRACT

Phenotypic and genotypic characterization of hulled and hulless winter barley (*Hordeum vulgare* L.) is necessary for improvement using traditional and molecular breeding techniques. Identification of genomic regions conferring resistance to Fusarium head blight (caused by *Fusarium graminearum*), leaf rust (caused by *Puccinia hordei* G. Otth), powdery mildew [caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *hordei* Em. Marchal], net blotch (caused by *Pyrenophora teres*) and spot blotch [caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler ex Dastur] will greatly aid in breeding for improved resistance. Determining factors that contribute to yield differences between hulled and hulless genotypes, and identification of markers associated with yield and yield related traits will greatly aid in improvement of hulled and hulless genotypes. The hulled cultivar Nomini, hulless cultivar Eve, and hulless line VA06H-48 were consistently resistant to Fusarium head blight (FHB) and had low deoxynivalenol (DON) accumulation. Screening with molecular markers on chromosomes 2H and 6H for FHB and DON identified quantitative trait loci (QTL) which may confer resistance in Virginia Tech germplasm. Evaluation of hulled and hulless full-sibs from four populations indicated that grain volume weight and protein concentration were significantly ($P \leq 0.05$) higher for hulless genotypes, while seedling emergence and grain ash concentration were significantly ($P \leq 0.05$) higher for hulled genotypes. In linear regression analysis, none of the assessed traits explained yield variation in all populations and environments.

Identification of hulless genotypes having yield potentials similar to those of their hulled sibs should be possible after adjusting for hull weight. A genome wide association study was used to identify chromosome regions governing traits of importance in six-rowed winter barley germplasm and to identify single nucleotide polymorphisms (SNPs) markers for use in a marker-assisted breeding program. Significant SNPs associated with previously described QTL or genes were identified for heading date, test weight, yield, grain protein, polyphenol oxidase activity, and resistance to leaf rust, powdery mildew, net blotch, and spot blotch. Novel QTL also were identified for agronomic, quality, and disease resistance traits. These SNP-trait associations provide the opportunity to directly select for QTL contributing to multiple traits in breeding programs.

DEDICATION

This dissertation is dedicated to my wife and best friend Bethany. Without her love and support I would not have been able to achieve my goals.

I would also like to dedicate my dissertation to my parents, Larry and Patricia Berger. Thank you for your love and support throughout the years. Whether it was making me wear a black bowtie at livestock shows or driving across the country to visit, you have always been there for me. Without your love and support I would not be the man I am today.

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ATTRIBUTIONS

Many colleagues aided in the collection, analysis and interpretation of data presented as chapters in my dissertation. A description of their contributions follows.

Chapter I: Characterization of Fusarium Head Blight (FHB) Resistance and Deoxynivalenol Accumulation in Hulled and Hulless Winter Barley.

Chapter I was submitted to the journal Plant Disease.

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Luciana Rosso, PhD is currently a research associate at Virginia Tech. Dr. Rosso aided in the collection of molecular data and contributed editorial comments.

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Carl Griffey, PhD is currently the W.G. Wysor professor of crop breeding and genetics at Virginia Tech. Dr. Griffey was a co-principal investigator for one of the grants supporting the research and contributed editorial comments.

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Chapter II: Yield potential of hulless versus hulled full-sib genotypes in four winter barley (*Hordeum vulgare* L.) populations.

Chapter II was submitted to the journal Crop Science.

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Wade Thomason, PhD is currently an associate professor at Virginia Tech. Dr. Thomason aided in management recommendations, experiment design, statistical analysis and contributed editorial comments.

Carl Griffey, PhD is currently the W.G. Wysor professor of crop breeding and genetics at Virginia Tech. Dr. Griffey was the principal investigator for one of the grants supporting the research, aided in management decisions and contributed editorial comments.

Chapter III: Marker-Trait Associations in Virginia Tech Winter Barley Identified Using Genome-Wide Mapping.

Chapter III was accepted for publication in the journal Theoretical Applied Genetics. The final publication is available at www.springerlink.com DOI: 10.1007/s00122-012-2011-7

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Gary Muehlbauer, PhD is currently a professor and endowed chair in molecular genetics in the department of agronomy and plant genetics at the University of Minnesota. Dr. Muehlbauer aided in securing funding, analysis and interpretation of data and contributed editorial comments. Additionally Dr. Muehlbauer was a co-principal investigator for one of the grants supporting the research.

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Brian Steffenson, PhD is currently a professor in the department of pathology at the University of Minnesota. Dr. Steffenson collected spot blotch and net blotch data analyzed in the study.

Carl Griffey, PhD is currently the W.G. Wysor professor of crop breeding and genetics at Virginia Tech. Dr. Griffey was a co-principal investigator for one of the grants supporting the research and contributed editorial comments.

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CHAPTER I

Characterization of Fusarium Head Blight (FHB) Resistance and Deoxynivalenol Accumulation in Hulled and Hulless Winter Barley

Berger, G., Green, A., Khatibi, P., Brooks, W., Rosso, L., Liu, S., Griffey, C., and Schmale, D., III.

Note: Chapter I was submitted to the journal Plant Disease.

Abstract

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is one of the most serious diseases impacting the U.S. barley (*Hordeum vulgare* L.) industry. The mycotoxin deoxynivalenol (DON) produced by the pathogen renders grain unmarketable if concentrations exceed threshold values set for end-use markets. Development of cultivars with improved FHB and DON resistance is necessary to ensure minimal losses. Elite hulled and hulless genotypes developed by the Virginia Tech winter barley breeding program were screened in inoculated, mist irrigated FHB nurseries over two years (2009-2010 and 2010-2011) at two locations (Blacksburg, VA and Mount Holly, VA) to validate resistance levels and stability over years and locations. Results demonstrated that barley genotypes varied significantly for resistance to FHB and DON accumulation. Stability of resistance across locations is highly desirable when developing FHB and DON resistant genotypes. The hulled cultivar Nomini, hulless cultivar Eve, and hulless line VA06H-48 were consistently resistant across locations to both FHB and DON accumulation. Screening the genotypes with molecular markers on chromosomes 2H and 6H for FHB and DON revealed QTL regions which may confer resistance in the Virginia Tech germplasm. Ongoing and future work with mapping populations seek to identify novel regions for resistance to FHB and DON accumulation unique to the Virginia Tech breeding program.

Introduction

Winter barley (*Hordeum vulgare* L.) genotypes developed in Virginia are widely adapted to the humid climate's variable rainfall patterns of the mid-Atlantic region of the United States (45). One of the major pathogens plaguing the U.S. barley industry is Fusarium head blight (FHB), caused by *Fusarium graminearu* Schwabe. The mycotoxin deoxynivalenol (DON) produced by the pathogen renders grain unmarketable if concentrations exceed threshold values set for end-use markets. Although hulled barley grown in the mid-Atlantic has historically been

utilized as animal feed (3), use of both hulled and hulless barley as a potential fuel ethanol feedstock has garnered recent interest (8). The hulless phenotype is controlled by a single recessive gene referred to as the *nud* locus on chromosome 7H (44). Hulled and hulless genotypes differ significantly in grain and kernel weight, starch, beta-glucan, oil and ash content (14). Hulless barley genotypes have higher starch concentrations and lower oil and ash concentrations than hulled genotypes (14). Selection of hulled and hulless barley genotypes with high starch and protein levels will help facilitate development of the next generation of barley cultivars suitable for both feed and fuel ethanol production (14).

Recent estimates suggest that the U.S. can produce two billion gallons of fuel ethanol per year from barley alone (37). Distiller's Dried Grains with Solubles (DDGS) is a nutrient rich byproduct of the ethanol fuel fermentation process that can be used as animal feed or to enrich human food products (15). Fuel ethanol producers rely on the sale of DDGS to bolster profits (*24).* In a series of small-scale fuel ethanol fermentation studies DON levels were concentrated 1.6 to 8.2 times in DDGS when compared with ground grain, depending on the barley genotype used in the fermentation mash (21). While the percentage increase in DON concentration in DDGS versus initial grain was highest for genotypes having lower initial DON levels, DDGS produced from these genotypes had the lowest overall DON concentrations (21).

Considering the importance of DDGS to fuel ethanol fermentation plants, breeding for FHB resistance is a critical objective in cultivar development. A key step in breeding for FHB resistance is characterizing levels of FHB and DON resistance in breeding material. Two types of FHB resistance described in wheat (*Triticum aestivum* L.) include: Type I resistance, which is a measure of initial pathogen infection and Type II resistance, which is a measure of the spread of the pathogen in the spike after initial infection (38). Although Type I resistance is used for

barley, Type II resistance is not measured as frequently since movement up and down the spike is not often observed (34). Previous studies have characterized barley as having varying levels of Type I resistance and high levels of Type II resistance (41). Resistance to DON production has also been proposed as a mechanism of resistance in wheat (41). Barley breeders focus on screening elite material for FHB and DON accumulation resistance to identify genotypes with improved levels of resistance (34).

Regions in the barley genome associated with FHB resistance have been characterized in spring barley. Quantitative trait loci (QTL) contributing to FHB resistance have been identified on all seven barley chromosomes (25). QTL for FHB resistance on chromosomes 2H and 6H have most commonly been identified among diverse germplasm (12, 13, 16, 17, 18, 25, 26, 35, 47). The spring barley cultivar Chevron (CI1111) introduced from Switzerland has been widely used as a source of resistance to FHB and kernel discoloration in the Midwest (13). De la Pena et al. (13) identified 10 QTL for FHB resistance, 11 QTL for DON accumulation, and 4 QTL for kernel discoloration in a population derived from Chevron and the elite breeding line M69. A major QTL located on chromosome 2H explained 13.5% of the variation for FHB severity and DON accumulation; however, resistance was linked in coupling to late heading date (13). Mesfin et al. (26) used a two-row by six-row population derived from a cross between the cultivars Fredrickson (two-rowed) and Stander (six-rowed) to determine the effect of heading date and the two-rowed (*Vrs1*) phenotype on FHB resistance. They detected three regions on chromosome 2H associated with FHB resistance with two of the regions being associated with DON accumulation (26). Among these QTL, one also was associated with heading date and another was associated with the *Vrs1* locus (26). A higher level of resistance to FHB has been observed for two-rowed compared to six-rowed barley (40). Nduulu et al. (30) determined that

the relationship between FHB severity, DON concentration and heading date for a coincidental QTL designated as *Qrgz-2H-8* (derived from Chevron) was likely due to tightly linked genes and not pleiotropy. Canci et al. (9) identified a large effect QTL on chromosome 6H which was estimated to decrease FHB severity and kernel discoloration by 56% and 73%, and increase heading date by 0.5 days. Molecular markers developed in spring barley mapping studies have the potential to be used to identify regions conferring resistance in winter barley.

Previous work by Khatibi et al. (22) reported variation for resistance to FHB and DON accumulation in the Virginia Tech winter barley germplasm. Variation in flowering date, FHB incidence, FHB index and DON accumulation were significant among hulled and hulless genotypes (22). However, only a few genotypes were tested over multiple years and many genotypes were only grown in a single location (22). Validation of response of putatively designated resistant and susceptible genotypes and stability to FHB in multiple geographic locations is necessary to determine reliable levels of resistance to FHB and DON in breeding genotypes. Further characterization of winter barley genotypes for FHB resistance using molecular markers also will elucidate the presence of QTL for FHB resistance.

Previous work by (22) identified genotypes as resistant, moderately resistant and susceptible based on performance within one environment. It was hypothesized that the level of resistance may change when tested over multiple years and environments due to changes in environmental conditions which may favor FHB infection and DON accumulation (2). Additionally, pedigree analysis suggests that sources of resistance within the Virginia Tech barley genotypes may be unique to those derived from Chevron (9, 30) and Fredrickson (26). Markers associated with novel sources of FHB and DON accumulation resistance would be of great value when developing new genotypes and selecting for FHB resistance. The specific objectives of the study

are to: 1) validate FHB resistance and its stability in resistant and susceptible genotypes of winter barley in multiple years and locations; 2) determine levels of resistance to DON accumulation in winter barley breeding genotypes and; 3) use phenotypic and genotypic data to aid in the selection of parents for traditional and marker assisted breeding programs, and for development of mapping populations for FHB resistance and DON accumulation.

Materials and Methods

Experimental

Nine hulled and nine hulless winter barley genotypes (Table 1) including three putatively resistant, moderately resistant and susceptible genotypes were selected from the Virginia Tech barley breeding program to further characterize and validate their response to FHB. Genotypes were planted in a randomized complete block with two replications in mist-irrigated nurseries at Blacksburg, VA and Mt. Holly, VA during the 2009-10 and 2010-11 growing seasons. Each experimental unit consisted of a seven-row plot planted at 13.4 m in length with 15.2 cm spacing between rows and a harvested plot area of 19 m^2 to produce sufficient grain for analysis of DON concentration in barley grain and for a detoxification study, as described by Khatibi et al. (21). *Fusarium graminearum* colonized corn (*Zea mays*) kernels were applied to plots at the boot stage in both locations during all years of the study, and plots at Blacksburg were spray inoculated using macroconidia (50,000 spores/ml) applied at 50% heading stage (10, 22). Isolates used in the study, mycotoxin genotype and source location are presented in Table 2 and inoculation protocols were performed as described by Khatibi et al. (22). Over-head mist irrigation was applied at 1-h intervals from 8:00 to 9:00 a.m. and from 4:00 to 5:00 p.m. daily for 3 weeks, except during precipitation events (10, 22). Plots were harvested at maturity (≤16% moisture) using Wintersteiger plot combines (Wintersteiger, Salt Lake City, UT) with the fan set at a low setting to reduce loss of infected grain.

Weather data

Temperature and rainfall data collected at the Kentland Research farm and Warsaw Eastern Virginia Agricultural Research Center (EVAREC) were used in the analysis. The Warsaw EVAREC weather station is the nearest available weather station to the Mt. Holly field location. In Warsaw minimum and maximum daily temperatures and total rainfall for a twenty-four hour period were recorded. Hourly temperature and rainfall for a twenty-four period were collected at the Kentland research farm. For both locations average daily temperatures and total daily rainfall were used in the analysis.

FHB INC, SEV, IND and quantification of DON in grain samples

Plots were rated for FHB incidence (INC) and severity (SEV) to determine FHB resistance. FHB incidence, the percentage of infected heads per 10 random heads sampled at three locations distributed throughout each plot, was measured twice each season (approximately three weeks and four to five weeks after heading). FHB severity, measured as the number of infected spikelets divided by the total number of spikelets for 30 diseased heads per plot, was determined at the same time of the second FHB incidence rating. FHB index (IND) was calculated as [(FHB INC × FHB SEV)/100] for each genotype and used as a measure of FHB resistance. After harvest a subsample of grain from each plot was cleaned on a screen cleaner without air which allowed grain to pass through and for removal of plant debris. Subsamples of grain were used for quantification of DON concentration and Fusarium damaged kernels (FDK), which was calculated as a percentage of infected kernels per random sample of 100 kernels. Concentration of DON was determined in ground whole grain flour subsamples from 100 g samples using gas chromatography and mass spectrometry as described by Khatibi et al. (22).

Molecular marker genotyping

In an effort to elucidate whether QTL for FHB resistance in Virginia Tech's winter barley germplasm are unique, genotypes were screened with markers for QTL *Qrgz-2H-8* (30), *QFhs.umn-2H.2, QFhs.umn-2H.4, QFhs.umn-2H.5 and QDon.umn-2H.1* (26) located on chromosome 2H. Genotypes also were screened with markers for a QTL located on chromosome 6H associated with FHB resistance (9). Leaf tissue was harvested at the two-leaf stage from genotypes planted in the study along with check cultivars Chevron (FHB resistant), Fredrickson (FHB resistant), and Stander (FHB susceptible). Leaf tissue was stored at -80^{o}C prior to grinding with a GenoGrinder (Spex CertiPrep, Metuchen, NJ). DNA isolation followed the protocol established by (36). Direct labeled primers were used for PCR amplifications following Saghai Maroof et al. (31). PCR products were resolved using the method described by (31). Genotypes were visualized by a 3130x*l* Genetic Analyzer (Applied Biosystems, Carlsbad, CA). Scoring of the genotypes was done manually based on allele size.

Statistical analyses

Normality of data was tested using the PROC UNIVARIATE procedure in SAS (version 9.2; SAS Institute, Cary, NC). The Shapiro-Wilk (W) test for normality in PROC UNIVARIATE was used to determine if data were distributed normally. Grand means and standard error of means were determined using PROC MEANS. Correlations between FHB INC and IND, DON and FDK were determined using PROC CORR. Analysis of variance for all data was tested using PROC GLIMMIX. Year, Location and Genotype were treated as fixed effects and Location by Year, nested within blocks, was treated as a random effect. Mean comparisons for genotype within location were calculated using the means procedure in SAS. The critical value for Tukey's Honest Significant Difference (HSD) ($\alpha = 0.05$) was calculated manually

using the method described by Zar (46). Stability of a genotype's response to FHB across locations is indicated by delta (Δ), which was calculated as the difference in respective values for FHB INC and IND, DON, and FDK between locations. Significance of the delta values was tested using Tukey's HSD. Genotypes which had significant values for Δ for the FHB INC and IND, DON and/or FDK were defined as unstable, as determined by Tukey's HSD and designated by p values associated with the tests of significance at alpha = 0.05.

Results

Analysis of variance

A consistent reduction in means occurred for all traits (FHB INC and IND, DON accumulation and FDK) from the 2009-2010 growing season to the 2010-2011 growing season (Table 3). During the 2009-2010 and 2010-2011 growing seasons, mean values were greater for Mt. Holly than Blacksburg for all traits. Mean values ranged from 67.8% to 73.5% for FHB INC, 17.8% to 29.9% for FHB IND, 18.6 ppm to 30.4 ppm and 13.7% to 21.5% for FDK in Mt. Holly. The lowest mean values for all traits were observed during the 2010-2011 growing season in Blacksburg (FHB INC = 63.0%; FHB IND = 14.2%; DON accumulation = 0.87 ppm; and FDK = 8.3%).

An analysis of variance for FHB INC, IND, DON accumulation and FDK is presented in Table 4. Barley genotypes varied significantly ($p \leq 0.05$) for FHB INC and IND, DON accumulation and FDK. There was a significant genotype x location interaction for FHB INC and IND, DON accumulation and FDK, thus prohibiting pooling of data between locations. In contrast, FDK was the only trait having a significant genotype x year interaction. The genotype x year interaction can be attributed to a change in magnitude of mean values in 2009-2010 versus 2010-2011 for moderately susceptible and susceptible genotypes. Although the interaction is

present, the genotype x location interaction for FDK is of more interest and likely has greater ramifications when breeding for resistance. Therefore, only the genotype x location interaction will be discussed further.

Correlations for FHB INC, FHB IND, DON accumulation and FDK

Pearson correlation coefficients were calculated across years for each location (Blacksburg and Mt. Holly) separately due to significance of the genotype x location interaction for all traits (Table 5). FHB INC was significantly ($p \leq 0.001$) and positively correlated with FHB IND at both locations ($r = 0.65$ to 0.70, $p \leq 0.001$). DON accumulation was significantly ($p \leq 0.05$) correlated with FHB INC and FHB IND at both locations. However, correlation values for Mt. Holly ($r = 0.60$ and $r = 0.76$, $p \leq 0.001$) were higher than those for Blacksburg ($r = 0.23$, $p \leq 0.05$ and $r = 0.31$, $p \leq 0.01$). In both locations DON accumulation was highly correlated with FDK ($r = 0.37$, $p \leq 0.01$ in Blacksburg and $r = 0.55$, $p \leq 0.001$ in Mt. Holly).

Resistance to FHB and DON accumulation

Genotypes varied significantly for FHB INC and IND, DON accumulation and FDK (Table 6). The hulled cultivar Nomini, the hulless cultivar Eve and the hulless experimental line VA06H-48 consistently had low values for FHB INC and IND, and DON accumulation over years at both locations. For Mt. Holly and Blacksburg, respectively, these three genotypes had means for FHB INC ranging from 32.5% to 53.3% and 49.2% to 60.8%; FHB Index from 3.5 to 5.5 and 8.6 to 13.8; DON accumulation from 5.6 ppm to 6.5 ppm and 0.3 ppm to 0.7 ppm; and FDK from 3.8% to 9.3% and 5.5% to 9.0%. In contrast, the hulled cultivar Thoroughbred, previously reported as being resistant to FHB and DON accumulation (22), was moderately susceptible to susceptible to FHB depending on location. For Thoroughbred FHB INC ranged from 75.0% to 81.7%; FHB SEV ranged from 31.2% to 37.1%; FHB Index ranged from 23.4 to

31.4; DON accumulation ranged from 30.5 ppm to 1.6 ppm; and FDK ranged from 23.3% to 11.8% at Mt. Holly and Blacksburg, respectively. Other cultivars and experimental genotypes varied from moderately resistant to susceptible at both locations. The experimental line VA06H-25 (FHB INC $\Delta = 20.0$, $p \leq 0.05$; FHB IND $\Delta = 21.8$, $p \leq 0.001$; DON accumulation $\Delta = 40.5$, $p \leq 0.001$; and FDK $\Delta = 13.0$, $p \leq 0.001$) and VA04B-125 (FHB INC $\Delta = 27.5$, $p \leq 0.01$; FHB IND $\Delta = 15.5$, $p \leq 0.05$; DON accumulation $\Delta = 44.6$, $p \leq 0.001$; and FDK $\Delta = 15.3$, $p \leq 0.001$) were unstable across locations for all traits measured in the study. Many moderately susceptible to susceptible genotypes were unstable across locations for DON accumulation. Due to the genotype x location interaction, means for all traits are presented by location.

Molecular characterization of genotypes

Initial markers screens indicated that resistant genotypes Nomini and VA06H-48 do not possess previously reported QTL for FHB resistance (Table 7 and 8). The fragment size in base pairs (146 bp) amplified in Eve for marker GMS03 associated with *Qrgz-2H-8* is identical to that of Chevron (146 bp). However, fragment sizes amplified by Eve for flanking markers identifying *Qrgz-2H-8* were not similar to Chevron (186 bp to 246 bp). Fragment sizes amplified in all genotypes except the hulless cultivar Doyce for marker MWG882, and in cultivars Doyce, Dan and Atlantic for marker ABG072 associated with QFhs.umn-2H.5 were identical to that of Fredrickson (414 bp and 273 bp, respectively). All other fragments amplified by the other six markers, especially in resistant sources Nomini, Eve and VA06H-48 were not similar to those of Fredrickson, suggesting that QTL for FHB resistance in the Virginia Tech breeding program may be unique.

Weather

Average daily temperatures for the period of April to June differed across years and locations. This variation is due to distinct differences in elevation between the research farm at Mt. Holly, VA (17 m elevation) and the Kentland research farm at Blacksburg, VA (531.5 m elevation) and the proximity of Mt. Holly to the Atlantic coast. Overall, Blacksburg had much cooler temperatures (13^{o}C to 23^{o}C for Blacksburg 2010; 13^{o}C to 21^{o}C for Blacksburg 2011; 17^{o}C to 27^{o}C for Mt. Holly 2010; and 17^{o}C to 25^{o}C for Mt. Holly 2011) for the period between heading and harvest when plants are prone to infection, FHB development and DON accumulation.

Daily rainfall varied greatly across locations. During 2010, both locations received heavy natural precipitation events between heading and harvest. While the amount of precipitation was lower at both locations in 2011, the rainfall patterns were similar to those in 2010. Infection and FHB development were greater in magnitude at Mt. Holly than Blacksburg in both years even though mist irrigation was applied twice a day in all test locations to ensure presence of adequate moisture for infection. Additional rainfall and high humidity combined with warmer temperatures at Mt. Holly during both years likely contributed to higher levels of infection and FHB development in this location.

Discussion

With renewed interest in development of barley for traditional and new markets, research is needed to contribute to the improvement of barley genotypes. Although many diseases such as leaf rust (caused by *Puccinia hordei*), powdery mildew (caused by *Blumeria graminis* f. sp. *hordei*), net blotch (caused by *Pyrenophora teres*) and spot blotch (caused by *Cochliobolus sativus*) are important in the mid-Atlantic region (42), these pathogens do not produce

mycotoxins which can accumulate in the grain and render it unmarketable. Damage caused by FHB manifests itself in reduced yields, discolored and shriveled kernels, mycotoxin contamination and reduction in seed quality (29). The current study evaluates a subset of winter barley genotypes classified as resistant, moderately susceptible and susceptible (22). Testing across multiple years and locations in the current study was conducted to validate and further characterize FHB resistance levels and stability of these genotypes. This work is important to characterize FHB and DON resistance in winter barley germplasm. The use of phenotypic and genotypic data also facilitates development of mapping populations and use of marker assisted breeding to further improve FHB resistance in barley. This work extends the work of (22) by characterizing resistance over multiple years and environments, and molecularly characterizing resistance using known markers for resistance QTL on chromosome 2H and 6H.

The interaction between genotype and location was significant for FHB INC, IND and DON accumulation, suggesting that the location of the nurseries played a significant role in determining these factors. For FHB INC and IND, DON accumulation and FDK the genotype x location interaction can be attributed to a change in rank among moderately susceptible and susceptible genotypes resulting from differing levels of infection and DON accumulation between locations. Differences in average daily temperature and to a lesser extent, daily precipitation, likely contributed to this interaction and instability of genotype responses to FHB across locations. Environmental factors such as temperature and high humidity are important to FHB development in cereals (1). Average temperatures (24^{o}C to 29^{o}C), extended periods of high humidity and frequent rainfalls provide the most favorable conditions for FHB development in cereals (20). It is possible that lower average daily temperatures reduced FHB infection and DON accumulation at the nursery location in Blacksburg. The magnitude of FHB and DON

accumulation under field conditions may be related to the amount and frequency of precipitation, humidity and/or dews as FHB infection requires moisture (29, 33). Higher temperatures and humidity combined with daily precipitation at the nursery location in Mt. Holly likely contributed to increased FHB infection and development. Even though spray inoculations of macroconidia at flowering were applied in tests at Blacksburg as well as application of colonized corn kernels, differences in natural background populations of the fungus (e.g., mycotoxin potential, aggressiveness, atmospheric concentrations of spores, etc.) could have contributed to higher FHB infection and development at Mt. Holly (27).

In the current study DON accumulation was highly correlated with FDK ($r = 0.37$, $p \leq 0.01$ in Blacksburg and $r = 0.81$, $p \leq 0.001$ in Mt. Holly) at both locations. Much debate has occurred regarding optimum timing of inoculation and rating for FHB resistance. FHB severity and DON accumulation can increase in barley any time between heading and maturity (33). McCallum and Tekauz (28) observed that the optimal timing of inoculation appeared to be 14 days after heading, which best distinguished levels of susceptibility between genotypes. Often FHB incidence and severity are used as measures of resistance. In general, barley has varying levels of Type I resistance (INC) and high levels of Type II resistance (SEV) (41). Fusarium damaged kernels (FDK) have been used as a measure of resistance to FHB and DON accumulation. Guides to identifying FDK (43) have aided researchers in determining the levels of FDK in grain samples. Jones and Mirocha (19) found a significant positive correlation between visually scabby kernels (synonymous with FDK) and DON concentration. However, inconsistencies in identification of FDK and environmental effects on grain quality often contribute to experimental error and genotype x location interactions for this trait. Due to difficulties in rating of FHB INC and IND, and environmental effects, comparison across

multiple years and locations is necessary to elucidate resistance in breeding material and stability of resistance across years and locations.

Both hulled and hulless genotypes ranged from resistant to susceptible for FHB INC and IND, and DON accumulation (Table 6). Results of the current study validate the high level and stability of resistance to FHB and DON accumulation in winter barley cultivars Nomini and Eve and elite line VA06H-48. Similar levels of resistance were reported for Nomini and Eve (22). However, the putatively resistant genotype Thoroughbred (22) was moderately susceptible to susceptible to FHB in the current study across years and locations. Thoroughbred was rated over multiple years for FHB INC, IND and DON accumulation by Khatibi et al. (22). However, only the scab nursery in Blacksburg, VA was used as a test environment. In that study, FHB INC and IND values for Thoroughbred ranged from 7.5% to 50.0% and 1.0% to 12.3%, respectively (22). When tested over multiple years and environments, in the current study, Thoroughbred had FHB INC values ranging from 75.0% to 81.7% and IND ranging from 23.4% to 31.4%. Similar to results presented for the Blacksburg environment in the current paper, DON values reported by (22) were low (< 6 ppm) for all genotypes tested. Of the ten genotypes tested from 2006 to 2010, DON values for Thoroughbred ranked sixth to tenth and ranged from 0.3 to 6 ppm (22). In the current study, DON values for Thorougbred ranged from 1.6 ppm in Blacksburg to 30.5 ppm in Mt. Holly. It should also be noted that DON values were only significantly correlated with FHB INC and IND in 2006 and 2010 (22). These differences highlight the difficulties faced when rating for FHB resistance and the need for multiple years of data from multiple environments to accurately assess a genotypes level of resistance.

Virginia Tech's barley program focuses on development of hulled and hulless cultivars and, thus, knowledge of variability for FHB resistance within and between these barley types is

critical for breeding progress. In a study by Legzdina and Buerstmayr (23), significant variation in FHB existed among all genotypes tested; however, the overall FHB mean, as measured by Area Under the Disease Progress Curve (AUDPC), did not differ significantly between hulled and hulless genotype classes. However, they did report that DON accumulation of hulled barley was significantly higher than that of hulless barley. The largest concentrations of DON have been reported to be present in the hulls (11) which thresh free from hulless barley. In the current study FHB INC and IND, and DON accumulation did not differ significantly between hulled and hulless genotypes within the Virginia Tech program. Differences in lineage within and among hulled and hulless genotypes in the current study likely contribute to variability in resistance to FHB and DON accumulation.

Differences in marker fragment sizes in Virginia's FHB resistant barley genotypes compared to those in FHB resistant sources reported in previous studies (9, 26, 30) suggests that unique QTL or alleles may be present in winter barley. Currently, little is known about the genomic regions contributing to FHB and DON accumulation in six-rowed winter barley. A majority of resistance sources used in previous mapping studies including Chevron, Fredrickson, Harbin (CiHo 10620) and Zhedar 2 are of European or Asiatic origin (12, 13, 26, 35). With the exception of Chevron, resistance sources used in mapping studies are of the *Vrs1* (two-rowed) phenotype. Crosses between Vrs1 (two-rowed) and *vrs1* (six-rowed) phenotypes increases segregation and complicates the identification of resistant six-rowed material. Consequently, QTL contributing to FHB resistance in two-rowed × six-rowed populations have been linked to the *Vrs1* locus (26) which is of little value to those focusing on development of six-rowed genotypes. Although multiple genotypes amplified the same fragment size for markers identifying *QFhs.umn-2H.5*, further validation is needed to determine if this QTL is present in

Virginia Tech genotypes. Identification of additional FHB resistant six-rowed material is of great value to breeding programs. This supports the need to develop mapping populations to find QTL and markers associated with regions contributing to FHB and DON accumulation resistance in winter barley. Use of association mapping to identify marker-trait associations is becoming widely used within the barley research community. Massman et al. (25) used genome-wide association analysis to identify SNP markers associated with FHB resistance. Currently, mapping populations are being developed by the Virginia Tech barley breeding program from crosses of Nomini × Thoroughbred, Eve × Doyce, and Eve × VA07H-35WS. Populations developed from these crosses will be genotyped with SNP markers and association mapping will be used to identify regions of the genome contributing to FHB and DON accumulation resistance. Markers identified in future studies will hopefully be integrated into marker-assisted selection (MAS) programs.

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Table 1: Barley genotypes tested for FHB resistance in Blacksburg and Mt. Holly, VA during the 2009-2010 and 2010-2011 growing seasons.

Genotype	Type	Pedigree	Year Of Release[a]
Nomini	Hulled	Boone/Henry//VA77-12-41	1991[39]
Callao	Hulled	Boone/Henry//Sussex	1994[32]
Price	Hulled	Callao/SC830366	2002[5]
Thoroughbred	Hulled	VA90-44-110/Plaisant	2003[4]
Atlantic	Hulled	VA97B-176/VA92-42-279	2011
VA04B-125	Hulled	VA97B-178/VA97B-283	Experimental
VA06B-32	Hulled	VA98B-112/VA99B-172	Experimental
VA07B-52	Hulled	VA97B-176//VA96B-113/VA96B-70	Experimental
VA96-44-304	Hulled	Callao/VA89-41-6	Experimental
Doyce	Hulless	CMB79-54//VA90-42-56/VA90-42-22/3/Pamunkey/4/H-585	2003[6]
Eve	Hulless	SC 860974/VA94-42-13	2007[8]
Dan	Hulless	VA96-41-17/ SC872143	2009[7]
VA05H-59	Hulless	VA96-41-35/VA95-41-26//VA92-42-46/3/SC860934	Experimental
VA06H-142	Hulless	Thoroughbred/Doyce	Experimental
VA06H-25	Hulless	Thoroughbred/SC 872143	Experimental
VA06H-31	Hulless	Thoroughbred/SC 872143	Experimental
VA06H-48	Hulless	Thoroughbred//VA96-41-25/SC890573	Experimental
VA07H-35WS	Hulless	Thoroughbred/SC 872143	Experimental

a: Cultivars released by the Virgnia Tech barley breeding program are indicated by their year of release and reference; experimental genotypes are designated as such.

Table 2: Isolate, mycotoxin genotype, and source locations of *Fusarium graminearum* isolates used for preparation of inoculum and infection of barley genotypes planted in Blacksburg and Mt. Holly,VA during the 2009-2010 and 2010-2011 growing seasons.

Growing Season	Isolate ID	TRI3/TRI12 genotype	Source location
2009-2010	VA8n12	15-ADON	Riner, VA
	VA11n9	3-ADON	
	VA12n18	15-ADON	
2010-2011	VA11n9	3-ADON	Riner, VA
	VA8n12	15-ADON	

Table 3: Grand means ± standard error of means for Fusarium head blight (FHB) incidence (INC) and index (IND), deoxynivalenol (DON) accumulation and fusarium damaged kernels (FDK) for genotypes grown in Blacksburg and Mt. Holly, VA during the 2009-2010 and 2010-2011 growing seasons.

	Blacksburg		Mt. Holly	
	2009-2010	2010-2011	2009-2010	2010-2011
Genotypes	18	18	18	18
FHB INC (%)	68.3 ± 1.3	63.0 ± 2.4	73.5 ± 3.6	67.8 ± 3.2
FHB IND (%)	28.2 ± 2.4	14.2 ± 1.4	29.9 ± 2.7	17.8 ± 2.0
DON accumulation (ppm)	0.93 ± 0.14	0.87 ± 0.12	30.4 ± 4.1	18.6 ± 1.7
FDK (%)	12.2 ± 1.2	8.3 ± 0.5	21.5 ± 2.7	13.7 ± 0.9

Table 4: Analysis of variance F-values for barley genotypes evaluated for FHB in Blacksburg and Mt. Holly, VA during the 2009-2010 and 2010-2011 growing seasons.

Effect	df	FHB INC[a]	FHB IND	DON	FDK
Year	1	3.48^{NS}	24.33**	2.68^{NS}	31.45**
Location	1	2.82^{NS}	0.96^{NS}	42.48**	70.77**
Location*Year	1	0.00^{NS}	0.14^{NS}	2.63^{NS}	0.24^{NS}
Location*Year(Block)	4	-	-	-	-
Genotype	17	9.42***	10.32***	3.23***	14.22***
Genotype*Year	17	1.41^{NS}	1.08^{NS}	1.22^{NS}	4.52***
Genotype*Location	17	1.86*	1.96*	2.99***	3.27***
Genotype*Location*Year	17	1.70^{NS}	1.72^{NS}	1.18^{NS}	1.75^{NS}
Residual	68	-	-	-	-
Total	143	-	-	-	-

Asterisks *, **, *** Significant at $p \leq 0.05$, 0.01 and 0.001, respectively.
NS: Non-significant
a: Incidence (INC), Index (IND), Deoxynivalenol (DON) and Fusarium damaged kernels (FDK)

Table 5: Pearson correlations for components of FHB resistance in barley genotypes evaluated in Blacksburg and Mt. Holly, VA averaged over years.

	Blacksburg (BBG)				Mt. Holly (MH)			
	FHB INC[a]	FHB IND	DON Accum.	FDK	FHB INC[a]	FHB IND	DON Accum.	FDK
FHB INC	-	0.74***	0.23*	0.45***	-	0.72***	0.60***	0.57***
FHB IND		-	0.31**	0.70***		-	0.76***	0.65***
DON Conc.			-	0.35**			-	0.55***
FDK				-				-

Asterisks *, **, *** Significant at $p \leq 0.05$, 0.01 and 0.001, respectively

a: Incidence (INC), Index (IND), Deoxynivalenol (DON) and Fusarium damaged kernels (FDK)

Table 6: FHB INC, IND, DON accumulation and FDK in barley genotypes evaluated at Blacksburg and Mt. Holly, VA averaged across years.

Genotype	Type	FHB INC [a] — % —			FHB Index — Value —			DON Accum. — ppm —			FDK — % —		
		MH[b]	BBG	Δ[c]	MH	BBG	Δ	MH	BBG	Δ	MH	BBG	Δ
Eve	Hulless	32.5	49.2	-16.7	3.5	13.8	-10.3	5.6	0.3	5.2	7.3	5.5	1.8
VA06H-48	Hulless	37.5	42.5	-5.0	5.0	8.6	-3.6	6.5	0.3	6.2	3.8	9.0	-5.3
Nomini	Hulled	53.3	60.8	-7.5	5.5	12.0	-6.5	5.8	0.7	5.1	9.3	5.5	3.8
VA06B-32	Hulled	73.3	64.2	9.2	13.3	14.6	-1.3	14.1	0.5	13.7	10.0	9.5	0.5
VA06H-31	Hulless	57.5	60.0	-2.5	19.0	13.3	5.7	13.5	1.1	12.4	7.8	5.0	2.8
Callao	Hulled	74.2	64.2	10.0	19.2	14.3	4.8	16.7	0.5	16.3*	11.5	7.8	3.8
VA07B-52	Hulled	69.2	60.8	8.3	20.7	14.4	6.2	25.1	0.7	24.4**	12.0	6.0	6.0*
Thoroughbred	Hulled	75.0	81.7	-6.7	23.4	31.4	-8.0	30.5	1.6	28.9***	23.3	11.8	11.5***
Dan	Hulless	60.8	59.2	1.7	24.3	24.7	-0.4	21.6	1.2	20.4*	20.0	13.5	6.5*
VA05H-59	Hulless	72.5	47.5	25.0**	25.8	16.2	9.5	23.7	1.4	22.3**	23.3	9.5	13.8***
Atlantic	Hulled	82.5	72.5	10.0	26.7	20.6	6.1	34.0	0.7	33.3***	13.3	7.3	6.0*
Price	Hulled	80.0	76.7	3.3	26.8	28.2	-1.4	29.9	0.3	29.6***	17.5	10.8	6.8*
VA06H-142	Hulless	69.2	75.8	-6.7	27.2	24.8	2.4	12.5	0.5	12.0	13.5	10.8	2.8
VA96-44-304	Hulled	90.0	77.5	12.5	32.4	29.3	3.1	37.8	0.9	37.0***	16.0	11.0	5.0
VA07H-35WS	Hulless	75.0	65.0	10.0	34.3	24.6	9.7	38.8	3.3	35.6***	18.0	14.3	3.8
VA04B-125	Hulled	95.8	68.3	27.5**	35.8	20.4	15.5*	45.2	0.6	44.6***	23.8	8.5	15.3***
Doyce	Hulless	80.0	82.5	-2.5	36.5	42.6	-6.2	37.5	0.7	36.8***	29.0	23.0	6.0*
VA06H-25	Hulless	93.3	73.3	20.0*	49.5	27.7	21.8***	42.1	1.7	40.5***	25.0	12.0	13.0***
Mean	-	70.6	65.6	1.2	23.8	21.2	2.6	24.5	0.9	23.6	15.8	10.0	5.8
Tukey's HSD[§]	-	29.1	29.9	17.5	17.9	22.6	12.0	35.8	1.3	14.9	11.1	7.4	5.6

Asterisks *, **, *** Significant at $p \leq 0.05$, 0.01 and 0.001, respectively

a: Incidence (INC), Index (IND), Deoxynivalenol (DON) and Fusarium damaged kernels (FDK)

b: Mt. Holly (MH) and Blacksburg (BBG)

c: Change across locations for component of FHB resistance (Δ = MH – BBG)

d: Tukey's Honest Significant Difference (HSD)

Table 7: Fragment size in base-pairs (bp) for markers associated with QTL on barley chromosomes 2H and 6H screened in Virginia Tech genotypes and known resistant genotype Chevron and susceptible genotype Stander.

QTL Name	Qrgz-2H-8[a]	Qrgz-2H-8[a]	Qrgz-2H-8[a]	Qrgz-2H-8[a]	Qrgz-2H-8[a]	QTL #10[b]	QTL #10[b]	QTL#10[b]
Marker[c]	EBmac0615	Bmag0015	GMS03	GBM1023	Bmac0132	Bmag0807	Bmag0870	Bmag0613
Chevron[d]	197	181	146	246	186	117	117	167
Stander[e]	195	177	144	237	NP	122	122	161
Eve	193	183	146	237	NP	121	118	165
VA06H-48	195	NP	144	238	NP	NP	117	163
Nomini	193	183	NP	239	NP	119	116	161
VA06B-32	193	183	NP	237	NP	122	120	161
VA06H-31	193	183	NP	237	NP	124	116	172
Callao	193	183	NP	237	NP	121	120	161
VA07B-52	193	183	146	239	NP	121	118	161
Thoroughbred	193	183	146	237	186	118	118	161
Dan	195	NP	144	239	NP	120	118	161
VA05H-59	195	183	144	237	NP	124	116	176
Atlantic	194	183	146	237	NP	120	118	161
Price	195	183	140	237	186	119	116	161
VA06H-142	196	NP	NP	237	NP	112	118	161
VA96-44-304	193	183	146	237	NP	119	118	161
VA07H-35	193	183	146	NP	NP	119	118	172
VA04B-125	193	183	146	238	NP	121	118	161
Doyce	195	NP	144	237	NP	121	118	161
VA06H-25	193	183	146	237	NP	102	NP	172

a: QTL described by (30) for Fusarium head blight (FHB) severity (SEV), deoxynivalenol accumulation and heading date on chromosome (chr.) 2H.

b: QTL described by (9) for FHB SEV and kernel discoloration on chr. 6H.

c: Marker identifying QTL in resistance source.

d: Chevron is known resistant genotype for these markers.

e: Stander is known susceptible genotype described by Canci et al. (2004).

NP: No peak

Table 8: Fragment size in base-pairs (bp) for markers associated with QTL on barley chromosomes 2H screened in Virginia Tech genotypes and known resistant genotype Fredrickson.

QTL Name[a]	QDon.umn -2H.1	QFhs.umn-2H.2	QFhs.umn -2H.2	QFhs.umn-2H.2	QFhs.umn-2H.4/5	QFhs.umn -2H.5	QDon.umn -2H.1	QFhs.umn-2H.2
Marker[b]	Bmag0125	EBmac521a	Bmag0140	HVBKasi	MWG882	ABG072	Bmag0125	EBmac521a
Fredrickson[c]	141	159	152	201	414	273	141	159
Eve	119	163	160	182	414	273	119	163
VA06H-48	131	163	157	171	414	273	131	163
Nomini	119	161	157	200	414	273	119	161
VA06B-32	131	163	161	201	414	273	131	163
VA06H-31	133	163	157	189	414	273	133	163
Callao	131	163	159	201	414	273	131	163
VA07B-52	131	163	159	189	414	273	131	163
Thoroughbred	131	163	157	202	414	273	131	163
Dan	133	163	157	182	414	274	133	163
VA05H-59	133	163	157	183	414	273	133	163
Atlantic	131	163	159	201	414	274	131	163
Price	131	NP	159	201	414	273	131	NP
VA06H-142	133	163	155	184	414	273	133	163
VA96-44-304	153	NP	159	189	414	273	153	NP
VA07H-35	154	NP	157	201	414	273	154	NP
VA04B-125	131	163	159	201	414	273	131	163
Doyce	133	163	155	143	416	NP	133	163
VA06H-25	131	NP	157	200	414	273	131	NP

a: QTL for Fusarium head blight severity and deoxynivalenol accumulation described by (26) on chromosome 2H.

b: Marker identifying QTL in resistant genotype.

c: Fredrickson is known resistant genotype.

NP: No peak

CHAPTER II

Yield potential of hulless versus hulled full-sib genotypes in four winter barley (*Hordeum vulgare* L.) populations

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ABSTRACT

Determining what factors contribute to the yield difference that exists between hulled and hulless winter barley (*Hordeum vulgare* L.) is necessary for continued yield improvement in the hulless barley germplasm pool. This yield difference is a major factor limiting the acceptance and production of hulless barley as an alternative to traditional hulled barley. Experiments were conducted in Warsaw, VA during 2010-11 and 2011-12 and in Blacksburg, VA during 2011-12. Traits assessed included seedling emergence, plants m^{-2}, normalized difference vegetative index (NDVI), heading date, spikes m^{-2}, plant height, lodging, yield, grain volume weight, 1000 kernel weight, spikelets $spike^{-1}$, seeds $spike^{-1}$, seed weight $spike^{-1}$, ash, crude fiber, fat, protein, and starch. Grain volume weight and protein concentration were significantly ($p \leq 0.05$) higher for hulless genotypes while seedling emergence and grain ash concentration were significantly ($p \leq 0.05$) higher for hulled genotypes. Other traits measured in the study varied by population and environment. On the basis of linear regression analysis, none of the traits explained yield variation in all populations and environments. Prior to adjustment for hull weight, hulless genotypes yielded significantly ($p \leq 0.05$) less than hulled genotypes on average in all populations at Warsaw and for population 1 at Blacksburg during the 2011-12 growing season. After adjustment for hull weight, yield potential of select hulless genotypes was statistically similar to that of hulled genotypes. Thus it is possible to identify hulless genotypes having yield potentials equal to those of their hulled sibs.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an early maturing cereal crop that is well adapted to diverse environments (Liu et al., 1996). It is especially suited to the mid-Atlantic region of the United States, comprised of many agricultural soils that have less water-holding capacity than

required for corn (*Zea mays* L.) production (Thomason et al., 2009). In the mid-Atlantic states, barley is frequently planted after soybeans (*Glycine max* L.) in a doublecrop system (Thomason et al., 2009). Although wheat (*Triticum aesitivum* L.) is also used in these doublecrop systems, soybean yields are generally higher following winter barley (Browning, 2011; Camper et al., 1972). This can be attributed to the earlier harvest date of barley which allows for an earlier planting date of soybeans (Camper et al., 1972). Soybeans grown after barley yielded 462 kg ha^{-1} to 955 kg ha^{-1} more than soybean double-cropped after wheat in three of the six environments in a study by Browning (2011).

The Virginia Tech breeding program has focused on development of hulled and hulless six-rowed winter barley cultivars for use in feed and biofuel industries. Hulled barley has a covered caryopsis where the hull, made up of the outer lemma and inner palea, remains firmly attached to pericarp endosperm at maturity (Taketa et al., 2008). In contrast, hulless barley has kernels with non-adhering hulls that are removed during threshing (Xue et al., 1997). The hulless trait is controlled by a single recessive gene commonly referred to as the *nud* gene (Liu et al., 1996). Taketa et al. (2008) reported that the covered/naked caryopsis is controlled by a single locus (*nud*) on chromosome 7HL.

Few studies have compared hulled and hulless barley genotypes for agronomic and quality traits. Hulless barley is more desirable than hulled barley for many uses because of higher digestible energy due to elevated starch and reduced fiber concentrations which can be attributed to removal of the hull (Griffey et al., 2010; Ingledew et al., 1995). Starch is the most abundant component of hulless barley accounting for 57% to 75% of grain on a dry weight basis (Bhatty, 1999; Griffey et al., 2010; Liu et al., 1996). Concurrently, hulless barley also has the potential to provide growers with a high protein source for food and animal feeds. Liu et al.

(1996) reported that hulless lines have lower neutral detergent fiber (NDF) and acid detergent fiber (ADF) than hulled lines and, therefore, have the potential to provide ruminants with more digestible energy. Research has shown that animals fed hulless barley had higher weight gains than those fed hulled barley (Gill et al., 1966). Alternatively, swine fed a hulled barley, high-fat ration had slightly improved feeding efficiency compared to pigs fed corn, hulless or hulled barley low-fat rations (Harper et al., 2004). However, digestibility of dry matter (DM), energy, and protein in the hulled barley low-fat diet was 3.9% to 6.9% less than in the other rations (Harper et al., 2004). Use of hulless barley in a pelleted grower pig diet resulted in greater DM, energy, and protein digestibility than in a similar diet using high quality hulled barley (Harper et al., 2004). Both hulled and hulless barley can be used at a relatively high inclusion rate (up to 41%) and produce growth performance comparable to similar diets based on corn or wheat.

Although the hulless trait has been associated with the aforementioned beneficial nutritional traits it has also been associated with decreased yield, seed weight, and seedling emergence (Choo et al., 2001). The hulless trait has not been associated with changes in heading date, maturity, or spike density (Choo et al., 2001). Decreased yield relative to that of hulled barley is the major factor limiting the acceptance and production of hulless barley as an alternative to hulled barley. It has been commonly reported that hulless barley yields on average are 10% to 30% less than those of hulled barley (Choo et al., 2001; Liu et al., 1996). When yields of hulless genotypes were adjusted by adding 15% to 19% to account for hull weight loss, they still were significantly lower than those of the hulled lines (Choo et al., 2001). The hulless (*nud*) gene was reported to explain 47% to 57% of the total variation for yield (Choo et al., 2001). Significantly higher grain volume weight and lower kernel weights have been reported for Virginia Tech hulless barley when compared to hulled barley (Griffey et al., 2010). The *nud*

gene was reported to contribute 85% to 95% of the total variation for grain volume weight (Choo et al., 2001). It has been hypothesized that the *nud* gene may have a pleiotropic effect on yield or might be linked to a yield QTL (Choo et al., 2001). A favorable allele contributing to an increase in yield is fixed in hulled genotypes but absent in hulless genotypes within the Virginia Tech barley program (Berger et al., 2012). A single nucleotide polymorphism (SNP) marker (2_0685) associated with the favorable allele mapped 1.09 centimorgans (cM) from the *nud* locus suggests that breakage of any negative linkages through recombination would be difficult (Szucs et al., 2009; Berger et al., 2012). Therefore, it is necessary to determine whether lower yields in hulless genotypes are due solely to the direct effect of the *nud* locus on hull attachment, or are also due to other negative pleiotrophic effects of this locus or other tightly linked loci on yield.

Current hulless cultivars yield 10% to 30% percent less than hulled cultivars. Thus emphasis has been placed on determining what traits and genes differ between hulled and hulless genotypes, and whether these factors can be altered to make hulless barley competitive in yield with traditional hulled barley. In the mid-Atlantic region, higher seeding rates to achieve higher plant densities in the field have been used to reduce yield differences between hulless and hulled barley cultivars (Thomason et al., 2009).

Determining which traits differ significantly between hulled and hulless genotypes and which traits contribute most to yield would aid in improving yields in both hulled and hulless barley. Analysis of yield components is an effective way to investigate relationships among traits and how they affect yield. In previous studies, yield components associated with yield included spikes per plant or unit area, kernels per spike, and kernel weight (Hockett and Nilan, 1985). In a study on genetic improvement of malting barley cultivars released since 1920, it was

determined that increases in yield could be attributed to improvements in lodging resistance, harvest index (HI), kernel weight, and spike number (Wych and Rasmusson, 1983). Similarly, Abeledo et al. (2003) reported that the main component associated with yield was number of grain m^{-2} due to variation in spikes m^{-2}. In a review of Western Canadian barley cultivars released from 1910 to 1987, the authors concluded that yield improvement was associated with increased harvest indices and reduced lodging rather than to a change in specific yield components (Jedel and Helm, 1994). Regarding selection strategies for yield, Puri et al. (1982) suggested that kernel weight per spike is an easily measurable trait associated with yield in barley. Rasmusson and Cannell (1970) demonstrated that spike number was consistently associated with yield while the effectiveness of selection for kernel weight and seeds $spike^{-1}$ varied by population and environment. Correlations between yield components and yield have been reported to vary between populations and environments (Abeledo et al., 2003; Bulman et al., 1993; Jedel and Helm, 1994; Puri et al., 1982; Wych and Rasmusson, 1983). Although multiple studies have been conducted to determine the factors contributing to yield improvement in hulled barley, few such studies have been conducted in hulless barley. Further research is needed to determine trait differences and alleles governing them in hulled and hulless barley and which of these factors contribute significantly to yield.

A reasonable hypothesis to ascertain is that development of hulless barley genotypes having similar or equal yield potential as hulled barley genotypes is possible when accounting for hull weight in yield comparisons. Albeit, breeding histories, timeframes, and efforts related to improvement of hulless versus hulled barley must be considered as well as differences in genetic backgrounds and diversity. In addition to the hulless trait, other physical and genetic factors likely contribute to yield differences between hulled and hulless genotypes. Studies

focusing on factors contributing to yield differences between hulled and hulless genotypes have been limited to date (Choo et al., 2001; Thomason et al., 2009). The current study builds on this body of literature and aims to identify factors that contribute to yield differences among full-sibs of hulled and hulless genotypes. The objectives of this study are to analyze yield potential, and determine which traits differ and how they contribute to yield differences in four full-sib populations of hulled and hulless barley.

MATERIALS AND METHODS

Experimental

Hulled and hulless barley populations 1 (Doyce x Spont03-44), 7 (Doyce x Spont03-48), 4 (VA04HDH-6 x Thoroughbred), and 5 (Thoroughbred x VA04HDH-19) were derived and advanced from four hulled x hulless crosses. 'Doyce' (PI 634932) was the first hulless cultivar developed by the Virginia Tech barley breeding program and represented the highest yield potential for hulless barley at the time of its release in 2003 (Brooks et al. 2005a). 'Thoroughbred' (PI 634933) released by the program in 2003, is a high yielding hulled cultivar (Brooks et al. 2005b). Hulless parental lines VA04HDH-6 and VA04HDH-19 are double haploid experimental lines derived from hulled x hulless crosses. Line VA04HDH-6 was derived from a cross of 'Price' (PI 632708), a short-awned, six-rowed, hulled winter barley (Brooks et al., 2005) to Doyce. Line VA04HDH-19 was derived from a cross of Price to VA00H-214, an experimental hulless line derived from 'H-585' (VA75-42-45/SC793556//'Lokian') / VA93-42-54 and VA93-42-57. Ancestry of VA75-42-45 is 'Jotun' (PI467357) / 4*'Rogers' (PI539127, Lambert, 1958) /3/ 'Cebada Capa' (PI539113) / 'Wong' (PI539117, Jensen, 1964a) // awnleted selection of 'Hudson' (CIho8067, Jensen, 1964b). Line SC793556 is a reselection from 'Redhill' (CIho15830, Graham et al., 1982), and 'Lokian' (CIho2457) is a hulless winter-habit

barley from China. VA93-42-54 and VA93-42-57 were derived from a series of crosses comprised of CI's 9623, 9658, 9708, BYDV Resistant 'Atlas' / 'Hanover' (CI13197, Starling et al., 1970) or 'Rapidan' (CI14006, Starling et al., 1973) /4/ 'Harrison' (CIho10667, Caldwell et al., 1966) /3/ Cebada Capa / Wong // awnleted selection of Hudson /5/ 'Barsoy' (CI 11904, Finker et al., 1968). Hulled parental lines Spont03-44 and Spont03-48 were derived from a *Hordeum spontaneum* L. selection (R9) backcrossed four times to Barsoy, a six-rowed hulled barley. Hulled and hulless sub-populations (F_4 generation) were derived from each of four populations segregating for the hulless trait grown at Warsaw and Blacksburg, Virginia in 2009. One hundred spikes from each population grown at both environments were harvested, threshed, and separated into hulled and hulless classes and planted in 1.2 m headrows along with parental and check genotypes at Warsaw, VA in fall 2009.

A total of 890 hulled and 848 hulless headrows were evaluated during the 2009-10 growing season for uniformity. Headrows segregating for spike type, awn type, awn length, and plant color were eliminated to facilitate selection of recombinant inbred lines (RILs) that visually were most homogenous for genotype. Headrows were harvested and threshed individually. Grain from each of the harvested lines was weighed and evaluated for seed type e.g. hulled or hulless. Hulled lines having less than 70 g of seed and hulless lines with less than 75 g were eliminated as these are the recommended seeding rates required to plant yield plots. Additionally, lines segregating for hulled and hulless seeds were identified and eliminated from the study. Hulless genotypes with >5% hull attachment were discarded. A total of 75 hulled and hulless genotypes randomly chosen from each of the four populations were evaluated in single yield plots during the 2010-11 growing season. From these initial 75 hulled and hulless genotypes, 25 hulled and hulless genotypes for each of the four populations were advanced and

evaluated in replicated yield tests during the 2011-12 growing season. Genotypes were advanced on the basis of line homogeneity and lodging resistance. Genotypes with weak straw are prone to excessive lodging which can confound yield measurements due to loss of grain prior to and at harvest. Lines segregating for hull type or hulless genotypes with >5% hull attachment were not advanced. The reduction in lines tested served to eliminate genotypes that might confound yield potential estimates due to segregation of hull attachment and/or lodging and reduce lines to a number that could be feasibly included in replicated yield tests at two locations.

Plots were planted on 17 and 18 October 2010 at Warsaw, on 29 September 2011 at Blacksburg, and 17 October 2011 at Warsaw. Each seven-row yield plot, which was 2.7 m in length with 15.2 cm row spacing, was designated as an experimental unit. The harvested plot area was 4.2 m^2. Hulled plots were seeded at a density of 473 seeds m^{-2} and hulless plots were seeded at 654 seeds m^{-2} based on the kernel weight of the seed source. Hulless plots were seeded at higher densities to compensate for poorer germination of hulless genotypes and to equalize plant stands for yield comparisons. Seed was treated with Raxil MD® fungicide (tebuconazole and metalaxyl, Bayer Crop Science) at a rate of 15.3 mL active ingredient (a.i.) per 45.5 kg to control seedling diseases. Weed control was achieved using Harmony-Extra SG® (DuPont) at Warsaw and Blacksburg, and Starane® (Dow Agro Sciences) at Warsaw at rates recommended by Hagood and Herbert (2012). Management practices, fall nutrient management and spring nitrogen (N) applications, were based on recommendations from the Virginia Cooperative Extension Soil Testing Laboratory and standard practices for winter barley production (Brann et al. 2000). The growth regulator Trinexipac-ethyl was applied between growth stage (GS) 25 and 30 (Zadoks et al., 1974) at a rate of 104.8 g a.i. ha^{-1} in both years and environments to minimize yield losses due to lodging. The foliar fungicides Tilt® (Propiconazole, Syngenta), primarily

used for control of powdery mildew [caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *hordei* Em. Marchal], and Prosaro® (Prothioconazole, Tebuconazole, Bayer Crop Science), primarily used for control of leaf rust [caused by *Puccinia hordei* G. Otth], were applied to plots during both years of the study to limit yield losses due to foliar plant pathogens. Tilt® was applied to plots at a rate of 126 g a.i. ha^{-1} on 4 April 2011 at Warsaw and 2 April 2012 at Blacksburg and at a rate of 63 g a.i. ha^{-1} on 3 March 2012 at Warsaw. Prosaro® was applied at a rate of 247 g a.i. ha^{-1} on 27 April 2011 and 3 April 2012 at Warsaw, and 17 April 2012 at Blacksburg.

Field evaluations

Plants m^{-2} was estimated by counting the number of seedlings in a uniform 0.305 m section of the three center rows at GS 12 in Warsaw and Blacksburg. The initial seeding rate m^{-2} and plants m^{-2} were used to estimate percent seedling emergence in both hulled and hulless genotypes. Tiller m^{-2} were estimated at GS 25 using the Normalized Difference Vegetative Index (NDVI) as described by Phillips et al. (2004) using a Greenseeker® optical sensor (NTech Industries, Trimble Agriculture, Sunnyvale, CA). Heading date was recorded as Julian (days from Jan 1) when spikes had fully emerged from the boot in 50% of the plot. In the 2011-12 tests, maturity date was recorded as the Julian date when 50% of the upper peduncles, at the point where it joins the spike (approximately 1 cm), in the plot were yellow and denoting physiological maturity. Grain fill period was calculated for 2012 as the number of days between the recorded heading date and maturity date. Spikes m^{-2} was estimated by counting spikes within 1 m of the plot at GS 90. Three representative spikes were removed from each plot and subsequently were used to determine spikelets $spike^{-1}$, seeds $spike^{-1}$, seed weight $spike^{-1}$, and floret fertility. Plant height and lodging were recorded at harvest (GS 92). Plant height was measured at two representative sections within each plot. Lodging was recorded on a 0 to 9 scale

(0 = no lodging and 9 = completely lodged). Entire plots were harvested using Wintersteiger (Salt Lake City, UT) plot combines. Grain yield was calculated from the total mass of grain harvested from each plot after adjustment to 13.5% moisture. A DICKEY-john® GAC 2100b machine (DICKEY-john, Minneapolis, MN) was used to determine moisture and grain volume weight for subsamples of grain taken from harvested plot seed. Subsamples were further cleaned using a Wintersteiger (Salt Lake City, UT) laboratory thresher to remove any extraneous material. Kernel weight was measured from a 500-kernel subsample taken from the cleaned subsamples. Percent hull attachment was visually estimated as the number of seed retaining hulls after threshing and cleaning of a 100 seed subsample.

Whole grain concentrations of ash, crude fiber, fat, protein, and starch were estimated on cleaned subsamples from each plot on an XDS Rapid Content Analyzer (Foss NIR Systems, Inc. Laurel, MD). Raw data values obtained from the XDS Rapid Content Analyzer were corrected using a bias adjustment based on calibrations derived from analysis of NIR versus actual wet chemistry values for these grain components from independent grain samples.

Statistical Analysis

In the 2010-11 test, plots were planted in a modified augmented design (MAD) (Type II) (Lin and Poushinsky, 1989) due to a lack of sufficient seed to plant a replicated study. Hulled and hulless lines were planted in separate parallel blocks to allow for harvesting of each type of barley using ideal threshing parameters. Within each block the MAD (type 2) design is structured as a split plot with whole plots arranged in rows and columns, with five rectangular subplots per whole plot (Lin and Poushinsky, 1989). The center subplot for each whole plot is designated as the control plot and has a line assigned to it as the control (Lin and Poushinsky, 1989). The hulled cultivar Thoroughbred and the hulless cultivar Doyce were assigned as

control lines in their respective blocks. A total of twenty whole plots per block were randomly derived and the hulled cultivars Price and Thoroughbred and hulless cultivars Doyce and Eve were assigned to random subplots within these whole plots. These designated control subplots were used to estimate subplot error. Experimental lines were then randomly allocated to remaining subplots in each whole plot based on population. The Glimmix procedure (proc) in SAS 9.2 (SAS Institute, Cary, NC) was used for analysis of variance as described by Lin and Poushinsky (1989).

During the 2011-12 growing season the experiment was planted as a split-split plot design with two replicates at Blacksburg and three replicates at Warsaw. The hulled and hulless groups were assigned to the main plot factor, populations were assigned to the sub-plots, and genotypes were assigned to the sub-sub-plots. Proc Glimmix was used for analysis of variance, with Environment, Block, Population, and Genotype treated as fixed effects and Environment nested within Replications, Block by Environment nested within Replications, and Block by Population by Environment nested within Replications as random effects.

Proc Means was used for pooled mean comparisons of experimental hulled and hulless genotypes within population for each year and environment. Prior to regression analysis, collinearity analysis was performed in Proc Reg (regression). Any trait exhibiting non-acceptable collinearity was removed from the dataset prior to further analysis. Proc Reg was used to develop multivariable models for yield within each population, year, and environment with a significance level of $\alpha=0.05$.

Least-Square (LS) Means obtained from Proc Glimmix were used for generating the plots of non-adjusted yields within populations and environment. In order to assess whether the genetic yield potential of the hulless genotypes was equal to those of their full-sib hulled

counterparts within population, yield values for hulless genotypes within populations and environments were adjusted for the hull weight difference between the hulled and hulless genotypes of a population. Previously, Choo et al. (2001) adjusted yields of hulless genotypes by determining the average seed weight difference between hulled and hulless genotypes within environments. The goal was to estimate the percent contribution of hull weight to total grain weight (Choo et al., 2001). Adjustments were then made by adding 15% and 19% to yields of hulless genotypes to account for the loss of hull weight at threshing when grown in two independent environments (Choo et al., 2001). In the current study, the adjustment value was determined by calculating the percent difference for 1000 kernel weight between hulled and hulless genotypes within populations and environments. Grain yield of hulless genotypes were then adjusted by adding the percent difference back to the non-adjusted grain yield value within populations and environments. The goal of the adjustment was to standardize the yield of hulled and hulless genotypes to determine if hulless genotypes could produce grain yields similar to hulled genotypes when accounting for differences arising from weight loss due to hull removal at threshing. Therefore this adjustment allows for estimation of yield potential differences while taking into account the inherent difference in yield due hull weight without the need to experimentally remove the hulls of hulled genotypes to compare yield potential. Previous estimates of hull weight contribution to total grain weight are between 7% and 19% (Anderson and Schroeder, 2010; Choo et al., 2001; Price and Parsons, 1979; Xue et al., 1997). Standard errors (S.E.) are displayed for comparison of adjusted yields of hulless genotypes with yields of hulled genotypes in the graphs.

RESULTS

Analysis of MAD Type II

Analysis of variance for the MAD Type II design for yield is presented in Table 1. Significant ($p \leq 0.05$) block effects were observed for all populations. Column effect, row × block, and column × block interactions also were significant for population 5, and the column × block interaction was significant for populations 4 and 7. Therefore, adjustment of yield values was necessary to account for variation.

Analysis of variance for replicated trials

Analyses of variance for agronomic and grain compositional traits of barley genotypes grown in replicated tests at Blacksburg and Warsaw, VA during the 2011-12 growing season are presented in Tables 2 and 3. Barley genotypes varied significantly ($p \leq 0.05$) for all traits except 1000 kernel weight. There was a significant genotype x environment interaction for all traits except 1000 kernel weight, plants m^{-2}, seedling emergence, and NDVI, which prohibited pooling of data across environments. The genotype × environment interaction can be attributed to changes in mean values which caused a change in ranking of genotypes across environments (data not shown). Barley yellow dwarf virus infection and late spring freeze at heading in Blacksburg likely contributed to increased variation between environments. Due to the genotype × environment interaction for a majority of the traits measured, further analysis and discussion are presented by environment.

Pooled mean comparisons of hulled and hulless genotypes

Pooled mean comparisons were calculated by environment (Warsaw and Blacksburg) and year (2011 and 2012) for hulled and hulless full-sib genotypes of the four populations. Yield (kg ha^{-1}), grain volume weight (g $liter^{-1}$), 1000 kernel weight (g), plants m^{-2}, seedling emergence

(%), NDVI (value), lodging (0-9), net blotch (0-9), ash (%), crude fiber (%), fat (%), and protein (%) varied significantly ($p \leq 0.05$) between hulled and hulless genotypes in all populations during the 2010-11 growing season (Table 4). Higher values for yield, 1000 kernel weight, seedling emergence, ash, and fat were observed for the hulled genotypes in all four populations. Higher values for grain volume weight, NDVI, lodging, net blotch, crude fiber, and protein were observed for the hulless genotypes in all four populations. Significance of all other traits varied between hulled and hulless genotypes in the four populations.

Similar to results from 2010-11, hulled and hulless genotypes in the four populations grown at Warsaw during 2011-12 differed for yield, grain volume weight, plants m^{-2}, seedling emergence, ash, and protein (Table 5). Additionally, the hulled and hulless genotypes in the four populations differed for seed weight $spike^{-1}$. Higher values for yield, seedling emergence, ash, and seed weight $spike^{-1}$ were observed for the hulled genotypes in all four populations. Higher values for grain volume weight, plants m^{-2}, and protein were observed for the hulless genotypes in all four populations. In the 2011-12 Blacksburg test, the hulled and hulless genotypes of the four populations differed for grain volume weight, 1000 kernel weight, plants m^{-2}, seedling emergence, ash, fat, protein, and starch (Table 6). Interestingly, only the hulled genotypes in population 1 had significantly higher yields than their hulless counterparts at Blacksburg. Higher values for 1000 kernel weight, seedling emergence, ash, and fat were observed for the hulled genotypes in all four populations. Higher values for grain volume weight, plants m^{-2}, and protein were observed for the hulless genotypes in all four populations.

In summary, grain volume weight and protein concentration were significantly ($p \leq 0.05$) higher for hulless genotypes in all populations while seedling emergence and grain ash concentration were significantly ($p \leq 0.05$) higher for hulled genotypes in all populations and

environments. Additionally, 1000 kernel weights were significantly ($p \leq 0.05$) higher for hulled genotypes in all populations and environments with the exception of population 1 at Warsaw in 2011-12. Yields of hulled genotypes were significantly higher than non-adjusted yields of hulless genotypes in all populations during both years at Warsaw, VA. However, yield was significantly different only for population 1 at Blacksburg, VA during the 2011-12 growing season.

Regression analysis for yield

All traits were regressed on yield in each of the four populations evaluated at Warsaw during the 2010-11 and 2011-12 growing seasons and at Blacksburg during 2011-12 to determine which traits contributed significantly ($p \leq 0.05$) to yield variation. Models containing all significant traits and the coefficient estimating their effect on yield are presented in Table 7. Percent variation explained for yield varied by population and environment. Variation explained by the models for yield ranged from 42% for population 4 in Warsaw 2011-12 to 84% for population 7 in Blacksburg 2011-12. None of the traits contributed to yield variation in all four populations in every environment. Grain volume weight had a significant ($p \leq 0.05$) positive effect on yield in population 1 in both environments during the 2011-12 growing season. Floret fertility significantly ($p \leq 0.05$) affected yield in population 7 at Warsaw in both years. Grain protein was negatively ($p \leq 0.001$) correlated with yield in populations 1, 7, and 4 in both environments during the 2011-12 growing season. Significance of other traits contributing to yield varied by population and environment.

Comparison of yield of hulled and hulless full-sibs

Comparison of yield of hulled lines versus non-adjusted and adjusted (for hull weight) yields of hulless full-sibs in the four populations grown at Warsaw during the 2010-11 growing season, and at Warsaw and Blacksburg, VA during the 2011-12 growing season are presented in

Figures 1, 2, and 3. Adjustment values ranged from 4% to 21% depending on population and environment. Adjustment values used in the current study varied slightly from previous estimates of 7% to 19% for hull weight contribution to total grain weight (Anderson and Schroeder, 2010; Choo et al., 2001; Price and Parsons, 1979; Xue et al., 1997). Differences in contribution of hull weight to total grain weight can be attributed to variation in genotypes tested and environmental factors where genotypes were grown.

Non-adjusted yields of hulless lines were significantly ($p \leq 0.05$) lower than those of their hulled sibs at Warsaw, VA during the 2010-11 growing season (Figure 1). Yield differences on average were 12% in population 4, 14% in population 5, 17% in population 7, and 18% in population 1 (Figure 1). Yields of hulless lines were adjusted by adding 8% to population 1, 9% to populations 5 and 7, and 12% to population 4 to account for the difference in grain weight due to hull weight loss. After adjusting yields in each population for the weight difference due to hull removal, yields of the hulless lines in populations 4 and 5 were not significantly ($p \leq 0.05$) different from those of their hulled sib lines. Adjusted yields of 17 hulless lines in population 1 and seven hulless lines in population 7 were not significantly different from those of their hulled sib lines.

Similar to the 2011-12 growing season, non-adjusted yields of hulless lines were significantly ($p \leq 0.05$) lower than those of their hulled sibs and on average differed by 7% in population 4, 11% in populations 5 and 7, and 19% in population 1 at Warsaw, VA (Figure 2). Yields of hulless lines were adjusted by adding 4% to populations 1, 4, and 7, and 6% to population 7 to account for the difference in grain weight due to hull weight loss. It should be noted that these estimates are on the low end for percent contribution of hull weight to total grain weight. Adjusted yields of hulless lines in populations 5 were not significantly different from

those of their hulled sib lines. Yields of five hulless lines in population 1, seventeen hulless lines in population 4, and fourteen hulless lines in population 7 were not significantly different from those of their hulled sib lines. Adjusted yields of the remaining hulless lines in populations 1, 4, and 7 were still significantly lower than their hulled sibs.

Differences between yields of hulled versus non-adjusted and adjusted yields of hulless sibs grown at Blacksburg (Figure 3) varied from those at Warsaw in 2011-12. In comparisons of hulled sib yields with non-adjusted yields of their hulless counterparts, yields differences on average were 1% for population 5, 3% for populations 7, 11% for population 4, and 12% for population 1 at Blacksburg, VA. Non-adjusted yields of hulled and hulless lines within populations 5 and 7 were not significantly ($p \leq 0.05$) different. Yields of hulless lines were adjusted by adding 15% to populations 4 and 5, and 21% to populations 1 and 7, to account for the average difference in grain weight due to hull weight loss. Only eleven hulless lines within population 1 and eight hulless lines in population 4 had adjusted yields that were still significantly lower than their hulled sibs.

DISCUSSION

Major issues limiting broad acceptance and production of hulless barley as an alternative to hulled barley are yield difference, primarily due to removal of the hull upon threshing, and reduced seedling emergence and vigor. Aside from hull removal at threshing, hulless barley is similar to hulled barley in most aspects in the field (Liu et al., 1996). Differences in experimental design in the 2010-11 and 2011-12 experiments and significant genotype x environment interactions in the 2011-12 tests stipulated analysis and reporting of data for each environment separately.

Pooled-mean comparisons were used to determine which traits differed significantly ($p \leq 0.05$) between hulled and hulless genotypes of a population. In tests at Warsaw in 2010-11 and 2011-12 and Blacksburg in 2011-12, traits that differed significantly between hulled and hulless genotypes in all populations included grain volume weight, plants m^{-2}, seedling emergence, grain ash, and protein concentrations (Tables 4, 5, and 6). Non-adjusted yields differed significantly between hulled and hulless genotypes in all populations at Warsaw during both years (Table 4 and, 5). All other measured traits varied in significance between populations and environments. Hullessness has been previously reported to be associated with poor seedling emergence, short plant height, high grain volume weight, low seed weight, and low grain yield (Choo et al., 2001). However, hullessness has not been associated with heading date, maturity, or spike density (Choo et al., 2001).

In the current study, seedling emergence was significantly ($p \leq 0.01$) higher for hulled than hulless genotypes for all four populations in all environments (Tables 4, 5, and 6). During the 2010-11 growing season seedling emergence was 3% to 30% higher than expected for hulled genotypes in all populations. Germination tests conducted prior to planting underestimated percent germination, thus the higher seeding rates used to adjust for reduced germination resulted in higher than expected seedling emergence in the field. Poor seedling emergence in hulless barley has been attributed to the fact that the germ of the exposed endosperm is more likely to be damaged during grain threshing and subsequent processing than in hulled barley (Thomason et al., 2009). Even at higher seeding rates, lower emergence and less early season vigor has been reported for hulless barley relative to hulled barley (Thomason et al., 2009). Conversely in the current study, plants m^{-2} at GS 12 was significantly higher for the hulless genotypes in the four populations at Warsaw and Blacksburg, VA during the 2011-12 growing seasons (Tables 5, and

6). At GS 25, NDVI, estimates of tiller density based on spectral reflectance, also were significantly higher for the hulless genotypes at Warsaw, VA in 2010-11 (Table 4). At equal seeding rates in a previous study, hulled lines exhibited higher plants m^{-2} at GS 25 (Thomason et al., 2009). The higher seeding rates used for hulless lines (654 seeds m^{-2} for hulless vs. 473 seeds m^{-2} for hulled) in the current study likely contributed to higher plants m^{-2} and NDVI values for hulless genotypes in the populations. Therefore, the seeding rate used in the current study probably was not optimal for the genotypes tested considering that the hulless genotypes had population densities that were higher than expected because emergence was less of a problem than reported previously. A consistent trend was not observed for the significance of spikes m^{-2} (Tables 4, 5, and 6), which varied by population and environment. Hulled genotypes have previously been reported to have higher plant m^{-2} at all seeding rates (278 seeds m^{-2} to 742 seeds m^{-2}) than hulless genotypes at GS 25 (Thomason et al., 2009). Differences for plants m^{-2} between hulled and hulless genotypes were larger at higher seeding rates (> 557 seeds m^{-2}) than at lower seeding rates (278 seeds m^{-2}) (Thomason et al., 2009). Although hulled lines were seeded at lower rates than hulless lines and had fewer plants m^{-2} at GS12 in the current study, the lack of or less than expected variability and significance of spikes m^{-2} suggests that hulled lines compensated for lower initial plants m^{-2} by producing more viable tillers which contributed to increased spikes m^{-2}.

Significantly ($p \leq 0.001$) higher grain volume weights were observed for hulless genotypes in all four populations and all environments and are consistent with previous reports for hulless barley (Bhatty et al., 1999; Choo et al., 2001; Griffey et al., 2010). Choo et al. (2001) concluded that the effect of the *nud* locus on test weight was likely a result of the presence or absence of the hull. On average, kernel weight of hulless lines was 4% to 12% less than that of

hulled lines within populations at Warsaw, VA during both growing seasons. This falls within the range previously reported by Bhatty (1999) and Thomason et al. (2009). However, the differences were greater at Blacksburg, VA where kernel weights of hulless lines were 15% to 21% less than those of hulled lines. Hull weight contribution to total grain weight has been previously estimated to be between 7% and 19% (Anderson and Schroeder, 2010; Choo et al., 2001; Price and Parsons, 1979; Xue et al., 1997). Therefore, the observed differences between kernel weight of hulled and hulless lines at Warsaw are consistent with the literature, while differences at Blacksburg were likely influenced by environmental factors.

Grain compositional traits varied between hulled and hulless genotypes in the four populations. Significantly ($p \leq 0.001$) higher protein concentrations were observed for the hulless genotypes in all four populations and all environments, while significantly higher ash concentrations were observed for the hulled genotypes (Tables 4, 5, and 6). Higher protein and lower ash concentrations in Virginia Tech's hulless vs. hulled barley have been previously reported by Griffey et al. (2010). Starch concentrations were significantly ($p \leq 0.001$) higher for hulless genotypes in population 5 in all environments and hulless genotypes in population 4 at Warsaw during both years. These results are consistent with previous reports in which hulless barley had significantly higher starch than hulled barley (Griffey et al., 2010). In the previous study (Griffey et al., 2010), Doyce had a four year average starch concentration of 64.08% versus 60.41% for Thoroughbred. In the current study Doyce had an average starch concentration of 59.6% at Warsaw in 2010-11, 60.4% at Warsaw in 2011-12, and 59.5% at Blacksburg in 2011-12, while Thoroughbred had an average starch concentration of 59.0% at Warsaw in 2010-11, 59.2% at Warsaw in 2011-12, and 59.1% at Blacksburg in 2011-12. It has been estimated that starch, protein, and β-glucan in barley make up about 80% of the weight of

barley grain (MacGregor and Fincher, 1993). Starch was estimated to constitute 50% to 60% of the dry weight, protein 9% to 12%, and β-glucans 3% to 5% (MacGregor and Fincher, 1993; Swanston and Ellis, 2002). Values reported in the current study are within these estimates for starch and protein concentrations (Tables 4, 5, and 6). In a regression analysis, Simmonds (1995) determined that a strong negative correlation exists between yield and grain protein concentration in many cereals including barley and wheat. Greater variability exists between hulled and hulless genotypes for grain protein concentration (Tables 4, 5, and 6) than for starch concentration in the current study. Grain starch concentrations differed from 0.1% to 2% between hulled and hulless genotypes across all populations and environments while grain protein concentration was 19% to 32% higher in hulless genotypes than hulled genotypes across all populations and environments. In the regression analysis, protein had a significant negative effect on yield in nine of the twelve population/environment combinations (Table 7). It is plausible that higher grain protein in hulless genotypes is contributing to decreased yields relative to hulled genotypes.

Significance of traditional yield components including seed weight spike^{-1}, seed spike^{-1}, spikelets spike^{-1}, and floret fertility varied between populations and environments (Tables 4, 5, and 6). Seed weight spike^{-1} was the most consistent of these traits with hulled genotypes in all four populations having significantly ($p \leq 0.01$) higher weights than hulless genotypes as expected when considering lack of hull weight in the latter case (Tables 4, 5, and 6). Previously, a greater number seed spike^{-1} was observed for hulled barley at all seeding rates when compared to hulless barley (Thomason et al., 2009). However, a similar trend was not observed among all populations and environments in the current study (Tables 4, 5, and 6). One explanation is that the hulled (Thoroughbred, Price, and Callo) and hulless (Doyce, Eve, VA00H-65, VA01H-122,

VA01H-124 and VA01H-125) barley lines included in the Thomason et al. (2009) study represented a more diverse group of barley genotypes within the Virginia Tech breeding program. The current study focuses on hulled and hulless lines developed from common crosses and it is likely that common alleles not associated with the *nud* locus control spike morphology traits such as seeds spike^{-1} and spikelets spike^{-1}.

Estimates for traits explaining variation in yield obtained from the regression analysis did not identify a single trait contributing significantly to yield variation across all populations and environments (Table 7). It should be noted that the largest coefficient obtained from regression analysis doesn't necessarily reflect that trait's importance in explaining yield variation. A decrease in the number of genotypes tested within populations from the 2010-11 (75 lines) to 2011-12 (25 lines) growing seasons likely decreased variation within the populations and potentially impacted significance of traits explaining yield variation. Although the same lines were tested in Warsaw and Blacksburg, VA during the 2011-12, significant genotype x environment interaction for most traits likely impacted the significance of traits explaining yield variation across environments. Concomitantly, barley yellow dwarf infection in all populations and net blotch in population 5 at Blacksburg decreased yields and negatively impacted the other measured traits. Further testing is needed to better understand which traits consistently impact yield within these populations.

Comparison of yields of hulled versus non-adjusted and adjusted yields of hulless full-sibs at Warsaw, VA during 2010-11, and Warsaw and Blacksburg, VA 2011-12 (Figures 1, 2, and 3) indicated that on average yield differences between hulled and hulless genotypes within the populations ranged from 1% to 19%. Yield differences between hulled and hulless genotypes of the populations were greater at Warsaw than at Blacksburg, while differences

between 1000 kernel weights were greater at Blacksburg than at Warsaw, VA. Concomitantly, non-adjusted yield differences between hulled and hulless genotypes of a population were not significant for populations 4, 5, or 7 at Blacksburg, VA (Table 6).

After adjusting grain yields for the difference in grain weight due to hull removal at threshing, yields of select hulless and hulled lines were similar at Warsaw in 2010-11, and in both of the 2011-12 environments (Figures 1, 2, and 3). Adjusted yields of hulless genotypes in population 5 did not differ significantly ($p \leq 0.05$) from hulled genotypes in all environments. Adjusted yields of 17 hulless lines in population 1 and seven hulless lines in population 7 at Warsaw, VA in 2010-11 were still significantly lower than those of their hulled sibs (Figure 1). During the 2011-12 growing season, five hulless lines in population 1, seventeen hulless lines in population 4, and fourteen hulless lines in population 7 at Warsaw (Figure 2), and eleven hulless lines in population 1, and eight hulless lines in population 4 at Blacksburg, VA (Figure 3) had adjusted yields that were still significantly ($p \leq 0.05$) lower than their hulled sibs. Interestingly, two hulless genotypes were identified in populations 5 and 7 that had adjusted yields similar to those of the hulled cultivar Thoroughbred at Warsaw, VA during the 2011-12 growing season.

Determining if yield differences between hulled and hulless genotypes can be attributed solely to hull removal at threshing, or are due to a combination of physical and genetic factors is strategic in further efforts to improve hulless barley. Traits differed between hulled and hulless genotypes in the four populations, yet none of the traits exhibited a consistent positive or negative effect on yield variation attributable to the presence or absence of the hull. If hull weight constitutes 7% to 19% of total grain weight (Anderson and Schroeder, 2010; Choo et al., 2001; Price and Parsons, 1979; Xue et al., 1997), adjusting yields of hulless genotypes for hull weight loss at threshing does not totally account for the yield difference between hulled and

hulless genotypes within these populations. Similarly, Choo et al. (2001) found that after adjusting yields of hulless genotypes for hull weight loss, the hulless class still yielded less than the hulled class and that the low yield of hulless barley could not be attributed solely to hull weight. Slight differences in contribution of hull weight to total grain weight can be attributed to parents used in formation of populations and the resulting differences in the genotypes tested.

When compared at similar plant densities hulless barley lines were previously reported to have 5% fewer spikes m^{-2} and 30% fewer grains $spike^{-1}$ (Thomason et al., 2009). In the current study significance of traits varied by population and environment, and hull removal at threshing had a negative yet nominal effect on 1000 kernel weight and seed weight $spike^{-1}$ in the hulless genotypes of a population relative to the hulled genotypes. An allele having a negative effect on yield mapped 1 cM from the *nud* locus and is fixed in hulless lines of the Virginia Tech barley breeding program (Berger et al., 2012). However, validation is necessary to determine whether the marker identified a unique allele or if it is associated with the *nud* locus. While hull weight accounts for a large portion of the yield difference between hulled and hulless genotypes, further research and genetic improvements in hulless germplasm are necessary to eliminate the current yield gap. Identification of the specific impacts of the *nud* locus and putative unfavorable linkages on agronomic and morphological traits was not possible in the current study due to inconsistent trait effects on yield and significant genotype x environment interactions. Nevertheless, it should be possible to breed for high yielding hulless barley lines through recombination and selection (Choo et al., 2001). Identification and selection of superior parents and additional favorable alleles not linked to the *nud* locus would greatly aid in the improvement and development of high yielding hulless barley genotypes.

CONCLUSIONS

Yield improvement is necessary to make hulless barley competitive with hulled barley. Although many quality-related traits make hulless barley ideal for use in food, feed, and fuel industries, decreased yield associated with hullessness deters acceptance of hulless barley as a viable alternative to hulled barley. Assessment of four full-sib populations revealed that grain volume weight and protein concentrations were significantly ($p \leq 0.05$) higher for hulless genotypes, while grain ash concentration was significantly ($p \leq 0.05$) higher for hulled genotypes. Grain protein concentration had a negative effect on grain yield in nine of the twelve population/environment combinations. Prior to adjustment for hull weight, hulless genotypes in the four populations yielded 4% to 19% less than the hulled genotypes. Yield potential of select hulless genotypes in all four populations were statistically similar to that of the hulled genotypes after adjusting for the hull weight loss at threshing. However, adjustment of hulless yields for hull weight loss at threshing did not totally explain the yield difference that existed between hulled and hulless lines. It is plausible that higher grain protein concentration in hulless genotypes is also contributing to the yield difference. Use of elite hulless and hulled parents in population development will facilitate selection of superior hulless genotypes having yields similar to hulled genotypes when accounting for hull weight. Further research is needed to determine whether any pleiotrophic effects due to *nud* locus exists or if other tightly linked loci influence yield potential of hulless genotypes.

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Table 1: Analysis of variance for yield of hulled and hulless barley lines and cultivars evaluated in a modified augmented design (Type 2) test at Warsaw in 2010-2011.

Source	Population 1	Population 7	Population 4	Population 5
	F			
Row	1.63	2.56	1.31	2.39
Column	1.91	0.16	0.42	6.09***
Block	152.37***	109.63***	200.15***	298.65***
Row*Block	1.85	0.32	0.62	3.11*
Column*Block	1.72	6.71***	4.57**	10.20***
MSE whole-plot	199360	171789.27	140402	64364
MSE subplot-error	145494	122262.42	110440	107337

* Significant at $p \leq 0.05$
** Significant at $p \leq 0.01$
*** Significant at $p \leq 0.001$

Table 2: Analysis of variance for hulled and hulless barley lines and cultivars evaluated in split-split plot design tests at Blacksburg and Warsaw, VA in 2011-2012.

Source	DF†	DDFM‡	Yield	Grain Volume Weight	1000 Kernel Weight	Plants m^{-2}	Seedling Emerg-ence	NDVI§	Spikes m^{-2}	Heading Date	Maturity Date	Grain Fill Period	Plant Height	Lodging	Seed weight $spike^{-1}$	Seeds $spike^{-1}$	Spikelets $spike^{-1}$	Floret fertility
			F															
Environment	1	3	55.02 **	565.03 ***	62.78 **	1.1	0.74	35.28 **	10.82 *	1419.87 ***	1948.38 ***	63.29 **	16.8 *	4.04	91.42 **	6.71	9.31	0.56
Environment(Replication)	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Block	1	3	4.00	43.56 **	0.86	0.26	0.14	0.39	0	1.31	6.99	3.75	2.01	1.82	3.27	2.34	0.01	2.77
Block*Environment	1	3	1.81	22.05 *	2.97	0.17	0.19	7.97	0.07	0.53	14.64 *	5.69	5.9	0.00	11.96 *	1.38	11.29*	0.1
Block*Environment(Replication)	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Population	3	18	1.14	2.5	0.03	2.1	2.41	1.99	0.73	4.6 **	3.06	0.02	0.05	0.74	2.63	0.41	1.09	0.64
Environment*Population	3	18	1.26	3.18 *	0.03	0.74	0.84	0.15	0.02	2.74	2.99	0.1	1.05	3.75	2.88	0.42	4.9	0.43
Block*Population	3	18	0.93	2.34	0.03	0.89	0.55	0.75	0.48	0.25	0.2	0.23	0.17	0.73	0.85	0.18	1.96	0.3
Block*Population*Environment	3	18	1.69	2.91	0.06	1.05	1.46	0.71	2.08	0.03	0.34	0.21	3	2.20	3.31 *	0.62	3.70 *	0.15
Block*Population*Environment(Replication)	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Genotype	213	814	12.90 ***	40.47 ***	1.17	2.16 ***	2.38 ***	1.23 *	1.76 ***	74.93 ***	15.27 ***	4.64 ***	5.61 ***	6.17 ***	2.66 ***	2.22 ***	3.33 ***	2.15 ***
Genotype*Environment	213	814	2.78 ***	2.81 ***	0.76	0.85	0.88	1.17	1.41 ***	5.59 ***	4.4 ***	2.22 ***	2.93 ***	3.40 ***	1.48 ***	2.30 ***	1.28 **	2.32 ***
Residual	814	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	1279	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Significant at $p \leq 0.05$
** Significant at $p \leq 0.01$
*** Significant at $p \leq 0.001$
†: Degrees of Freedom
‡: Denominator Degrees of Freedom
§: Normalized Difference Vegetative Index

Table 3: Analysis of variance for grain composition traits for hulled and hulless barley lines and cultivars evaluated in split-split plot design tests at Blacksburg and Warsaw, VA in 2011-2012.

Source	DF†	DDFM‡	Ash	Crude Fiber	Fat	Protein	Starch
			F				
Environment	1	3	0.08	82.55 **	1.63	143.78 **	0.73
Environment(Replication)	3	-	-	-	-	-	-
Block	1	3	0.24	2.67	0.23	2.8	0.00
Block*Environment	1	3	0.06	0.81	1.3	1.06	6.10
Block*Environment(Replication)	3	-	-	-	-	-	-
Population	3	18	0.74	0.95	1.25	2.22	1.63
Environment*Population	3	18	1.08	0.7	1.69	1.72	0.26
Block*Population	3	18	1.31	0.9	0.36	0.72	0.51
Block*Population*Environment	3	18	0.53	0.08	0.45	0.43	0.01
Block*Population*Environment(Replication)	18	-	-	-	-	-	-
Genotype	213	814	14.24 ***	12.33 ***	10.62 ***	16.21 ***	10.89 ***
Genotype*Environment	213	814	2.22 ***	1.54 ***	2.22 ***	2.52 ***	3.34 ***
Residual	814	-	-	-	-	-	-
Total	1279	-	-	-	-	-	-

* Significant at $p \leq 0.05$
** Significant at $p \leq 0.01$
*** Significant at $p \leq 0.001$
†: Degrees of Freedom
‡: Denominator Degrees of Freedom

Table 4: Pooled mean comparisons of agronomic, spike component, and grain composition traits for hulled and hulless barley genotypes in four populations tested at Warsaw, VA in 2010-2011.

Traits	Hulless-1	Hulled-1	Hulless-7	Hulled-7	Hulless-4	Hulled-4	Hulless-5	Hulled-5
Yield (kg ha^{-1})	7445	9080***	7327	8874***	7633	8672***	7726	9000***
Grain volume weight (g $liter^{-1}$)	751.2***	637.7	760.8***	633.7	749.9***	648	759.5***	642.4
1000 Kernel weight (g)	30.9	33.5***	30.7	33.6***	33.5	38.0***	34.7	38.3***
Plants m^{-2}	431**	399	425	497***	385	472***	353	438***
Seedling emergence (%)	80.0	103.0***	79.0	128.0***	72.0	122.0***	66.0	113.0***
NDVI†	0.86***	0.77	0.86***	0.77	0.86***	0.76	0.85***	0.77
Heading date (Julian)	112	112	112	112	115	115	114**	113
Plant height (cm)	87.4	90.4***	86.8***	82.9	94.3	94.8	93.3	92.8
Lodging (0-9)	5.5*	4.7	5.5***	3.4	4.5***	3.2	5.0***	3.8
Spikes m^{-2}	880***	790	891	861	515	550	656***	556
Net blotch (0-9)	3.2***	1.9	4.1***	2.3	5.4*	4.8	5.4***	3.9
Ash	1.98	2.60***	1.98	2.65***	1.99	2.43***	1.97	2.41***
Crude fiber	3.86***	3.47	3.79***	3.4	3.84***	3.41	3.74***	3.31
Fat	1.83	2.67***	1.83	2.75***	1.79	2.59***	1.82	2.53***
Protein	10.00***	6.78	9.81***	7.08	9.67***	7.59	9.27***	7.38
Starch	58.9	59.0	59.8***	59.1	59.5***	59.1	59.7***	59.1
Seed weight $spike^{-1}$ (g)	1.48	1.59***	1.48	1.62***	1.60	1.86***	1.65	1.71
Seeds $spike^{-1}$	47	47	48	49	48	48	47***	43
Spikelet $spike^{-1}$	56	57	58	59	56	57	54	52
Floret fertility (%)	84	82	83	83	85	85	89***	84

* Significantly different at $p \leq 0.05$
** Significantly different at $p \leq 0.01$
*** Significantly different at $p \leq 0.001$
†: Normalized Difference Vegetative Index

Table 5: Pooled mean comparisons of agronomic, spike component, and grain composition traits for hulled and hulless barley genotypes in four populations tested at Warsaw, VA in 2011-2012.

Traits	Hulless-1	Hulled-1	Hulless-7	Hulled-7	Hulless-4	Hulled-4	Hulless-5	Hulled-5
Yield (kg ha^{-1})	5903	7256***	6572	7402***	6630	7140**	6623	7409***
Grain volume weight (g $liter^{-1}$)	746.2***	637.8	759.3***	636.2	735.3***	650.1	753.2***	644.8
1000 Kernel weight (g)	34.9	36.2	34.1	35.6*	39.5	41.1**	39.2	41.5***
Plants m^{-2}	425***	357	414***	358	411***	375	432***	395
Seedling emergence (%)	65.9	75.5***	64.2	75.6***	63.7	79.3***	67.1	83.4***
NDVI†	0.63	0.67**	0.67	0.68	0.64	0.63	0.64	0.68***
Heading date (Julian days)	91	95*	88	94***	98	97	94	94
Maturity date (Julian days)	131	134*	130	134***	135	134	133	133
Grain fill period (Julian days)	41**	39	42*	39	37	37	39	39
Plant height (cm)	74.5	72.5	75.1	74.5	77	74	78.8	77.9
Lodging (0-9)	0.36*	0.09	0.44*	0.16	0.07	0.04	0.12	0.11
Spikes m^{-2}	702	723	726	774*	717	776***	775***	611
Ash	1.99	2.65***	1.97	2.67***	2.01	2.42***	2.01	2.52***
Crude fiber	4.44	4.57	4.39	4.70***	4.48	4.45	4.37***	4.08
Fat	2.38	2.37	2.39	2.35	2.17	2.17	2.22	2.22
Protein	8.85***	6.09	8.68***	6.01	8.48***	6.23	8.33***	6.28
Starch	59.5	59.8	59.8	59.6	59.9**	59.3	60.6***	59.4
Seed weight $spike^{-1}$ (g)	1.47	1.68***	1.39	1.69***	1.58	1.68**	1.51	1.69***
Seed $spike^{-1}$	45	47	45	48*	42	41	41	41
Spikelets $spike^{-1}$	50	55**	50	55***	46	46	45	47**
Floret fertility (%)	90**	86	91***	86	92	89	92***	88

* Significantly different at $p \leq 0.05$

** Significantly different at $p \leq 0.01$

*** Significantly different at $p \leq 0.001$

†: Normalized Difference Vegetative Index

Table 6: Pooled mean comparisons of agronomic, spike component, and grain composition traits for hulled and hulless barley genotypes in four populations tested at Blacksburg, VA in 2011-2012.

Traits	Hulless-1	Hulled-1	Hulless-7	Hulled-7	Hulless-4	Hulled-4	Hulless-5	Hulled-5
Yield (kg ha^{-1})	5040	5757**	5401	5591	5308	5931	6463	6504
Grain volume weight (g $liter^{-1}$)	703***	615	707***	589	607***	602	711***	615
1000 Kernel weight (g)	28.0	35.5***	28.4	35.8***	32.2	37.9***	33.6	39.5***
Plants m^{-2}	463***	370	453***	370	465***	386	482***	395
Seedling emergence (%)	71.8	78.1**	70.3	78.1***	72	81.6***	74.7	83.5***
NDVI†	0.75	0.75	0.72	0.76**	0.75	0.75	0.73	0.75
Heading date (Julian days)	100	101	98	103**	108	108	104	104
Maturity date (Julian days)	144	145	142	147***	147	149*	147	147
Grain fill period (Julian days)	44	44	44	45	39	41**	43	44
Plant height (cm)	78.6	82.3*	71	78**	81	84*	82	84*
Lodging (0-9)	0.4	0.08	0.96	0.58	1.2	0.64	0.96	1.52
Spikes m^{-2}	1075***	894	995	1003	966	907	954	945
Net blotch (0-9)	2.4	2.5	2.9*	2.2	2.9	3	2.98	3.18
BYDV‡ stunting (%)	6.2	2.1	9.2	4.5	7.8	7.5	1	1.8
BYDV discoloration (%)	24.5	17.1	17.7	21.1	18	15.1	8.1	14.1*
Ash	1.96	2.77***	1.94	2.66***	1.96	2.44***	1.97	2.55***
Crude fiber	2.06	2.39***	4.02	4.30**	3.9	3.96	3.96**	3.62
Fat	4.05	4.31**	2.21	2.44***	2.07	2.31**	2.17	2.33*
Protein	11.5***	7.9	11.1***	8.4	11.5***	8.7	10.3***	8.3
Starch	58.8	60.0***	59.1	60.1***	59.0	59.8***	60.5***	59.6
Seed weight $spike^{-1}$ (g)	1.21	1.33**	1.25	1.28	1.35	1.32	1.31	1.40*
Seeds $spike^{-1}$	47	41	43*	40	40	38	40	39
Spikelets $spike^{-1}$	44	45	48	46	44**	41	45	43
Floret fertility (%)	100	91	90	86	91	92	89	92

* Significantly different at $p \leq 0.05$
** Significantly different at $p \leq 0.01$
*** Significantly different at $p \leq 0.001$
†: Normalized Difference Vegetative Index
‡: Barley Yellow Dwarf Virus

Table 7: Significant traits with coefficients from linear regression for yield for barley genotypes within populations grown at Warsaw, VA during 2010-2011 and 2011-2012, and at Blacksburg, VA during 2011-2012.

		Warsaw 2010-2011				Warsaw 2011-2012				Blacksburg 2011-2012			
Variable	+ / -[†]	Pop. 1	Pop. 7	Pop. 4	Pop. 5	Pop. 1	Pop. 7	Pop. 4	Pop. 5	Pop. 1	Pop. 7	Pop. 4	Pop. 5
Intercept	-	26959	5385	-1082	-9110	-4965	-10312	3114	-19346	807	9537	11362	4742
Plant height	5/0	40.97**	-	25.10**	30.84**	49.60***	-	-	-	-	60.67***	-	-
Heading date	4/0	-	142.47**	-	150.63***	37.37**	-	-	107.95***	-	-	-	-
Floret fertility	4/0	-	2964.18***	1737.39*	-	-	24612*	-	-	-	-	-	4474.89***
NDVI[‡]	3/0	-	-	-	-	-	4954.69*	3467.99*	5115.91**	-	-	-	-
Plants m^2	3/0	-	-	-	-	-	27.76***	4.92**	7.40*	-	-	-	-
Grain fill period	2/0	-	-	-	-	-	-	-	126.73*	-	60.44**	-	-
Spikes m^{-2}	2/0	1.21*	-	-	0.99**	-	-	-	-	-	-	-	-
Seed weight $spike^{-1}$	2/0	-	-	470.30**	-	-	-	-	1176.16**	-	-	-	-
Spikelets $spike^{-1}$	1/0	-	-	-	-	-	510.48**	-	-	-	-	-	-
Lodging	1/0						322.20*						
Grain volume weight	3/3	-	6.33**	-	-5.50***	6.87*	-	-	-	15.23***	7.92**	-	-2.27*
1000 kernel weight	3/1	-	-	52.63**	53.63**	69.27*	-	-	-	-	-66.86*	-	-
Fat	3/1	878.59***	1061..03***	870.77***	-					-	-	-885.73*	-
Seeds $spike^{-1}$	2/2	-	-25.08*	-	-	46.79**	-547.00**	60.06**	-	-	-	-	-
Ash	2/1	-	-	-	-	-	-	-	2713.65***	-	-1344.34**	-	-
Seedling emergence	1/2	-	-	-	492.25*	-	-139.06***	-	-42.82*	-	-	-	-
Crude Fiber	1/1	-	-	388.76*	-	-	-	-	-	-	-734.97**	-	-
Protein	0/9	-275.65***	-403.43***	-	-144.96*	-660.58***	-1415.69***	-387.38***	-	-529.32***	-807.54***	-290.86***	-
Net Blotch	0/3	-	-	-124.91***	-89.81**	-	-	-	-	-	-	-	-146.10**
BYDV[§] discoloration	0/3	-	-	-	-	-	-	-	-	-15.38***	-	-52.53***	-32.01***
Starch	0/2	-389.65	-300.93**	-	-	-	-	-	-	-	-	-	-
BYDV stunting	0/1	-	-	-	-	-	-	-	-	-	-28.04***	-	-
R^2	-	0.60	0.63	0.63	0.67	0.81	0.71	0.48	0.68	0.83	0.84	0.69	0.70

* Significant at $p \leq 0.05$

** Significant at $p \leq 0.01$

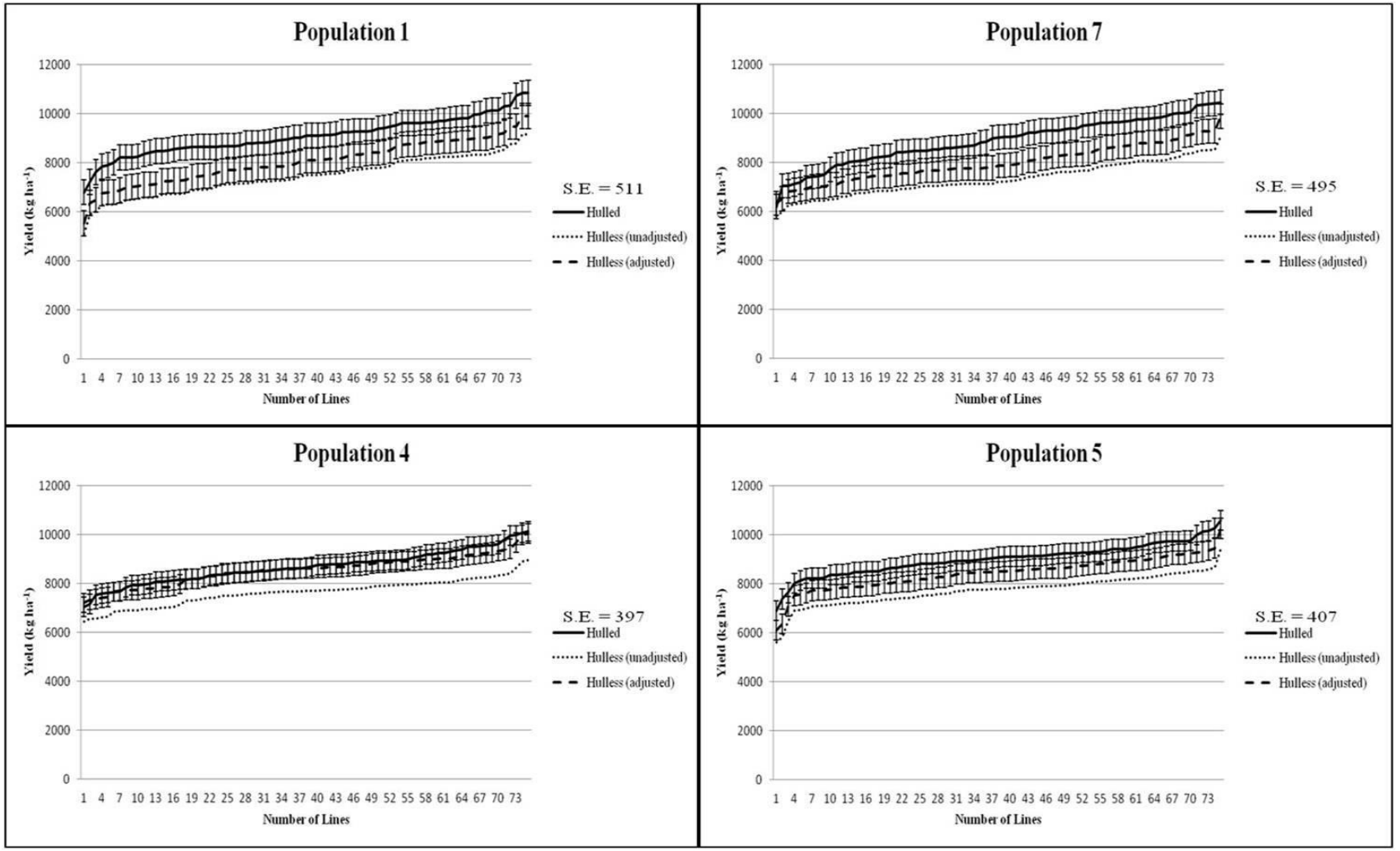
*** Significant at $p \leq 0.001$

†: + / - designate the number of environments where a significant trait had a positive or negative effect on yield in the regression analysis.

‡: Normalized Difference Vegetative Index

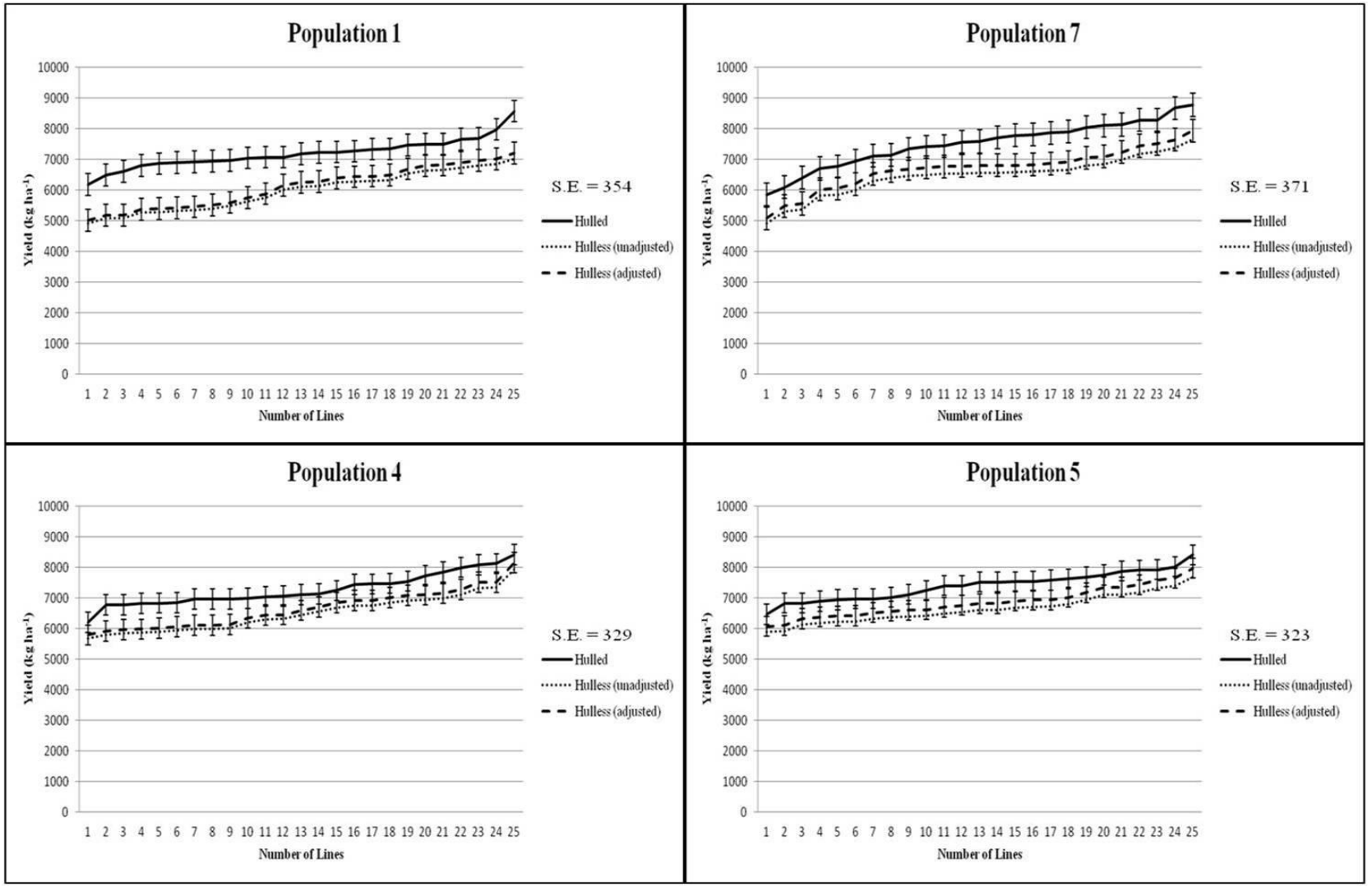
§: Barley Yellow Dwarf Virus

Figure 1: Yields of hulled barley versus non-adjusted and adjusted† yields of hulless full-sibs in four populations planted at Warsaw, VA in 2010-2011.



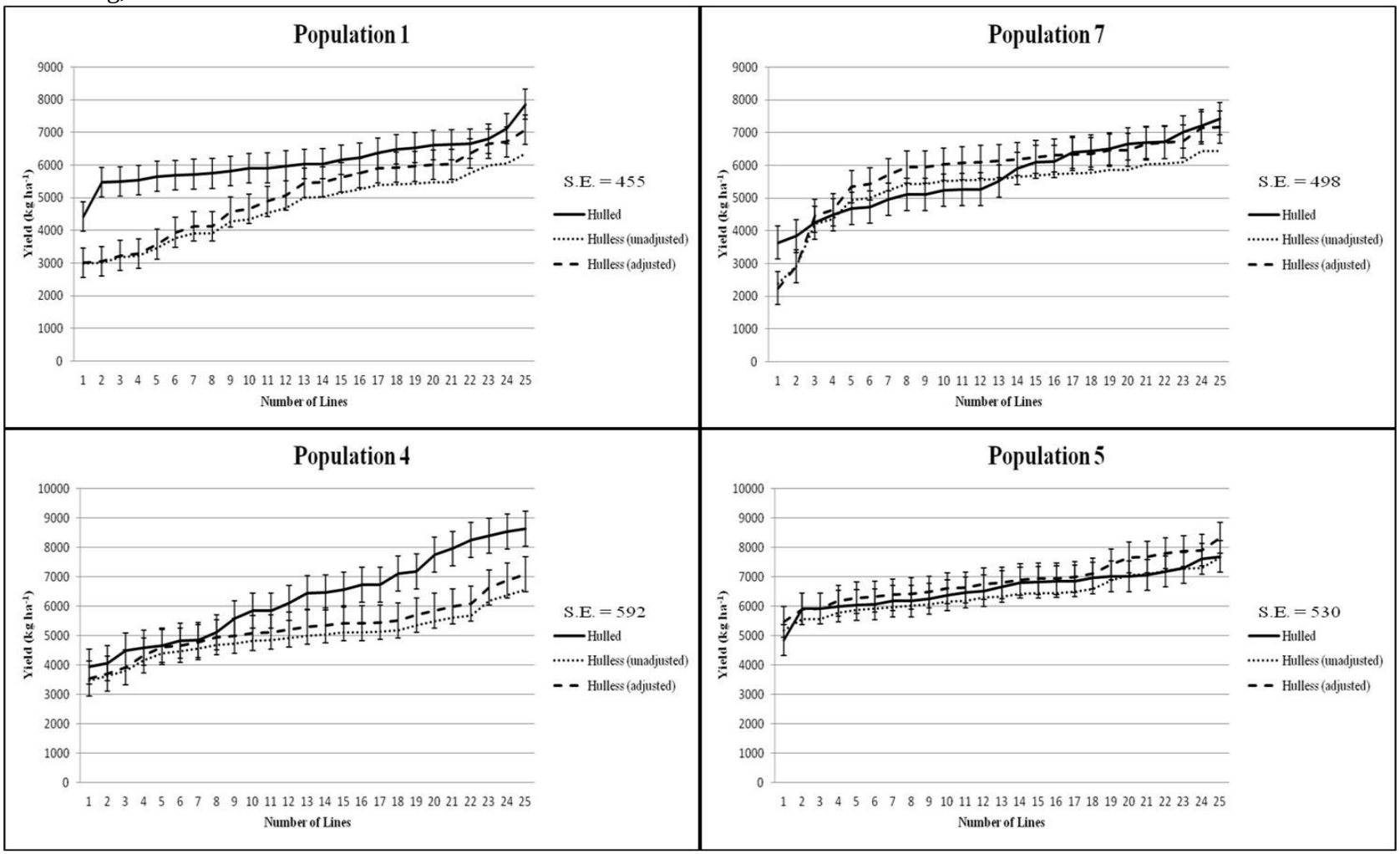
†: Adjustments of hulless yields are as follow: Population 1, adjustment = 8%; Population 7, adjustment = 9%; Population 4, adjustment = 12%; and Population 5, adjustment = 9%

Figure 2: Yields of hulled barley versus non-adjusted and adjusted† yields of hulless full-sibs in four populations planted at Warsaw, VA in 2011-2012.



†: Adjustments of hulless yields are as follow: Population 1, adjustment = 4%; Population 7, adjustment = 4%; Population 4, adjustment = 4%; and Population 5, adjustment = 6%

Figure 3: Yields of hulled barley versus non-adjusted and adjusted† yields of hulless full-sibs in four populations planted at Blacksburg, VA in 2011-2012.



†: Adjustments of hulless yields are as follow: Population 1, adjustment = 21%; Population 7, adjustment = 21%; Population 4, adjustment = 15%; and Population 5, adjustment = 15%

CHAPTER III

Marker-Trait Associations in Virginia Tech Winter Barley Identified Using Genome-Wide Mapping

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ABSTRACT

Genome wide association studies (GWAS) provide an opportunity to examine the genetic architecture of quantitatively inherited traits in breeding populations. The objectives of this study were to use GWAS to identify chromosome regions governing traits of importance in six-rowed winter barley (*Hordeum vulgare* L.) germplasm and to identify single nucleotide polymorphisms (SNPs) markers that can be implemented in a marker-assisted breeding program. Advanced hulled and hulless lines (329 total) were screened using 3,072 SNPs as a part of the U.S. Barley Coordinated Agricultural Project (CAP). Phenotypic data collected over four years for agronomic and food quality traits and resistance to leaf rust (caused by *Puccinia hordei* G. Otth), powdery mildew [caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *hordei* Em. Marchal], net blotch (caused by *Pyrenophora teres*) and spot blotch [caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechsler ex Dastur] were analyzed with SNP genotypic data in a GWAS to determine marker-trait associations. Significant SNPs associated with previously described quantitative trait loci (QTL) or genes were identified for heading date on chromosome 3H, test weight on 2H, yield on 7H, grain protein on 5H, polyphenol oxidase activity on 2H and resistance to leaf rust on 2H and 3H, powdery mildew on 1H, 2H and 4H, net blotch on 5H and spot blotch on 7H. Novel QTL also were identified for agronomic, quality, and disease resistance traits. These SNP-trait associations provide the opportunity to directly select for QTL contributing to multiple traits in breeding programs.

Keywords

Barley, Association mapping, Quantitative trait loci, Disease resistance, Quality traits, Single nucleotide polymorphism

INTRODUCTION

The use of genome-wide association mapping (GWAS) to identify marker-trait associations in plants has become a popular alternative to traditional bi-parental mapping studies. Association mapping has been used to study yield and yield stability and agronomic and morphological traits in barley (Kraakman et al. 2004; Inostroza et al. 2009). In barley, use of association genetics has been made possible by the availability of high parallel SNP assay platforms (Waugh et al. 2009). These platforms have allowed for sufficient marker densities for genome-wide scans and LD gene identification (Waugh et al. 2009). In a panel of 190 barley cultivars, GWAS was used to identify SNP markers associated with row type (Ramsay et al. 2011), which provide an initial starting point for isolating the *INTERMEDIUM-C* gene. Wang et al. (2012a) found significant associations for sixteen morphological and nine agronomic traits in GWAS of 615 United Kingdom barley cultivars. Steffenson et al. (2007) used GWAS to detect significant chromosome regions conferring resistance to stem rusts (*Puccinia graminis* Pers.: Pers. f. sp. *tritici* Eriks. & E. Henn and also Pers. f. sp. *secalis* Eriks. & E. Henn) in Sharon goatgrass (*Aegilops sharonensis*) and wild barley (*Hordeum vulgare* ssp. *spontaneum*).

GWAS have been used successfully in diverse crops to identify genomic regions contributing to numerous traits. Tian et al. (2011) used genome-wide nested association mapping to detect genes associated with leaf architecture traits including upper leaf angle, leaf length and width in maize (*Zea mays* L.). Mamidi et al. (2011) identified loci involved in iron deficiency chlorosis tolerance in soybean (*Glycine max* L.). Yao et al. (2009) identified significant marker-trait associations for yield, thousand kernel weight, spikelets per spike, grains per spike, spikelet density, and plant height on chromosome 2A in wheat (*Triticum aestivum* L.). Adhikari et al. (2012) utilized GWAS to identify genomic regions associated with resistance to

bacterial leaf streak (*Xanthomonas translucens* pv. *undulosa*) and spot blotch (*Cochliobolus sativus*) in spring wheat. Poland et al. (2011) used genome-wide nested association mapping to identify candidate genes of a quantitative nature related to plant defense in a study focusing on resistance to northern leaf blight (*Setosphaeria turcica*) in maize. These studies clearly demonstrate the effectiveness of GWAS to dissect complex genetic traits and identify markers associate with these traits.

The Barley CAP project focused on analysis of genotypic and phenotypic data for diverse barley germplasm in 10 U.S. breeding programs in order to identify marker-trait associations using GWAS (Blake et al. 2012; Waugh et al. 2009). Genotypic data was generated using two barley Oligo Pool Assays (OPAs) containing 3,072 SNPs with Illumina's Golden Gate assay (Close et al. 2009; Fan et al. 2006). The two barley OPAs were developed by Close et al. (2009) from barley expressed sequence tags (ESTs). Recently, a new consensus SNP map was developed by Munoz-Amatriain et al. (2011), and contains 2,994 SNP loci in 1,163 marker Bins covering a genetic distance of 1,137 centimorgans (cM).

Germplasm from the 10 U.S. programs participating in the Barley CAP was comprised of two-rowed and six-rowed, winter and spring habit, and malt, feed, and food grade barley. Only the Virginia Tech and Oregon State University programs focus on winter barley. Unique structure of the programs allowed for the determination of LD within U.S. barley programs as well as mapping of multiple traits through GWAS (Hamblin et al. 2010; Lorenz et al. 2010; Massman et al. 2010; Roy et al. 2010; Wang et al. 2012b). Limited exchange between the Virginia Tech program and other U.S. programs is likely due to differences in growth habit, adaptation, and end use (Hamblin et al. 2010). The Virginia Tech program had the lowest LD of any program (Hamblin et al. 2010). The Virginia Tech program is also unique in development of

both hulled and hulless germplasm. Identification of QTL within the Virginia Tech program through GWAS is valuable for furthering marker assisted selection (MAS) within the program as well as dissemination of unique alleles to other breeding programs with similar breeding objectives.

GWAS has been used successfully to identify marker-trait associations within the barley CAP material. Using single SNP and haplotype analysis Lorenz et al. (2010) detected heading date QTL in 1,824 barley lines from CAP years 2006 and 2007. Wang et al. (2012b) detected three major heading date QTL in a panel of 766 spring barley breeding lines and a subset of 384 lines. GWAS has been used to identify chromosome regions conferring resistance to Fusarium head blight [(*Fusarium graminearum* (Schwabe)] and spot blotch in barley (Massman et al. 2010; Roy et al. 2010). These studies highlight the potential of GWAS to identify markers associated with both novel and previously described QTL for agronomic traits and disease resistance.

The Virginia Tech barley breeding program is unique within the U.S in that its primary focus is on the development of winter hulled and hulless six-rowed varieties for use in feed and fuel industries. While several breeding programs focus on two- and six-rowed spring malting barley, only two programs (Virginia Tech and Oregon State University), with very unique and diverse germplasm, breed six-rowed winter feed barley (Hamblin et al. 2010). Greater diversity exists among the 6-rowed winter feed barley germplasm, which is attributed to a more lax selection criteria for end-use quality than that required for malting barley (Hamblin et al. 2010). Currently, very few markers are being used in the Virginia Tech program for MAS and allele enrichment. The main markers currently being used for MAS are ones for selection of the *nud* locus which controls the hulless phenotype (Taketa el al. 2008). Previous mapping studies

pertaining to Virginia Tech barley have focused on development of markers for leaf rust (caused by *Puccinia hordei*) and net blotch resistance (caused by *Pyrenophora teres*) (Mammadov et al., 2007; O'Boyle 2009). Identification and validation of markers for additional agronomic, quality, and disease resistance traits in Virginia hulled and hulless barley germplasm would facilitate implementation of an effective marker-assisted variety development program. Other programs lacking resistance genes/QTL present in the Virginia Tech germplasm could benefit from markers identified in this study for introgression of QTL into their breeding material. Effectiveness of GWAS to identify novel and previously described regions associated with traits of interest in barley has been well documented in previous studies (Lorenz et al. 2010; Massman et al. 2010; Roy et al. 2010; Wang et al. 2012b). Validation of markers is essential to eliminate potential false positive associations and to identify previously reported or novel sources of QTL/genes within a breeding program. Identification of markers mapped to previously reported regions containing QTL/genes give validation of true marker-trait associations, while markers identifying novel QTL need further validation. Concurrently, understanding the underlying genetic architecture helps to determine which alleles can be selected for without simultaneously selecting for deleterious alleles. The research findings presented here are the first large-scale attempt to identify markers for critical traits within the 6-rowed hulled and hulless winter barley germplasm.

Yields of hulless barley are 10% to 30% less than hulled barley on average (Choo et al. 2001; Liu et al. 1996). Thus, improving yield potential of hulless barley is an important priority. Higher starch and lower fiber and ash concentrations in hulless versus hulled barley make it desirable for use in food, feed, and biofuel production (Griffey et al. 2010). Concurrently, the hulless trait in barley has been associated with poor seedling emergence and low seed weight

(Choo et al. 2001). Hulless lines yielded less than hulled lines even when adjusting for hull weight loss by adding 15% to 19% to yield of hulless lines (Choo et al. 2001). The *nud* gene which controls the hulless phenotype contributed 47% to 57% of the total variation for yield (Choo et al. 2001). Conversely, hulless lines have about 20% greater test weight than hulled lines with the *nud* gene contributing 85% to 95% of total variation for test weight (Choo et al. 2001). Significantly higher test weights and lower kernel weights for hulless lines have been reported for Virginia Tech barley (Griffey et al. 2010). It is hypothesized that the *nud* gene might have a pleiotropic effect on yield or might be linked to a yield QTL (Choo et al. 2001).

Quality traits of barley are important for brewing, food, feed, and fuel industries. Both positive and negative quality attributes are associated with hulled and hulless barley. Hulless barley has been shown to contain significantly higher starch concentrations than hulled and malting type barley (Griffey et al. 2010). Starch content of hulless lines ranged from 59.1% in 2003 to 63.5% in 2005, while that of hulled lines ranged from 53.5% in 2003 to 59.2% in 2002 (Griffey et al. 2010). Hulless barley has also been shown to contain higher levels of β-glucan which is desirable in food grade barley (Bhatty, 1999; Griffey et al. 2010). Grain protein of hulled and hulless lines was reported to be similar and ranged from 7.98% to 10.96% in Virginia winter barley (Griffey et al. 2010). Barley grain contains higher levels of phenolic compounds than other cereal grains, which makes it more susceptible to discoloration by polyphenol oxidase (PPO) (Bendelow and LeBerge, 1979; Baik et al. 1995). Oxidation of phenolic compounds by PPO has a negative effect on the aesthetic properties of products made from barley (Baik et al. 1995; Quinde-Axtell et al. 2005). Gutierrez et al. (2011) used GWAS to detect genes and QTL for beer and malting quality.

Foliar fungal pathogens such as leaf rust, powdery mildew (caused by *Blumeria graminis* f. sp. *hordei*) and net blotch are prevalent diseases in the mid-Atlantic region of the U.S. where winter feed barley is grown. They impact productivity by reducing green leaf area, photosynthesis, and transpiration which negatively affect yield and grain quality. Although many studies have focused on identification and mapping of resistance genes in spring type barley, few have focused on genes contributing to resistance within the Virginia Tech winter barley breeding material. Mammadov et al. (2007) developed and validated markers for leaf rust resistance genes *Rph5* and *Rph7*. O'Boyle et al. (2011) characterized net blotch resistance in the six-rowed winter barley cultivar Nomini and in two spring barley lines concluding that each carried single dominant resistance genes. Using a bi-parental population, O'Boyle (2009) mapped the resistance gene in Nomini near the centromere of chromosome 6H.

This study contributes to the growing use of GWAS to identify marker-trait associations in a variety of important agronomic plants. Use of improved methodology helps to limit false positives that can occur due to population structure. Currently, little marker information is available for genes or QTL contributing positively to agronomic, quality, and disease resistance traits in the six-row winter barley germplasm. Identification of markers through GWAS would greatly aid in MAS. The objectives of this study are to use genome-wide association mapping to: 1) identify chromosome regions governing traits of importance in six-rowed winter barley germplasm; 2) identify SNP markers that can be implemented in a MAS breeding program and; 3) identify the genetic architecture for five key traits.

MATERIALS AND METHODS

Field evaluations

A set of 329 hulled and hulless barley cultivars and advanced lines from the Virginia Tech program were evaluated for agronomic, disease resistance, and quality traits over a four year period. During each year of the study, barley lines in preliminary (F_7, F_8 and F_9 generations) and advanced tests (F_9 and greater generations) were planted in a randomized complete block design with three replications at Warsaw and Blacksburg, VA. Plots were planted at standard seeding rates for hulled and hulless barley and managed according to standard practices recommended in the mid-Atlantic region (Thomason et al. 2009). Each experimental unit consisted of a seven-row yield plot, 2.7 m in length with 15.2 cm spacing between rows and a harvested plot area of 2.9 m^2. Common hulled (Callao, Price, Nomini, Thoroughbred, Wysor, VA92-42-46 and VA96-44-304) and hulless [Doyce, Eve (VA01H-68), Dan (VA03H-61) and VA01H-125] checks were used in each year of the study to provide consistent comparisons. These checks are used extensively within the program as parents and common checks in field trials. Agronomic data was collected for plant height, stem length, lodging resistance, grain yield, and test weight. Disease reaction under natural field infection was assessed using an ordinal 0 to 9 rating scale (0=Absence of disease symptoms and 9=Severe disease) related to percent disease severity for leaf rust, net blotch, and powdery mildew. All genotypic and phenotypic data for the Virginia Tech barley program used in this study are available at The Hordeum Toolbox (Blake et al. 2012).

Quality data

To measure polyphenol oxidase (PPO) activity, de-hulled barley kernels were ground using a Cyclone sample mill (UDY Corporation, Fort Collins, CO) fitted with a screen having

0.5 mm openings. PPO activity was determined using a modified protocol of American Association of Cereal Chemists International (AACCI) Approved Method 22-85.01 (2010). Whole barley flour (0.20 - 0.22 g) was reacted with 1.5 ml of L-Dihydroxyphenylalanine (L-DOPA) for one hour on a Labquake rotator (Barnstead Thermolyne, Dubuque, IA) at room temperature, followed by centrifugation at 5,000 x *g* (times gravity) for 10 min. Absorbance was read at 475 nm with L-DOPA as the blank and used as the estimate of PPO activity.

Greenhouse screening

Lines were screened in the greenhouse each year for seedling reaction to leaf rust, powdery mildew, and spot blotch. Virulence/avirulence formulas for *P. hordei* isolates ND8702 (Race 8) and VA90-34 (Race 30) used in the study are described in Mammadov et al. (2007). Inoculation, screening, and rating methods are as described by Brooks et al. (2000). Scores from greenhouse ratings were transformed to a numeric scale to account for infection type (IT) in the association analysis (Steffenson, personal communication).

Bulk isolates of powdery mildew collected from infected volunteer barley plants in the field were used in greenhouse disease screening tests. Isolates were increased and maintained on the susceptible barley cultivars Thoroughbred and Dayton. Inoculation, screening and rating methods are as described by Moseman et al. (1984). Scores from greenhouse ratings were transformed to a numeric scale to account for infection type (IT) in the association analysis (Steffenson, personal communication).

Seedling resistance to spot blotch was assessed using spot blotch isolate ND85F. Planting, inoculation, screening, and rating methods are as described by Roy et al. (2010).

Genotyping

Samples were genotyped at the USDA-ARS Biosciences Research Lab in Fargo, ND with two barley Oligo Pool Assays (OPAs) containing 3,072 SNPs using Illumina's Golden Gate assay (Fan et al. 2006). Data analysis of SNPs from the two barley OPAs was performed using Illumin's Bead Studio software with manual inspection. The two barley OPAs were developed by Close et al. (2009) from barley expressed sequence tags (ESTs). The consensus SNP map developed by Munoz-Amatriaín et al. (2011) containing 2,994 SNP loci in 1,163 marker Bins covering a genetic distance of 1,137 centimorgans (cM) was used in the study to determine chromosome location of traits.

Statistical analysis

The Mean (Proc Mean) and GLM (Proc GLM) procedures in SAS version 9.2 (SAS Institute, Cary, North Carolina, 2008) were used to analyze phenotypic data and to generate trait means and analyses of variance. Means within location and year, as well as across years and locations, were used in association analyses, which were conducted using TASSEL v. 3.0 (Bradbury et al. 2007). A total of 2,242 SNPs were initially investigated. Marker data were filtered for presence in a minimum of 246 (75% of 329 lines) lines to be present in filtered data set. A minimum allele frequency (MAF) of 0.1 was used to further filter the data set. Thus, markers with a MAF of less than 10% were removed from the data set prior to analysis. After filtering, a total of 1,213 SNPs were used in the association analysis. A mixed linear model (MLM) was used to determine marker trait associations (Yu et al. 2006). The mixed linear model combines information for population structure (*Q*) and kinship (*K*) (Bradbury et al. 2007). Population structure (*Q*) was accounted for using a principle component analysis (PCA) (Price et al. 2006). The PCA was based on filtered SNP datasets generated in TASSEL. Kinship (*K*),

which is the average relatedness between lines, was calculated in TASSEL using filtered SNP datasets. Prior knowledge of the breeding populations suggested that three major sub-populations exist in the Virginia Tech barley breeding program. While the hulled barley breeding program has existed for over 60 years, the hulless barley breeding program was only initiated during the past 20 years. Crosses between hulled x hulless parents were made initially and to a lesser extent presently to develop superior hulless genotypes. Most of the lines evaluated in the current study were derived from hulled x hulled and hulless x hulless crosses, while fewer lines were derived from hulled x hulless crosses. Sub-populations arising from differences in hulled and hulless genotypes, and different elite parents used in development of hulled x hulless crosses account for the observed sub-population divisions.

Post association analysis corrections are necessary to adjust for statistical confidence intervals based on the number of tests performed (Noble 2009). The Bonferroni (BON) option in Proc MULTTEST in SAS version 9.2 (SAS Institute, Cary, North Carolina, 2008) was used to correct for multiple testing. Significant *P* values from the BON correction were used to identify significant marker-trait associations. Allele substitution effects presented in tables and text are the difference between the two genotypes for a given marker. Positive or negative signs for allele substitution effects designate which genotype was associated with a higher or lower value.

Linkage disequilibrium

LD (r^2) values were calculated from SNP datasets in HAPLOVIEW (Barret et al. 2005). Only SNP alleles with a MAF > 0.1 were used in calculations. LD values were plotted against the genetic distance (cM) to estimate LD decay and average distribution of r^2 values between SNP marker pairs in the full set of Virginia Tech barley germplasm. Additionally, LD was

assessed between significant markers (after BON correction) to define boundaries of potential QTL identified in the association analysis.

RESULTS

Analysis of phenotypic data

Phenotypic data for significant marker-trait associations are presented in Table 1. The full list of data analyzed in the GWAS is available in supplemental Table S1. Due to the larger number of traits and datasets analyzed, only those for which significant marker-trait associations were identified are presented herein. The number of genotypes (N) varied per analyzed dataset. Significant ($P \leq 0.05$) differences between genotypes existed for all analyzed traits. Mean values, standard deviations, minimum and maximum values show the variability of traits for lines analyzed in the study.

Population structure and LD within Virginia Tech barley germplasm

Accounting for population structure prior to an association analysis is important to account for subdivisions that may lead to spurious marker-trait associations. Population substructure was accounted for using PCA (Figure 1). Prior knowledge of the breeding program's composition suggested the existence of three sub-populations consisting of hulled, hulless, and hulled x hulless genotypes. A screen plot indicated that three principle components (PC) accounted for 31% of total observed variation (data not shown). PC1 accounted for 16% of the variation and separated hulled lines from the remainder of lines in the study. PC2 accounted for 11% of the variation and separated hulless genotypes derived primarily from crosses among hulled and hulless parents. PC3 accounted for 4% of the total observed variation and further subdivided hulless genotypes derived from hulled x hulless crosses. Wang et al. (2012b) demonstrated the effectiveness of PCA to account for population structure prior to GWAS.

LD (r^2) was assessed in the full set of Virginia Tech lines using 2,242 SNPs. LD was found to decrease below the basal level (r^2<0.1) over a distance of 5 to 10 cM (Figure 2). At 0 cM average LD among the 2,243 SNPs was r^2 = 0.35. Concurrently, 49.9% of SNP pairs had an average LD below the basal level (r^2<0.1). At 50 cM marker pairs are not considered to be linked. Average LD among SNP pairs at 50 cM was r^2 = 0.04. A high portion of SNP pairs (8.9%) have an LD greater than r^2>0.1.

GWAS for QTL contributing to agronomic traits

GWAS was conducted on yield, test weight, heading date, plant height, lodging, and stem length. Marker-trait associations for QTL contributing to heading date, plant height, test weight, and yield were identified on chromosomes 1H, 2H, 3H, 5H, 6H, and 7H (Table 2). Marker-trait associations were mapped to regions of the barley genome previously described in the literature as containing QTL for these traits as well as to previously unreported regions that were classified as novel QTL.

QTL *QHd-3H.102* for heading date was identified on chromosome 3H by marker 1_0583 which explained 6% of the variation. The mean difference in heading date between genotypes having different marker alleles was 2.5 days. This region corresponds to a previously mapped QTL for heading date *QHd.StMo-3H.2* described by Hayes et al. (1993) and *QHd3H.100* described by Wang et al. (2012b). A single significant novel QTL (*QPh-1H.30*) for plant height was identified on chromosome 1H by marker 1_0238 which explained 4% of the variation. There was a 4.7 cm mean difference in plant height between genotypes having different marker alleles.

The test weight QTL *QTwt-2H.164* identified by marker 2_1436 mapped to the same region containing *QTw.IgDa-2H* described by Backes et al. (1995). This marker explained 6%

of the variation, with the favorable allele being associated with an increased test weight of 18.0 g liter^{-1} relative to the alternative allele in the Warsaw across year's dataset. Markers on chromosomes 2H, 5H, and 7H were associated with potentially novel QTL that explained 4% to 10% of the variation in test weight. A total of three markers (3_0216, 1_0138 and 1_1307) associated with the novel QTL *QTwt-2H.107-109* on chromosome 2H explained 7% to 10% of the variation in the Warsaw across year's dataset with allele substitution effects ranging from 21.1 to 24.0 g liter^{-1}. The novel QTL *QTwt-7H.91-94* on chromosome 7H explained 4% to 7% of the variation in test weight when data was analyzed across years and environments. Unfavorable alleles were associated with a decrease in test weight of 51.9 and 104.2 g liter^{-1} which was noted for markers 1_0143 and 2_0685 associated with this QTL.

The yield QTL *QYld-7H.94* identified on chromosome 7H was identified by marker 2_0685 and explained 5% of variation for yield in the Warsaw across year's dataset. The favorable allele was associated with an increase in yield of 1693.3 kg ha^{-1} for marker 2_0685. The region containing 2_0685 corresponds to the region containing the previously described yield QTL *QYld.HaTR-7H* (Tinker et al. 1996; Rostoks et al. 2005). A novel QTL *QYld-6H.7* was identified on chromosome 6H by two markers (3_0651 and 2_1204). The markers explained 3% of variation with allele substitution effects ranging from 539.0 to 580.2 kg ha^{-1} for yield when data was analyzed across years and environments.

GWAS for QTL contributing to grain quality traits

Association mapping was conducted for amylose, beta glucan and grain protein concentration, grain weight, grain hardness (SKCS), hull proportion, phenolic compound content, and polyphenol oxidase activity. Significant QTL for grain protein and polyphenol oxidase activity were identified on chromosomes 2H, 5H, and 6H (Table 3). QTL were not

detected for amylose, beta glucan concentration, grain weight hardness (SKCS), hull proportion, or phenolic compound content. Lack of QTL identified for these traits is due to little phenotypic variation among lines or lack of significant associations.

A single marker (3_1417) identified QTL *QGp-5H.96* on chromosome 5H. This marker mapped to a region that contained the previously described QTL *QGpc.HaMo-5H* for grain protein described by Marquez-Cedillo et al. (2000). Marker 3_1417 described 15% of the variation for grain protein across environments in 2009. The favorable allele at marker 3_1417 was associated with an increase in grain protein of 2.55% relative to the alternate allele. A novel QTL *QGp-6H.142* for grain protein was identified on chromosome 6H by marker 2_0537 and described 13% of the variation for grain protein across environments in 2009. The unfavorable allele associated with *QGp-6H.142* was associated with a 2.03% decrease in grain protein.

A QTL *QPPO-2H.119-125* for polyphenol oxidase activity was identified on chromosome 2H. This QTL mapped to the region described by Taketa et al. (2010) as containing duplicate *PPO* genes. Markers associated with the region explained 6% to 15% of the variation observed for polyphenol oxidase activity in the across years dataset. Allele substitution effect for absorbance ranged from -0.15 to -0.27 for favorable alleles associated with the PPO gene containing region. Markers 2_0099, 2_1184, and 20064 were the most significant markers associated with polyphneol oxidase activity explaining the greatest amount of variation (15%). Reductions in absorbance are indicative of reductions in discoloration in products made from barley grain.

GWAS for QTL contributing to disease resistance

Identification of regions of the genome contributing to disease resistance is important for development of cultivars resistant to multiple pathogens. Association mapping was conducted

for resistance to leaf rust, powdery mildew, net blotch, and spot blotch. Significant ($P \leq 0.05$) marker-trait associations were identified on chromosomes 2H, 3H, 6H, and 7H for leaf rust resistance (Table 4), 1H, 2H, and 4H for powdery mildew resistance (Table 5), 5H for net blotch, and 7H for spot blotch (Table 6).

Seedling resistance to leaf rust was assessed for two *P. hordei* races (8 and 30). Significant ($P \leq 0.05$) marker-trait associations were identified for chromosome regions containing previously described *Rph* genes which confer resistance to *P. hordei* race 8, race 30, and races contributing to natural field infections (Table 4). Markers 2_1398 and 3_0297 identified QTL *QRph-3H.11* which confers resistance to *P. hordei* race 8. These markers map to the 3H chromosome region containing gene *Rph7* (Parlevliet, 1976). Three markers on chromosomes 2H and 3H were associated with regions containing previously reported genes conferring resistance to *P. hordei* race 30. The unfavorable allele for 2_0159 identifying QTL *QRph-3H.6* was associated with an increase in leaf rust scores by 1.83 relative to the alternative allele. Markers 1_0376 and 1_1227 identified the QTL *QRph-2H.149* on chromosome 2H and both explained 23% of the variation with favorable alleles being associated with a reduction in leaf rust scores of 0.61 relative to the unfavorable alleles. These markers mapped to the region containing *Rph15* (Weerasena et al., 2004). Novel QTL for seedling leaf rust resistance were found on chromosomes 7H for race 8 and on 6H for race 30. A single marker (1_0713) was associated with the novel QTL *QRph-7H.81* for seedling resistance to race 8, while marker 3_1274 was associated with *QRph-6H.49*, which contributes to race 30 resistance.

QTL were identified for seedling and field resistance to powdery mildew (Table 5). The QTL *QPm-1H.8-11* was identified by four markers (2_1174, 2_1226, 3_0950 and 3_0951) on chromosome 1H. Markers identifying *QPm-1H.8-11* explained 6% to 8% of the variation for

powdery mildew resistance and were associated with allele substitution effects ranging from -0.95 to -1.06 across years in the greenhouse. These markers map to the region containing the multi-allelic *Mla* resistance gene cluster (Wei et al. 1999). Marker 1_1050 mapped to the region containing the previously described resistance gene *MlLa* (Giese et al., 1993). Markers 3_0142, 1_0785 and 3_1139, associated with the QTL *QPm-4H.100-114*, were the only markers associated with field resistance to powdery mildew. Favorable alleles for marker 3_0142 and 1_0785 were associated with a consistent reduction in powdery mildew scores of 3.17 to 3.39 relative to the alternative alleles for these markers and explained 23% to 26% of the variation for powdery mildew resistance in the Warsaw 2008 dataset. Markers identifying *QPm-4H.100-114* mapped to the region containing the previously described *mlo* resistance gene (Buschges et al., 1997). The unfavorable allele for marker 3_1139 identifying the novel QTL *QPm-4H.114* resulted in an increase of 2.94 in powdery mildew scores across environments in 2009 relative to the alternative allele. Marker 1_1050 was associated with QTL *QPm-2H.169* on chromosome 2H and explained 6% of the variation for powdery mildew resistance across years in the greenhouse. Presence of the unfavorable allele in barley genotypes was associated with an increase of 1.05 in powdery mildew scores relative to the alternative allele, thus the deleterious effect of *QPm-2H.169* on powdery mildew resistance needs to be evaluated further.

Marker-trait associations ($P \leq 0.05$) for field resistance to net blotch and seedling resistance to spot blotch were identified on chromosomes 5H and 7H, respectively (Table 6). Marker 2_0987 identified the QTL *QNb-5H.38* for net blotch resistance and explained 8% of the variation across environments with the favorable allele being associated with a reduction in net blotch score by 3.69. Marker 2_0987 mapped to the region containing a previously identified QTL *QNb.StMo-5H* (Steffenson et al. 1996) for net blotch resistance. A novel QTL *QNb-5H.60*

associated with net blotch resistance was identified on chromosome 5H by marker 3_0007, which explained 9% of the variation for net blotch resistance across environments. The unfavorable allele for this marker was associated with an increase in net blotch scores of 1.97 relative to the alternative allele.

Markers 2_0495 and 1_0451 identified the QTL *QSb-7H.18-27* associated with seedling resistance to spot blotch on chromosome 7H. These markers explained 6% to 7% of variation for spot blotch resistance. Unfavorable alleles for these markers were associated with increases in disease scores of 0.77 and 0.93, respectively. These markers mapped to a region containing a previously described QTL associated with spot blotch resistance (Steffenson et al. 1996; Roy et al. 2010).

LD pattern for QTL in hulled and hulless germplasm

The pattern of LD (R^2) between markers identifying regions containing QTL detected in the association analysis was investigated between all pair-wise combinations of markers. Regions of interest included the multiple markers identifying the test weight QTL *QTwt-2H.107-109* on chromosome 2H, yield and test weight QTL (*QYld-7H.94* and *QTwt-7H.91-94*) on chromosome 7H, polyphenol oxidase activity QTL *QPPO-2H.119-125* on chromosome 2H, leaf rust resistance QTL (*QRph-3H.6* and *QRph-3H.11*) on chromosome 3H, and powdery mildew resistance QTL *QPm-1H.8-11* on chromosome 1H (Figure 3a, b, c, d and e). Investigating LD within the regions allowed for the identification of boundaries for QTL and detection of other markers in LD with significant markers identified in the association analysis. Markers in LD (R^2>0.2) were identified for these regions of interest. A novel QTL for test weight (*QTwt-2H.107-109*) was identified by markers 3_0216, 1_0138, and 1_1307 on chromosome 2H. A high degree of LD (R^2 = 0.37 to 0.95) existed between these markers, and they are considered to

identify the same test weight QTL. These markers also share varying degrees of LD with flanking markers. However, no other markers in the region were significant for test weight in the association analysis. A novel QTL for test weight (*QTwt-7H.91-94*) was identified by two markers (1_0143 and 2_0685) that were in high LD (R^2 = 0.89) and considered to detect the same QTL. Additionally marker 2_0685 identified the yield QTL (*QYld-7H.94*), suggesting that the test weight and yield QTL were coincidental. Markers 1_10143 and 2_0685 were in moderate to high LD (R^2= 0.25 to 0.63) with seven other markers; however, these markers were not significantly associated with yield or test weight in the association analysis. A total of seven markers identified the QTL *QPPO-2H.119-125* for polyphenol oxidase activity on chromosome 2H. Markers in this region showed moderate to high LD (R^2 = 0.23 to 1.0) and are considered to identify the same QTL. Varying degrees of LD were shared between these markers and additional markers in the area that were not significant for polyphenol oxidase activity. Two QTL (*QRph-3H.6* and *QRph-3H.11*) for resistance to *P. hordei* races 30 and 8, respectively, were identified 5 cM apart on chromosome 3H. Mammadov et al. (2007) estimated the distance between *Rph5* and *Rph7* to be around 5 cM. A single marker 2_0159 identified *QRph-3H.6* (same region as gene *Rph5*) while two markers 2_1398 and 3_0297 identified *QRph-3H.11* (same region as *Rph7*). Markers identifying *QRph-3H.6* and *QRph-3H.11* are not in LD (R^2 = 0.03 to 0.04) and these QTL are considered to be independent. Four markers identified the QTL *QPm-1H.8-11*, which mapped to the same region as the previously described *Mla* resistance cluster (Wei et al. 1999). Markers 2_1174, 2_1226, and 3_0950 exhibited moderate to high LD (R^2 =0.27 to 0.63), while marker 3_0951 exhibited high LD (r^2 = 0.36) with 3_0950 but was not in LD (R^2 = 0.04 to 0.06) with markers 2_1174 and 2_1226. Markers 2_1174 and 2_1226 share

a high degree of LD with multiple markers on chromosome 4H. However, these markers were not significant for powdery mildew resistance.

DISCUSSION

Barley CAP

The purpose of barley CAP was to develop SNP markers for identification of QTL in breeding programs to be utilized for MAS. A total of 96 lines were submitted during all four years of the study by the Virginia Tech winter barley program. Although it was possible to genotype 384 unique individuals, common checks and individuals from a separate mapping study were submitted for genotyping. Therefore, a total of 329 lines were used for the GWAS. A total of 318 experimental lines represented promising breeding material being evaluated for agronomic performance, quality traits, and disease resistance. Genotyping of common checks and experimental lines allowed for the determination of LD within the breeding program and detection of QTL through GWAS. A total of 23 traits (supplemental Table S1) were investigated in the current study.

GWAS of agronomic traits

GWAS of agronomic traits in the Virginia Tech breeding program revealed common and novel QTL for heading date, plant height, test weight, and yield. Of the markers identified, 15 were mapped to previously described QTL for heading date, test weight, and yield. The QTL *QHd.3H-102* for heading date mapped to the same region as the previously reported QTL *QHd.StMo-3H.2* (Hayes et al. 1993). Restriction fragment length polymorphism (RFLP) markers ABG453 and ABC307B were the initial significant flanking markers identifying *QHd.StMo-3H.2* which spanned an 8.5 cM region on the Barley consensus SNP map (Rostoks et al. 2005). Marker 1_0583 associated with the region containing *QHd.StMo-3H.2* mapped less

than 1 cM from ABC453 on the 2009 SNP consensus map (Close et al. 2009). The favorable allele was associated with a reduction in heading dates of 2.5 days relative to the alternative allele. The marker identifying *QHd.3H-102* would be useful in development of earlier heading varieties.

Of the 10 markers associated with test weight QTL, two mapped to regions containing previously described QTL *QTw.nab-2H* (Marquez-Cedillo et al. 2000) and *QTw.IgDa-2H* (Backes et al. 1995) on chromosome 2H. Marquez-Cedillo et al. (2000) mapped *QTw.nab-2H* to a region on chromosome 2H flanked by markers *vrs1* and MWG503. In addition to *QTw.nab-2H*, QTL for kernel plumpness, grain protein concentration, soluble protein to total protein (S/T) ratio and diastatic power, which is the joint action of α-amylase and β-amylase and any other carbohydrate-degrading enzymes, were also mapped to the region flanked by *vrs1* and MWG503. These QTL are thought to be coincidental to the *vrs1* locus (Marquez-Cedillo et al. 2000). The *vrs1* locus is known to control the six row phenotype in barley (Ubisch, 1916; Lundqvist et al. 1997). Marquez-Cedillo et al. (2000) hypothesized that inflorescence architecture controlled by *vrs1* could determine grain size and partitioning of proteins and enzymes to kernels and that grain protein concentration, S/T protein ratio and diastatic power could be controlled by a gene or genes linked to *vrs1*. Komatsuda et al. (2007) determined that *Vrs1* (two rowed phenotype) encodes for a member of the homeodomain-leucine zipper (HD-ZIP) class of transcription factors. Concurrently, it was shown that the six rowed phenotype controlled by *vrs1* arose due to a mutation in the HD-ZIP I-class homeobox gene (Komatsuda et al. 2007). We identified markers in the *vrs1* region associated with *QTw.nab-2H* for test weight. Interestingly, the Virginia Tech program is entirely of six row (*vrs1*) morphology. Marker 1_0196 mapped 17.56 cM proximal of *vrs1* (Szucs et al. 2009). For marker 1_0196 two alleles

are present in the breeding materials, with neither being fixed within the hulled or hulless subpopulations. Thus, either there are two or more *vrs1* alleles segregating in the breeding materials with one of them contributing directly to increased test weight. Alternatively, it is possible that the same *vrs1* allele is present in all breeding lines but it was from different sources that carry closely linked alternative alleles for the test weight QTL. Backes et al. (1995) mapped *QTw.IgDa-2H* to a region on chromosome 2H flanked by RFLP markers MWG866 and MWG829. Variation explained for this QTL was low (Backes et al. 1995). Of the two markers identifying previously described QTL for test weight, marker 2_1436 was significant in the Warsaw dataset across years suggesting that it may be useful in selecting for improved test weight in this environment.

The QTL *QYld-7H.94* for yield mapped to the same position as the previously described QTL *QYld.HaTR-7H* (Tinker et al. 1996; Rostoks et al. 2005). Tinker et al. (1996) mapped *QYld.HaTR-7H* to a region on chromosome 7H flanked by RFLP markers MWG626 and MWG571D. These markers flank Amy2 and ABC154 on the 2nd Barley consensus map (Qi et al. 1996). Marker ABG701 mapped 8.3 cM proximal to the QTL on the short arm of chromosome 7H, ABC154B mapped to the same position as *QYld.HaTR-7H* and AMY2 mapped 3.8 cM distal from the QTL on the agronomic QTL consensus map (Rostoks et al. 2005). Additionally, QTL for dormancy and spikes per unit area map to the same region as *QYld.HaTR-7H* on the consensus map (Rostoks et al. 2005). The SNP marker 2_0685 identifying the region containing *QYld.HaTR-7H* in the current study maps 2 cM from ABG701 and 34 cM from Amy2 on the 2009 consensus map (Close et al. 2009). Therefore, the SNP marker identifying *Qyld-7H.94* falls within a 10-30 cM region of the markers identifying *QYld.HaTR-7H*. The favorable allele for the marker identifying *QYld-7H.94* was associated with a 1693.3 kg ha^{-1} yield increase

in Warsaw across years. Hulless barley lines yield 10% to 30% less than hulled cultivars on average (Liu et al. 1996; Choo et al. 2001) and constitute a large portion of the Virginia Tech barley program. Conversely, test weights of hulless lines are significantly higher than those of hulled lines (Griffey et al. 2010). Due to the inverse relationship between yield and test weight and the presence of hulled and hulless lines in the Virginia Tech program, it can be hypothesized that *QTwt-7H.91-94* is a coincident QTL with *QYld.HaTR-7H*. Adversely, the unfavorable allele for marker 2_0685 associated with the yield QTL is fixed within the hulless population. The true effect of the *nud* locus on this QTL is not known. Rostoks et al. (2005) positioned *QYld.HaTR-7H* at 91 cM which is approximately 3.8 cM from Amy2 and 13 cM proximal of the *nud* locus (104 cM). Szucs et al. (2009) positioned 2_0685 marker 1.09 cM distal of the nud locus. Based on the position of 2_0685 relative to the nud locus it is likely that recombination between marker 2_0585 and the nud locus is unlikely. Therefore, breakup of any negative linkages that exist between the *nud* locus and *QYld.7H.94* within the hulless subpopulation would be difficult. Screening for *QYld-7H.94* within the hulled subpopulation would be beneficial for continued yield improvement. Markers 2_1204 and 3_0651 identified a novel QTL *QYld-6H.7-8* for yield on chromosome 6H in the across environments dataset. Favorable alleles for markers associated with *QYld-6H.7-8* were associated with an increase in yield of 539.0 and 580.2 kg ha^{-1} relative to the alternative allele for these markers. Unlike alleles for marker 2_0685 identifying *QYld-7H.94*, the favorable alleles for markers 2_1204 and 3_0651 identifying *QYld-6H.7-8* are not fixed within the hulled or hulless subpopulations. These alleles would be useful for selecting improved yields in both subpopulations. Identification of parents within the hulled and hulless subpopulations possessing favorable alleles prior to crossing would be of great benefit in development of improved germplasm and varieties. In hulled x hulless populations, selection of

parents from the hulled and hulless subpopulations containing multiple favorable alleles for yield would aid in the development of both hulled and hulless lines possessing superior yield potential. Use of these marker alleles would be beneficial in selecting for improved yield in both subpopulations.

GWAS for quality traits

GWAS of quality traits in the Virginia Tech breeding program revealed common and novel QTL for grain protein and polyphenol oxidase activity. Hulled and hulless barley genotypes have been reported to contain similar levels of grain protein (Griffey et al. 2010). Improvement of grain protein in hulled and hulless genotypes would greatly improve their marketability for use in health beneficial foods and ethanol production (Griffey et al. 2010). Markers associated with grain quality QTL were identified on chromosomes 2H, 5H, and 6H. The marker 3_1417 identified *QGp-5H.96* on chromosome 5H mapped to the region containing the previously described QTL *QGpc.HaMo-5H* (Marquez-Cedillo et al. 2000). Marquez-Cedillo et al. (2000) mapped *QGpc.HaMo-5H* to a region on chromosome 5H flanked by RFLP markers ABC302A and MWG635D. Subsequently, *QGpc.HaMo-5H* was positioned between SNP markers 2_0818 and 1_0183 at 91 cM on the Oregon Wolfe Barley 2008 consensus map (Szucs et al. 2009). The SNP marker 3_1417 which identified *QGp-5H.96* in the current study maps between 20 and 30 cM from the SNP markers flanking *QGpc.HaMo-5H* as positioned by Szucs et al (2009) on the current SNP consensus map (Muñoz-Amatriaín et al. 2011). Although markers identifying these QTL map to similar positions, further evaluation is needed to determine if the QTL identified within this region are unique. A novel QTL was indentified on chromosome 6H by marker 2_0537. The unfavorable allele for marker 2_0537 was associated with a reduction in grain protein of 2.03%. Selection of this QTL may be beneficial in malt

barley breeding programs as lower protein concentration is desirable. Within the Virginia Tech germplasm the allele for decreased grain protein is found in a high proportion of both hulled and hulless genotypes suggesting that selection for the allele may be useful in reduction of grain protein when developing malt barley genotypes. However, for genotypes developed for feed and fuel production, decreased grain protein is undesirable. QTL for grain protein have been previously identified on chromosome 6H by See et al. (2002) and Canci et al. (2003). The QTL identified by See et al. (2002) is near marker hvm74, while the QTL identified by Canci et al. 2003 is flanked by markers MWG916 – Bmag0807. Comparison of positions for these markers relative to 2_0357 suggests that *QGp-6H.142* is a novel QTL.

Polyphenol oxidase activity is associated with discoloration of products made from barley grain (Jerumanis et al. 1976; Baik et al. 1995; Quinde et al. 2004; Quinde-Axtell et al. 2006). Quinde et al. (2004) reported that variation exists among barley genotypes for polyphenol oxidase activity. In the current study, seven markers identified a single QTL *QPPO-2H.119-125* for polyphenol oxidase activity on chromosome 2H, which mapped to the region of previously reported duplicate *PPO* genes (Taketa et al. 2010). These duplicate genes were mapped to the long arm of chromosome 2H and were flanked by the RFLP marker MWG882 and microsatellite marker Bmac0415 (Taketa et al. 2010). Taketa et al. (2010) determined that *PP01* and *PPO2* are physically separated on chromosome 2H. *PPO1* controlled phenol reactions in the hull, caryopses and rachis, and *PPO2* controlled phenol reaction in the crease of the caryopses (Taketa et al. 2010). A high degree of LD was observed for all markers associated with *QPPO-2H.119-125* in the current study. No other traits were in LD with markers identifying *QPPO-2H.119-125* thus, selection for this trait alone will be possible. Favorable alleles for selection of reduced polyphenol oxidase activity exist in both the hulled and hulless subpopulations. Selection for

reduced values in both subpopulations should aid in the development in grain with improved quality.

GWAS for disease resistance

Markers associated with resistance to leaf rust, powdery mildew, net blotch, and spot blotch are of extreme importance to the Virginia Tech program. GWAS identified both common and novel QTL for disease resistance. Currently, 19 *Rph* genes conferring resistance to leaf rust have been identified in barley. In Virginia, two primary *P. hordei* races (8 and 30) are used for screening material for leaf rust resistance (Brooks et al. 2000). Prior work focusing on identification of leaf rust resistance genes has provided knowledge of genes within the program. Mammadov et al. (2007) validated markers for *Rph5* and *Rph7* within the Virginia Tech program; however, a recent race change rendered *Rph7* ineffective. In the current study seven markers identified QTL for resistance to *P. hordei* races 8 and 30. Markers identifying common QTL in the current study mapped to regions containing resistance genes *Rph5*, *Rph7* and *Rph15* (Parlevliet, 1976; Roane and Starling, 1967; Weerasena et al. 2004). Mammadov et al. (2007) mapped Rph5 to the short arm of chromosome 2H with markers ABG70 and TC2863-12.4 and Rph7 with AY6242926-C11. The distance between *Rph5* and *Rph7* was estimated to be around 5 cM (Mammadov et al. 2007) which is consistent with our results for *QRph-3H.6* and *QRph-3H.11*. Furthermore, these genes were considered to be linked in repulsion in a majority of Virginia Tech barley lines screened by Mammadov et al. (2007). LD was extremely low (R^2 = 0.03 to 0.04) between markers associated with *Rph5* and *Rph7*, further suggesting they are inherited independently. Incorporating both *Rph5* and *Rph7* into a single genotype is tedious without the use of molecular markers. Although LD is low between *Rph5* and *Rph7*, use of SNP markers to pyramid these resistance genes into a single genotype will improve the ability to

successfully identify genotypes carrying both genes. Gene *Rph7* confers resistance to race 8, while genes *Rph5* and *Rph15* confer resistance to race 8 and race 30. Unfavorable alleles for markers identifying QTL which mapped to the regions containing *Rph5* and *Rph7* were associated with increases in leaf rust scores. These unfavorable alleles associated with regions containing *Rph5* and *Rph7* were present in a large number (~80 %) of lines included within the study suggesting that favorable alleles are in low frequency within the Virginia Tech program. Favorable alleles for markers identifying the region containing *Rph15* are present in 10% of lines for 1_0376 and 60% of lines for 1_1227. The effectiveness of markers identifying the region containing *Rph15* needs to be further validated to determine their usefulness in MAS. Identification of these QTL also confirms the possibility to detect multiple loci in a relatively small region within the genome using association mapping.

Relatively little is known about powdery mildew resistance in the Virginia Tech barley program. Identification of markers allowing for selection of powdery mildew resistance is of great interest. A total of eight markers identified QTL conferring resistance to powdery mildew. Markers identifying common QTL in the current study mapped to regions containing the *Mla* resistance gene cluster, the *MlLa* resistance gene, and the *mlo* resistance gene (Buschges et al. 1997; Geise et al. 1993; Wei et al. 1999). Wei et al. (1999) mapped alleles *Mla6*, *Mla14*, *Mla13* and *Ml-Ru3* to the region flanked by Hor1 and Hor2 on chromosome 1H. The 2005 SNP consensus map has markers Hor5 (13.89 cM), MWG938 (14.58 cM), Hor2 (14.76 cM) and Hor1 (26.8 cM) on the short arm of chromosome 1H (Rostoks et al. 2005). Markers Hor5 (2.08 cM) and MWG938 (6.37 cM) mapped to the same region as the SNP markers 2_1174, 2_1226, 3_0950, and 3_0951 which identified the *Mla* region in the current study (Close et al. 2009). With as many as 32 resistance genes/alleles being identified in the *Mla* gene cluster (Jorgensen,

1992, 1994; Kintzios et al. 1995; Wei et al. 1999), it is possible that individual markers displaying low LD values may be associated with different resistance genes at this locus. Favorable alleles associated with markers identifying the region containing the *Mla* resistance gene cluster were found in 38 to 64% of lines in the study. Wei et al. (1999) concluded that the *Mla* region investigated in their study contained 11 copies of nucleotide-binding site of leucine-rich repeat (NBS-LRR) resistance-gene homologues (*RGHs*) which are present in three distinct families. Of particular interests to the barley breeding community are markers mapping to the region known to contain the *mlo* resistance gene. The *mlo* resistance gene is resistant to all known races of powdery mildew which makes it of great value to programs in areas affected by this disease (Jorgensen 1992). LD between markers identifying *QPm-4H.100-102* (*mlo*) was high (R^2 = 0.98), while 3_1139 identifying *QPm-4H.114* was not in LD (R^2=0.01) with markers 3_0142 and 1_0785. The favorable alleles associated with marker identifying the region containing *mlo* were found in 66% of lines within the study and were associated with a reduction in powdery mildew ratings of 3.17 and 3.39 relative to the alternative alleles, respectively. Among the markers associated with powdery mildew in the current study, the unfavorable allele of marker 3_1139 associated with novel QTL *QPm-4H.114* was associated with an increase in powdery mildew scores relative to the alternative allele. The unfavorable allele for marker 3_1139 was found in 22% of the lines in this study. *QPm-4H.100-102* and *QPm-4H.114* are considered to be independent QTL for powdery mildew resistance. Thus, simultaneous selection for *QPm-4H.100* and selection against the unfavorable allele for the marker 1_1050 associated with *QPm-4H.114* should be possible.

O'Boyle et al. (2011) determined that the hulled cultivar Nomini carries a single dominant gene for net blotch resistance. In a bi-parental mapping study O'Boyle et al. (2009)

mapped the resistance gene in Nomini to the centromeric region of chromosome 6H. The study by O'Boyle et al. (2009) was the first effort to map net blotch resistance in Virginia Tech winter barley. In the current study QTL for resistance to net blotch was identified on chromosome 5H. A single marker 2_0987 identified *QNb-5H.38* which mapped to the region containing the previously described net blotch QTL *QNb.StMo-5H* (Steffenson et al. 1996). Presence of the unfavorable allele for marker 3_0007 identifying QTL *QNb-5H.60* was associated with an increase in net blotch scores by 1.97 relative to the alternative allele. Thus further evaluation of the presence and effects of this QTL in barley germplasm likely are needed to eliminate its adverse effect on resistance.

A QTL *QSb-7H.18-27* for seedling resistance to spot blotch was identified on chromosome 7H with a high degree of LD (R^2 = 0.59) between markers 2_0495 and 1_0451 (Data not shown). These markers mapped to a region containing previously described QTL for spot blotch resistance (Steffenson et al. 1996; Roy et al. 2010). Roy et al. (2010) identified QTL *Rcs-qtl-7H-bPb-4584* for spot blotch resistance using a DArT marker on chromosome 7H through association analysis. This QTL is located at the putative *Rcs5* locus on chromosome 7H (Roy et al. 2010). The *Rcs5* gene is considered to be a major effect locus which confers both seedling and adult plant resistance to spot blotch, while Roy et al. (2010) reported *Rcs-qtl-7H-bPb-4584* to be a low effect QTL explaining only 3.1% of the variation. In the current study, *QSb-7H.18-27* has a slightly larger effect explaining 6% to 7% of the variation for spot blotch resistance. Roy et al. (2010) hypothesize that smaller effects reported in association mapping studies versus those reported in previous mapping studies may be due to their smaller population sizes which overestimated QTL effects or the presence of a resistance allele at more than one QTL which could affect QTL effect estimates.

Conclusions

Rapid advances in molecular and statistical techniques have paved the way for new mapping techniques such as association mapping. The current study highlights the usefulness of association mapping in identify marker-trait associations for numerous agronomic, quality and disease resistance traits in a breeding program. Furthermore, it serves as a valuable reference for researchers interested in the application of association genetic techniques. Concurrently, this is the first large-scale mapping project to identify markers associated with regions where novel QTL and previously described QTL/genes reside in Virginia Tech hulled and hulless winter barley germplasm. Of particular interest are markers associated with known QTL/genes which will allow for selection of important agronomic, quality, and disease resistance traits. Further validation of novel QTL and use of diagnostic markers for previously identified genes and QTL will facilitate improvement of these traits in a marker-assisted selection breeding program.

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Table 1: Means of phenotypic data for barley traits having significant marker-trait associations.

Source[a]	Trait[b]	N[c]	Mean[d]	Std.Dev.[e]	Min.[f]	Max.[g]	*P*[hi]
Across Years and Envs.	HD	654	110.27	5.0	99.3	123	<0.0001
06 Across Envs.	PH	192	82.7	7.8	57.7	103	0.0214
Across Years and Envs.	TWT	752	651.2	76.39	452.17	804.14	<0.0001
06 Across Envs.	TWT	192	672.6	69.8	514.8	804.1	<0.0001
Warsaw Across Years	TWT	376	661.4	71.8	524.2	787.2	<0.0001
Warsaw Across Years	Yield	376	5026	1587	2627	9221	<0.0001
Across Years and Envs	Yield	752	5036	1768	1784	9221	<0.0001
09 Across Envs.	GP	178	11.33	1.2	4	13.45	<0.0001
Across Years	PPO	376	0.46	0.29	0.13	1.52	0.05
08 GH	LR Race 8	96	1.17	1.04	0	3.6	<0.01
08 GH	LR Race 30	82	1.73	0.84	0	2.6	<0.01
Across Years GH	PM	368	2.1	1.3	0.5	5	<0.0001
09 Across Envs.	PM	184	1.3	1.8	0	8.3	<0.0001
08 Warsaw	PM	93	1.6	2	0	8	<0.0001
Across Years and Envs	NB	667	3.4	1.6	0.3	9	<0.0001
Across Years	SB	373	5	1.3	1.5	8	0.0012

a: Source of data analyzed; 2006 (06), 2008 (08), 2009 (09), Environments (Envs.), Greenhouse (GH), Across Years and Envs. (Data from 2006-2009 for Blacksburg and Warsaw, VA), Warsaw Across Years (Data from 2006-2009 for Warsaw, VA), Across Envs. (Data from Blacksburg and Warsaw, VA for a given year), Across Years GH (Data from 2006-2009 GH screenings).
b: Trait analyzed and units: heading date (HD, days to heading), plant height (PH, cm), test weight (TWT; g liter^{-1}), yield (kg ha^{-1}), grain protein (GP; %), polyphenol oxidase activity (PPO; absorbance, dry weight basis), leaf rust race 8 (LR Race8; 0-3.6), leaf rust race 30 (LR Race 30; 0-3.6), powdery mildew GH (PM; 0-5), powdery mildew field (PM; 0-9), net blotch (NB; 0-9), spot blotch (SB; 1-10).
c: Number of individuals analyzed in the dataset.
d: Mean value for trait analyzed.
e: Standard deviation (Std. Dev.) for trait analyzed.
f: Minimum value observed for trait analyzed.
g: Maximum value observed for trait analyzed.
h: *P*-value as determined from analysis of variance for each dataset.
i: *P*-values for leaf rust race 8 and race 30 datasets based on repeated checks throughout years.

Table 2: Significant marker-trait associations for Quantitative Trait Loci (QTL) associated with agronomic traits in the Virginia Tech barley germplasm.

Data[a]	Trait[b]	Marker[c]	Chr.[d]	cM[e]	Bin[f]	r^{2g}	BON[h]	QTL[i]	Effect[j]	Type[k]	Ref.[l]
Across Years and Envs.	HD	1_0583	3H	102.66	101	0.06	0.0061	*QHd-3H.102*	-2.5	*QHd.StMo-3H.; QHd3H.100*	Hayes et al. 1993; Wang et al. 2012b
06 Across Envs.	PH	1_0238	1H	30.15	34	0.04	0.0500	*QPh-1H.30*	4.7	Novel	Novel
Across Years and Envs.	TWT	3_1284	2H	15.98	18	0.03	0.0062	*QTwt-2H.15*	-42.8	Novel	Novel
06 Across Envs.	TWT	1_0196	2H	89.68	106	0.08	0.0026	*QTwt-2H.89*	-61.7	*QTw.nab-2H*	Marquez-Cedillo et al. 2000
Warsaw Across Years	TWT	3_0216	2H	107.92	123	0.07	0.0089	*QTwt-2H.107-109*	21.1	Novel	Novel
Warsaw Across Years	TWT	1_0138	2H	108.58	124	0.10	<0.0001	*QTwt-2H.107-109*	24.0	Novel	Novel
Warsaw Across Years	TWT	1_1307	2H	109.29	125	0.09	0.0003	*QTwt-2H.107-109*	23.0	Novel	Novel
Warsaw Across Years	TWT	2_1436	2H	164.35	181	0.06	0.0210	*QTwt-2H.164*	18.0	*QTw.IgDa-2H*	Backes et al. 1995
Warsaw Across Years	TWT	1_1448	5H	131.64	131	0.06	0.0322	*QTwt-5H.131*	-16.3	Novel	Novel
Across Years and Envs.	YLD	3_0651	6H	7.87	8	0.03	0.0039	*QYld-6H.7-8*	580.2	Novel	Novel
Across Years and Envs.	YLD	2_1204	6H	8.74	8	0.03	0.0082	*QYld-6H.7-8*	539.0	Novel	Novel
Warsaw Across Years	TWT	1_1014	7H	54.32	63	0.06	0.0127	*QTwt-7H.54*	17.7	Novel	Novel
Across Years and Envs.	TWT	1_0143	7H	91.12	99	0.04	0.0002	*QTwt-7H.91-94*	-56.5	Novel	Novel
Across Years and Envs., Warsaw Across Years	TWT	2_0685	7H	94.34	101	0.07, 0.19	<0.0001, <0.0001	*QTwt-7H.91-94*	-102.9, -121.6	Novel	Novel
Warsaw Across Years	YLD	2_0685	7H	94.34	101	0.05	0.0500	*QYld-7H.94*	1693.3	*Qyld.HaTR-7H*	Tinker et al. 1996; Rostoks et al. 2005

a: 2006 (06) and Environments (Envs.); Data set where significant marker-trait association for QTL were found.
b: Heading date (HD), plant height (PH), test weight (TWT) and yield (YLD); Traits for which significant marker-trait association exists.
c: Marker showing significant association with QTL for agronomic traits.
d: Chromosome where QTL was located.
e: Position on chromosome in (cM) where significant markers were located according to Munoz-Amatriain et al. (2011).
f: Bin locations of barley chromosome according to Munoz-Amatriain et al. (2011).
g: Percent of phenotypic variation explained by the marker identified as r^2.
h: Corrected *P*-value after Bonferroni correction.
i: QTL identified in association analysis. QTL name consist of trait, chromosome and region identified.
j: Allele substitution effect relative to alternate allele present at the locus detected in association mapping on HD (days to heading), PH (cm), TWT (g liter^{-1}) and YLD (kg ha^{-1}).
k: Previous identification for QTL/gene in a region.
l: QTL previously identified in region are identified by their reference. QTL identified but not previously described in literature are identified as novel.

Table 3: Significant marker-trait associations for Quantitative Trait Loci (QTL)/genes associated with quality traits in Virginia Tech barley germplasm

Data[a]	Trait[b]	Marker[c]	Chr.[d]	cM[e]	Bin[f]	r^{2g}	BON[h]	QTL[i]	Effect[j]	Type[k]	Ref.[l]
09 Across Envs.	GP	3_1417	5H	96.12	97	0.15	0.0023	*QGp-5H.96*	2.55	*QGpc.HaMo-5H*	Marquez-Cedillo et al. 2000
09 Across Envs.	GP	2_0537	6H	142.2	126	0.13	0.0016	*QGp-6H.142*	-2.03	Novel	Novel
Across Years	PPO	1_1094	2H	119.72	135	0.06	0.0296	*QPPO-2H.119-125*	-0.15	*PPO*	Taketa et al. 2010
Across Years	PPO	2_0099	2H	124.29	136	0.15	<0.0001	*QPPO-2H.119-125*	-0.25	*PPO*	Taketa et al. 2010
Across Years	PPO	2_1184	2H	124.98	138	0.15	<0.0001	*QPPO-2H.119-125*	-0.26	*PPO*	Taketa et al. 2010
Across Years	PPO	2_0064	2H	124.98	138	0.15	<0.0001	*QPPO-2H.119-125*	-0.27	*PPO*	Taketa et al. 2010
Across Years	PPO	1_0988	2H	124.98	138	0.06	0.0276	*QPPO-2H.119-125*	-0.22	*PPO*	Taketa et al. 2010
Across Years	PPO	1_0128	2H	124.98	138	0.06	0.0465	*QPPO-2H.119-125*	-0.15	*PPO*	Taketa et al. 2010
Across Years	PPO	1_0989	2H	125.97	139	0.06	0.0276	*QPPO-2H.119-125*	-0.22	*PPO*	Taketa et al. 2010

a: 2009 (09) and Environments (Envs.); Data set where significant marker-trait association for QTL were found.
b: Grain protein (GP) and Polyphenol oxidase activity (PPO); Traits for which significant marker-trait association exists.
c: Marker showing significant association with QTL/qene for quality traits.
d: Chromosome where QTL/gene was located.
e: Position on chromosome in (cM) where significant markers were located according to Munoz-Amatriain et al. (2011).
f: Bin locations of barley chromosome according to Munoz-Amatriain et al. (2011).
g: Percent of phenotypic variation explained by the marker identified as r^2.
h: Corrected *P*-value after Bonferroni correction.
i: QTL/gene identified in association analysis. QTL name consist of trait, chromosome and region identified.
j: Allele substitution effect relative to alternate allele present at the locus detected in association mapping on grain protein (%) and polyphenol oxidase activity (absorbance, dry weight basis).
k: Previous identification for QTL/gene in a region.
l: QTL/gene previously identified in region are identified by their reference. QTL identified not previously described in literature are identified as novel.

Table 4: Significant marker-trait associations for Quantitative Trait Loci (QTL)/genes associated with leaf rust resistance in Virginia Tech barley germplasm.

Data[a]	Race[b]	Marker[c]	Chr.[d]	cM[e]	Bin[f]	r^{2g}	BON[h]	QTL[i]	Effect[j]	Type[k]	Ref.[l]
08 GH	Race 30	1_0376	2H	149.27	164	0.23	0.0468	*QRph-2H.149*	-0.61	*Rph15*	Weerasena et al. 2004.
08 GH	Race 30	1_1227	2H	149.27	164	0.23	0.0500	*QRph-2H.149*	-0.61	*Rph15*	Weerasena et al. 2004.
08 GH	Race 30	2_0159	3H	6.31	4	0.31	0.0038	*QRph-3H.6*	1.83	*Rph5*	Roane and Starling. 1967
08 GH	Race 8	2_1398	3H	11.01	8	0.27	0.002	*QRph-3H.11*	1.65	*Rph7*	Parlevliet, 1976
08 GH	Race 8	3_0297	3H	11.01	8	0.27	0.002	*QRph-3H.11*	1.65	*Rph7*	Parlevliet, 1976
08 GH	Race 30	3_1274	6H	52.85	40	0.25	0.002	*QRph-6H.49*	-0.69	Novel	Novel
08 GH	Race 8	1_0713	7H	81.78	88	0.31	0.0035	*QRph-7H.81*	0.43	Novel	Novel

a: 2008 (08) and Greenhouse (GH); Data set where significant marker-trait association for QTL were found.
b: *P. hordei* isolates ND8702 (Race 8) and VA90-34 (Race 30) were used in greenhouse screenings; Traits for which significant marker-trait association exists.
c: Marker showing significant association with QTL/qene for leaf rust resistance.
d: Chromosome where QTL/gene was located.
e: Position on chromosome in (cM) where significant markers were located according to Munoz-Amatriain et al. (2011).
f: Bin locations of barley chromosome according to Munoz-Amatriain et al. (2011).
g: Percent of phenotypic variation explained by the marker identified as r^2.
h: Corrected *P*-value after Bonferroni correction.
i: QTL/gene identified in association analysis. QTL name consist of trait, chromosome and region identified.
j: Allele substitution effect relative to alternate allele present at the locus detected in association mapping on LR (Race 8 and Race 30) scores (0-3.6).
k: Previous identification for QTL/gene in a region.
l: QTL/gene previously identified in region are identified by their reference. QTL identified not previously described in literature are identified as novel.

Table 5: Significant marker-trait associations for Quantitative Trait Loci (QTL)/genes associated with powdery mildew resistance in Virginia Tech barley germplasm.

Data[a]	Marker[b]	Chr.[c]	cM[d]	Bin[e]	r^{2f}	BON[g]	QTL[h]	Effect[i]	Type[j]	Ref.[k]
Across years GH	2_1174	1H	8.96	14	0.07	0.009	*QPm-1H.8-11*	-1.00	*Mla* Resistance cluster	Wei et al. 1999
Across years GH	2_1226	1H	9.37	15	0.08	0.0006	*QPm-1H.8-11*	-1.03	*Mla* Resistance cluster	Wei et al. 1999
Across years GH	3_0950	1H	11.35	17	0.06	0.0168	*QPm-1H.8-11*	-1.06	*Mla* Resistance cluster	Wei et al. 1999
Across years GH	3_0951	1H	11.35	17	0.06	0.0321	*QPm-1H.8-11*	-0.95	*Mla* Resistance cluster	Wei et al. 1999
Across years GH	1_1050	2H	169.66	186	0.06	0.0281	*QPm-2H.169*	1.05	*MlLa*	Giese et al., 1993
08 Warsaw	3_0142	4H	100.97	117	0.23	0.0179	*QPm-4H.100-102*	-3.17	*mlo*	Buschges et al. 1997
08 Warsaw	1_0785	4H	102.93	119	0.26	0.0103	*QPm-4H.100-102*	-3.39	*mlo*	Buschges et al. 1997
09 Across Envs.	3_1139	4H	114.61	133	0.16	0.001	*QPm-4H.114*	2.94	Novel	Novel

a: 2008 (08), 2009 (09), Environments (Envs.) and Greenhouse (GH); Data set where significant marker-trait association for QTL were found.
b: Marker showing significant association with QTL/qene for powdery mildew resistance.
c: Chromosome where QTL/gene was located.
d: Position on chromosome in (cM) where significant markers were located according to Munoz-Amatriain et al. (2011).
e: Bin locations of barley chromosome according to Munoz-Amatriain et al. (2011).
f: Percent of phenotypic variation explained by the marker identified as r^2.
g: Corrected *P*-value after Bonferroni correction.
h: QTL/gene identified in association analysis. QTL name consist of trait, chromosome and region identified.
i: Allele substitution effect relative to alternate allele present at the locus detected in association mapping on powdery mildew GH (0-5) and powdery mildew field (0-9).
j: Previous identification for QTL/gene in a region.
k: QTL/gene previously identified in region are identified by their reference. QTL identified not previously described in literature are identified as novel.

Table 6: Significant marker-trait associations for Quantitative Trait Loci (QTL)/genes associated with net blotch and spot blotch resistance in Virginia Tech barley germplasm.

Data[a]	Trait[b]	Marker[c]	Chr.[d]	cM[e]	Bin[f]	r^{2g}	BON[h]	QTL[i]	Effect[j]	Type[k]	Ref.[l]
Across Years and Envs.	NB	2_0987	5H	38.78	34	0.08	0.0464	*QNb-5H.38*	-3.69	*QNb.StMo-5H*	Steffenson et al. 1996
Across Years and Envs.	NB	3_0007	5H	60.21	62	0.09	0.0330	*QNb-5H.60*	1.97	Novel	Novel
Across Years	SB	2_0495	7H	18.73	25	0.06	0.0433	*QSb-7H.18-27*	0.77	*QSb.StMo-7H; Rcs-qtl-7H-bPb-4584*	Steffenson et al. 1996; Roy et al. 2010
Across Years	SB	1_0451	7H	27.09	31	0.07	0.0088	*QSb-7H.18-27*	0.93	*QSb.StMo-7H; Rcs-qtl-7H-bPb-4584*	Steffenson et al. 1996; Roy et al. 2010

a: Environments (Envs.); Data set where significant marker-trait association for QTL were found.
b: Disease for which significant marker-trait association were found; net blotch (NB) and spot blotch (SB).
c: Marker showing significant association with QTL/qene for net blotch and spot blotch resistance.
d: Chromosome where QTL/gene was located.
e: Position on chromosome in (cM) where significant markers were located according to Munoz-Amatriain et al. (2011).
f: Bin locations of barley chromosome according to Munoz-Amatriain et al. (2011).
g: Percent of phenotypic variation explained by the marker identified as r^2.
h: Corrected *P*-value after Bonferroni correction.
i: QTL/gene identified in association analysis. QTL name consist of trait, chromosome and region identified.
j: Allele substitution effect relative to alternate allele present at the locus detected in association mapping on net blotch (0-9) and spot blotch (1-10) scores.
k: Previous identification for QTL/gene in a region.
l: QTL/gene previously identified in region are identified by their reference. QTL identified not previously described in literature are identified as novel.

Figure 1: Principal Component Analysis (PCA) of 329 hulled and hulless barley lines from the Virginia Tech program. Principle component 1 (PC1) explained 16% of variation and separated hulled (black) lines from the remainder of the lines in the study. PC2, accounted for 11% of the variation and separated hulless lines (red and green) in the study. PC3 (not shown), accounted for 4% of the total observed variation and provided further subdivision between lines derived from hulled x hulless crosses (red and green).

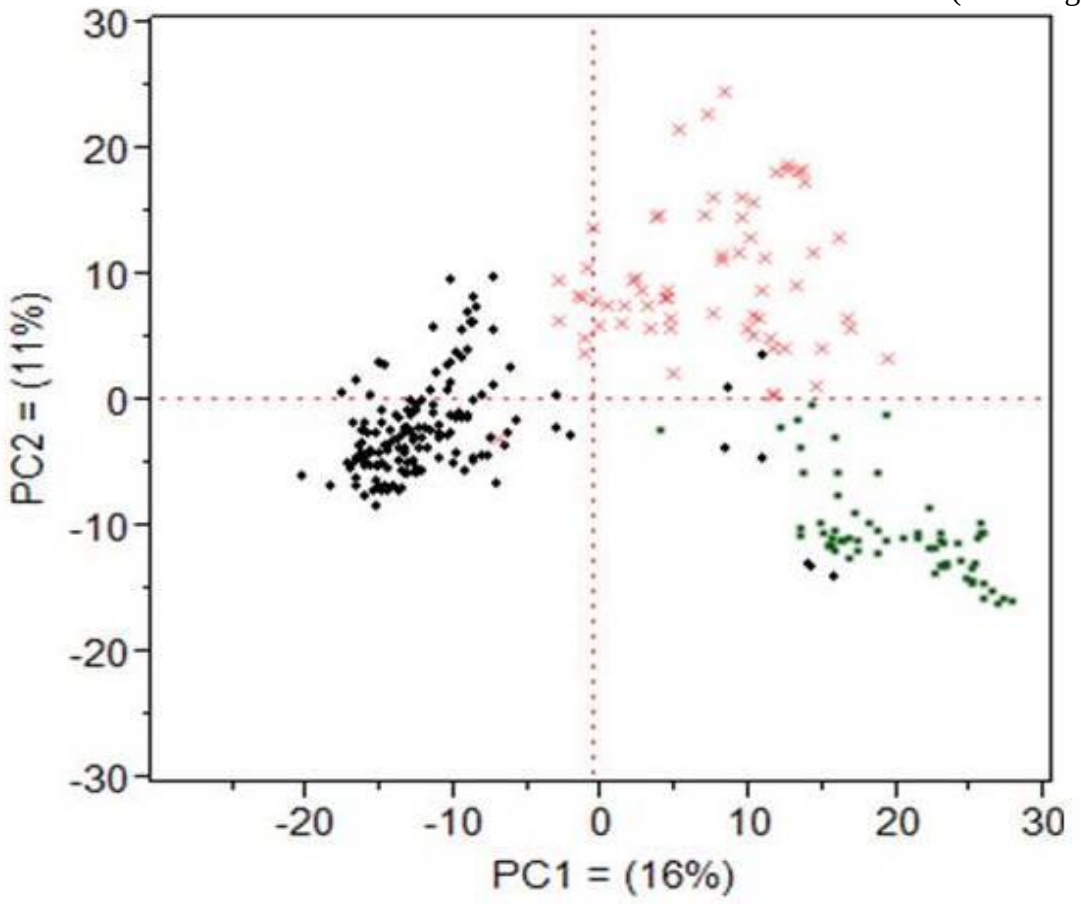


Figure 2: Linkage Disequilibrium (LD) decay in Virginia Tech barley.

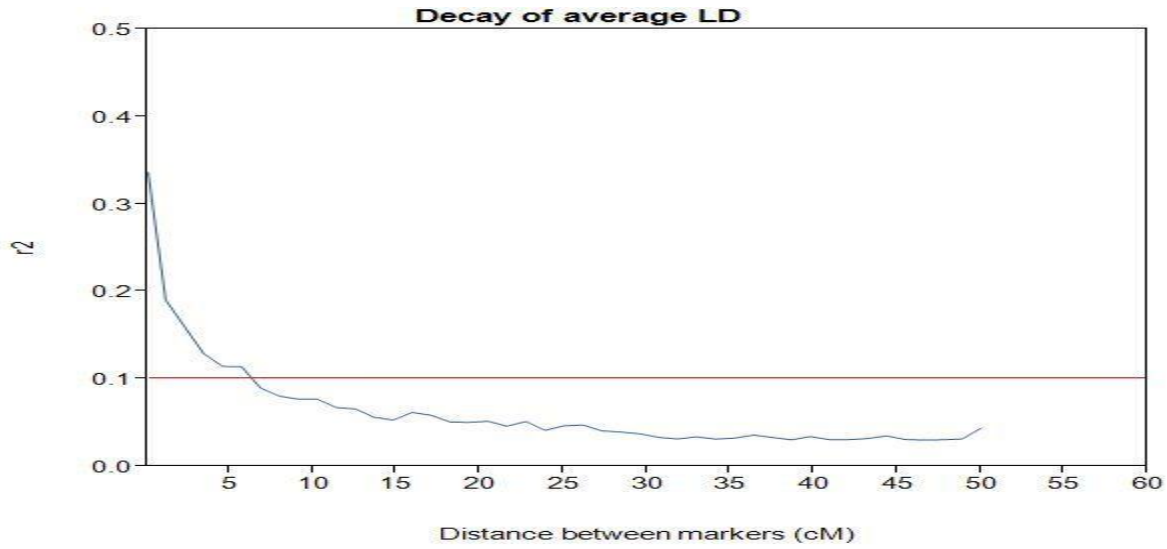
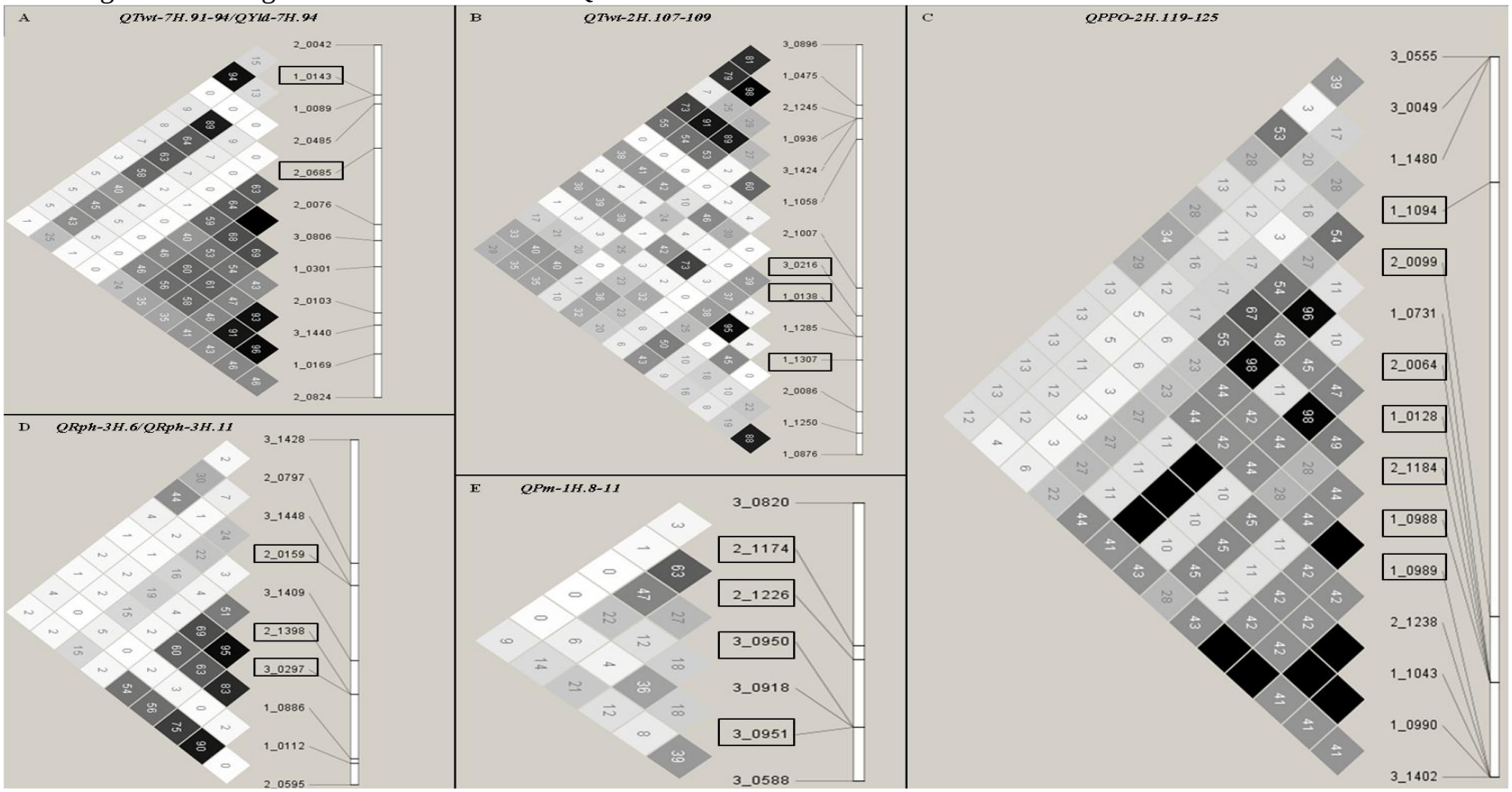


Figure 3: Linkage Disequilibrium (LD) pattern for markers identifying QTL: A) *QTwt-7H.91-94* and *QYld-7H.94* on chromosome 7H; B) *QTwt-2H.107-109* on chromosome 2H; C) *QPPO-2H.119-125* on chromosome 2H; D) *QRph-3H.6* and *QRph-3H.11* on chromosome 3H; and E) *QPm-1H.8-11* on chromosome 1H. Pairwise r^2 values are shown in shaded boxes with solid black boxes indicating $R^2 = 1.0$. Significant markers for each QTL are outlined in black.



CHAPTER IV

FUTURE DIRECTIONS

Future Directions

Use of both traditional breeding and molecular techniques is necessary for continued improvement of hulled and hulless winter barley (*Hordeum vulgare* L.). Phenotypic characterization of Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, and deoxynivalenol (DON) accumulation in hulled and hulless genotypes over four environments confirmed the presence and stability of resistance in Virginia Tech's winter barley germplasm. Screening genotypes with molecular markers on chromosomes 2H and 6H associated with resistance to FHB and DON revealed that the resistant genotypes Nomini, VA06H-48, and Eve likely are novel sources of resistance. Mapping populations, Eve × Doyce, Eve × VA07H-35, and Nomini × Thoroughbred, are being developed in an effort to identify markers associated with quantitative trait loci (QTL) conferring resistance to FHB and DON accumulation.

Yield improvement is necessary to make hulless barley competitive with hulled barley. Decreased yield associated with hullessness deters acceptance of hulless barley as a viable alternative to hulled barley. After adjusting for hull weight, yield potential of select hulless genotypes was similar to that of hulled genotypes. Thus it is possible to identify hulless lines having yield potentials equal to those of their hulled sibs. Use of elite hulless and hulled parents in population development will facilitate selection of superior hulless genotypes having yields similar to hulled genotypes when accounting for hull weight differences. Further research is needed to determine whether any pleiotrophic effects due to *nud* locus exists or if other tightly linked loci influence yield potential of hulless genotypes. A subset of 192 lines was genotyped with the barley 9K SNP chip to further elucidate if tightly linked loci are present.

The Barley Coordinated Agricultural Project (CAP) association mapping study was the first large-scale mapping project to identify markers associated with regions where novel QTL and previously described QTL/genes reside in Virginia Tech hulled and hulless winter barley germplasm. Markers associated with genes *Rph 5*, *7*, and *15*, *mlo*, *MlLa*, the *Mla* resistance cluster, *PPO*, and QTL for grain protein, test weight, yield, and resistance to spot blotch and net blotch are being validated for their usefulness in marker-assisted selection (MAS). Diagnostic markers will be implemented in a MAS program which aims to improve the effectiveness of selection within the barley program.