Organic Multiuse Naked Barley Characterization and Genomic Studies

By

Chris Massman

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The dissertation is approved by the following members of the Final Oral Committee:

Lucia Gutiérrez, Associate Professor, Agronomy

Jeffrey Endelman, Associate Professor, Horticulture

Juan Zalapa, Professor, Horticulture

Julie Dawson, Associate Professor, Horticulture

William Tracy, Professor, Agronomy

DEDICATION

This dissertation is dedicated to my mother and father without who's love and support, I would not be where I am today.

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ABSTRACT

Barley is an important cereal grain with many end-uses and health benefits. The hulless, or "naked," trait provides additional opportunity to the industry in that the normally tightly adhering hull will thresh freely during harvest and cleaning. The production and selection of organic naked barley germplasm in this study combines traditional plant breeding and genomic methods. The first step is the characterization of advanced lines in a regional yield trial and assessment of the genotype by environment interactions acting on the panel. Two groups of environments with similar genotypic rankings were identified in this work. This information will allow the tailoring of breeding approaches to specific regions as well as the use of characterized lines as potential parents in the breeding process. The second portion of this work delves more specifically into the quantitative nature of the free-threshing character of naked barley. The propensity of the hull to fall from the grain is defined here as threshability. Threshability is critical in naked barley as genotypes that fail to shed the hull will require the same dehulling that is used in hulled grains. This work used a diverse panel of 350 genotypes grown in nine environments to identify two QTL, one on chromosome 2H and one on chromosome 3H, relevant to threshability. GWAS was used to identify these loci, while accounting for population structure and linkage. Extending this genomic work, genomic predictions were performed to test the utility of genomic selection for increasing genetic gain of threshability. A predictive ability of .84 for threshability was obtained for this study. Additional covariates, including plump percentage, thin percentage, test weight, and marker data from the associated QTL were included in the genomic prediction model; however, they were not effective in further increasing accuracy.

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PREFACE

I have written this dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy in plant breeding and plant genetics at the University of Wisconsin – Madison. The overall research has been a part of the OREI project: “Multi-use naked barley for organic systems.” Chapter one details my literature review of barley, the naked trait, plant breeding, organic production, genotype by environment interactions, marker assisted selection and genomic prediction. The second chapter features the published work “Genetic Characterization of Agronomic Traits and Grain Threshability for Organic Naked Barley in the Northern US.” The third chapter, “Genome Wide Association Study and Genomic Prediction for Grain Threshability,” focuses on the naked trait threshability and is intended for publication Crop Science. Chapter four is a research summary for a broad audience written with the support of the Wisconsin Initiative for Science Literacy. Chapter five broadly summarizes my work, it’s implications, and possible avenues of future research. It is my hope that these research findings and results will assist future scientists and plant breeders working in naked barley.

CHAPTER 1. LITERATURE REVIEW

Information on barley, the naked trait, organic systems, genotype by environment interactions, marker assisted selection, and genomic prediction are found in this chapter. An understanding of these topics will provide the context required to follow the subsequent research activities.

Barley

Barley (*Hordeum vulgare* L.) is a cereal grain domesticated over ten thousand years ago in the fertile crescent from its wild ancestor, *Hordeum spontaneum* L. (Badr et al., 2000). There is also evidence to suggest additional sites of domestication further east in Tibet and Central Asia (Morrell and Clegg, 2007; Wang et al., 2015). Regardless of whether the origins of barley are monophyletic or polyphyletic, high levels of diversity were found among barley cultivars throughout the Middle East and Asia (Dai and Zhang, 2016). Barley was originally grown for food and malt purposes and was a mainstay in the diets of early civilizations throughout the world (Newman and Newman, 2006).

Today, barley ranks fourth in crop production worldwide following wheat, rice, and maize (FAO, 2020) with a number of end-uses in feed, food, and malting (Ullrich, 2011). More than half of barley currently produced worldwide is used in animal feed (Blake et al., 2010; Ulrich, 2011). Malting is barley's second most common end use (Akar et al., 2004) and is a primary focus of many breeding programs in the United States (Horsley and Harvey, 2011). Food, in contrast to the historical use of barley, makes up a relatively small proportion of the barley used worldwide. A contributing factor to the lowered popularity of barley as a food crop is the presence of a husk that adheres tightly to the grain, known as the hull. The hull must be removed prior to the grain's use in food, taking with it a portion of the bran and nutritional value that would otherwise be provided in the grain (Baik et al. 2008). Cereals such as wheat, rice, and maize which do not have hulls that adhere to the grain are now the primary food crops worldwide (FAO, 2020).

The hull is composed of the lemma and palea of the barley spikelet (Guerfali et al., 2017). The palea is the smaller of these organs and results from the fusion of two prophylls, while the lemma is the larger organ resulting from the leaf subtending the axillary meristem (Grant et al., 2021; Kellogg 2001). The hull has high concentrations of indigestible cellulose and lignin (Guerfali et al., 2017), and makes up 10-13% of the grain by weight (Meints et al. 2015). At maturity the hull of covered barley cannot be removed from the caryopsis due to the presence of an adhesion layer that fuses the hull with the caryopsis (Grant et al., 2021). Figure 1.1 outlines the structure of the barley caryopsis and hull.

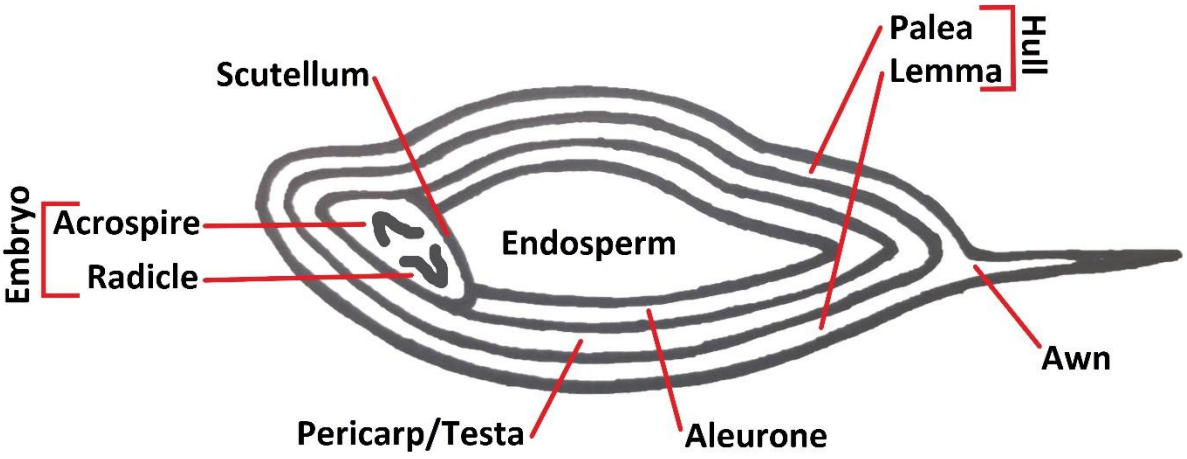

Figure 1.1 Diagram of the barley caryopsis.

The pericarp and testa are the outermost layers of the caryopsis and consist of maternally derived ovary tissue (Freeman and Palmer, 1983). Barley aleurone is a three-cell thick layer that makes up the outermost layer of the endosperm (Ho, 1979) and serves as an important germination regulator (Roustan et al., 2018). The aleurone is not only maternally derived and is

instead the result double fertilization. The pericarp and aleurone layers can be cumulatively referred to as the bran, which has high concentrations of starch, β-glucan, anthocyanins, and a variety of beneficial minerals (Zeng et al., 2020). Beneath the aleurone the underlying endosperm can be subdivided into the subaleurone and starchy endosperm layers (Roustan et al., 2018). Both layers are the result of double fertilization and contain high levels of seed storage proteins; however, the starchy endosperm has a higher concentration of starch compared to the subaleurone layer (Roustan et al., 2018; Moore et al., 2016). The barley embryo consists of the developing radicle and acrospire which will form the seedling after germination. The embryo is attached to the endosperm via a modified cotyledon known as the scutellum, which facilitates transfer of nutrients from the endosperm to the embryo (Reid, 1985).

Overall objectives in barley breeding include higher yield and resistance to diseases (Anderson and Reinbergs 1985). Specific breeding objectives of barley programs aimed at malting, feed or food are more varied. Selection of feed barley focuses on increasing starch and total digestible energy (Meints and Hayes 2019; Rossnagal, 1999). Breeding programs for food barley heavily select for high β-glucan, which has been found to have a number of health benefits including reduced cholesterol (Kalra et al., 2000; Ho et al., 2016) and lowered risk for heart disease (FDA, 2020). However, high levels of β-glucan present challenges during the malting process (Bamforth and Martin, 1981). Selection is against high β-glucan when genotypes are intended for malting purposes, because high β-glucan reduces malt extract levels (Meints and Hayes 2019). Similarly, protein has been found to be negatively correlated with starch content (Yu et

al., 2016). Starch is necessary to feed the microbes in the malting process and would be a desirable characteristic in malting barley (Bhatty, 1986). Grain not meeting specifications for malting or for food is sold as feed. Feed barley receives a lower price at market compared to maize, putting growers at a disadvantage when selling barley grain in this market (Meints and Hayes 2019, Blake et al. 2010). A moderate protein and β-glucan target when selecting barley varieties could allow for grain that fits a variety of end-uses. These multi-use varieties would ideally be suitable for at least two of the three primary end-uses of barley. A barley variety suitable for multiple markets would present growers multiple options and higher flexibility when selling barley grain.

Naked Barley

Traditionally barley varieties grown in the US are hulled. However, there are lines with very low levels of hull adherence where the hull can become detached during harvesting or threshing. This type of barley is hulless, or “naked” (Bhatty, 1986). The naked trait is simply inherited and under single gene control by the “nud” locus on chromosome arm 7HL (Taketa et al., 2008). This is a recessive trait that causes a loss of function in the gene that leads to an adhesive substance being excreted by the caryopsis after flowering to cement the hull on the grain (Taketa et al., 2008; Baik et al. 2011). Without this function, the hull does not fuse with the underlying caryopsis and becomes prone to falling off. When the hull is successfully abscised, there is no longer a need for processing steps like pearling to remove the hull mechanically (Baik et al, 2011). Since this processing step does not need to occur, the grain maintains a whole grain status and its full nutritional value. This increases the grain’s usability for food

purposes (Pourkheirandish and Komatsuda, 2007). Naked barley flour, when used to enrich wheat flour, significantly increases the β-glucan and antioxidant content of the blend (Narwal et al., 2017). Naked barley also has benefits in animal feed. Studies have found that the digestible energy in naked barley is greater than hulled barley when fed to swine (Bhatty et al., 1979). Finally, the loss of the hull reduces the levels of tannins, a detrimental compound in malting that originates in the hull, when the grain is used for malt (Edney and Rossnagel, 2000). The removal of the intermediate processing step as well as the higher accessibility of grain nutrients makes naked barley a promising idea for both growers and consumers.

Despite the hull no longer being adhered to the caryopsis, not all naked barley is successful in abscising the hull. In this way, the naked trait is required for loss of the hull, but not sufficient for total, consistent abscission of the hull. The degree of hull loss in naked genotypes is a continuous trait defined here as threshability. Threshability is a distinct trait from the naked phenotype and is critical in the production of naked barley for food uses (Meints and Hayes 2019). Naked barley failing to shed the hull must undergo the same dehulling processes as hulled barley, offsetting the benefits naked grains can provide. Although there is no current standard in the US, naked grains in Canada must have fewer than 5% hulls still attached to make food-grade and fewer than 15% for feed-grade (Legzdiņa and Gaile, 2008). Selection of threshability in Canadian breeding programs has been done phenotypically, often by selecting rounder grains with thinner hulls (Rossnagel, 2000). These grain size and shape characteristics are correlated with threshability and are useful for indirect selection techniques.

The genetic and biochemical architecture behind the threshability trait in barley is not well described. An analysis of four crosses of naked barley of varying threshability conducted by Ram and Singh (1996) determined that the high-threshing trait was controlled by two recessive genes of additive effect. On the other hand, threshability in wheat has been researched in greater depth than in barley. The major domestication locus (Q) on chromosome 5A and the tenacious glume locus (Tg) on chromosome 2D are the genes primarily responsible for the high threshability of hexaploid wheat (Simons et al. 2006; Jantasuriyarat et al., 2004; Sood et al., 2009). These findings would suggest that threshability in barley is a polygenic trait; however, is controlled by relatively few loci of large effect.

Organic Production

Organic markets have seen rapid growth in the past decade with sales of organic products increasing by 12.4% in 2020 (OTA, 2021), and organic beers seeing expanding markets (AB InBev, 2020; Allagash Brewing, 2020; New Belgium Brewing, 2020). Despite lower average yields on organic farms (Seufert et al. 2012), premiums on price for organically produced goods can make their production economically competitive with conventional systems (Crouder et al., 2015).

The rising popularity of organic systems is attributable to a number of factors. The belief that organic products provide health and environmental benefits is one reason consumers are increasingly purchasing these products (Lotter 2003). This holistic approach to food, health, and the environment is a core aspect of organic agriculture, and organic production does address

key sustainability goals (Gomiero et al., 2011). Sustainable farms are those who can produce a large quantity of food economically while contributing to environmental wellbeing and sustainability (Reganold and Wachter, 2016). This is done through an emphasis on ecologically friendly management practices, barring the use of synthetic chemicals that can pollute groundwater, and increasing biodiversity (FAO, 2019; Reganold and Wachter, 2016). Increased biodiversity and varied crop rotations are of particular importance. A diverse cropping rotation has been shown to suppress weed and pest infestations (Weisberger et al., 2019; Leroux et al., 1996). Because synthetic herbicides and pesticides are not used in certified organic systems, this biological suppression of pests is very important. From an environmental perspective, these rotations also have a higher potential for carbon sequestration which can help mitigate the causes of climate change (Scialabba and Muller-Lindenlauf, 2010).

Organic cropping systems are a possibility for adding value to barley production as well. Despite the current, relatively low area used to grow certified organic barley in the US (NASS, 2016), barley grain has the potential to fill new demands in the expanding organic food, feed, and malting industries (Meints and Hayes, 2019). Barley, with its low nitrogen use and adaptability to multiple soil types, would also provide effective ways for farmers to diversify their cropping rotations (Baker et al., 2020). Because of the lack of synthetic pesticides or fertilizers in organic operations, crops grown in these environments will face a different range of biotic and abiotic challenges than those from conventional farms (Crespo-Herrera and Ortiz, 2015; Wolfe et al 2008). Many commercially available organic barley varieties were bred under nonorganic conditions, which can lead to underperformance in organic environments (Wolfe et al., 2008).

Experiments by Murphy et al. show that the top performing wheat genotypes in conventional trials are not the top performing genotypes in organic systems (2007). Additional studies by Miko et al. showed that both grain yield and test weight performance were distinct between genotypes bred under conventional systems and those bred under organic systems when planted in a side-by-side experiment to compare these two systems (2014). Even within organic systems, there is a high degree of variability between growing environments (Barberi, 2002). These genotype by management interactions can represent significant barriers to selecting genotypes that show optimal performance across the range of environmental conditions (Cooper et al., 2001). Putting resources into breeding barley in and for organic production would increase the productivity of the system. This includes selecting for better weed suppression and grain yield stability (Lammerts van Bueren et al., 2002).

Traditional Plant Breeding Approaches

Cultivar improvement is based on the selection of superior germplasm from a diverse population. In a crop such as barley where the end goal is a pure line, this involves the creation of a segregating population through crossing, self-pollination of these individuals to reach homozygosity, selection for important traits, and evaluation of the resulting inbred lines over a wide area to make a final determination of suitable lines for release (Fehr, 1987). The gains made via this process are usually quantified by the breeder's equation or:

[1.1] $$R = h^2 S$$

where R is the response to selection, h^2 is the heritability of the trait being selected on, and S is the selection differential (Lush, 1937). The selection differential is difference between the mean

of the selected individuals and the population mean. Historically, phenotypic selection has been used to drive progress in plant breeding.

The overall schemes used to apply selective pressure can vary and there are a number of common strategies used in self-pollinated crops like barley. Recurrent selection is a general cyclical method where selected individuals from a population are intermated and used to produce the next generation. This process is repeated to increase the frequency of positive alleles in the population (Orf, 2008; Foster, 1987). Pedigree selection is a commonly used method in plant breeding where records of a line's ancestry are recorded from the initial cross to the finished line (Foster, 1987). Single seed descent is a method where a single seed from each plant in a segregating population is forwarded to the next generation until each line reaches homozygosity (Foster, 1987; Funada et al., 2012). This maximizes the genotypic variance in the final generation. A major drawback to all of these methods is the time required to produce finished lines. It can take upwards of a decade from initial cross to final release of a superior genotype (Foster, 1987; Wanga et al., 2021). Finished lines represent a significant investment of time and money on the part of plant breeders and seed companies.

Genotype by Environment Interactions

Genotype by environment interactions (GEI) are a major factor in conducting multi-environment trials (MET; Burgueno 2012; van Eeuwijk et al. 2016). Relative differences in the performances of lines across the environments in a trial make selection for a single, optimal genotype challenging. The traditional means to manage GEI are to ignore, reduce, or exploit it

(Eisemann et al., 1990). Ignoring or reducing the GEI effect on a multi-environment trial are most effective when the observed genetic variance is high compared to the GEI variance (DeLacey et al., 1996). There are a number of strategies for reducing the effect of GEI on selection. Additive mixed models are a staple in the analysis of multi-environment trials (van Eeuwijk, 1995). They can be used to estimate genotypic and environmental main effects; however, explicitly modeling the GEI component is difficult, particularly when dealing with a large target set of environments where the potential impacts of GEI could be large (Malosetti et al., 2013). Regressing genotypic values on the environmental mean, also known as Finlay-Wilkinson regression, is another way to address the effects of GEI in multi-environment trials (Finlay and Wilkinson 1963, Becker and Leon, 1988). Regression methods allow for more explicit characterization of the GEI component (Malosetti et al., 2013). Stability analysis via Finlay-Wilkinson regression is a method of finding genotypes that have consistent performance across a range of environments. Genotypes showing high levels of stability over many environments could be selected to balance performance and adaptability. Alternatively, genotypes that have a heightened response to changes in the mean performance of an environment, can be selected to leverage the increased productivity of these high yielding environments. When genetic variance is low compared to the effects of GEI, reducing the impacts of GEI becomes less effective (DeLacey et al., 1996). In these cases, it may be more effective to select within subgroups of the target set of environments. The construction of mega environments (ME) from sets of locations is a method for overcoming high levels of GEI in multi-environment trials (Braun et al., 1996). Locations within an ME are not geographically linked by definition, rather they share similar genotypic rankings (CIMMYT, 1989). In other words, relative performance of

genotypes will be consistent across environments within ME. Incorporating ME information into plant breeding can allow for a more tailored approach to selection and research. When differences in genotypic rankings are high between mega-environments, locally adapted lines specific to their target ME should be developed (Pswarayi et al., 2008). Delimitation of ME can be achieved through the use of genotypic main effect plus genotype by environment interaction (GGE) or location-grouping (LG) models (Yan, 2019). GGE and LG models are used in the construction of biplots to visualize patterns and trends in multi-environment data (Yan et al.,2000; Yan et al., 2001; Yan, 2019). GGE models subject the two-way table of genotypic means by environment to single value decomposition and the resulting principal components (PCs) can be projected onto a biplot to create a powerful visual tool for the delineation of ME (Yan et al., 2000). However, due to the unbalanced nature of many plant breeding activities, detection of repeatable GEI patterns can be difficult (Yan, 2019). If GEI patterns are not repeatable, they are nor useful when determining testing locations and ME. The LG methodology makes visualizing the relationship between environments and within locations easier by using a table of Pearson correlations between environments and locations in place of the standard two-way table (Yan 2019). As this type of analysis is not dependent on individual genotypic means, it is more robust to unbalanced datasets.

QTL Mapping

Tools available to breeders now include DNA markers. The wide availability of genomic sequencing and mapping have made this technology accessible across the world. These methods have proven especially useful in the breeding of quantitative traits (Bernardo, 2020;

Perez-de-Castro et al., 2012). Quantitative traits are under the control of multiple quantitative trait loci (QTL) and have continuously distributed phenotypes. Phenotypic values for these traits are hard to describe using Mendelian genetics, but rather a relatively large number of QTL with varying effects (Falconer and Mackay, 1996). QTL with a large impact on the trait in question are considered major QTL.

There are a number of strategies for identifying QTL. One QTL discovery method is by linkage mapping. Interval or composite interval mapping have been used to identify QTL in segregating populations where phenotype and genotype information is available (Zeng, 1994; Jansen 1994). A drawback of linkage mapping is the construction of appropriate segregating populations can be an expensive and time-consuming process (Shi et al. 2017). Identification of QTL can also be achieved by performing a genome wide association study (GWAS; Jannink et al., 2001). GWAS has the capability to identify relevant chromosomal regions to a given trait in diverse populations (Shi et al. 2017; Locatelli et al. 2012; Kraakman et al., 2004). It has been found that large, balanced data sets are optimal for detecting QTL and reducing false positive results (Wang et al., 2011). Factors such as population structure and kinship have a large impact on the effectiveness of GWAS and can lead to false positives, due to a nonrandom assortment of alleles between subpopulations rather than true associations to a specific trait (Thornsberry and Buckler, 2003; Newell et al., 2010; Gutierrez et al., 2015). The GWAS methodology can be expanded to account for both population structure and kinship using the Q+K model proposed by Yu et al. (2006). The Q matrix is an *nxp* matrix where *n* is the number of individuals and *p* is the number of subpopulations. Values in this matrix quantify the relatedness of each genotype

to each subpopulation. The K matrix is an *nxn* matrix defining the covariance of genotypes resulting from polygenic background effects (Zhang et al., 2010; Endelman, 2011; Yu et al., 2006). The GWAS methodology is commonly used and has been successful in identifying QTL in a variety of crops, including barley (Korte and Farlow, 2013; Locatelli et al., 2012; Gutierrez et al., 2011).

Marker assisted Selection (MAS) is a selection strategy for selecting genotypes based on marker scores at QTL linked to the trait of interest (Tanksley, 1983). MAS can be useful in selection of traits that are inefficient to phenotype or in the pyramiding of multiple QTL into a single genotype (Xu and Crouch, 2008). A MAS pipeline is most effective when selection is based on QTL of major effect, otherwise gains on selection will be marginal compared to phenotypic selection alone (Bernardo, 2020). Before use, a QTL must also be validated to ensure its effect on a trait remains constant in a variety of genetic backgrounds (Langridge et al., 2001). One limitation that breeding programs encounter with MAS is its inability to account for complex traits with many QTL (Korte and Farlow 2013, Heffner 2009). As the number of QTL increases, effects of individual QTL become marginal and either hard to detect or not worth pursuing in an intentional breeding pipeline (Bernardo, 2020).

Genomic Selection

Genomic selection is a relatively new technique that has been successfully implemented in plant breeding to increase genetic gain and decrease breeding cycle time (Bernardo, 2009; Heffner et al., 2010). GS overcomes some of the limitations of MAS by using the scores for all

available markers, rather than single or few loci (Heffner et al., 2009). By using a training population that has both phenotypic and genotypic data, a model can be fit to predict the breeding values of untested lines based on their genotype (Heffner et al., 2010). Accuracy of the predictions are affected by a variety of factors including trait heritability, population size (Lorenz et al., 2012), population structure (Isidro et al., 2015; Berro et al., 2019), marker density (Lorenzana and Bernardo, 2009), and model (Heslot et al., 2012). By modeling and understanding GEI effecting the population, estimates tailored to individual breeding programs in different locations can be produced (Burgueño et al., 2012; Lado et al., 2016). Additionally, mega-environment information and environmental covariates can be used to predict genotypic performance in new or untested environments (Lado et al., 2016; Heslot et al., 2013). The utility of genomic selection is maximized when the traits being predicted are difficult to phenotype, when many individuals must be phenotyped quickly, or when insufficient samples are available for phenotyping (Lado et al. 2018). Genomic selection is also effective at increasing genetic gain per unit of time (Bernardo, 2020).

The genomic best linear unbiased prediction (gBLUP) procedure is one method of predicting genotypic performance based on information from relatives (Bernardo, 1994). The gBLUP procedure uses the genomic relationship matrix, also known as the realized relationship matrix, to determine relatedness and what information is shared between genotypes (Bernardo 2020). An alternative to the gBLUP procedure is the ridge regression BLUP (rrBLUP) procedure, which estimates marker effects and predicts genotypic values based on these estimates (Endelman, 2011; Bernardo 2020). Research has found gBLUP and rrBLUP to be equivalent; however, not

optimal when modeling QTL of large effect (Tan et al., 2017; Bernardo, 2020). The effect of major QTL can be better accounted for in these methods by the inclusion of those QTL as a fixed effects in the model to ensure their predicted effects are not shrunken (Bernardo 2014; Li et al., 2019; Bian and Holland, 2017). The gBLUP procedure can also be extended to a multi-trait model that uses correlated traits to increase prediction accuracy (Lado et al., 2018). Multi-trait models have proven useful in increasing predictive ability for even complex traits such as yield when the traits used in making predictions are highly correlated (Bhatta et al., 2020; Hayes et al., 2017)

Objectives

Given the importance of barley, the utility of naked grains in multiple markets, and the advances in plant breeding made over the past several decades, the objectives of this work were two-fold. The first objective was to characterize available naked barley genotypes in terms of their individual genotypic performance and the effects of GEI that act on them. This provides a starting point for the characterized genotypes to be used in future crosses. The GEI characterization can be useful in defining regions where genotypes perform similarly. The second objective of this work was to apply GWAS and GS in the improvement of the naked barley breeding pipeline. Threshability is a key trait where rapid progress is required for the utility of the crop. Modern genomic methods can identify chromosomal regions with significant association with threshability and predict genotypic performance based on the performance or relatives. The above research activities will aid in the production of optimal naked barley genotypes that will be useful to growers and farmers.

CHAPTER 2. GENETIC CHARACTERIZATION OF AGRONOMIC TRAITS AND GRAIN THRESHABILITY FOR ORGANIC NAKED BARLEY IN THE NORTHERN U.S.

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Authors: Chris Massman, Brigid Meints, Javier Hernandez, Karl Kunze, Patrick M. Hayes, Mark E. Sorrells, Kevin P. Smith, Julie C. Dawson, and Lucia Gutierrez

Affiliations: C. Massman, L. Gutierrez, Department of Agronomy, University of Wisconsin – Madison, 1575 Linden Dr, Madison, WI 53706, USA; J. Dawson, Department of Horticulture, University of Wisconsin – Madison, 1575 Linden Dr, Madison, WI 53706, USA; B. Meints, J. Hernandez, P.M. Hayes, Department of Crop and Soil Science, Oregon State University, 3050 SW Campus Way Corvallis, OR 97331; K. Kunze, M.E. Sorrells, School of Integrative Plant science, Plant Breeding and Genetics Section, Cornell University, Ithaca, New York 14853; K. Smith, Department of Agronomy and Plant Genetics, 1991 Upper Buford Circle St. Paul, MN 55108

Author Contributions BM, PMH, MES, KPS, and LG conceptualized the study. LG designed the specific hypothesis and supervised the research including formal data analysis. CM, KK, BM, PMH, MES, KPS, and LG performed the investigation associated to the field experiments. CM performed the formal data analysis with assistance from JH. CM, BM, JH, and LG wrote the original draft. All authors revised and edited the manuscript and agreed to the final version of the manuscript. CM and BM contributed equally to the manuscript.

ABSTRACT

Naked barley grain threshes freely from its hull during harvesting and cleaning. Much of the available naked barley germplasm is unadapted to U.S. barley growing regions, and few genotypes have been selected to thrive under organic systems. The goal of this research was to characterize a set of spring naked barley genotypes for agronomic traits in the northern U.S. under organic conditions and estimate the degree of genotype by environment interaction (GEI). To achieve this goal, a multi-environment trial was conducted over three years. Experiments were grown under organic conditions where traits including grain yield, test weight, plant height, heading date, and threshability were evaluated. Contributions of environment, genotype, and GEI to the phenotypic variance were calculated. In the tested germplasm, test weight, plant height, heading date, and threshability were found to have higher variance attributed to genotypic effects compared to GEI. Grain yield variance attributed to GEI was five times greater than that of genotype alone. GGE and LG biplots showed two sets of environments where naked barley genotypes performed similarly. Sensitivity analysis by Finlay-Wilkinson regression found that grain yield was highly sensitive to changes in environment mean yield. These results show that selection for grain yield should be conducted within mega-environments to leverage GEI patterns. Heading date, plant height, test weight, and threshability can be selected across mega-environments.

INTRODUCTION

Barley (*Hordeum vulgare L.*) is the fourth most widely grown cereal crop in the world (FAO, 2017). The grain has three primary end-use markets: food, malt, and feed. Barley grain is low in

fat but high in fiber and other important nutrients including iron, potassium, and B vitamins (Meints et al. 2016). There are various nutritional benefits associated with eating barley grain, many of which are attributed to its high protein and β-glucan levels. This includes lowered cholesterol and a reduced risk of heart disease (FDA, 2020; Kalra and Jood, 2000; Keogh et al., 2003). Despite this, a relatively small market share of the barley produced worldwide is for food (FAO, 2017). This is due, in part, to the sometimes-conflicting breeding targets of the three end uses. High levels of β-glucan are desirable in food barley for a nutritious grain; however, this leads to lower extract levels in malting, and digestive problems in chicks (Classen et al., 1985; Meints and Hayes 2019). Targeting moderate levels of β-glucan that leverage the health benefits in food uses while minimizing the negative impacts in malting or feed uses could provide a multi-use grain that is able to address needs in the food, feed, and malting markets (Meints and Hayes, 2019). Another contributor to the minimal use of barley as a food crop is the presence of a hull that tightly adheres to the grain. This unpalatable outer husk is primarily made up of insoluble fiber and is typically removed before the grain can be used for food (Yadav and Hicks, 2015). Mechanical removal of the hull, also known as pearling, strips away part of the bran and germ, in addition to the hull, and makes the grain ineligible for whole grain status (Baik and Ullrich, 2008; Peterson, 1994, Meints and Hayes, 2019). This contrasts with a crop such as wheat where the grain threshes clean. Naked barley genotypes lose their hull naturally during harvesting or threshing, like wheat. This grain does not require pearling and, as a result, maintains its whole grain status (Meints and Hayes, 2019).

Naked barley arose by spontaneous mutation from domesticated covered barley genotypes over 8,000 years ago (Lister and Jones, 2012). This trait is the result of a single recessive allele at the *nud* locus on the long arm of chromosome 7H (Taketa et al., 2008). Minimal grain processing along with the well substantiated health benefits of barley and whole grains, make naked barley a desirable crop for use in food. Naked grain can provide benefits to the malting and feed industries as well. In terms of animal feed, studies in swine and chickens have found that the digestible energy in naked barley is greater than covered barley (Classen et al., 1985; Bhatty et al., 1979; Griffey et al., 2010). Covered barley is typically used for malting and brewing because the hull provides protection for the growing acrospire during the malting process and a natural filtration system during brewing. However, in malting, the use of naked grain has been shown to result in higher levels of malt extract and reduced levels of tannins, an undesirable compound in malting, that would normally originate in the hull (Edney and Rossnagel, 2000; Meints and Hayes, 2019). Despite the advantages of naked grain over covered types, covered barley is the dominant grain type grown worldwide. There is a need for high performance naked barley genotypes to meet demands in the food, malting, and feed markets. Selecting for genotypes that combine the desired characteristics of all three markets into multi-use varieties while also keeping in mind a moderate β-glucan target increases the flexibility and marketability of the grain for growers.

Organic farming systems benefit from diverse rotations to improve soil health, maintain fertility, and break pest, disease, and weed cycles. Barley can offer several advantages for organic farmers. It requires less nitrogen, establishes more quickly, and matures earlier than

other small grains, and it can be grown in a wide range of soil types and conditions (Baker et al., 2020). Despite its advantages, organic barley production has been limited. Roughly 809,000 hectares of barley are grown annually in the US, but according to the 2016 Certified Organic Survey, only 20,742 hectares were certified organic (NASS, 2016). The limited area is in part due to scarce market opportunities: organic beer appeals to a niche market (Waldrop and McCluskey, 2018), organic barley feed cannot compete with the price of imported organic corn (Reaves et al., 2019), and food barley accounts for only 3.7% of total barley production in the U.S., so the organic production and market opportunities are further limited (AMBA, 2020). However, new and expanding organic beer markets may offer new opportunities for farmers to grow organic barley (AB InBev, 2020; Allagash Brewing, 2020; New Belgium Brewing, 2020) and in the U.S., organic product sales climbed 12.4% in 2020, breaking the $60 billion mark for the first time and more than doubled the previous year's growth, according to the Organic Trade Association (OTA, 2021), so opportunities exist within the organic food barley market.

Genotype by environment interactions (GEI) are a major factor in plant breeding that confound selection because no single optimal genotype can be determined when multiple, distinct environments are being considered (Burgueno, 2012; van Eeuwijk et al., 2016). Given the diverse geographical regions where barley is produced in the U.S., improvement of naked barley genotypes cannot be done without considering GEI. The use of mixed models in data analysis can model and determine the significance of GEI acting on the panel (van Eeuwijk, 1995). Genotypic main effect plus genotype–environment interaction (GGE) as well as location-grouping (LG) models have also been used to characterize genotype and GEI effects (Yan, 2019).

These strategies lead to clearer estimates of genotypic performance, both in specific environments and in overall performance across environments. Finlay-Wilkinson regression, or the linear regression of a genotypic value on the environmental mean, is a useful methodology for assessing stability of genotypic performance over multiple environments (Finlay and Wilkinson 1963, Becker and Leon, 1988). GEI can further be reduced by dividing the target set of environments into more uniform subgroups known as mega-environments (ME; Braun et al., 1997). Selecting genotypes within ME is a way to tailor selection to the regional level. Biplots constructed from GGE or LG models can be used to visualize multi-environment data and define these ME (Yan et al., 2000). When multiple management systems are considered, GEI can be confounded with genotype by management interactions (GMI; Cooper et al., 2001). Genotype by management is also particularly relevant to organic systems as the wide variety of management practices can lead to high variability between organic environments (Barberi, 2002). For this reason, genotypes intended for organic systems should be assessed under organic conditions. As with most crops, modern varieties are selected under conditions that include the use of synthetic fertilizers and pesticides and are not necessarily best adapted for organic farming conditions. Barley genotypes bred under conventional systems do not show optimal performance when grown in organic conditions (Wolfe et al., 2008). New varieties bred and selected for organic systems that meet quality standards to be sold into multiple markets may encourage farmers to add barley to their rotation. Investing resources into breeding naked barley genotypes in and for organic production is an important step to improving productivity. This includes not only breeding for better grain yield and quality, but for stability across environments as well (Lammerts van Bueren et al., 2002).

This study had three objectives. i) Characterize a panel of naked barley genotypes spring planted on certified organic ground for plant height, heading date, test weight, grain yield, and threshability. ii) Determine the magnitude of genotype by environment effects acting on these traits and define ME. iii) Assess the stability of the naked barley genotypes present in the panel across environments in the northern United States. A better understanding of the genetic and environmental factors affecting the measured traits that result from this experiment will facilitate decision making for future selection and experimental design.

MATERIALS AND METHODS

Germplasm

The naked barley spring regional trial (SRT) panel consisted of eighteen genotypes of naked barley and one covered check (Table 2.1). The panel included two and six row barley as well as spring and facultative growth types (Table 2.1). Not all genotypes were tested in all environments (Table 2.1). Location specific check lines (both naked and covered) were planted in each environment. A full breakdown of genotypes planted in each environment as well as their performance can be found in supplemental figures 2.2-2.6.

Table 2.1 Description of 18 naked barley genotypes and one covered check evaluated in fifteen environments included in the spring regional trial.

Genotype	Row type	Growth habit	Grain color	Starch type	Breeding program[a]	# Locations per year		
						2018	2019	2020
10.0655	2	Facultative	White	Waxy	OSU	6	5	4
10.0662	2	Facultative	White	Waxy	OSU	6	5	4
10WAN-129.6	2	Spring	White	Normal	WSU	6	5	-
12WAN-106.12	2	Spring	White	Normal	WSU	6	5	2
BB28	6	Spring	Black	Normal	OSU	6	5	4
BB5	6	Spring	Black	Normal	OSU	6	5	4
BBB528	6	Spring	Black	Normal	OSU	-	5	2
CDC Ascent	2	Spring	White	Waxy	USask	-	5	4
CDC Carter	2	Spring	White	Normal	USask	-	5	4
CDC Clear	2	Spring	White	Normal	USask	6	5	4
DH133529	6	Facultative	White	Normal	OSU	6	5	4
DH133535	6	Facultative	White	Normal	OSU	6	5	4
Havener	2	Spring	White	Waxy	WSU	6	5	2
Meg's song	2	Spring	White	Waxy	WSU	6	5	2
MS10S4111-01	6	Spring	White	Normal	UMN	6	5	4
MS10S4115-03	6	Spring	White	Normal	UMN	6	5	4
Purple Valley	6	Spring	Purple	Normal	Landrace	6	5	4
Tamalpais	6	Spring	White	Normal	UC Davis	-	5	2
Full Pint[b]	2	Spring	White	Normal	OSU	6	5	4

[a] Indicates the breeding program that developed the genotype. Breeding program codes are as follows: UMN, University of Minnesota; WSU, Washington State University; USask, University of Saskatchewan Crop Development Centre; UC Davis, University of California at Davis; and OSU, Oregon State University.

[b] Full Pint is a covered genotype used as a check.

Both commercially available and unreleased genotypes were solicited from project participants and were assessed for their performance in environments across the northern U.S. Previous selection of these naked barley genotypes has generally been for food end-uses and high β-

glucan. Additionally, like many genotypes grown in organic systems, previous breeding efforts were directed towards conventional, rather than organic, environments. Unreleased genotypes from this panel were screened for potential release and genotypes that perform well in this set of environments could be selected as parents in a future breeding pipeline.

Experimental Design and Locations

Genotypes were evaluated in a randomized complete block design (RCBD) with three replications of twenty genotypes at each of 15 environments (i.e. combination of location and year). Trials were grown in seven locations between the 2018 and 2020 spring growing seasons including Arlington and Madison, WI; Ithaca (Caldwell farm), Freeville, and Aurora (Musgrave farm), NY; Corvallis, OR; and Lamberton, MN (see Table 2.2 for a characterization of the growing environments). Trials at Arlington and Madison, WI were not planted in 2020 due to the COVID-19 pandemic. At least nineteen of the genotypes within a given growing season were the same across all locations with at least one local check genotype. Four genotypes were discontinued after the 2018 season (i.e. *10WA-121.7*, *BB25*, *DH133532*, and *X07G26-T35*) due to low grain yield, and new genotypes were added (*BBB528*, *CDC Ascent*, *CDC Carter*, and *Tamalpais*, Table 2.1). After the 2019 season, genotype *10WAN-129.6* was also removed from the panel due to covered smut contamination. This created an unbalanced dataset where each experimental naked genotype was evaluated in seven to fifteen environments (Table 2.1).

Table 2.2 Description of fifteen testing environments over eight locations, four states and three growing seasons where the SRT panel was evaluated.

Location	Year	Environment	Latitude	Longitude	Planting date	Harvest date	Grain yield (kg ha^{-1})[c]	Heading date (DAP)[bc]
Arlington, WI	2018	ARL18	43.30°N	-89.38°W	27-Apr	30-Jul	594	55
Arlington, WI	2019	ARL19	43.30°N	-89.38°W	26-Apr	30-Jul	1427	65
Ithaca, NY	2019	ITH19	42.44°N	-76.46°W	24-Apr	29-Jul	1247	61
Ithaca, NY	2020	ITH20	42.44°N	-76.46°W	7-May	-	-	55
Corvallis, OR	2018	COR18	44.56°N	-123.26°W	26-Apr	28-Aug	4653	52
Corvallis, OR	2019	COR19	44.56°N	-123.26°W	26-Apr	14-Aug	2618	52
Corvallis, OR	2020	COR20	44.56°N	-123.26°W	16-Apr	10-Aug	3301	54
Freeville, NY	2018	FRE18	42.50°N	-76.30°W	9-May	26-Jul	792	54
Freeville, NY	2019	FRE19	42.50°N	-76.30°W	23-Apr	29-Jul	4025	60
Freeville, NY	2020	FRE20	42.50°N	-76.30°W	5-May	21-Jul[a]	1860	53
Lamberton, MN	2018	LAM18	44.23°N	-95.26°W	5-May	17-Aug	630	63
Lamberton, MN	2020	LAM20	44.23°N	-95.26°W	23-Apr	10-Aug	3040	61
Madison, WI	2018	MAD18	43.07°N	-89.40°W	24-Apr	27-Jul	934	53
Madison, WI	2019	MAD19	43.07°N	-89.40°W	16-Apr	17-Jul	2319	61
Aurora, NY	2018	AUR18	42.73°N	-76.65°W	25-May	6-Aug†	424	54

[a] Plots were harvested at maturity starting at indicated date

[b] DAP, days after planting

[c] Mean grain yield and heading date at each location are results from this study, shown here as a characterization of each growing environment.

Field Management

All trials were conducted on certified organic land using organic practices except the Ithaca trials in 2019 and 2020 that were on transitional ground but were still managed under organic practices. In the Madison 2019 and Arlington 2019 environments, the area between plots was cultivated before tillering (Zadoks 20/Z20; Zadoks et al., 1974). This occurred on the 7th of June, 2019 and the 10th of June, 2019 respectively. In the Ithaca 2020 environment, tine weeding was

conducted two weeks after planting (approximately Z20). Similarly, a rotary hoe and tine weeder were used in Lamberton 2020 to control weeds one to two weeks after planting. Trials at Corvallis, OR were not mechanically weeded, but did receive supplemental irrigation until after grain-filling according to local practice. The Freeville 2020 trial was also irrigated to account for a particularly dry season (Supplemental table 2.1). Trials in other environments were not irrigated. At Corvallis, OR organic compost derived from food scraps and yard trimmings as well as a Nutri-Rich 8-2-4 blood/feather meal granular fertilizer was applied prior to planting. Trials in other locations received no supplemental fertilizer; however, several of the trials followed nitrogen-fixing legumes (Supplemental table 2.1). All trials were hand weeded as needed, and approximate people-hours spent hand weeding are found in supplemental table 2.1. Additional soil fertility notes can be found in supplemental table 2.1.

Phenotypic Evaluation

Phenotypic data for grain yield (YLD), test weight (TWT), heading date (HD), plant height (PH), and threshability (THR) were recorded. YLD is the total weight of harvested grain converted to kilograms per hectare and yield data were recorded for fourteen of the fifteen tested environments. TWT is the grams per liter of the grain and test weight data were recorded for twelve of the environments. HD was the date when 50% of the plants in the plot have reached complete ear emergence (Z59). HD is expressed as days after the planting date and heading date data were recorded for all fifteen tested environments. For twelve environments, plant height was measured on a whole plot basis in centimeters at physiological maturity (Z89), from the ground to the tip of the spike, not including the awns. THR was estimated using a visual

score representing the proportion of grains that have lost the hull post-cleaning. Samples with all hulls still attached received a score of zero and samples with no hulls attached received a four based on protocol from Legzdina and Mezaka (2008). Threshability data were recorded for thirteen of the environments. See supplemental tables 2.2 through 2.6 for a breakdown of genotypic means for each environment.

Statistical Analysis

Variance Component Analysis

Plot level observations for the eighteen naked barley genotypes were used in a single-step random effects model for the purpose of estimating variance components and broad sense heritability. Covered checks are not included in the variance component analysis, as the intent was to characterize the naked lines specifically. This analysis was run using PROC VARCOMP in the SAS software version 9.4 (SAS Institute, 2020). The random effects model included genotype as well as year and location effects.

[2.1] $$\underline{y_{ijkl}} = \mu + \underline{G_i} + \underline{L_j} + \underline{A_k} + \underline{GL_{ij}} + \underline{GA_{ik}} + \underline{LA_{jk}} + \underline{GLA_{ijk}} + \underline{\beta_{l(jk)}} + \underline{\varepsilon_{ijkl}}$$

where y_{ijkl} is the plot level observation, μ is the overall mean; G_i is the random effect of the ith genotype with $G_i \sim N(0,\sigma^2_G)$; L_j is the random effect of the jth location with $L_j \sim N(0,\sigma^2_L)$; A_k is the random effect of the kth year with $A_k \sim N(0, \sigma^2_{A)}$; GL_{ij} is the interaction term between the ith genotype and the jth location with $GL_{ij} \sim N(0,\sigma^2_{GL})$; GA_{ik} is the interaction term between the ith genotype and the kth year with $GA_{ij} \sim N(0,\sigma^2_{GA})$; LA_{jk} is the interaction term between the jth location and the kth year with $LA_{jk} \sim N(0,\sigma^2_{LA})$; GLA_{ijk} is the random three way interaction between genotype, location, and year with $GLA_{ijk} \sim N(0, \sigma^2_{GLA})$; $\beta_{l(jk)}$ is the random effect of the

lth block nested in the jth location and kth year with $\beta_{k(j)} \sim N(0,\sigma^2_\beta)$; and ε_{ijkl} is the random residual term with $\varepsilon_{ijkl} \sim N(0,\sigma^2_\varepsilon)$. Also, where σ^2_G, σ^2_L, σ^2_A, σ^2_{GL}, σ^2_{GA}, σ^2_{LA}, σ^2_{GLA}, σ^2_β, and σ^2_ε are the variance components of genotype, location, year, genotype by location, genotype by year, location by year, genotype by location by year, block nested within location and year, and the residual, respectively. The covariance among random effects is equal to zero. Heritability for each trait was calculated *ad hoc* following Schmidt et al. (2019) based on Holland et al. (2010) using harmonic means for years, locations, environments, and replications. This measure of heritability was expressed as the proportion of the total phenotypic variance attributed to genotypic effects where the harmonic means of years, locations, environments, and replications were used to approximate the phenotypic variance or:

[2.2] $$H^2 = \frac{\sigma^2_g}{\sigma^2_g + \frac{\sigma^2_{ga}}{\bar{n}_a} + \frac{\sigma^2_{gl}}{\bar{n}_l} + \frac{\sigma^2_{gal}}{\bar{n}_{al}} + \frac{\sigma^2_{plot}}{\bar{n}_{alr}}}$$

where H^2 is the estimate of the heritability. σ^2_g, σ^2_{ga}, σ^2_{gl}, σ^2_{gal}, and σ^2_{plot} are the variance component estimates of genotype, genotype by year, genotype by location, genotype by year by location, and the residual error respectively. $\bar{n}_a$, $\bar{n}_l$, $\bar{n}_{al}$, and $\bar{n}_{alr}$ are the harmonic means of the number of years, locations, environments, and plots per environment per genotype, respectively.

Genotypic Performance

Empirical best linear unbiased estimates (BLUEs) for each genotype were estimated using a two-step phenotypic analysis accounting for spatial and environmental variation. This analysis was performed using PROC MIXED in the SAS software version 9.4 (SAS Institute, 2020). The

first-step utilized plot level information from all genotypes planted at a given location to account for spatial variation at the environment (i.e. location-year combination) level following the model:

[2.3] $$\underline{y_{ijkl}} = \mu + G_i + \underline{\beta_j} + \underline{R_k} + \underline{C_l} + \underline{\varepsilon_{ijkl}}$$

where y_{ijkl} is the plot level observation, μ is the overall mean, G is the effect of the ith genotype, β is the random effect of the jth block with $\beta_j \sim N(0,\sigma^2_\beta)$, R_k is the random effect of the kth row with $R_k \sim N(0,\sigma^2_R)$, C_l is the random effect of the lth column with $C_l \sim N(0,\sigma^2_C)$, and ε_{ijkl} is the random residual term with $\varepsilon_{ijkl} \sim N(0,\sigma^2_\varepsilon)$. Also, where σ^2_R and σ^2_C are the variance components of row and column respectively. The covariance among random effects are equal to zero. Row and column effects in equation [2.3] were considered a post-blocking control of spatial variation and were only considered when their inclusion improved model fit (i.e., The AIC and BIC of the model was lower when post-blocking was included). See supplemental tables 2.2-2.6 for a breakdown of which environments included this post-blocking.

Results from model [2.3] produced an incomplete two-way table of genotypes by environments as not all eighteen genotypes were evaluated in all environments. Using BLUEs from step one for only the eighteen experimental naked barley genotypes, a second step of the analysis was performed to predict the genotypic grain yield performance of untested genotypes in tested environments and predict missing values from the two-way table. Predicted values were later used in the construction of GGE biplots to further examine GEI impacting the tested naked barley germplasm. As the HD, TWT, PH, and THR traits were influenced far more by genetic, rather than GEI effects, only YLD was analyzed in step two. Covered checks were not included in

the second step of the analysis, as the intent was to characterize the naked lines specifically. This second step used the following model:

[2.4] $$\underline{y_{ij}} = \mu + G_i + E_j + \underline{GE_{ij}}$$

where y_{ij} is the genotypic BLUE from step one, μ is the overall mean, G_i is the effect of the ith genotype, E_j is the effect of the jth environment (i.e. location-year combination), GE_{ij} is the random effect of the ith genotype in the jth environment with $\boldsymbol{ge} \sim \mathrm{N}(\mathbf{0}, \sum \sigma^2_{\mathrm{ge}})$, and where σ^2_{ge} is the variance component of the genotype by environment interactions. A first-order factor analytic covariance structure (Σ) was used to model genotype by environment effects.

GGE and LG Biplot Analysis

The naked barley grain yield BLUEs calculated in the second step of the analysis (model [4]) were fit using a genotype plus genotype by environment interaction (GGE) model. The environment centered GGE model following Yan (2019) is:

[2.5] $$P_{ij} = (d\lambda_1^{\alpha}\zeta_{i1})\left(\lambda_1^{1-\alpha}\tau_{1j}/d\right) + (d\lambda_2^{\alpha}\zeta_{i2})\left(\lambda_2^{1-\alpha}\tau_{2j}/d\right) + \varepsilon_{ij}$$

where P_{ij} is the standardized two way genotype by environment table, ζ_{i1} and ζ_{i2} are the eigenvalues of principal component (PC) one and two for the ith genotype, τ_{1j} and τ_{2j} are the eigenvalues of PC1 and PC2 of the jth environment, λ_1 and λ_2 are the singular values of PC1 and PC2, α is the singular value partitioning genotypes, and ε_{ij} is the residual term. The largest two principal components that result from the single value decomposition (SVD) were plotted to create GGE biplots (Yan et al., 2000). GGE models were run and biplots were constructed using the *gge* package version 1.6 (Wright and Jean-Louis, 2020) in R version 4.0.2 (R Core Team, 2020).

To further visualize repeatable patterns of GEI present in this study, LG biplots were also constructed following Yan (2019). Producing an LG biplot follows the same SVD as producing a GGE biplot (model [5]); however, the two-way genotype by environment table (P_{ij}) is replaced by a location by environment table of Pearson correlations (Supplemental Table 2.7). The SVD was performed using the gge package version 1.6 (Wright and Jean-Louis, 2020) and the resulting principal components were plotted using the ggplot2 package version 3.3.3 (Wickham et al., 2020) in R version 4.0.2 (R Core Team, 2020). As correlations were determined across genotypes, LG biplots are more robust to unbalanced data. As a result, LG biplots were constructed using correlations between the genotypic BLUEs estimated in step one of the two step analysis rather than the predicted values estimated in step two.

Sensitivity Analysis

The naked barley grain yield BLUEs estimated in step one of the analyses (model [3]) were used in Finlay-Wilkinson regression to assess sensitivity of experimental naked genotypes. The standard FW model is:

[2.6] $$\underline{y_{ij}} = \mu_i + \beta_i E_j + \underline{\varepsilon_{ij}}$$

where μ_i is the expected genotypic performance of the ith genotype in an average environment, β_i is the sensitivity of genotype *i* to changes in environment quality, E_j is the environmental effect (i.e. location-year combinations) used as a regressor, ε_{ij} is the random residual term with $\varepsilon_{ij} \sim N(0,\sigma^2_\varepsilon)$ (Finlay and Wilkinson, 1963). FW regression was performed in the FW package version 0.0 (Lian 2014) in R version 4.0.2 (R Core Team, 2020).

RESULTS

Variance Component Analysis

Grain yield, test weight, heading date, plant height, and threshability of the eighteen experimental naked genotypes were assessed for the proportion of phenotypic variance attributed to genotype, location, year, location by year, and GEI. In this context, GEI is considered the combined effect of the genotype by location, genotype by year, and genotype by location by year variance components from the random effects model (equation [2.1]). The genotype:GEI ratio shows the relative sizes of these two effects. The ratio between genotypic and GEI effects was highest for heading date (6.46), indicating that the genetic differences between lines had an effect on the observed phenotypic variance more than six times that of GEI. This ratio was lowest for grain yield (0.18), indicating that the genetic differences between lines had an effect on the observed phenotypic variance less than one fifth than that of GEI. The genotype to GEI ratio of plant height, threshability and test weight fell between these two traits (3.63, 5.34, and 2.57 respectively). Agronomic traits had an *ad hoc* heritability of 0.40 for grain yield, 0.90 for test weight, 0.94 for heading date, 0.81 for plant height, and 0.93 for threshability.

Table 2.3 Variance component estimates as a proportion of the total variance and *ad hoc* heritability of 18 naked barley genotypes evaluated in up to fifteen location-year combinations in the northern U.S.

Covariance Parameter	HD	PH	THR	TWT	YLD
Genotype	0.36	0.15	0.66	0.44	0.02
Location	0.17	0.37	0.00	0.02	0.03
Year	0.15	0.11	0.00	0.00	0.08
Location*Year	0.21	0.20	0.13	0.29	0.71
Genotype*Location	0.01	0.00	0.04	0.03	0.02
Genotype*Year	0.01	0.00	0.00	0.01	0.02
Genotype*Location*Year	0.03	0.04	0.08	0.13	0.07
Rep(Location*Year)	0.01	0.02	0.00	0.01	0.01
Residual	0.03	0.09	0.07	0.08	0.04
# Locations	7	6	7	6	6
# Years	3	3	3	3	3
# Environments	15	13	13	12	14
Genotype:GEI	6.46	3.63	5.34	2.57	0.18
Heritability	0.94	0.81	0.93	0.90	0.40

Genotypic Performance

Heritability of grain yield for individual environments was between 0.82 and 0.99 at single environments (supplemental table 2.2). Mean grain yield ranged between 402 at Aurora 2018 and 4699 kg ha^{-1} at Corvallis 2018. Naked genotypes *MS10S4115-03*, and *MS10S4111-01* were either top or not significantly different from the top performing genotypes in nine of the fourteen environments (supplemental table 2.2).

Test weight had a minimum heritability of 0.88 and a maximum of 0.99 at single environments (supplemental table 2.3). Mean test weight ranged from 504.7 at Arlington 2018 to 741.3 g L^{-1}

at Corvallis 2018. The naked genotype *Purple Valley* had the highest or was not significantly different from the highest test weight genotype in eleven of the twelve environments. The only exception was Arlington 2018, where grain yield of *Purple Valley* was too low to assess test weight (supplemental table 2.3).

Heading date was highly heritable in the trials with a low of 0.95 and a high of 0.99 at individual environments (Supplamental Table 2.4). Average heading date occurred between 52 days after planting in Corvallis 2019 and 64 days after planting in Arlington 2019. The naked genotype *CDC Clear* was the latest heading genotype or not significantly different from the latest genotype in twelve environments out of fifteen (supplemental table 2.4).

The naked genotype *MS10S4115-03* was the tallest or not significantly different from the tallest genotype in eleven of the twelve environments. Mean plant height ranged between 48 cm in Aurora 2018 and 98 cm in Freeville 2019. Heritability for plant height fell between 0.82 and 0.99 (supplemental table 2.5).

In terms of threshability, mean scores in each environment for the naked barley genotypes ranged from 1.7 in Arlington 2019 to 3.2 in Aurora 2019. The naked genotype *Purple Valley* consistently scored four (i.e. all hulls detached) and was the genotype with the highest threshability in all twelve environments. Heritability for threshability in individual environments ranged from 0.76 to 0.99 (supplemental table 2.6).

Mean genotypic performance across all environments for traits with low GEI (HD, PH, TWT, and THR) are reported in table 2.4 below. Overall, genotype *Purple Valley* had the highest threshability and highest test weight. Genotype MS10S4115-03 was the tallest. Genotypes CDC Carter, CDC Clear and 10WAN-106.12 were last to reach heading.

Table 2.4 Mean genotypic HD, PH, TWT, and THR performance across all environments with available data for the 18 experimental naked barley genotypes.

Genotype	HD (DAP)	PH (cm)	TWT (g L^{-1})	THR (0-4)
10.0655	59	61.7	699	3.3
10.0662	57	66.8	665	3.1
10WAN-129.6	62	73.6	664	2.9
12WAN-106.12	57	69.6	645	2.4
BB28	51	64.9	577	1.6
BB5	53	72.6	537	1.5
BBB528	52	68.1	553	1.7
CDC Ascent	61	73.3	722	3.4
CDC Carter	62	76.0	706	3.2
CDC Clear	62	75.9	699	3.3
DH133529	55	68.4	695	3.2
DH133535	60	68.3	583	2.3
Havener	61	69.2	649	2.7
MS10S4111-01	55	71.6	625	2.3
MS10S4115-03	58	78.0	616	2.3
Meg's song	59	72.1	669	2.9
Purple Valley	54	76.6	747	4.0
Tamalpais	52	50.9	506	1.4
Mean	57	69.6	639	2.5
Standard Error	1	1.5	16	0.2
# of Environments	15	13	12	13

GEI Analysis

GGE and LG biplots were used to visualize GEI patterns affecting the eighteen naked barley genotypes. Based on the variance component analysis, grain yield was the only trait where the effects of genotype on the observed phenotypic variance were outweighed by GEI (Genotype:GEI ratio of 0.17; Table 2.3). For this reason, modeling, and visualization of GEI through biplots was only conducted for grain yield.

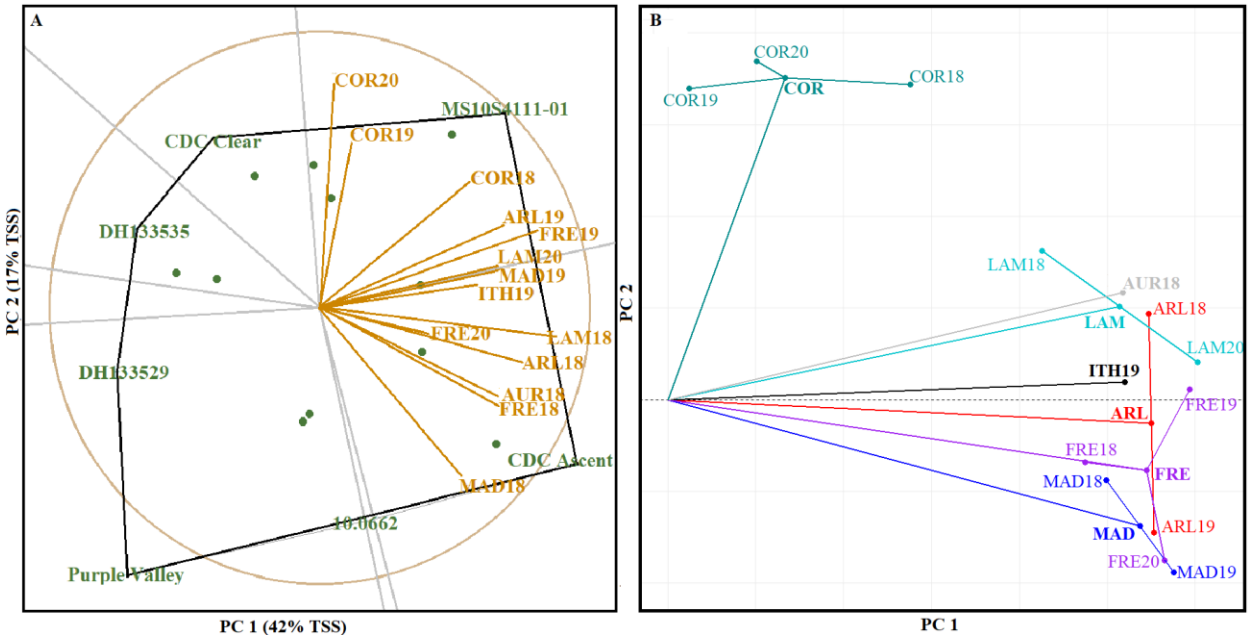

Figure 2.1 GGE biplot (A) and LG biplot (B) of grain yield for 18 naked barley genotypes repeated in more than seven environments in the spring regional trial. In the GGE biplot, genotypes are shown in green and environments are displayed in gold. ME are delineated by dashed lines originating from the plot's origin. In the LG biplot, Locations are indicated by their respective three letter code, while years within a given location are indicated by their letter code and year number.

Two MEs were identified for grain yield based on the GGE biplot (figure 2.1). ME1, containing COR20, COR19, COR18, ARL19, FRE19, and LAM20, shows the genotype *MS10S4111-01* as the top performing genotype. Genotype *CDC Ascent* outperforms other genotypes in ME2, containing the environments MAD18, MAD19, ITH19, ARL18, FRE18, FRE20, AUR18, and LAM18. The first two principal components plotted in the GGE biplot for grain yield account for 59% of the total observed phenotypic variance, or total sum of squares (TSS).

Two distinct groupings of environments are also shown by the LG biplot (figure 2.1). The first contains only the environments located in Corvallis, OR. The second contains all other environments assessed in this study. The LG biplot is similar to the GGE biplot in that Corvallis, OR seems to be distinct in both; however, the LG biplot grouped the ARL19, FRE19, and LAM20 environments with the second ME rather than the first.

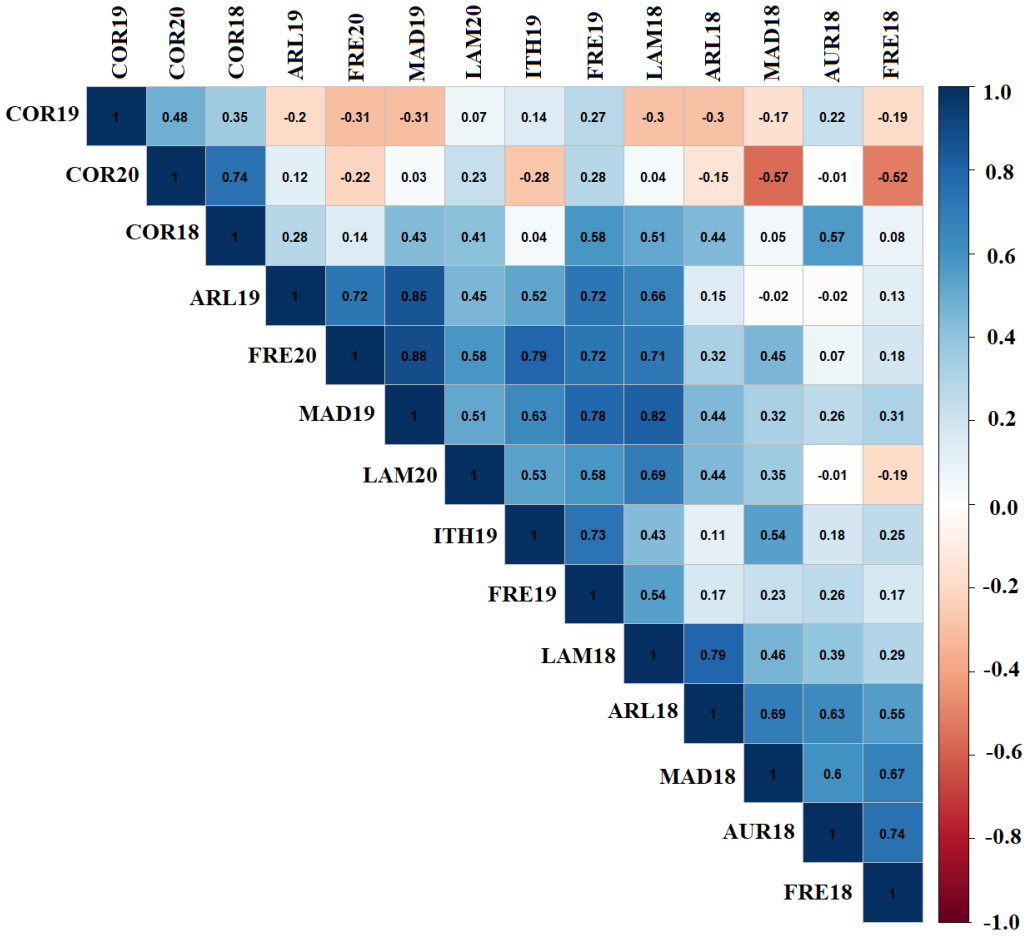

Figure 2.2 Correlation matrix of grain yield across the fourteen environments with available yield data assessed in the spring regional trial. Environments are arranged by mega-environment membership as determined by the LG biplot analysis (figure 2.1).

A Pearson correlation matrix presents the information from the LG biplot in a different way. Environments located in Corvallis (ME1), particularly COR20 and COR19, are overall negatively or not correlated with the other environments (ME2) in terms of grain yield.

Sensitivity Analysis

Based on a Finlay-Wilkinson regression it was found that many genotypes were sensitive to changes in the environmental mean grain yield. This means that as the environmental mean increases, genotypes show increased responses in grain yield (slope greater than one). The genotypes *MS10S4111-01* and *Tamalpais* in particular show above average responses to increases in environment quality (slopes of 1.26 and 1.44 respectively). The genotype *Purple Valley* displayed a below average response (slope of 0.49). While this lower response led to a reduced ability to leverage high-quality environments, the yield penalty was reduced in environments yielding less than 630 kg ha^{-1}.

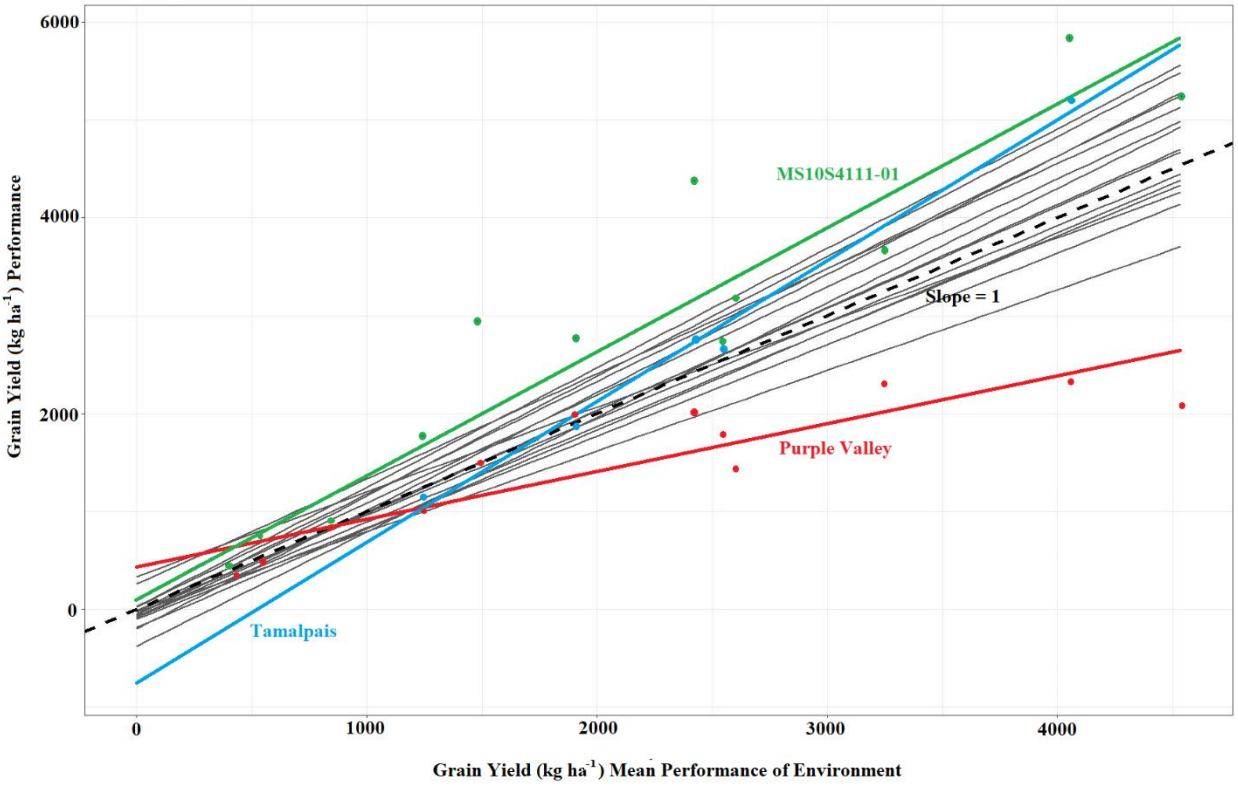

Figure 2.3 Plot displaying the results of the Finlay-Wilkinson regression for grain yield (kg ha^{-1}) for the 18 naked barley genotypes in fourteen environments. Genotypes Purple Valley, MS10S4111-01, and Tamalpais are colored in red, green, and blue, respectively. The remaining genotypes are in gray.

DISCUSSION

Historically plant breeders have chosen to manage GEI by ignoring, reducing, or exploiting GEI (Eisemann et al., 1990). Modeling and accounting for GEI in multi-environment trials can reduce its effects on selection and help determine the best genotype for each environment; however, when the genotypic effects on the observed phenotypic variance are less than the effects of GEI, the options of ignoring or reducing GEI become less effective (DeLacey et al., 1996). The exploration and identification of GEI affecting grain yield, test weight, plant height, and heading date in naked barley is an important aspect of the crop's improvement. The effect of GEI was trait dependent. Grain yield had high GEI effects compared to genotypic effects and lower GEI was observed for heading date, plant height, test weight, and threshability.

The traits that were measured as a part of this study were selected because they were important for either naked barley production (THR), organic barley production (HD and PH), or both (YLD and TWT). In a survey that collected responses from 81 organic barley producers around the U.S., grain yield was identified as the most important trait for breeders to select for by 48% of the respondents and was rated as one of the top three traits by 82% of respondents (Baker et al., 2020). Additionally, because the hull accounts for 10 to13% of the weight of the grain in covered barley, breeding for grain yield in naked barley is especially important for it to be competitive with covered barley (Meints et al., 2015). Because of the often-complex rotation systems that organic farmers use, an early heading barley can result in either a longer grain filling period with higher grain yield, or earlier harvest that can be valuable for growers who may be planting another crop into the field post-harvest. Plant height is an important trait to

measure because of its relationship with grain yield, lodging, and weed competitive ability (Mason et al., 2007).

Threshability is an example of a trait where GEI had a low impact on the observed phenotypic variance. *Purple Valley* was the most threshable genotype in all locations, and samples of the grain were consistently free of hulls (a threshability score of 4.0). *Purple Valley* is a landrace that has been collected in several locations around the world (Canada, Peru, and Australia) and deposited into USDA-GRIN under four different accessions (PI 281852, PI 477844, PI 510561, CIho 15270) as early as the 1960s (data not shown). *Purple Valley* seed for this study was sourced from Lonesome Whistle (Junction City, Oregon). Because of the pearling or additional processing required to have the hulls removed (Meints and Hayes, 2019), threshability is a key trait in naked grains and naked landraces, which would have been used predominately for food and likely were selected for this trait. Genotypes with low threshability retain some proportion of grain with hulls still attached (Meints and Hayes, 2019; Bleidere et al., 2012). While the naked phenotype is caused by a single gene (*Nud*, Taketa et al., 2008), the ease with which the hull is removed during harvest is likely a function of multiple genes that are correlated with grain shape and hull thickness (Ram and Singh, 1996). Selecting for thinner hulls and rounder grains has been an effective strategy to increase threshability indirectly in Canadian breeding programs (Rossnagel, 2000). Based on the low GEI observed for this trait, the threshability of genotypes could be assessed in early generations and few environments.

Heading date had low GEI, as indicated by the low proportion of GEI variance compared to the genotypic variance. This is similar to what has been found in other studies of covered barley, where genetic main effects contribute more to the observed variance than GEI (Mut et al., 2010). Due to the low impact of GEI on the observed variance of heading date, ranking of genotypes was similar across locations. However, regional requirements for heading date may be different from location to location. Flowering induction is impacted largely by photoperiod and temperature, and the testing locations in this study were all in the Northern U.S. at a similar latitude with a 15 to 15.5 hour day-length at flowering induction.

Plant height was also impacted less by GEI compared to genotypic effects. This has been found in other studies of covered barley where the relative contribution of GEI variance was low compared to the variance of the genotypic main effect (Rodriguez et al., 2007). Based on the low impact of GEI on plant height in this naked barley germplasm, rankings of plant height in one environment would be relevant for all the tested environments. However, because of the impact of plant height on other plant characteristics such a susceptibility to lodging, regional requirements for plant height may be different particularly for those environments where intense precipitation and storms can increase lodging (Telkar et al., 2012; Berry, 2019). Environments in the Northwestern U.S. (i.e. Corvallis, OR) have fewer severe weather events on average (Climate.gov, 2021) and therefore, less lodging. This means that taller genotypes might not have a disadvantage in the Northwestern U.S.

The relative contribution of GEI to the observed phenotypic variance of test weight was lower compared to genotypic effects. *Purple Valley* was one of the top genotypes in terms of test weight and was consistently a top performing line for test weight in this study. Naked lines tend to have higher test weight due in part to the loss of the hull which accounts for 10 to 13% of grain weight (Agu et al., 2009; Rey et al., 2009). Griffey et al. (2010) found significant increases of 16% in the test weight of naked genotypes compared to covered feed genotypes. In this study, the test weight of the covered check *Full Pint* was significantly lower than the highest test weight line in eleven of twelve environments. The one environment where *Full Pint* was not significantly different than the highest test weight line was Arlington 2018, where low yields prevented test weight data being recorded for several genotypes, including *Purple Valley*. The higher impact of genotypic effects relative to GEI on test weight in naked barley, along with the presence of high test weight genotypes that consistently performed well regardless of environment, suggests that it is possible to select for stable lines that have high test weight in all environments.

Grain yield was the trait with the highest relative GEI. GEI has been found to have a greater impact on grain yield relative to genotypic effects in other multi-environment trials in barley (Rodriguez et al., 2007; Nowosad et al., 2018). This GEI can be controlled in a variety of ways. Identifying genotypes that are stable for grain yield across a target set of environments is one option. Emphasizing stability is a key aspect to plant breeding in the face of climate change (Snowdon et al., 2020). Genotypes will need to remain stable in the face of increasing climate extremes and variability (Heisey and Rubenstein, 2015; EPA, 2021). In the tested naked barley

germplasm, few genotypes displayed low sensitivity to the environment. The least sensitive genotype, *Purple Valley*, has low overall performance in terms of grain yield. Another method to reduce the impact of GEI is to select for regional adaptation of genotypes. (Mathews et al., 2008; Lado et al., 2016). Mega-environment delimitation is an important step in defining groups of environments that have low GEI within. Based on the GGE biplot constructed for grain yield in this study, MEs were not geographically defined. All of the Corvallis environments fall under the same ME (ME1), as do all Madison environments (ME2). However, there is a strong genotype by location by year interaction driven mainly by the year effect at Arlington and Freeville that separate these locations into two MEs (ARL19 and FRE19 in ME1 and ARL18, FRE18, and FRE20 in ME2). Large genotype by year interaction is common in many species (Lado et al., 2016; Monteverde et al., 2018; Monteverde et al., 2019; Kucek et al. 2019; Gonzalez-Barrios et al. 2019) including barley (Gutierrez et al., 2015; Bhatta et al., 2020; Neyhart et al., 2021a,b). A possible explanation for this interaction is weed pressure. In 2018, during the first year of this study, high weed pressure was likely responsible for lower grain yields in all locations except for Corvallis (Supplemental table 2.2). Weed management was kept to a minimum early in the study, with the intent of screening for weed competitive ability. Weed management practices were changed for the following seasons to avoid crop losses and more accurately reflect organic growing environments. The improvement and optimization of management practices used over the course of this study introduced genotype by management effects between year one and the remaining years of the study. Those genotype by management effects were likely responsible for some of the GEI patterns associated with the year effect (Cooper et al., 2001). Repeatable GEI patterns can be more easily observed

following the LG biplot procedure (Yan 2019). The LG biplot of grain yield (figure 2.2) displays two MEs: one with all Corvallis environments and the other with all remaining environments at Lamberton, Madison, Arlington, Freeville, Aurora, and Ithaca. As opposed to the GGE biplot, the LG biplot suggested a geographical separation of MEs between the Northwestern U.S. and the upper Midwest/Northeast U.S. However, this separation was likely a result of both the supplemental irrigation and fertilizer that was applied at Corvallis, but not at any of the other locations. Improving not only the overall productivity and stability of naked barley grain yield, but also selecting for weed competitive ability displayed by individual genotypes should therefore be breeding priorities. The high level of GEI found in naked barley grain yield should be leveraged to breed genotypes that are stable and have high performance within a target set of environments.

SUMMARY

Improving existing naked barley germplasm is critical and cannot be done without properly accounting for the impacts of GEI. For traits where genetic effects dominate (test weight, plant height, heading date, and threshability), the effects of GEI can be ignored without extensively impacting the ability to make genetic progress. For traits such as grain yield where the effects of GEI drive the observed variance, selection within ME where the impact of GEI can be exploited would benefit productivity. Additionally, when breeding for organic systems and organic markets other traits, such as weed competitive ability, resistance to biotic and abiotic stressors, and end-use quality are important selection targets and deserve more study. In this study, eighteen naked barley genotypes were assessed for agronomic performance across fifteen environments and used to identify two ME in the Northern U.S. for organic naked barley production. This information can be used to tailor selection to the regional level and release optimally performing, stable genotypes for use by organic growers.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Phenotypic data is available on T3/Barley.

ACKNOWLEDGMENTS

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Supplemental Table 2.1: Description of the 15 environments where the spring regional trial was grown including precipitation from planting until harvest, soil series, available phosphorus, available potassium, soil pH, previous crop in rotation, time hand weeding, and principal foliar disease

Environment	Precipitation (in)	Soil Series	Soil Test Year	%OM	P (ppm)	K (ppm)	pH	Previous Crop in Rotation	Time Hand Weeding (hrs)	Principal Foliar Disease
ARL18	15.4	Plano silt loam	2017	3.9	92	203	7.2	Soybeans	52	-
ARL19	17.4	Plano silt loam	2017	4.2	59	94	7.2	Corn	11	Barley Yellow Dwarf
ITH19	13.1	-	-	-	-	-	-	Forage	-	Spot Blotch
ITH20	8.9	-	-	-	-	-	-	Fallow	-	Spot Blotch
COR18	1.3	Chehalis silty clay loam	2018	3.1	27.7	290.8	6.5	Vegetables	2	Leaf Rust
COR19	3.4	Chehalis silty clay loam	2019	5.2	37	314	6.3	Vegetables	2	Leaf Rust
COR20	6.6	Chehalis silty clay loam	2020	3.38	30	256	6.4	Vegetables	2	Leaf Rust
FRE18	12.5	-	-	-	-	-	-	Vegetables	12	Spot Blotch
FRE19	13.4	-	-	-	-	-	-	Kale	12	Spot Blotch
FRE20	4.9	-	-	-	-	-	-	Oats	12	Spot Blotch
LAM18	19.9	Normania loam	2015	7.4	6	97	6.9	Soybeans	10	-
LAM20	15.3	Normania loam	2019	4.0	11	168	6.5	Winter Rye	10	-
MAD18	18.4	Kegonsa silt loam	2017	2.3	29	94	7.1	Vegetables	19	Spot Blotch
MAD19	16.4	batavia silt loam	2018	3.6	57	205	7.0	Alfalfa	24	Barley Yellow Dwarf
AUR18	9.9	-	-	-	-	-	-	Clover	-	Spot Blotch

Supplemental Table 2.2: BLUEs of grain yield (in kg/ha) for each of the 18 experimental naked genotypes and 20 covered or check genotypes in 14 environments of the spring regional trial

Genotype	ARL18	ARL19	ITH19	COR18	COR19	COR20	FRE18	FRE19	FRE20	LAM18	LAM20	MAD18	MAD19	AUR18
Experimental Genotypes														
10.0655	245.2	1158.8	1269.5 *	4307.9	2616.4	2907.6	566.6	3960.8	2351.9 *	440.9	2827.8 *	909.3	2421.7	377.9 *
10.0662	554.0	1288.6	1580.6 *	4052.2	2199.1	2055.1	807.8 *	3666.3	2352.7 *	674.2	3830.5	1666.9 *	2903.6	479.7 *
10WAN-129.6	728.1	1758.5	1399.5 *	5389.7 *	3565.3 *		831.2 *	4055.6		511.2		776.9	1703.8	401.2 *
12WAN-106.12	741.8	2061.5	1720.2 *	5383.4 *	3550.4 *		1042.9 *	5054.6 *	1629.6	764.5		606.2	3215.4	441.7 *
BB28	504.1	1490.7	902.8	5282.2 *	2396.7	3739.3	557.0	3072.0	1913.6	742.9	3160.0 *	545.2	2765.0	417.8 *
BB5	567.2	1538.9	847.9	5879.0 *	2524.0	3825.2	677.2	4004.0	1792.8	654.6	3850.7 *	881.6	2430.9	424.7 *
BBB528		1286.0	947.4		2406.7			3746.1	1586.4				2692.8	
CDC Ascent		2056.1	1347.3 *		2422.3	2529.0		5077.1 *	2634.0 *		4196.9 *		3115.7	
CDC Carter		1455.8	1013.9 *		2090.4	1782.7		4425.1 *	2602.3 *		4062.8 *		2385.1	
CDC Clear	149.2	1623.3	1390.5 *	3660.7	2585.3	3176.7	393.6	4154.6	2281.3 *	453.2	4308.6 *	564.5	2529.5	171.2
DH133529	234.6	899.0	966.3	4115.3	2523.8	2665.9	822.8 *	3273.4	1609.3	213.6	1380.5	750.0	1988.2	433.1 *
DH133535	231.7	776.1	1216.7 *	4180.0	3659.9 *	3092.9	633.6	3295.8	1415.3	274.6	2916.9	837.2	938.6	436.7 *
Full Pint†	327.1	2009.1	1164.1 *	6033.7 *	3598.4 *	4508.2	911.6 *	3862.3	2513.2 *	865.1 *	3105.9	1397.6 *	1950.8	382.4 *
Havener	567.1	1293.7	1509.0 *	5434.2 *	1649.4		942.7 *	3741.8	840.4	682.2		958.8	1518.3	531.7 *
Meg's song	849.1 *	1685.4	1411.7 *	5277.2 *	2940.8		844.0 *	5058.5 *	1450.6	773.9		1292.7 *	2674.2	466.9 *
MS10S4111-01	337.2	2963.2 *	1750.4 *	5242.3 *	2726.1	3171.0	788.1 *	5844.7 *	2773.4 *	805.5	3662.8 *	869.1	4379.7 *	443.6 *
MS10S4115-03	805.9	1596.5	1561.0 *	5023.7	2545.9	2149.3	878.4 *	4737.8 *	2638.4 *	904.7 *	4214.2 *	1601.8 *	3509.0 *	519.0 *
Purple Valley	359.4	1529.3	1013.8 *	2080.3	1785.5	1429.0	736.3 *	2342.7	1992.1	481.6	2316.2	867.0	2011.9	286.6
Tamalpais		1490.0	1141.3 *		2667.9			5202.6 *	1889.8				2758.0	
Check Genotypes														
120341†				6029.0 *										
10WAN-121.7	715.0			4209.1			963.4 *			1236.6 *		760.0		405.3 *
5-36-OCOLOR					2623.4	2521.8			1924.2		2094.1			
AAC Azimuth							678.7							498.2 *

BB25	567.4			5650.1 *			666.9			771.9		833.5		376.4 *
Copeland†					3102.8 *	3347.6								
DH130910†			765.3					2957.9	1615.5					
DH133532	377.3			3735.6			783.3 *			305.9		1316.1 *		254.6
DH140212						4509.1			241.6		2152.0			
DH140450						5160.3			1538.8		1871.1			
DH160767						6183.5 *			1598.8		2594.9			
DH160813						4733.1			1786.6		3991.5 *			
Jet					1904.9									
Lacey†										1339.2 *	4854.2 *			
Oscar					2659.9									
PPWQ						3795.7			1936.5		2472.9			
Purple Prince					2256.2									
Quest†	1363.0 *	3433.4 *										2222.4 *	4076.6 *	
X07G26-T35	688.4			5473.5 *			1055.0 *			755.0		1029.6		531.6 *
Trial Mean	477.3	1574.6	1212.2	4698.8	2569.9	3157.0	759.6	3982.7	1733.7	620.3	3039.8	964.5	2462.7	401.6
Heritability	0.89	0.95	0.84	0.98	0.92	0.99	0.91	0.9	0.97	0.92	0.91	0.82	0.97	0.89
Row Effect	X	X			X	X		NA		X	X		X	
Column Effect	X	X	X		X	X	X	NA	X	X	X	X	X	X

† indicates a hulled genotype

* Indicates top performing or not significantly different from top performing genotype

Supplemental Table 2.3: BLUEs of grain test weight (in g/L) for each of the 18 experimental naked genotypes and 20 covered or check genotypes in 12 environments of the spring regional trial

Genotype	ARL18	ARL19	ITH19	COR18	COR19	COR20	FRE18	FRE19	FRE20	MAD18	MAD19	AUR18
Experimental Genotypes												
10.0655		668.9 *	763.2 *	791.4 *	676.1	732.5	686.0 *	757.3 *	715.7 *	613.3	723.8 *	664.2 *
10.0662	599.5 *	626.6	706.1 *	774.4 *	597.6	666.0	668.1 *	710.3	706.3 *	603.8	677.6 *	641.0
10WAN-129.6	546.0 *	700.8 *	717.3 *	781.1 *	684.8		640.0 *	675.3		656.7 *	619.7	623.3
12WAN-106.12	506.2 *	646.2	706.1 *	737.0	642.1		627.9 *	725.7	558.3	556.8	674.1 *	684.1 *
BB28	459.1 *	466.6	564.3	690.9	546.9	593.4	593.1	669.0	537.7	516.9	610.0	674.0 *
BB5	407.8	394.9	528.4	683.6	567.3	559.5	569.2	623.7	495.3	438.4	511.5	666.2 *
BBB528		452.1	589.5		538.1			646.3	513.0		576.4	
CDC Ascent		732.7 *	770.2 *		671.7	757.5 *		779.3 *	743.3 *		629.0 *	
CDC Carter		691.7 *	735.0 *		652.2	708.3		767.7 *	724.3 *		694.0	
CDC Clear		700.7 *	745.5 *	783.5 *	659.4	777.8 *	642.4 *	759.0 *	722.3 *	613.0	658.7 *	
DH133529		697.3 *	731.9 *	775.4 *	664.5	731.6	697.3 *	766.7 *	678.3 *	634.8	685.7 *	682.6 *
DH133535		618.8	600.4	700.2	570.0	724.9	504.1	576.0	421.9	543.2	597.9	661.3 *
Full Pint†	505.8 *	549.2	590.1	684.0	589.5	654.1	558.9	584.3	563.3	536.1	553.3	531.9
Havener	540.2 *	648.4	738.6 *	811.5 *	495.6		705.3 *	740.0 *	487.4	582.8	677.2 *	677.6 *
Meg's song	570.5 *	684.2 *	742.3 *	778.6 *	595.7		680.8 *	739.3 *	549.3	625.6	673.7 *	687.3 *
MS10S4111-01		586.2	656.3	735.9	559.9	607.0	647.7 *	694.3	626.7	593.8	583.8	680.6 *
MS10S4115-03	514.6 *	633.6	636.3	728.0	507.5	597.9	609.4	700.7	570.0	576.5	627.0 *	689.5 *
Purple Valley		724.4 *	719.1 *	808.4 *	781.6 *	807.7 *	772.3 *	781.7	750.0 *	711.6 *	764.5 *	698.5 *
Tamalpais		429.4	561.4		501.3			567.3	473.0		503.2	
Check Genotypes												
120341†				681.1								
10WAN-121.7	473.8 *			761.1			635.3 *			549.3		644.6
5-36-OCOLOR					745.7 *	788.5 *			690.0 *			
AAC Azimuth							588.4					682.9 *

BB25	422.1			660.3			634.5 *			534.9		672.9 *
Copeland†					552.3	601.6						
DH130910†			518.7					587.3	544.0			
DH133532				738.2			673.3 *			663.9 *		719.0 *
DH140212						734.1						
DH140450						733.1			612.3			
DH160767						575.8			482.0			
DH160813						560.7			480.7			
Jet					700.6							
Lacey†												
Oscar					768.5 *							
PPWQ						779.5 *			712.7 *			
Purple Prince					713.4 *							
Quest†	521.7 *	565.5								554.7	619.7	
X07G26-T35	530.2 *			749.8			651.8 *			558.8		653.9 *
Trial Mean	504.7	601.6	660.8	741.3	618.8	679.6	636.6	688.8	589.6	580.2	629.6	663.8
Heritability	0.9	0.99	0.97	0.98	0.97	0.99	0.9	0.98	0.98	0.97	0.88	0.95
Row Effect	X	X		X	X	X		NA		X	X	
Column Effect	X	X	X	X	X	X	X	NA	X	X	X	X

† indicates a hulled genotype

* Indicates top performing or not significantly different from top performing genotype

Supplemental Table 2.4: BLUEs of heading date (in DAP) for each of the 18 experimental naked genotypes and 20 covered or check genotypes in 15 environments of the spring regional trial

Genotype	ARL18	ARL19	ITH19	ITH20	COR18	COR19	COR20	FRE18	FRE19	FRE20	LAM18	LAM20	MAD18	MAD19	AUR18
Experimental Genotypes															
10.0655	60*	67	63	55	53	55*	53	57*	62	53	64	60	57	63	56*
10.0662	55	66	61	54	52	52	52	55	61	53	63	62	53	60	54
10WAN-129.6	61*	72*	67*		56	56*		57*	64*		70*		59	68*	60*
12WAN-106.12	54	64	62	58*	54	52		54	59	52	63		53	63	55
BB28	47	59	55	48	46	47	46	51	54	46	56	57	45	53	46
BB5	49	59	58	55	49	49	50	51	56	49	58	58	46	56	47
BBB528		59	55	51		48			55	47				56	
CDC Ascent		70*	65*	58*		55*	60		64*	56		64		66*	
CDC Carter		69*	67*	59*		55*	59		65*	56		66		67*	
CDC Clear	61*	69*	67*	60*	60*	55*	59	60*	65*	56	69*	64	61*	67*	58*
DH133529	53	62	60	53	51	51	52	53	60	52	61	58	54	61	50
DH133535	59*	67	65*	56	54	54*	59	56	64*	55	67*	65	59	62	55
Full Pint†	60*	68*	64*	55	53	54	56	56	62	52	67*	65	56	67*	55*
Havener	58*	70*	66*	62*	56	54		56	63	57	66		56	66*	57*
Meg's song	56	67	64*	58	55	54*		55	60	55	64		53	63	59*
MS10S4111-01	54	59	60	54	52	51	51	55	57	51	61	58	54	57	52
MS10S4115-03	55	66	65*	56	55	53	56	55	63	53	62	60	56	62	55
Purple Valley	51	61	56	49	52	51	51	49	56	47	65	55	50	55	57*
Tamalpais		59	55	49		47			57	47				56	
Check Genotypes															
120341†					52										
10WAN-121.7	60*				51			53			64		52		54
5-36-OCOLOR				48		48	49			46		56			
AAC Azimuth								54							51

BB25	50				50			51			58		49		49	
Copeland†				54	57											
DH130910†			63	55					60	54						
DH133532	52				49			54			62		52		50	
DH140212						66*				70*		69*				
DH140450				56			53			53		63				
DH160767				55			53			52		61				
DH160813				53			51			49		57				
Jet						49										
Lacey†											57	57				
Oscar						51										
PPWQ				53			52			49		60				
Purple Prince						56*										
Quest†	52	60											49	56		
X07G26-T35	56				52			55			64		54		56*	
Trial Mean	55	64	62	54	53	52	54	54	60	52	63	61	53	61	54	
Heritability	0.96	0.97	0.98	0.97	0.99	0.99	0.99	0.95	0.99	0.99	0.98	0.98	0.99	0.99	0.96	
Row Effect	X	X			X	X	X		NA		X	X	X	X		
Column Effect	X	X	X	X	X	X	X	X	NA	X	X	X	X	X	X	

† indicates a hulled genotype

* Indicates top performing or not significantly different from top performing genotype

Supplemental Table 2.5: BLUEs of plant height (in cm) for each of the 18 experimental naked genotypes and 20 covered or check genotypes in 13 environments of the spring regional trial

Genotype	ITH19	ITH20	COR18	COR19	COR20	FRE18	FRE19	FRE20	LAM18	LAM20	MAD18	AUR18
Experimental Genotypes												
10.0655	46.9	51.7	61.0	67.3	70.3	59.7	92.0	64.3	51.9	55.1	60.2	42.3
10.0662	54.3 *	54.3	64.7	68.0	70.3	66.6	97.3 *	68.9	55.2	68.2	69.4 *	43.5
10WAN-129.6	55.6 *		77.3 *	80.7		73.0 *	103.0 *		65.7 *		79.6 *	56.1 *
12WAN-106.12	54.8 *	52.0	69.8	77.3		68.5	97.7 *	82.1 *	60.1		67.8	47.7
BB28	48.1	49.0	69.0	69.0	70.0	56.8	101.3 *	63.1	64.9 *	56.5	66.8	46.3
BB5	49.3	60.0 *	79.6 *	79.7	86.9 *	65.6	108.3 *	79.3 *	70.5 *	65.3	66.3	50.4
BBB528	49.2	52.0		77.3			105.0 *	68.3				
CDC Ascent	51.4	58.7 *		78.7	76.2 *		101.7 *	81.4 *		74.6 *		
CDC Carter	59.8 *	61.7 *		82.0	82.4 *		107.3 *	85.0 *		74.8 *		
CDC Clear	59.6 *	57.7 *	80.7 *	85.3 *	74.8	69.0	110.0 *	87.6 *	65.8 *	73.1 *	81.5 *	61.4 *
DH133529	55.5 *	52.3	69.7	71.0	71.4	67.9	101.3 *	78.7 *	62.2 *	63.1	67.9	44.3
DH133535	51.6	51.3	64.9	76.3	82.8 *	62.3	97.7 *	75.3	62.2 *	60.2	69.2 *	42.8
Full Pint†	36.1	52.7	54.6	61.3	61.3	57.1	75.0	67.9	54.2	59.1	65.5	44.8
Havener	51.2	50.3	70.0	73.7		66.9	96.3	80.0 *	65.2 *		70.4 *	46.7
Meg's song	48.6	56.0 *	70.5	78.0		64.4	100.7 *	86.9 *	71.2 *		75.1 *	50.0
MS10S4111-01	53.1 *	60.7 *	74.9 *	80.7	82.4 *	74.6 *	102.0 *	73.4	69.0 *	62.0	66.9	51.1
MS10S4115-03	56.7 *	64.7 *	85.2 *	85.5 *	77.1 *	79.5 *	110.3 *	86.3 *	78.0 *	73.7 *	78.1 *	50.5
Purple Valley	66.9 *	66.3 *	86.5 *	84.3 *	86.1 *	80.0 *	98.7 *	85.0 *	81.5 *	62.6	65.9	55.0 *
Tamalpais	37.6	41.3		53.3			74.3	47.3				
Check Genotypes												
120341†			50.6									
10WAN-121.7			69.9			69.5 *			72.9 *		74.5 *	51.8
5-36-OCOLOR		68.7 *		94.3 *	90.6 *			94.0 *		72.7 *		
AAC Azimuth						77.4 *						47.0

BB25			71.5			63.9			69.8 *		64.4	48.7
Copeland†				83.7 *	86.7 *							
DH130910†	45.6	49.3					90.3	65.4				
DH133532			68.0			65.7			57.1		64.4	44.2
DH140212		50.0			81.9 *			67.9		66.6		
DH140450		40.0			63.5			52.0		38.1		
DH160767		54.0			73.1			67.7		52.1		
DH160813		47.0			60.7			58.0		55.4		
Jet				83.7 *								
Lacey†									73.2 *	66.2		
Oscar				93.3 *								
PPWQ		70.3 *			87.2 *			97.0 *		84.2 *		
Purple Prince				100.0 *								
Quest†											79.0 *	
X07G26-T35			72.7			72.9 *			65.3 *		74.6 *	48.2
Trial Mean	51.1	54.4	70.0	77.8	76.3	67.8	98.0	73.4	65.4	63.3	70.1	48.4
Heritability	0.99	0.87	0.93	0.92	0.92	0.93	0.94	0.92	0.82	0.93	0.86	0.95
Row Effect			X	X	X		NA		X	X	X	
Column Effect	X	X	X	X	X	X	NA	X	X	X	X	X

† indicates a hulled genotype

* Indicates top performing or not significantly different from top performing genotype

Supplemental Table 2.6: BLUEs of threshability (on a 1-4 scale) for each of the 18 experimental naked genotypes and 14 naked checks in 12 environments of the spring regional trial

Genotype	ARL18	ARL19	ITH19	COR18	COR19	COR20	FRE18	FRE19	FRE20	LAM18	MAD18	MAD19	AUR18
Experimental Genotypes----------													
10.0655	2.7*	3.5*	3.5*	3.4*	2.9	3.3*	4.0*	3.5*	3.5*	3.0*	3.2*	3.5*	3.5*
10.0662	2.5	2.8	3.5*	3.6*	2.0	3.3*	3.5*	3.3*	3.3*	3.3*	2.9*	3.5*	3.5*
10WAN-129.6	2.8*	3.5*	3.5*	3.5*	3.0		3.0	2.8		2.2	2.8	2.3	3.0
12WAN-106.12	1.3	2.0	3.0	2.9	2.3		2.5	3.3*		2.3	1.4	2.5*	3.3*
BB28	0.5	1.0	1.8	2.3	1.0	1.5	2.0	2.8	1.3	0.5	1.2	2.0	3.0
BB5	0.5	1.0	1.5	2.0	1.0	1.5	2.0	2.0	1.0	2.1	0.5	1.6	2.8
BBB528		1.0	2.0		1.0			2.0				2.5*	
CDC Ascent		3.5*	3.5*		2.6	3.1*		3.8*	3.6*			3.5*	
CDC Carter		3.5*	3.5*		3.0	2.7		3.5*	3.5*			3.0*	
CDC Clear	3.0*	3.5*	3.5*	3.7*	3.0	3.5*	3.5*	3.5*	3.3*	3.0*	3.2*	3.2*	2.8
DH133529	2.2	3.5*	3.5*	3.6*	3.0	3.1*	3.5*	3.5*	3.3*	2.4	2.9*	3.3*	3.5*
DH133535	1.7	2.5	2.5	2.8	2.4	3.3*	2.5	2.0	2.0	1.7	1.7	1.7	2.5
Havener	2.0	2.3	3.5*	3.6*	1.9		3.5*	3.0*		2.4	1.9	3.0*	3.3*
Meg's song	2.5	3.0*	3.5*	3.0	2.2		3.0	3.5*		3.3*	2.3	2.9*	3.3*
MS10S4111-01	1.5	1.8	2.8	2.9	1.7	2.0	2.8	2.8	2.5	2.0	2.3	2.6*	2.8
MS10S4115-03	1.8	2.5	2.5	3.0	1.5	1.8	2.8	3.0*	1.8	2.0	2.2	2.5*	3.3*
Purple Valley	4.0*	4.0*	4.0*	4.0*	4.0*	4.0*	4.0*	4.0*	4.0*	4.0*	4.0*	4.0*	4.0*
Tamalpais		1.3	2.0		1.1			1.3				1.5	
Check Genotypes----------													
10WAN-121.7	1.3			3.5*			3.0			2.5	2.2		3.3*
5-36-OCOLOR					3.5*	3.6*			3.6*				
AAC Azimuth							2.0						3.0
BB25	0.5			1.9			3.0			1.1	1.9		3.3*
DH133532	3.0*			3.3			3.3*			2.6	3.3*		3.5*

DH140212						3.1 *							
DH140450						3.2 *			2.5				
DH160767						1.5			1.0				
DH160813						1.7			1.3				
Jet					3.7 *								
Oscar					3.4 *								
PPWQ						3.5 *			3.5 *				
Purple Prince					3.4 *								
X07G26-T35	2.3			3.0			3.0			3.1 *	1.8		3.0
Trial Mean	1.7	2.3	2.9	3.1	2.2	2.6	2.9	2.9	2.4	2.2	2.1	2.6	3.2
Heritability	0.97	0.97	0.99	0.98	0.97	0.98	0.93	0.94	0.98	0.97	0.97	0.92	0.76
Row Effect	X	X		X	X	X		NA		X		X	
Column Effect	X	X		X	X	X		NA		X	X	X	

* Indicates top performing or not significantly different from top performing genotype

Supplemental Table 2.7: Pearson correlations of grain yield between environments across genotypes for each of the 14 harvested environments of the spring regional trial

Environments	ARL	MAD	COR	FRE	AUR	LAM	ITH
ARL18	1	0.69	0.44	0.55	0.63	0.79	-
MAD18	0.69	1	0.05	0.67	0.6	0.46	-
COR18	0.44	0.05	1	0.08	0.57	0.51	-
FRE18	0.55	0.67	0.08	1	0.74	0.29	-
AUR18	0.68	0.6	0.57	0.74	1	0.39	-
LAM18	0.79	0.46	0.51	0.29	0.29	1	-
ARL19	1	0.85	-0.2	0.72	-	-	0.52
MAD19	0.85	1	-0.31	0.78	-	-	0.63
COR19	-0.2	-0.31	1	0.27	-	-	0.14
FRE19	0.72	0.78	0.27	1	-	-	0.73
ITH19	0.52	0.63	0.14	0.73	-	-	1
COR20	-	-	1	-0.22	-	0.23	-
FRE20	-	-	-0.22	1	-	0.58	-
LAM20	-	-	0.23	0.58	-	1	-

CHAPTER 3. GENOMIC PREDICTION OF THRESHABILITY IN NAKED BARLEY

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Authors: Chris Massman, Brigid Meints, Javier Hernandez, Karl Kunze, Patrick M. Hayes, Mark E. Sorrells, Kevin P. Smith, and Lucia Gutierrez

Affiliations: C. Massman, L. Gutierrez, Department of Agronomy, University of Wisconsin – Madison, 1575 Linden Dr, Madison, WI 53706, USA; B. Meints, J. Hernandez, P.M. Hayes, Department of Crop and Soil Science, Oregon State University, 3050 SW Campus Way Corvallis, OR 97331; J. Hernandez, Departamento de Acuicultura y Recursos Agroalimentarios, Universidad de Los Lagos, Región de los Lagos, Osorno; K. Kunze, M.E. Sorrells, School of Integrative Plant science, Plant Breeding and Genetics Section, Cornell University, Ithaca, New York 14853; K. Smith, Department of Agronomy and Plant Genetics, 1991 Upper Buford Circle St. Paul, MN 55108

Author Contributions: BM, PMH, MES, and LG conceptualized the study. LG designed the specific hypothesis and supervised the research including formal data analysis. CM, KK, BM, PMH, MES, and LG performed the investigation associated to the field experiments. CM performed the formal data analysis. CM, BM, JH, and LG wrote the original draft. All authors revised and edited the manuscript and agreed to the final version of the manuscript.

ABSTRACT

Threshability, defined here as the propensity of grains to lose their hull after harvest, is a key trait in naked barley (Hordeum vulgare L.). While threshability is a defining characteristic of naked grains and has been found to be associated with grain size and shape, its genetic architecture is poorly described. The goals of this study were to identify quantitative trait loci (QTL) associated with threshability and evaluate their utility as covariates in genomic prediction (GP) models. A genome wide association study (GWAS) identified two loci on chromosomes 2H and 3H associated with threshability. The locus on chromosome 2H accounted for 9.9% of the phenotypic variance explained (PVE). The locus on chromosomes 3H accounted for 7.8% of the PVE. With effects on threshability of 0.18 and 0.29 for each marker, these markers could have a limited impact when implemented in marker assisted selection (MAS). Predictive ability for threshability was 0.84 using genomic best linear unbiased prediction (gBLUP). Incorporation of the markers with significant associations as covariates in the model did not improve predictive ability. Likewise, predictive ability was not improved by the inclusion of grain test weight, percentage of thin grains, or percentage of plump grains as covariates in the gBLUP model. The high predictive ability for threshability overall indicate that genomic selection (GS) would be useful in selection for threshability in naked barley.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the fourth most widely grown cereal in the world (FAO-STAT, 2020). Originally grown as a food crop as early as 10,000 years ago, barley's primary end uses have shifted toward animal feed and malting in the last two centuries (Baik and Ulrich, 2008); however, barley has seen renewed interest as a food crop resulting from its many nutritional benefits including high protein and β-glucan levels (Keogh et al., 2003; Meints et al., 2016). Most barley grown in the United States has covered (hulled) grain with a tightly adhering husk that must be removed before the grain can be used in food (Meints and Hayes, 2019). The removal of the hull is an additional processing step that also removes part of the bran and germ, compromising the nutritional value and making the grain ineligible for whole-grain status (Baik and Ulrich, 2008). An alternative to this is naked (hull-less) barley grain types where the hull threshes freely during harvest and cleaning, thus, the grain maintains its whole-grain status and nutritional benefits. Naked barley is believed to have first appeared in cultivated covered barley over 8,000 years ago and resulted from a single mutation at the *nud* locus on the long arm of chromosome 7H (Swanston et al., 2011; Taketa et al., 2008; Lister and Jones, 2012). The recessive *nud* mutation is a loss of function that prevents the formation of a lipid layer on the surface of the caryopsis which would otherwise lead to adhesion of the hull to the caryopsis, while the functional *NUD* allele allows for this adhesion to take place (Duan et al., 2015; Taketa et al., 2008). The absence of the hull leads to higher digestible energy in livestock feeding and reduced levels of off-flavor inducing tannins in brewing (Bhatty et al., 1979; Griffey et al., 2010; Edney and Rossnagel, 2000; Meints and Hayes, 2019). These benefits together make naked barley promising from a multi-use perspective. Naked barley selected for use in the feed, food,

and malting industries allows the grain to meet multiple end-uses and provides growers multiple market options when selling the grain.

While the recessive *nud* allele prevents adhesion of the hull to the underlying caryopsis, abscission of the lemma and palea that comprise the hull may not occur. In some cases, the hull will not be adhered to the grain but will remain intact and lightly attached. In this way, the recessive *nud* allele is required, but not sufficient for complete removal of the hull. The mechanisms behind this phenomenon are not fully understood. Ideally, in naked germplasm, the lemma and the palea are abscised at the base of the grain and, not being adhered to the caryopsis as a result of the *nud* mutation, they fall away. Threshability is only observable in naked grains, because in covered grains, the lemma and palea remain affixed as a result of their adhesion to the underlying caryopsis. The proportion of grain having successfully shed the hull after harvest and cleaning in naked grains is what is defined here as threshability. There is phenotypic variation in threshability, with highly threshable grain being free of attached hulls (Figure 3.1). Low threshability grain does not have an advantage over covered grains and still requires pearling before it can be used in food. With the renewed interest in barley as a food crop in the past several decades, researchers, bakers, and consumers have focused on the ability of naked grains to shed their hull without the intermediate processing steps that would otherwise remove part of the bran (Baker et al., 2020). Threshability is a key trait in naked barley (Meints and Hayes, 2019), and the release of high threshability genotypes is critical to the adoption of naked barley.

Because naked barley is grown on minimal acreage in the US, selecting for naked barley has not been a high priority for breeding programs. However, breeding programs in Canada have been

targeting the naked trait for multiple end-uses for the last 50 years (Rossnagel, 2000) and therefore have set standards for the percentages of hulls remaining on naked barley for different end-uses: 5% in food barley and 15% in feed barley (Legzdiņa and Gaile, 2008). Breeding programs in Canada have historically used phenotypic selection to achieve genetic gain (Rossnagel, 2000). This includes selecting based on high weight per volume, as this has been found to be positively correlated with threshability (Legzdiņa and Gaile, 2008; Rossnagel 2000). Because threshability is based on the propensity of the lemma and palea to release the grain, hull strength and thickness as well as grain size and shape characteristics play a large role in the threshability of a naked barley genotype (Ram and Singh, 1996). Despite the importance of threshability, the genetic and biochemical mechanisms behind this trait are poorly described. Ram and Singh (1996) reported that threshability in naked grains is under the control of two unlinked recessive genes with the homozygous recessive configuration conferring easy threshing. Understanding the genetic architecture of threshability is an important aspect to consider when breeding naked barley germplasm.

Figure 3.1. Threshability variation in barley on a 0-4 scale. A score of zero indicates 100% of hulls remain attached to the grain, one indicates 75% attached, two indicates 50% attached, three indicates 25% attached, and four indicates 0% of hulls remain attached.

Although threshability is defined exclusively for naked germplasm that carry the *nud* allele, the hull can also be lost in covered (hulled) germplasm in a phenomenon known as skinning. Skinning in covered barley leads to poor hull adhesion and occasionally loss of the hull entirely. Skinning is not related to the recessive *nud* allele (Romero et al., 2018). Like threshability, environmental effects play a large roll in skinning, along with the physical characteristics of the hull including hull thickness and grain shape (Grant et al., 2021; Brennan et al., 2017). Existing

studies have identified QTL for skinning in barley on chromosomes 2H, 3H, 5H, 6H, and 7H (Rajasekaran et al., 2004; Romero et al., 2018).

Threshability in other cereals such as wheat has been researched in more depth than in barley. The major domestication locus (Q) and the tenacious glume locus (Tg) are the genes primarily responsible for the high threshability of hexaploid wheat (Simons et al., 2006; Jantasuriyarat et al., 2004; Sood et al., 2009). The Q locus on wheat chromosome 5A has a wide range of effects including glume shape. The *Tg* locus on chromosome 2D controls glume toughness (Sood et al., 2009) and the dominant allele confers free-threshing seeds and loose glumes (Simons et al., 2006). Additional studies have identified QTL relevant to glume tenacity and rachis fragility on chromosomes 2A, 2B, 6A, 6D, and 7B of hexaploid wheat (Jantasuriyarat et al., 2004). These findings highlight the polygenic nature of threshability in other cereals.

Methods such as marker assisted selection (MAS) can be used to identify breeding lines or genotypes with favorable alleles based on marker scores at QTL linked to the trait of interest rather than phenotypic values alone (Tanksley, 1983). This method requires the identification of QTL with large effects on the trait being selected (Bernardo, 2020; Collard et al., 2005). GWAS has been found to be an effective tool for identifying relevant QTL in diverse populations (Jannink et al., 2001). The GWAS approach to QTL discovery can be expanded to account for underlying population structure as well as marker-based kinship that could otherwise lead to spurious associations (Bernardo, 2020) by using the Q+K model outlined by Yu et al. (2006).

The MAS approach can be limited by its relative inability to account for complex or multigenic traits (Korte and Farlow, 2013; Heffner et al., 2009). The genomic prediction (GP) approach overcomes some of the limitations of MAS by using the scores for all available markers, rather than single or few loci (Heffner et al., 2009; Meuwissen et al., 2001). This allows for the effects of all markers to be utilized in making predictions, rather than just those markers that passed the threshold for statistical significance (Bernardo, 2020). Alternatively, using information on the genomic relationship between genotypes, the genetic values of individuals that have not been phenotyped can be predicted from the performance of a training set of related genotypes using the gBLUP framework (Bernardo, 1994; VanRaden, 2008). Some studies showed that predictive ability could be increased by the incorporation of major QTL as fixed effects in the gBLUP model (Spindel et al., 2016; Brøndum et al., 2015; Li et al., 2019; Rutkoski et al., 2014). However, if the QTL used represent a small percentage (<10%) of the phenotypic variation explained or there are many QTL, accuracy could be unaffected or even decreased by the use of those QTL (Bernardo, 2020; Rice and Lipka, 2019). Multi-trait models have also been used as an extension of GP models to leverage phenotypic data that plant breeders may already have available (Lado et al., 2018). Studies have shown that the use of highly correlated traits could improve predictive ability (Arojju et al., 2020; Bhatta et al., 2020; Rutkoski et al., 2014). The use of additional covariates, either major QTL or correlated traits, can further improve on the utility of the GP methodology.

Grain with good threshability is critical for the utilization of naked barley genotypes, particularly in a food context. Improving the threshability of naked barley germplasm will aid the crop's adoption. Genomic approaches in plant breeding have been shown to increase genetic gain per

unit of time (Heffner et al., 2010) and have the potential to enhance the phenotypic selection already performed in naked barley. The objectives of this work were to (i) use GWAS to identify QTL significantly associated with threshability, (ii) test the utility of incorporating the identified QTL as fixed effects into a gBLUP model for GP to increase predictive ability, and (iii) test the use of plump/thin grain percentages and test weight as covariates to increase predictive ability for threshability. Identified QTL will help elucidate the underlying genetic architecture of threshability and have the potential to be incorporated into a MAS breeding scheme. Assessing the potential of GP for threshability through gBLUP as well as the efficacy of incorporating additional covariates in GP models will provide potential alternatives to phenotypic selection.

MATERIALS AND METHODS

Germplasm

We used the Naked Barley Diversity (DIV) panel consisting of 350 unique naked barley genotypes. The DIV panel contains spring, winter, and facultative genotypes as well as six and two row spike types. In order to test for winterhardiness and differentiate spring and facultative genotypes, the full panel was fall planted and the subset of genotypes (223) without vernalization requirements were also spring planted. For the purposes of GWAS and GP for threshability, the full panel of 350 genotypes was used.

Environments

Trials were conducted in three locations over three seasons for a total of nine environments (i.e. location-year combinations, Table 3.1). Both fall and spring planted panels were evaluated. Trials in Corvallis, OR (44.56°N, -123.26°W) used a type-2 modified augmented experimental design described by Lin and Poushinsky (1985) with double five-foot rows in spring 2018 and fall 2019 but changed to growing mini-plots in the spring 2019, fall 2020, and spring 2020 growing seasons as more seed became available. The trial in Madison, WI (43.07°N, -89.40°W) used the augmented design proposed by Federer (1956) with double-row, five-foot plots. Trials in Freeville, NY (42.52°N, -76.33°W) used a type 2 modified augmented experimental design with double-row, five-foot plots. Trials were conducted on certified organic ground using organic practices.

Table 3.1. Description of nine environments over three locations and three growing seasons where the DIV panel of naked barley was evaluated. Proportion of genotypes with more than one replication (Rep), number of genotypes tested (#G), mean threshability (THR), and trial heritability for threshability (H^2) are reported.

Location	Year	Season	Rep	#G	THR	H^2
Corvallis, OR	2018	Spring	0.05	221	2.69	0.91
Corvallis, OR	2019	Spring	0.05	219	3.04	0.91
Corvallis, OR	2020	Spring	0.05	230	2.99	0.94
Corvallis, OR	2019	Fall	0.04	350	2.82	0.98
Corvallis, OR	2020	Fall	0.04	349	2.80	0.99
Freeville, NY	2019	Fall	0.04	350	3.09	0.91
Freeville, NY	2020	Fall	0.04	335	3.04	0.71
Freeville, NY	2020	Spring	0.05	224	2.66	0.88
Madison, WI	2019	Spring	0.69	226	2.81	0.73

Phenotyping

Genotypes were assessed for threshability, test weight, and plump/thin grain percentages. Threshability is visually scored by observing the proportion of grain that loses its hull after harvest and cleaning. Grain was cleaned on a Pfeuffer MLN Sample Cleaner and then threshability was scored on a zero to four scale, where samples with no attached hulls received a four and grain with all hulls still attached received a zero-score based on protocol by Legzdina and Mezaka (2008). Test weight was measured as grams per liter of cleaned grain. The measurement of test weight pre- and post- cleaning is one way that breeders have indirectly selected for genotypes with high threshability because lines that have fewer attached hulls

typically have higher test weights (Rossnagel, 2000). Plump and thin grain values are the percentages by weight of the grain sample falling through the 6/64" or 5/64" sieves of a Pfeuffer Sortimat, respectively. Because naked barley grain with no hulls attached are often inherently smaller than grain with a hull (the hull accounts for 10-13% of the weight and volume of the grain), lower plump scores may indicate genotypes that have higher threshability. Test weight, plump grain percentage, and thin grain percentage were only recorded for each genotype in the Corvallis 2019 and 2020 environments due to smaller plot sizes and therefore lower grain weights in Madison, Freeville, and Corvallis 2018.

Genotypic best linear unbiased estimates (BLUEs) for each trait were calculated in the SAS software version 9.4 (SAS Institute, 2022) using a two-step mixed model. The first step accounts for spatial variation at the trial level following the model (underlined effects are considered random):

[3.1] $$\underline{y_{ij}} = \mu + G_i + \underline{\beta_j} + \underline{\varepsilon_{ij}}$$

where y_{ij} is the observed value of a trait for the ith genotype and jth replication, μ is the overall mean of a trait, G_i is the effect of the ith genotype, β_j is the random effect of the jth replication with $\beta_j \sim N(0, \sigma^2_\beta)$, and ε_{ij} is the residual error term with $\varepsilon_{ij} \sim N(0, \sigma^2_\varepsilon)$. The variance components of replication and error are σ^2_β and σ^2_ε respectively. The covariance among random effects is equal to zero. The second step of the mixed model accounts for environmental and genotype by environment effects following the model:

[3.2] $$\underline{y_{ij}} = \mu + G_i + E_j + \underline{GE_{ij}}$$

where y_{ij} is the genotypic BLUE calculated in model 1, E_j is the effect of the jth environment, and GE_{ij} is the random effect of the ith genotype in the jth environment with $GE \sim N(0, \sigma^2_{GE})$. Also, where σ^2_{GE} is the variance component of the genotype by environment effect.

Broad-sense heritability was calculated at each environment following Cullis et al. (2006) to account for the unbalanced number of observations resulting from the augmented experimental design with the following model:

[3.3] $$H^2_{Cullis} = 1 - \frac{\bar{V}_{\Delta}^{BLUP}}{2\sigma_g^2}$$

where H^2_{Cullis} is the broad-sense heritability, $\bar{V}_{\Delta}^{BLUP}$ is the average standard error of genotypic BLUPs, and σ_g^2 is the genotypic variance. The genetic variance and BLUPs were calculated following model [1], but with genotype as a random effect.

Genotyping and Data Filtering

Genotyping was done using the Illumina 50K SNP Chip at the USDA-ARS genotyping lab in Fargo, ND. Monomorphic markers as well as markers with more than 10% missing data were filtered out. Markers with a minor allele frequency less than 5% were also filtered out. Finally, linkage disequilibrium (LD) between pairs of markers was calculated as the correlation coefficient in the R package, *SNPRelate* (Zheng et al., 2022). Markers in perfect LD (r^2=1.0) were removed from the analysis. After filtering, there were marker data available for 349 individuals at 32,208 loci.

Population structure was assessed via Ward's hierarchical clustering method and the STRUCTURE software (Pritchard et al., 2000) using a subset of 325 uncorrelated markers

distributed across the barley genome. Six subpopulations were identified consisting of 38, 128, 62, 37, 44, and 38 genotypes respectively (data not shown). These subpopulations were verified via visualization in biplots plotting the first two principal components of a principal component analysis as well as comparison with features shared between genotypes within each subpopulation (vernalization requirement, row type, and origin).

Correlation Between Grain Traits

Correlations between threshability, percentage of thin grain, percentage of plump grain, and test weight were calculated and displayed via a Pearson correlation matrix. As these observations were only made in the Corvallis location, this correlation is the relationship between the genotypic mean threshability across Corvallis environments resulting from model [3.2] compared to the mean percentage of plump grain, percentage of thin grain, and test weight also calculated across the Corvallis environments using model [3.2].

Genome Wide Association Study

GWAS for threshability was performed using the *GWASPoly* package by Rosyara et al. (2016) in R version 4.0.3 (R Core Team, 2022). The QK model described by Yu et al. (2006) was used to control for both population structure and kinship based on marker-relatedness. The mixed model equation for the QK model is:

[3.4] $$\mathbf{y} = \mathbf{S\alpha} + \mathbf{Qv} + \mathbf{Zu} + \mathbf{e}$$

where **y** is the vector of genotypic BLUEs estimated from model [3.2], **α** is the vector of fixed SNP effects with incidence matrix **S**, **v** is the vector of fixed population effects with incidence matrix **Q**, **u** is the vector of polygenetic background effects with incidence matrix **Z**, and the

vector of residual effects is **e**. Also, where Var(u)=2Kσ_g^2 and Var(e)=Rσ_e^2. K is an nxn realized additive relationship matrix. R is an nxn matrix where the diagonal elements are the reciprocal of the number of observations for each phenotypic data point, and the off-diagonal elements are equal to zero (Yu et al., 2006). The Q matrix is the result of the structure analysis using the *Structure* software (Pritchard et al., 2000) that found six subpopulations present in the assessed naked barley germplasm. The probability of a genotype belonging to each of the six subpopulations was used to construct the Q matrix. In the case of the GWAS analysis, the genotypic BLUEs for threshability across all nine environments resulting from model [2] were used to detect significant QTL.

Manhattan plots and qq plots were constructed to visually search for markers associated with threshability QTL at a family-wise error rate (FWER) of α=0.05. Phenotypic variance explained (PVE) for each of the identified markers significantly associated with a QTL were calculated using multiple regression with backwards elimination also in the *GWASpoly* package.

Genomic Prediction

Genomic prediction for threshability was performed using the *rrBLUP* package (Endelman, 2011). The gBLUP method for genomic prediction proposed by Bernardo (1994) was used to leverage available marker data in making predictions. The mixed model for the gBLUP method is:

[3.5] $$\mathbf{y} = \mathbf{1}\mu + \mathbf{Zu} + \mathbf{X\beta} + \mathbf{e}$$

where **y** is the vector of genotypic BLUEs estimated from model [3.2], **1** is a vector with all elements equal to one, μ is the overall mean, **u** is the vector of marker effects with incidence

matrix **Z**, **β** is the vector of fixed effects with incidence matrix **X**, and **e** is the vector of residual effects. Fixed effects included as covariates are *in-silico* markers determined to be significant in the GWAS analysis, grain test weight, percentage of plump grain, or percentage of thin grain.

Predictive ability was calculated as the correlation between the observed and predicted genotypic value for threshability. Each fixed effect was tested independently for its effect on predictive ability. Markers associated with QTL for threshability were used as fixed effects to prevent possible shrinkage of their estimated effects (Bernardo, 2014). The use of significant markers as covariates was tested with genotypic BLUEs for threshability based on all nine environments. Additionally, because of the documented effects of barley grain size and shape on threshability (Ram and Singh, 1996), plump/thin percentages and test weight values were also used as covariates. Because test weight, plump grain percentages, and thin grain percentages were only available in Corvallis, genotypic BLUEs for threshability from model [3.2] but based only on Corvallis environments were used to test these covariates. Predictive ability and mean prediction error variance for each model were calculated through 1,000 iterations of seven-fold cross validation.

Effects of Population Structure

The impact of subpopulation structure on the predictive ability observed in this study was also investigated. In a study by Berro et al (2019), it was found that predictive ability could be overestimated when the trait of interest is associated with underlying population structure. In these cases, GS models accurately predict group membership rather than performance within subpopulations. The main effect of subpopulation on the threshability of a genotype was

assessed using analysis of variance. Then Fisher's least significant difference test was used to detect significant differences between the threshability of subpopulations at the α=0.05 level. One iteration of the seven-fold cross validation scheme was assessed to create a predicted versus observed scatterplot of threshability values, color coded by subpopulation membership using the ggplot2 package version 3.3.3 (Wickham et al., 2020) in R version 4.0.2 (R Core Team, 2020). Calculating the predictive ability within subpopulations, rather than across subpopulations provides information on differences between subpopulations. Expanding on this, the threshability values of each of the six subpopulations was predicted using the other five subpopulations in a leave-one-out type of analysis (i.e. threshability values of individuals belonging to subpopulation one were predicted using information from subpopulations two, three, four, five, and six). Finally, predictive ability when a randomly selected subset of individuals of equal size to the given subpopulation is predicted. This allows for comparisons between predictions made on a single subpopulation and predictions made on a randomly selected group of individuals of equal size.

RESULTS

Correlation Between Grain Traits

Test weight was found to be highly correlated with threshability in this study; however, plump percentage and thin percentage were not correlated with threshability (Figure 3.2).

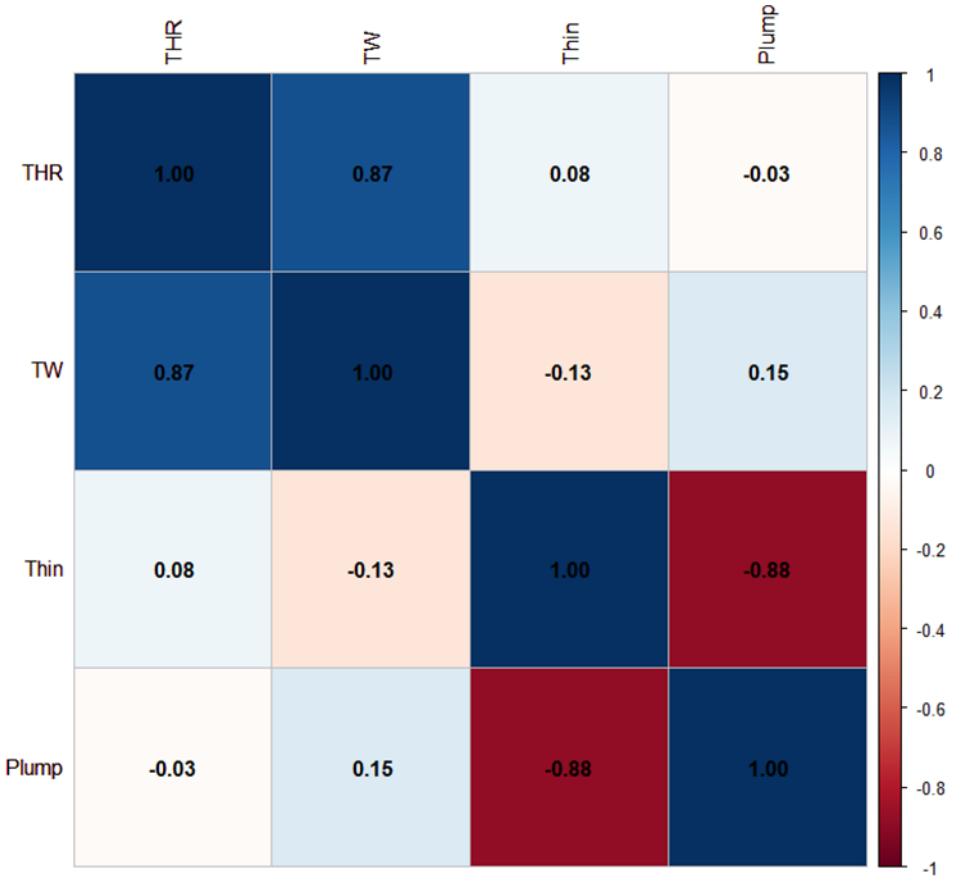

Figure 3.2. Pearson correlations between threshability (THR), test weight (TW), thin grain percentage (Thin), and plump grain percentage (Plump) in the Corvallis environments.

Genome Wide Association Study

The GWAS identified one marker significantly associated with threshability on chromosome 3H at 109.84 cM (JHI-Hv50k-2016-205614), and one on chromosome 2H at 15.37 cM (JHI-Hv50k-2016-71750; figure 3.3). Linkage maps are based on the barley reference genome presented by Mascher et al. (2017).

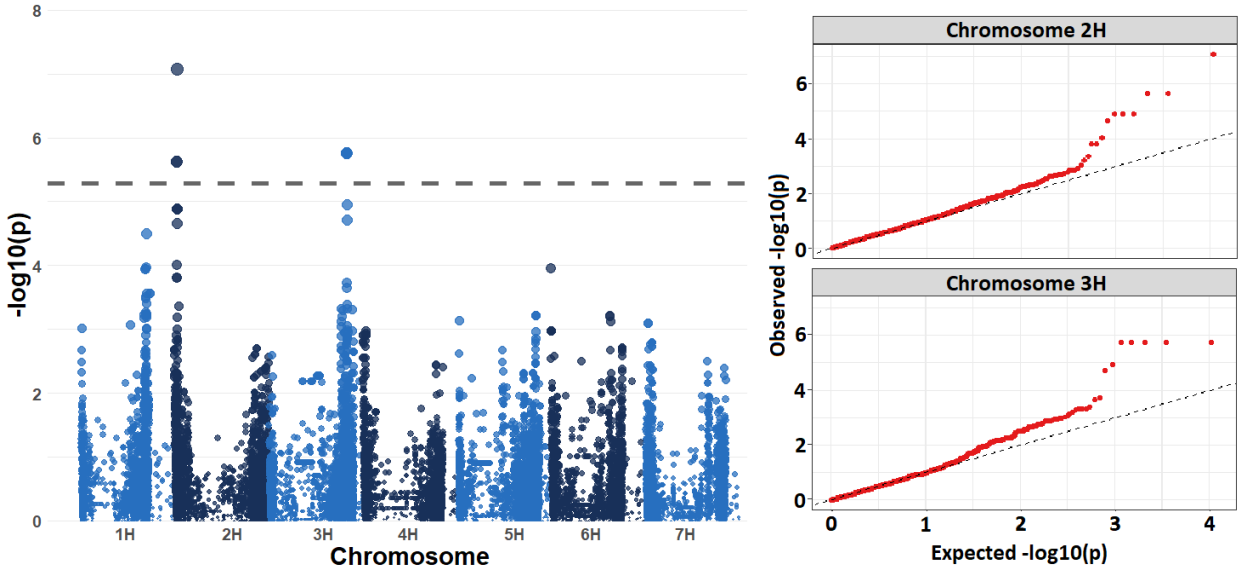

Figure 3.3. Manhattan plot and the associated qq-plots from the GWAS for threshability with an α=0.05 FWER threshold.

Marker JHI.Hv50k-2016-71750 accounted for 9.9% of the observed phenotypic variance using multiple regression with backwards elimination with the alternate allele having an effect of a 0.18 increase in a genotype's threshability. Marker JHI.Hv50k-2016-205614 accounted for 7.8%

of the observed phenotypic variance with the alternate allele having an effect of a 0.29 increase in a genotype's threshability.

Table 3.2. Marker, position, effect, frequency of the allele causing high threshability (Freq.), and percentage of the phenotypic variance explained (PVE) information for the two markers identified with significant associations to threshability.

Marker	Chromosome	Position (bp)	Position (cM)	Effect	Freq.	PVE
JHI.Hv50k-2016-71750	2H	23431465	15.37	0.18	0.48	9.9
JHI.Hv50k-2016-205614	3H	634927181	109.84	0.29	0.83	7.8

The allele conferring high threshability at marker JHI.Hv50k-2016-205614 is widespread in the assessed germplasm and was the most frequent allele in all subpopulations except for subpopulation six. The allele conferring high threshability at marker JHI.Hv50k-2016-71750 is primarily found in subpopulations one, two, and four. In total, 165 individuals from this population carry the high threshability allele at both loci with genotypes *PI 286261*, *PI 294734*, *VA16H-160*, *PI 190646*, *PI 190661*, *PI 190694*, and *PI 565714* showing consistently high threshability.

Genomic Prediction

Performing GP through gBLUP yielded a predictive ability of 0.843 for threshability when no covariates were used (Table 3.3). Inclusion of markers significantly associated with QTL for threshability did not improve the predictive ability in the combined GS analysis. Predictive ability was 0.814 and 0.807 when markers JHI.Hv50k-2016-71750, and JHI.Hv50k-2016-205614 were incorporated as fixed effects, respectively. Similarly, the use of grain test weight, plump grain percentage, and thin grain percentage as covariates in the GP model did not increase predictive ability (0.718, 0.798, and 0.801 respectively) in the Corvallis environments compared to a predictive ability of 0.801 when no additional covariates were used (Table 3.3).

Table 3.3. Model information, mean predictive ability (PA), standard deviation (SD) of PA, and mean prediction error variance (PEV) for a baseline gBLUP model and gBLUP models with either *in-silico* markers or grain traits as covariates.

Model	Covariate	PA	SD	PEV
All Environments----------				
1	None	0.843	0.006	0.102
2	JHI.Hv50k-2016-71750	0.814	0.006	0.086
3	JHI.Hv50k-2016-205614	0.807	0.007	0.093
Corvallis Environments----------				
4	None	0.801	0.007	0.118
5	Percent Thin	0.801	0.006	0.119
6	Percent Plump	0.798	0.006	0.118
7	Test Weight	0.718	0.009	0.041

Effects of Population Structure on Genomic Prediction

Subpopulation membership does have a significant effect on the threshability of a given genotype. Subpopulation one shows significantly higher threshability than any other subpopulation and subpopulation six shows significantly lower threshability than any other subpopulation (Table 3.4; Figure 3.4).

Table 3.4. Summary of the Fisher's LSD test comparing each of the six subpopulations with mean threshability of each subpopulation (THR), and the group membership of each subpopulation

Subpopulation	THR	Group
One	3.6	A
Two	3.1	B
Three	3.0	BC
Four	2.8	CD
Five	2.7	D
Six	2.2	E

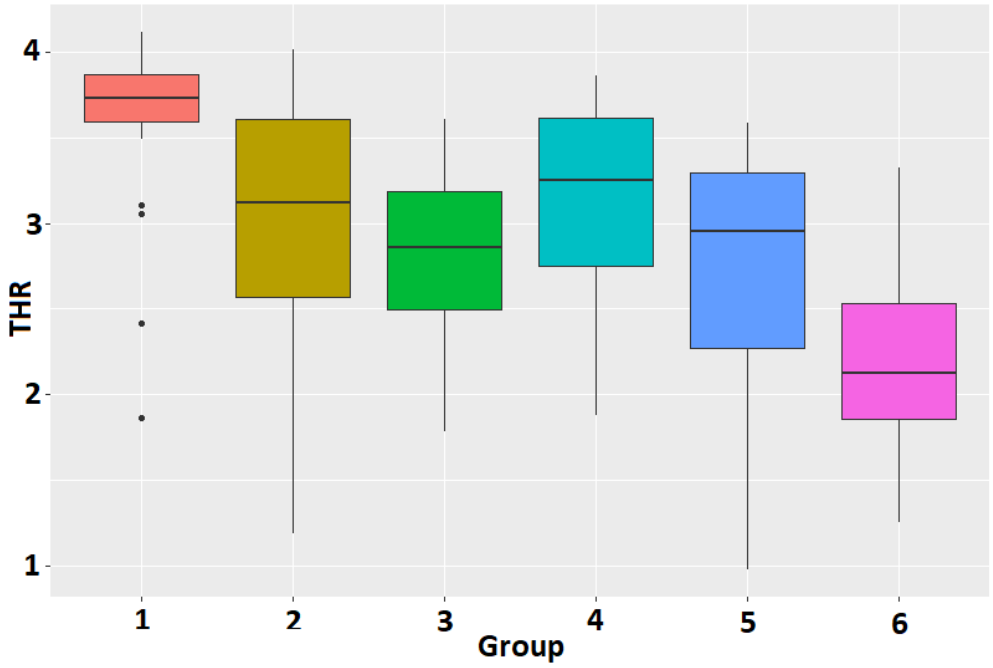

Figure 3.4. Histogram of observed threshability scores within each of the identified subpopulations.

Observed versus predicted threshability scatterplots by subpopulation are displayed in figure 3.5. Figure 3.5A shows the predicted versus observed threshability for one iteration of the seven-fold cross validation scheme color coded by subpopulation membership. Figure 3.5B shows the predicted versus observed threshability of subpopulation three in the leave one subpopulation out analysis.

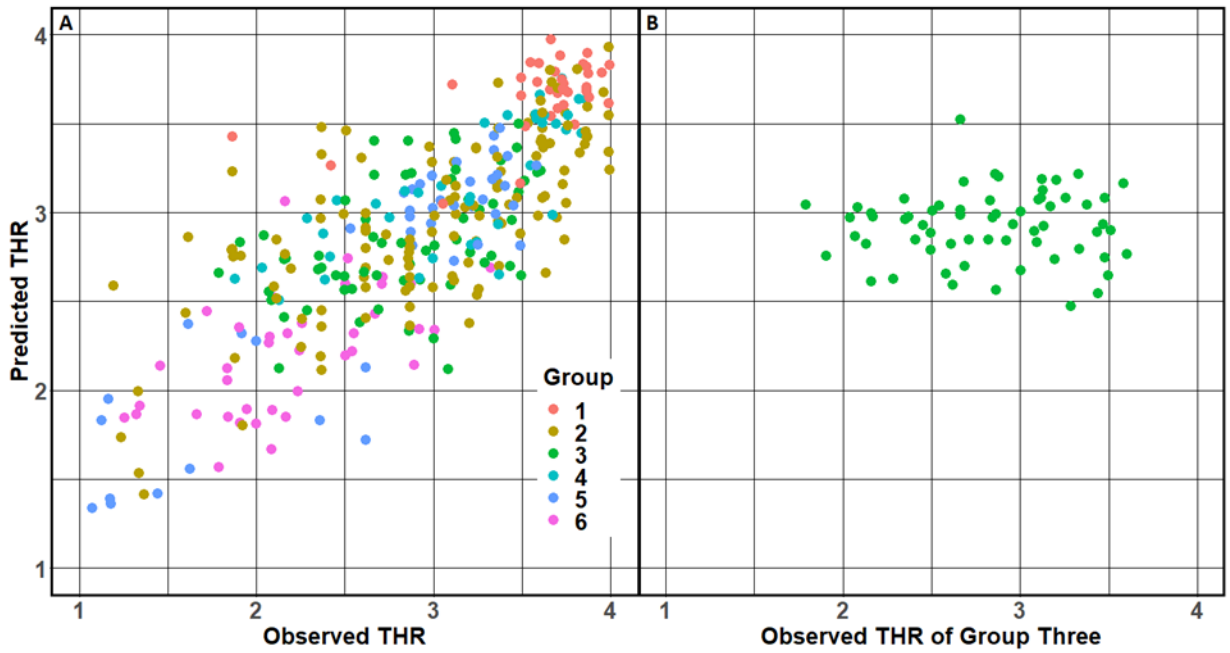

Figure 3.5. Scatterplot of observed threshability by predicted threshability resulting from A) one iteration of the gBLUP using all available subpopulations with seven-fold cross validation B) predicting only subpopulation three using the other five subpopulations.

Both predictive ability within subpopulation and predictive ability when an individual subpopulation was predicted using information from the other five were lower than the observed predictive ability when a randomly selected subset of individuals of equal size to the given subpopulation is used as the testing population (Table 3.5). Subpopulation three in particular shows an extreme decline in predictive ability when groups one, two, four, five, and six are used in making the predictions (Table 3.5).

Table 3.5. Percentage of the total population represented by each subpopulation (%), predictive ability within each subpopulation under the seven-fold cross validation scheme (PA_{within}), predictive ability when one subpopulation was predicted using information from the other five (PA_{loo}), and predictive ability when a randomly selected subset of individuals of equal size to the given subpopulation is predicted (PA_{random}).

Subpopulation	**%**	**PA_{within}**	**PA_{loo}**	**PA_{random}**
One	10.9	0.57	0.72	0.84
Two	36.5	0.72	0.67	0.83
Three	17.7	0.47	0.09	0.84
Four	10.6	0.77	0.70	0.84
Five	12.6	0.89	0.73	0.84
Six	10.9	0.58	0.57	0.84

DISCUSSION

Significant Marker-Trait Associations

Two significant marker trait associations were identified in our study, one on chromosome 3H at 634.9Mb (JHI-Hv50k-2016-205614), and one on chromosome 2H at 23.4 Mb (JHI-Hv50k-2016-71750; figure 3.2). Multiple grain size and shape QTL have previously been identified on chromosome 3H in covered barley, including one for grain width between 631.86 and 641.54 Mb and one for grain length between 604.52 and 606.01 Mb (Wang et al., 2019). While the marker significantly associated with threshability on 3H is outside the range of the previously identified QTL for grain length, it does fall into the interval of the grain width QTL. These characteristics are positively correlated with threshability with elongated grains showing reduced threshability (Ram and Singh, 1996). Previous QTL discovery studies have also identified QTL for hull thickness on the short arm of chromosome 2H in covered barley (Wang et al., 2015; Collins et al., 1999). Ram and Singh (1996) have reported that hull thickness plays a role in the ability of the grain to shed its hull with thinner hulls threshing freely. Grain length, width, and hull thickness were not directly measured in the present work, so additional research would be required to determine if QTL for these grain traits would have an impact on the observed threshability of a naked barkey genotype.

Given the high genetic component and heritability of threshability identified in this and other studies (Massman et al., 2021), the hope would be that variants with large effect could be identified. The alleles significantly associated with threshability identified in this study account for a combined 17.7% of the observed PVE in the population and a 12% increase in the

threshability of a given genotype. These are QTL of minor effect that account for little of the observed differences in threshability. If there are many loci that have an impact on threshability, these effects may even be overestimated due to the Beavis Effect where small population sizes and undetected QTL affect the observed effect of identified QTL (Beavis 1998). Related to the Beavis Effect is the "missing heritability" of QTL discovery projects. (Maher, 2008). Missing heritability is an artifact of using single markers or polymorphisms to characterize complex quantitative traits of small effects (Gymrek and Goren, 2021; Gibson, 2021; Maher, 2008). The threshability trait could be under the control of many loci, each with minor effects that would not be detectable in our study.

Potential of Marker Assisted Selection

Marker assisted selection based on large effect QTL has the potential to increase genetic gain in plant breeding programs. As marker technologies become more available and methods for QTL discovery become more advanced, this potential can be realized (Xu and Crouch, 2008; Collard et al., 2005). In this analysis, marker JHI.Hv50k-2016-71750 individually accounts for 9.9% of the observed phenotypic variance and has an effect of 0.18 on threshability. Additionally, marker JHI.Hv50k-2016-205614 accounts for 7.8% of the observed phenotypic variance with an effect of 0.29 on threshability. The larger effect but smaller PVE of marker JHI.Hv50k-2016-205614 relative to JHI.Hv50k-2016-71750 is likely due to the high frequency of this marker's high threshing allele. This marker has a large effect, but the allele conferring high threshability is already present in the majority of the population. Both of these QTL require validation to verify their association with threshability in a variety of genetic backgrounds (Langridge et al., 2001). If the QTL from the present study were to be validated, they could be used to select genotypes

pre-harvest and increase efficiency. They could also be used to pre-select germplasm for integration into a breeding program. The identification of the haplotype conferring high threshability could allow for this pre-selection even in covered germplasm, where threshability is not directly observable. Previous research shows that MAS is effective at increasing genetic gain compared to phenotypic selection alone when there are few QTL of major effect (Bernardo, 2020; Knapp, 1998). This method is less effective as the genetic architecture of a trait becomes more complex. Previous research on threshability, in barley and other cereals, indicated that the genetic architecture is controlled by relatively few major loci (Ram and Singh, 1996; Simons et al., 2006; Jantasuriyarat et al., 2004; Sood et al., 2009). Whether or not the QTL identified in this study constitute loci of major effect is a more complicated question. The QTL have a combined effect of 0.47 on threshability. This represents a roughly 12% change in the threshability of a given genotype. The cost of validation and deployment of QTL in a MAS breeding scheme for threshability may outweigh the benefits when compared to GS or phenotypic selection alone. If multiple minor-effect QTL are the underlying cause of threshability in naked barley, GS may be the more efficient option because GS leverages information from all available markers.

Genomic Prediction

Predictive ability was found to be very high (0.843) using the gBLUP model with no additional covariates included in the GP analysis (Table 3.3). Predictive ability was not increased using information from associated markers, test weight, percent plump grain, or percent thin grain as covariates in the model. Based on the high correlation of test weight with threshability, we would expect that its inclusion in GP models would increase predictive ability, but that was not

the case. Inclusion of test weight in the GP model did reduce the prediction error variance (PEV, Table 3.3), indicating a higher level of precision in making predictions. In this case, the lower predictive ability could be the result of naked genotypes with either higher or lower test weight than would have otherwise been expected based on their threshability score.

Predictive ability of GS is affected by several factors including heritability and marker density (Bernardo, 2020). Heritability for threshability in this study was very high (Table 3.1). Other studies have found threshability to be a highly heritable characteristic in barley (Ram and Singh, 1996; Massman et al., 2021). High heritability and relatively few QTL of major effects would both lead to higher prediction accuracies as were found in our work (Bernardo, 2020; Zhang et al., 2019). High marker coverage is an additional factor that increases genomic predictive ability (Lorenz et al., 2012; Lorenzana and Bernardo, 2009). The 32,208 SNPs used in this study provided excellent coverage of the genome and allowed for more accurate predictions.

Effects of Population Structure on Genomic Prediction

Given the significant effect of subpopulation membership on the threshability of a given genotype, population structure was investigated more in depth. Predictive ability within each subpopulation was less than the predictive ability across subpopulations (except subpopulation five) when the seven-fold cross validation scheme was used. Both the predicative ability within each subpopulation, and the predictive ability when genotypes of a single subpopulation were used as the testing population were lower than predictive abilities of randomly selected testing populations of equal size. This lowered predictive ability indicates there may be an overestimation of the true predictive ability in this study. The large drop in predictive ability

when subpopulation three was predicted using all other groups would indicate that subpopulation three in particular is genetically distinct from the other groups. The genotypes found in subpopulation three primarily originated from the Washington State University breeding program, which has focused on selection for a waxy starch type. Starch type was not measured in this panel and this trait represents a possible confounding factor. All genotypes in subpopulation three also have a two-row spike type. All other subpopulations contain either six-row or a mix of six and two-row germplasm. Historically, two and six-row barleys have been selected for different uses. Six-row barley, with a higher protein content on average, was primarily a feed and food grain, while two-row was primarily used for malt purposes (Miralles et al., 2021). The difference between the selection history of these grain types may cause the differences observed in this study. Based on the assessment of the effects of subpopulation membership in the present study, the use of a structured gBLUP model proposed by de los Campos and Perez (2013) would yield a more accurate estimation of the true predictive ability. This method includes group specific effects when making predictions, so the overestimation of predictive ability found in the present study would be avoided (de los Campos and Perez, 2013; Berro et al. 2019).

Potential of Genomic Prediction

While the heritability for threshability is high and phenotypic selection can reliably be used to improve the trait (Ram and Singh, 1996; Rossnagel, 2000), GP would allow for the assessment of more genotypes than could be efficiently phenotyped. Additionally, predictions of threshability in untested genotypes could be achieved using information from relatives and methodology similar to what was used in this study. Resource allocation is an important aspect

of plant breeding, with phenotyping being a major resource sink (Bernardo, 2008; Lado et al., 2018). Because threshability is measured post-harvest, phenotyping can lead to delays in planting the next generation, particularly when the goal is to plant multiple generations in quick succession like in a speed breeding context (Wanga et al., 2021; Bhatta et al., 2021; González-Barrios et al., 2021; Watson et al., 2018). Additionally, in field trials, threshability can be selected alongside agronomic traits using GP. Using these methods, and accounting for the effects of population structure, GP can be successfully used to increase gain from selection per unit of time compared to phenotypic selection alone (Bernardo, 2020). The prospects of implementing GP for selection of threshability moving forward are very good.

SUMMARY

This study used a set of 349 genotypes in nine environments to identify QTL relevant to threshability in organic naked barley and used them as covariates in GP to increase accuracy. A GWAS using the QK model and 32,208 SNP markers identified two loci with a combined effect of a 12% increase in the threshability of a genotype. Incorporation of these loci as fixed effects in the gBLUP model did not increase predictive ability; however, predictive ability was as high as 0.84 overall. The use of grain test weight, as well as plump/thin percentages as covariates in the GP models had no impact on predictive ability. Closer investigation of the underlying population structure indicated that the observed predictive ability could be overestimated and a structured gBLUP model may be more appropriate for making predictions. The identified QTL of small effect may not be ideal for use in MAS; however, with the high predictive ability observed in this study, GS would be useful in selection for threshability as long as population structure was accounted for. Genetic improvement of threshability in naked barley will increase its usefulness as a food crop and remove the need for additional processing steps to remove the hull.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Phenotypic data will be available on T3/Barley.

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CHAPTER 4. COMMUNICATING RESEARCH TO A GENERAL AUDIENCE

My objective in writing this portion of my dissertation is to describe my goals, methods, and results to a broader audience. Each person on this planet relies on agriculture and, by extension, plant breeding. It is therefore the duty of researchers to make an intentional effort in the reporting and communication of their work to the non-scientific public. It is my hope that by the end of this chapter, readers will have an understanding of plant breeding and how it relates to the improvement of naked barley. This chapter has been written with the support of the Wisconsin Initiative for Science Literacy.

Barley: An Overview

Barley is an ancient grain used in the feed, food, and malting industries. While it was first grown 10,000 years ago for food purposes, today we primarily see it in malting and animal feed products. A reason for this change is due to the presence of a husk, also known as the hull, that tightly adheres to the surface of the grain. People and animals cannot digest this hull and we must remove it before the grain can be used in food. This additional processing increases cost and removes a portion of the underlying bran that is full of nutrients. Compare barley to a crop like wheat where the hull falls off naturally after harvest, and it is easy to see why wheat is the more popular for food. Fortunately, there is a type of barley that has an easily removable hull like wheat. This is known as hulless, or "naked," barley. The ease with which the hull falls off the grain is known as threshability, and there is some difference in threshability ratings between genotypes of naked barley (a genotype is how we refer to a group of plants with an identical genetic makeup). Naked barley with high threshability does not need additional processing to remove the hull; however, it is not often grown in the United States. There are very few naked barley genotypes that are well suited to planting in the US. There are even fewer that are properly adapted for organic production. With the high use of barley in organic systems, organic naked barley would be beneficial for farmers and consumers alike. This then begs the question; how do we get good genotypes of naked barley that are suitable for planting in organic systems? The answer lies in the science of plant breeding.

Plant Breeding Basics

Plant breeding is a topic I find fascinating. It sits at the intersection of many different schools of science including plant science, genetics, and statistics. In general plant breeding is the systematic improvement of plant species by humans for use in a variety of contexts. The food we eat, some of the fibers we wear, and the plants we plant in our front yard are the result of careful, intentional selection by plant breeders conducted over thousands of years. Historically, improvement of plant species has been achieved by phenotypic selection, that is, selecting which plants to grow based on the observable expression of different traits. If I were phenotypically selecting for the yield of barley grain, I would develop a set of diverse barley genotypes (either through intermating genotypes I already have, or collecting new ones), weigh the grain each genotype produces, and select those that produced the most. I would then evaluate my selected genotypes in a variety of environments to choose which were the most productive and consistent. This has been the primary method for crop improvement since agriculture first began; however, the process from start to finish can take over a decade.

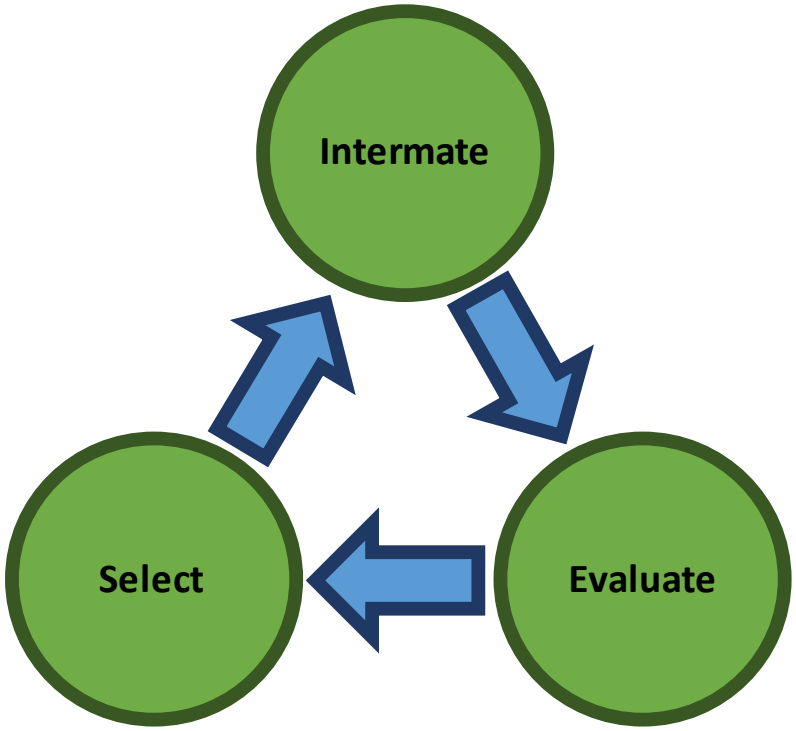

Figure 4.1 A general Outline of the breeding process.

A challenge that plant breeders face in their work is a phenomenon called genotype by environment interaction. Simply put, a genotype's performance will change depending on the environment it is grown in, and not every genotype responds in the same way to changes in the environment. This makes selecting the best plants a difficult job when we consider that these genotypes will be grown over a wide range of farms, environments, and management practices.

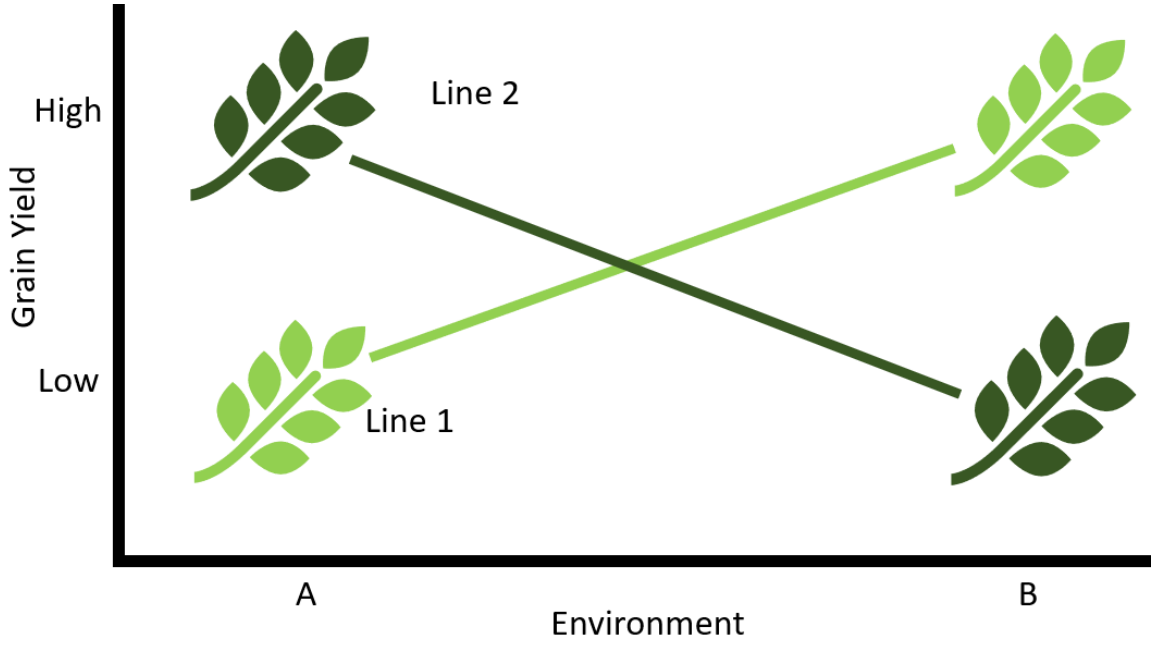

Figure 4.2 An example of genotype by environment interaction in grain yield where genotype 2 has the highest yield in environment A, but the rank is flipped in environment B.

Plant breeders today use a variety of statistical and graphical methods to make the best selections possible. These methods help to characterize, reduce, or even leverage genotype by environment interactions. Another challenge plant breeders face is that not all traits are easily observed. Some are expensive to measure, while others may be too labor intensive to efficiently test.

Modern Plant Breeding

Modern breeding methods include ways for plant breeders to supplement phenotypic selection. With the advent of genetic and molecular technologies in plant breeding, we can now

better understand and select for traits in plants. This is particularly useful for those traits that are hard to observe. With genetic markers, we can identify DNA sequences at specific locations on a plant's genome. A genome is all of the genetic information that makes up a plant. Areas of the genome that are associated with a particular complex trait are known as quantitative trait loci or "QTL." These bits of genetic code can have large impacts on the phenotype of a plant depending on their configuration. Once we identify QTL it opens the possibility of selecting these complex traits based on genotypic data, in addition to phenotypic data. We can also use all available markers, not just individual QTL, to predict an untested line's performance based on genotypic data. These predictions are like building what we think the plant will look like, marker by marker, like building a tower out of blocks. These methods make selecting for hard to observe traits easier. When used in combination with phenotypic selection, they have the potential to increase the capacity for breeders to improve the crop they are working on.

Implications for Naked Barley

While phenotypic selection alone could reasonably be done to improve naked barley for organic systems in the US, I believe that genomic methods have the potential to greatly increase our ability to make accurate and informed selections. My goals in this work were twofold. First, I wanted to characterize available naked barley genotypes and assess the genotype by environment interactions that act on them when planted across the northern US. This information helps us get to know the genotypes we were starting with and allows us to better understand how they perform. My second goal was to identify QTL for the trait of

threshability and determine if it is possible to select high threshability individuals by predicting the performance of untested genotypes.

Experimental Setup

This work was conducted across the northern United States. Researchers at Cornell in New York, the University of Wisconsin-Madison, University of Minnesota, and Oregon State University planted naked barley trials during the 2018 to 2020 growing seasons.

Figure 4.3 A map of the six experimental locations where trials were planted during the 2018, 2019, and 2020 growing seasons

Two experiments were conducted side by side at each of these locations in each year. The first, the spring regional trial, was a group of nineteen barley genotypes (eighteen were naked and one was hulled for comparison). These genotypes were being tested for overall performance and those that performed well could have been recommended to farmers or used as parents in future generations.

Figure 4.4 An example of the spring regional trial grown in Madison, Wisconsin. Each plot is a distinct genotype that was tested in all the experimental locations.

The second experiment, the diversity panel, was a set of 350 naked barley genotypes that had diverse origins and characteristics. There was marker data available for each of these genotypes that would be used later in the study. Markers are a useful tool in that when we know a specific DNA sequence that lies in a particular position on a plant's genome is associated with a trait, we can use the marker to select or compare plants.

Figure 4.5 An example of the diversity trial grown in Madison, Wisconsin. Each row is a distinct genotype that was tested in all the experimental locations.

The Analysis

The Spring Regional Trial

My goal with the spring regional trial was to assess how the different naked barley genotypes changed in performance across the range of environments in the northern US. I did this in three

steps. First, I estimated the genetic, environmental, and genotype by environmental variation present in the performance of this group of genotypes. This step gives an idea of the relative importance of genotype by environment interactions to each measured trait. Second, I created graphical representations of grain yield performance of each genotype across the testing area. This allowed us to see patterns in performance and locations where genotypes tended to be ranked similarly based on their performance. Third, I assessed how an individual genotype's performance changed from one location to another.

The Diversity Panel

I used the diversity panel (the 350 naked barley genotype panel) to better understand the basis of the grain threshability trait. There is not a large amount of information available on the genetic pathways that control threshability in naked barley, so this part of the work was particularly interesting. First, I performed a genome wide association study to identify QTL that were important to threshability. This analysis uses the marker data and identifies markers that are significantly associated with threshability. Second, I used all available marker data to predict the threshability of genotypes based only on their genotype. This method uses the threshability scores and genotype data from a set of naked barley lines and predicts the threshability of new, untested lines. Recent research indicates that including additional information in our prediction, such as correlated traits or QTL from the genome wide association study, will allow us to make even better predictions.

Findings and Discussion

The Spring Regional Trial

The results from the spring regional trial indicated that naked barley grain yield was highly impacted by the effects of genotype by environment interactions. This means that across all of our environments, no single genotype was the "best." The top yielding naked barley line in Wisconsin was not the top yielding line in Oregon. This is true of many crops and highlights the need for careful selection and a solid understanding of the target set of environments. Other traits measured in this study were much less affected by genotype by environment interactions. When a trait is primarily determined by genotype and not influenced much by genotype by environment interactions, the ranking of genotypes will remain relatively consistent across environments. In this case, the environment itself may still impact the trait, but not the relative performance of the genotypes. For example, in Oregon barley genotypes grew taller on average than in Wisconsin, but the tallest genotype in Oregon was still the tallest line in Wisconsin. Focusing on grain yield for the remainder of the analysis, we identified two groups of environments where genotype by environment interactions were minimized. One of the groups was made up of the Oregon locations. The other group was made up of the Minnesota, Wisconsin, and New York locations. Within each of these groups individually, lines were similarly ranked in terms of their performance, but the rankings changed between groups.

The Diversity Panel

My analysis of the diversity panel led to the discovery of two QTL for the threshability trait. Additionally, predicting the threshability of naked barley individuals based on their genotype was very accurate. This means we can reliably predict a naked barley genotype's threshability without ever seeing the grain. However, using additional information (i.e. correlated traits or QTL data) when making my predictions did not increase the accuracy of these predictions.

Moving forward from this research, we can implement these findings in a number of ways. Knowing how naked barley genotypes perform across a variety of environments, we can tailor our selections to better fit our target environments. Because we know genotypes planted in Oregon perform differently than the ones in Minnesota, Wisconsin, or New York, we can make recommendations and selections tailored to these sets of environments. This allows us to get the best possible plants to farmers growing naked barley. My research on QTL discovery and genomic prediction will allow us to implement genomic methods in our selections for threshability. Selection for threshability will be of great importance moving forward, and these methods will increase our ability to make genetic progress.

Education and Extension

My work with naked barley extends beyond scientific research. In 2018 and 2019, the Cereals Breeding and Quantitative Genetics Lab at the University of Wisconsin-Madison paired with classes from Kromrey Middle School to teach lessons on agriculture, science, and biology using naked barley as a model.

In 2018, a class of fifth grade students designed, executed, and analyzed their own experiments that involved manipulating the light availability of a barley plant's environment. My fellow researchers and I helped visiting students formulate the experiment, select their seeds for planting, and set up the experiment. For the experiment, a set of plants were grown in standard greenhouse conditions while a set were planted but covered to prevent light from reaching the plants. Researchers from the cereals breeding team recorded plant height data and maintained the plants on behalf of the 5th grade students. On a follow-up visit to campus, students reviewed plant height data from the experiment and drew conclusions about why the plants in the standard conditions outperformed the experimental conditions. They also found that plants in the dark conditions did grow to a certain extent, indicating that light was not required for germination, but was required for proper development of the plant.

Figure 4.6 Scientists from Kromrey Middle School setting up their experiment in the greenhouse

In 2019, the entire 6th grade from Kromrey Middle School (almost 250 students!) visited UW Madison to have a full day of scientific lessons and seminars, again using naked barley as a model. Throughout the day, students visited a variety of stations that taught different lessons on plant breeding and genetics. Researchers and I from the Cereals Breeding Lab led these stations. One station addressed crop science and agronomy, walking students through what plants are used for and what they need to grow. Another station taught some basic concepts in plant breeding including complex traits and selection. The final station walked students through some basic genetic questions. The students at this final station learned about the difference between a phenotype and a genotype, then used Punnett squares to determine the results of a

cross between two plants. I primarily worked at the plant breeding station. I was amazed how quickly students were able to tackle quantitative genetics.

Figure 4.7 6th grade students meet with researchers at UW-Madison to discuss science, plant breeding, and agriculture. The group pictured is working through the agronomy station.

The goal of both the 5th and 6th grade visits was not just to increase awareness for naked barley, but also to increase their awareness of scientific principles, and possibly inspire them to become scientists one day.

CHAPTER 5. SUMMARY AND FUTURE AVENUES OF RESEARCH

This chapter includes a brief summary of the work and implications presented in this dissertation. It also touches on areas of future research that can be pursued to further expand our understanding of breeding for naked barley and how to implement modern plant breeding methods.

This work has dealt with the characterization of a panel of spring planted naked barley as well as a more in-depth investigation of the genetic basis and selection for the threshability trait. From the characterization of advanced lines in the regional yield trial, two mega environments for naked barley grain yield were identified, one made up of environments in the Northwestern US and another composed of the Northeastern and North Central US experimental environments. The traits of plant height, heading date, test weight, and threshability were less affected by genotype by environment interactions. This information will allow the tailoring of selections and recommendations to specific regions. Additionally, characterized lines can be used as potential parents in the breeding process. Delving more deeply into the free threshing character of naked barley, two QTL relevant to threshability, one on chromosome 2H and one on chromosome 3H, were identified via GWAS while accounting for population structure and linkage. The combined effect of these alleles was a 12% change in the threshability of a line; however, neither allele could explain more than 10% of the phenotypic variance. While the implementation of these markers in a MAS scheme may not represent the best use of resources, it does provide insight into the quantitative nature of the threshability trait. Extending this genomic work, genomic predictions were performed to test the utility of genomic selection for increasing genetic gain of threshability. A predictive ability of .84 for threshability was obtained for this study using the gBLUP model. Additional covariates, including plump percentage, thin percentage, test weight, and marker data from the associated QTL were included in the genomic prediction model; however, they were not effective in further increasing accuracy. With the high predictive ability of threshability found in this study, genomic selection could potentially be used to increase gain from selection. The cost of

implementing GS should be balanced with the effectiveness of traditional phenotypic selection for threshability. The cost of genotyping and construction of a training population may outweigh the benefits of selecting for threshability alone. If genomic selection for threshability could be conducted alongside other, ongoing selection efforts, or using more cost-effective low-density marker panels, it may represent a better use of resources than phenotypic selection alone.

In terms of future directions of study resulting from this work, there are a number of options. The genotype by management impacts found in the regional trial presented in this work certainly merit further investigation. There are as many organic management strategies as there are organic farms. Even within locations of our study, there was a large level of variability depending on a variety of biotic and abiotic factors including planting date, rainfall, and weed pressure. A more direct comparison of organic versus conventional management could shed light on which of these factors creates the most variability in certified organic systems where naked barley is grown. Genomic selection for threshability has the highest potential in cases where rapid advancement of generations is desired, such as a speed breeding scheme. Implementing this methodology and determining the realized response to selection would provide a better idea of the overall utility of genomic selection for threshability. As mentioned previously, the cost of genotyping and performing genotypic selection is not inconsequential. In depth analyses into the use of low-density marker panels could provide one method or reducing this cost. Naked barley is a promising crop for uses in food, feed, and malt. This

combination of applied plant breeding and genomic methods has the ability to create new, productive varieties that will in turn add value to barley production.

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