Genome-Wide Association and Prediction of Grain and Semolina Quality Traits in Durum Wheat Breeding Populations

Abstract

Grain yield and semolina quality traits are essential selection criteria in durum wheat breeding. However, high phenotypic screening costs limit selection to relatively few breeding lines in late generations. This selection paradigm confers relatively low selection efficiency due to the advancement of undesirable lines into expensive yield trials for grain yield and quality trait testing. Marker-aided selection can enhance selection efficiency, especially for traits that are difficult or costly to phenotype. The aim of this study was to identify major quality trait quantitative trait loci (QTL) for marker-assisted selection (MAS) and to explore potential application of genomic selection (GS) in a durum wheat breeding program. In this study, genome-wide association mapping was conducted for five quality traits using 1184 lines from the North Dakota State University (NDSU) durum wheat breeding program. Several QTL associated with test weight, semolina color, and gluten strength were identified. Genomic selection models were developed and forward prediction accuracies of 0.27 to 0.66 were obtained for the five quality traits. Our results show the potential for grain and semolina quality traits to be selected more efficiently through MAS and GS with further refinement. Considerable opportunity exists to extend these techniques to other traits such as grain yield and agronomic characteristics, further improving breeding efficiency in durum cultivar development.

Core Ideas

- Genome-wide association and prediction for grain and semolina quality traits were performed using elite durum wheat breeding populations.
- Several QTL for test weight, semolina color, and gluten strength were detected.
- Forward prediction accuracies of 0.27–0.66 were obtained for the quality traits using genome-wide markers.
- Implementation of genomics-assisted breeding could enhance selection efficiency for grain and semolina quality traits in durum wheat breeding program.
over 35 million tons per year (International Grain Council, 2013). Over 14 million hectares of durum wheat are planted each year, primarily in Mediterranean basin countries and North America. Most commonly, durum wheat is milled into a granular product called semolina, which is utilized for pasta production. Suitable end-use quality including milling, pasta processing, cooking, and nutrition characteristics is required in durum wheat cultivars.

The primary process components of durum wheat grain milling are semolina, flour, bran, and germ. A high semolina extraction rate, defined as the percentage of grain milled into semolina, is preferred in the durum milling industry. Grain test weight, a measure of grain density, is positively correlated with semolina yield (Matsumo and Dexter, 1980). Compared with semolina extraction rate, test weight is easier to measure. Semolina quality affects various pasta qualities, including color, pasta firmness, and cooking loss. High semolina protein content provides more nutrients and also determines pasta-cooking quality such as firmness and cooking loss. Glutenins and gliadins are major storage proteins. Glutenins and gliadins are each separated into multiple groups of peptides encoded by multigene families (Troccoli et al., 2000). The composition of glutenins and gliadins contribute to gluten strength and pasta-cooking qualities in addition to total protein content (Troccoli et al., 2000). Consumers favor pasta exhibiting a bright yellow color conferred by lutein, a yellow carotenoid pigment found in durum endosperm and semolina. Carotenoids are also beneficial to human health as antioxidants that can reduce the risk of some chronic degenerative diseases (Abdel-Aal et al., 2008; Nishino et al., 2009). Some carotenoids have provided A activity, which provides protection against ocular diseases (Ribaya-Mercado and Blumberg, 2004).

Testing semolina quality traits is expensive and time consuming. For example, it takes 3 d to test 100 samples and costs $63.5 per sample at the NDSU durum wheat quality and pasta processing laboratory (unpublished data, Dr. Frank Manthey, 2017). For this reason, semolina quality selection is generally performed on a limited number of lines in late generations. For example, only approximately 200 F4, breeding lines from replicated-plot yield trials (replications pooled per breeding line within a location) in the NDSU durum wheat breeding program are tested for semolina quality traits. Deferring phenotypic selection for quality traits to late generations results in the advancement of numerous undesirable lines, consequently wasting money and resources. Utilization of molecular markers linked to genes could enable selection conducted on a larger number of lines prior to costly yield trials, and therefore enhance selection efficiency.

Numerous biparental QTL mapping studies have been conducted on quality traits in wheat. Major QTL for test weight (Huang et al., 2006; Patil et al., 2013; Sun et al., 2009; Zhang et al., 2008), gluten strength (Patil et al., 2009; Conti et al., 2011; Kumar et al., 2013), semolina color (Blanco et al., 2011; Patil et al., 2008; Pozniak et al., 2007; Zhang et al., 2008) and protein content (Conti et al., 2011; Joppa et al., 1997; Patil et al., 2009; Olmos et al., 2003; Uauy et al., 2006) have been detected on all chromosomes. However, few identified QTL have been utilized for MAS by breeding programs. Population specificity, environment specificity, and lack of polymorphism or diagnostic markers for the QTL detected from wide-cross mapping populations are major factors limiting MAS utilization (Bernardo, 2008; Holland, 2007; Wang et al., 2012). Genome-wide association studies (GWAS) for traits evaluated over multiple years and locations using locally adapted breeding populations could be more effective toward identifying population-specific QTL suitable for MAS directly in breeding populations (Pozniak et al., 2012; Begum et al., 2015). Advances in single nucleotide polymorphism (SNP) discovery and high-throughput genotyping have facilitated GWAS in plants, including wheat, a polyploid species with a large complex genome (Poland et al., 2012; Wang et al., 2014). Major QTL and candidate genes for agronomic and quality traits have been found in wheat breeding populations using GWAS (Begum et al., 2015; Bentley et al., 2014; Würschum et al., 2016). However, lack of major QTL for some complex traits in breeding populations may constrain MAS utilization in breeding programs. In GWAS, testing association between a single marker and phenotype is a common method used to identify QTL. Compared with single marker association, it was proposed that haplotype-based association analysis has several advantages, including increased power, ability to capture epistatic interactions, and reduced type I error rate (Hamblin and Jannink, 2011; Morris and Kaplan, 2002; Zhao et al., 2007). More loci or stable haplotype associations across environments were detected from haplotype-based association in empirical studies than SNP-based association analysis (Contreras-Soto et al., 2017; N’Diaye et al., 2017).

Unlike MAS, which focuses on a small number of markers linked to major genes, GS is based on predicted performance from genome-wide markers, which could potentially capture minor- to medium-effect QTL (Meuwissen et al., 2001). Many studies have documented the superiority of GS to MAS for predicting complex traits, and its potential to enhance genetic gain (Heffner et al., 2011; Meuwissen et al., 2001; Zhong et al., 2009).

The objectives of this study were to (i) understand the genetic architecture of grain and semolina quality traits in the NDSU durum breeding populations; (ii) identify major QTL and facilitate MAS; (iii) compare SNP- and haplotype-based GWAS; and (iv) explore potential application of GS in the NDSU durum breeding program.

Materials and Methods

Plant Materials and Genotyping

In total, 1220 F4, breeding lines from the NDSU durum wheat breeding program were genotyped using genotyping-by-sequencing (GBS). DNA was isolated with the Wizard Genomic DNA Purification Kit (A1125; Promega) per the manufacturer’s instructions and quantified with a Quant-iT PicoGreen dsDNA assay kit (P7589; Thermo.
and entered into EDA for further testing in the subsequent year (e.g., 60 lines from 2012 AYTs were selected and tested in 2013 URDNs). Therefore, some of the 1184 breeding lines were tested in multiple environments (year-location combinations) in this study up to the year 2016 (Supplementary Table S3). Majority of lines were tested in two environments (Supplementary Table S3). The experimental design was an augmented block design with four replications for AYTs and EDAs, and randomized complete block designs for URDNs. Four to six cultivars (Alkabo, Carpio, Divide, Joppa, Lebsock, Mountrail, Strongfield, and/or Tioga) were used as common checks in each trial.

Grain samples from four replications for each breeding line within an individual trial were pooled to test grain and semolina quality traits. The traits evaluated were test weight, semolina extraction rate, semolina protein content, semolina color (or semolina yellowness), and sedimentation volume. Test weight was measured using the AACC method 55-10.01 (AACC, 2000). Grain samples were then milled using the Quadrumat Junior (C.W. Brabender Instruments, Inc., South Hackensack, NJ) according to AACC method 26-50.01 (AACC, 2000). Using near infrared spectroscopy, semolina protein was measured with an Infratec 1241 grain analyzer (FOSS Analytical, Hoganas, Sweeden) and adjusted to a 14% moisture basis. Semolina color was quantified as CIE b-values using the Chroma Meter CR-410 (Konica Minolta, Ramsey, NJ). Gluten strength was determined using the microsedimentation test (Dick and Quick, 1983). Gluten strength was measured using the sedimentation volume method, which correlates highly (0.918) with mixogram scores and explains variation in cooking quality of pasta (Payne, 1987).

Phenotypic Data Analysis

The 1184 breeding lines were tested in 29 unbalanced historical trials across three locations in 5 yr (total of 15 location-year combinations). Up to three trials were tested in a year-location combination. The number of breeding lines per trial ranged from 8 to 352 (Supplementary Table S2). Two-stage analysis of phenotypic data was performed for the trials. In the first stage, best linear unbiased estimations (BLUEs) were estimated for all breeding lines within each individual trial using the PROC MIXED procedure in SAS v.9.3 (SAS Institute, 2011). Experimental design of AYTs and EDAs used in this study were augmented design. The model was

\[
y = \mu + g_i + b_j + \varepsilon_{ij}
\]

where \(y\) is the vector of unadjusted phenotypes, \(\mu\) is the overall mean, \(g_i\) is the fixed effect of the \(i\)th genotype, and \(b_j\) is the random effect of the \(j\)th block. For URDNs with randomized complete block design, observed phenotypic values were used in further analysis. To identify outlier trials with poor heritability, additive variance components (\(Va\)) and error variance components (\(Ve\)) were calculated using the relationship matrix with the \texttt{mixed.solve} function in the package \texttt{rrBLUP} (ridge regression best linear unbiased prediction, Endelman,
The estimated BLUEs or observed phenotypic values were used as phenotypic training data. Genotypic data was the 19,863 markers with missing values < 50%. Heritability ($h^2$), the proportion of variance due to additive genetic effects, was calculated as $Va/(Va + Ve)$ and was used to eliminate trials with a $h^2$ score < 0.1.

In the second analysis stage, we estimated BLUEs of breeding lines across the remaining trials using the PROC MIXED procedure in SAS v.9.3 (SAS Institute, 2011). The model was

$$y^* = \mu + g_i + t_j + e_{ij}$$  \hspace{1cm} (2)

where $y^*$ represents the estimated BLUEs of breeding lines within AYTs or EDAs calculated in the first stage or observed phenotypic values within URDNs, $\mu$ is the overall mean, $g_i$ is the fixed effect of the $i$th genotype, and $t_j$ is the random effect of the $j$th trial. The estimated BLUEs were further used in GWAS to identify major QTL. By considering genotype as random effect, best linear unbiased predictors (BLUPs) were estimated from the model [2] and used for genomic prediction analysis.

**GWAS for QTL Detection**

SNP-based GWAS was performed using TASSEL v. 5 (Bradbury et al., 2007). Based on the Scree plot (Supplementary Fig. S1), the first five PCs were chosen as covariates to capture population structure in association analysis. A centered kinship ($K$) matrix was calculated based on the 19,863 SNPs using TASSEL. Four statistical models were tested: (i) simple association analysis using general linear model (naïve model); (ii) general linear model including the first five PCs as covariates ($P$ model); (iii) linear mixed model including kinship matrix ($K$ model); and (iv) linear mixed model including population structure and kinship matrix ($PK$ model). The mean of the squared difference (MSD) between observed and expected $p$-values of all SNP markers was estimated for each model. A high MSD value means greater deviation of the observed $p$-values from expected $p$-values with a uniform distribution. The best model for each trait was determined as the model returning the smallest MSD value. Haplotype blocks were constructed from the 19,863 SNPs using the four-gamete method (Wang et al., 2002) implemented in the software Haploview (Barrett et al., 2005). Haplotypes with frequency > 5% were used for association analysis with $PK$ statistical model using TASSEL v.3 (Bradbury et al., 2007). The false discovery rate (FDR) was calculated from $p$-values using the R function $p.adjust$ (method = fdr; Benjamini and Hochberg, 1995). Significance of marker-trait association or haplotype-trait association is defined by FDR as a $q$-value < 0.01.

**Candidate Gene Analysis**

Candidate genes for the biosynthesis of glutenins and gliadins including Glu-1, Glu-3, Gli-1, and Gli-2 were identified from the literature (Anderson et al., 1984; Dong et al., 2016; Forde et al., 1985; Harberd et al., 1985). Orthologous genes for *Triticum aestivum* were retrieved from the UniGene Cluster database at NCBI by searching keywords “gliadin” and “glutenin.” In a recent study, a set of carotenoid biosynthetic and catabolic genes were identified and used for association analysis of grain yellow pigments in tetraploid wheat collections (Colasuonno et al., 2017). The same genes were used for the candidate gene analysis here in this study. Physical positions of candidate genes were identified by sequence alignment with BLASTn against the wheat reference genome. The QTL and significant markers identified in this study were screened against those candidate genes.

**Genomic Prediction Model Development and Validation**

Genomic prediction was evaluated with five statistical models including rrBLUP, Bayesian Lasso (BL), Bayesian ridge regression (BRR), reproducing kernel Hilbert space (RKHS), and genomic best linear unbiased prediction (GBLUP). Nonlinear models RKHS and GBLUP could capture nonadditive effects. The rrBLUP model was constructed using R package rrBLUP (Endelman, 2011). The other models were constructed using R package BGLR (Perez and de los Campos, 2014).

Prediction accuracies were validated using five-fold cross-validation and forward prediction methods. Cross-validation predictions were conducted on all breeding lines across all trials, in which 80% of individuals were randomly selected as the training population and the remaining 20% of individuals were used to validate the genomic prediction accuracy. Genomic prediction accuracy was estimated as the Pearson correlation ($r$) between genomic estimated breeding values (GEBVs) and BLUPs of phenotypic values. Random sampling training and validation sets were repeated 100 times, and the mean of correlations was defined as the genomic prediction accuracy. Forward prediction validations were conducted using lines from earlier years’ AYT lines as training populations to predict the following years’ AYT lines (e.g., 2012–2014 lines as training population to predict 2015 lines, 2012–2015 lines as training population to predict 2016 lines).

Using the 1184 lines, additive variance components ($Va$) and error variance component ($Ve$) were calculated using the relationship matrix $A$ with the mixed. solve function in the package rrBLUP (Endelman, 2011). Heritability ($h^2$) was calculated as $Va/(Va + Ve)$. Relative genetic gain of GS compared with phenotypic selection for each quality trait was estimated as described by Battenfield et al. (2016):

$$\frac{CR}{R} = \frac{i_x r}{i_x h^2}$$  \hspace{1cm} (3)

where $CR$ is the response to GS, $R$ is the response to phenotypic selection, $i_x$ is the selection intensity for phenotypic selection, $i_y$ is the selection intensity for GS, and $r$ is the prediction accuracy of the GS model estimated as the Pearson correlation between GEBVs and BLUPs of phenotypic values.
Results

Marker Genotyping, Linkage Disequilibrium, and Population Structure
In total, 1184 breeding lines from the NDSU durum wheat breeding program were genotyped using GBS at 96-plex. The 1184 lines had an average of 1.8 million reads per line, ranging from 0.6 to 4.9 million reads. A total of 19,863 SNP markers with a minor allele frequency > 5% and a call rate > 50% were obtained; 1699 of which had a call rate > 80%. A PC analysis was performed with the 1699 SNPs. The first five PCs explained 9.9, 7.2, 6.1, 5.8, and 5.1% of the total variation, respectively. Scatter plots of PC1 against PC2 for the 1184 lines were made to evaluate population structure. There was no clear population structure, and breeding lines from different years’ AYT’s were intermixed (Supplementary Fig. S2). We measured LD as $r^2$ between a pair of markers based on marker data sets having missing values for <50% of the genotypes. Mean of LD decayed to 0.37 between markers, with distance < 1 Mb and 0.25 with distance < 5 Mb (Supplementary Fig. S3). Given Subgenome A and B size of ~12 Gb (Mayer et al., 2014; Chapman et al., 2015), over 37% of genetic variation could be captured by the 19,863 SNPs in this study.

Phenotypic Data
Heritability was estimated for each trait at each of the 29 trials (data not shown). In total, three trials for test weight, one trial for sedimentation volume, two trials for semolina extraction, one trial for semolina protein content, and four trials for semolina color, respectively, were made to evaluate population structure. There was no clear population structure, and breeding lines from different years’ AYT’s were intermixed (Supplementary Fig. S2). We measured LD as $r^2$ between a pair of markers based on marker data sets having missing values for <50% of the genotypes. Mean of LD decayed to 0.37 between markers, with distance < 1 Mb and 0.25 with distance < 5 Mb (Supplementary Fig. S3). Given Subgenome A and B size of ~12 Gb (Mayer et al., 2014; Chapman et al., 2015), over 37% of genetic variation could be captured by the 19,863 SNPs in this study.

SNP-Based GWAS for QTL Identification
GWAS was conducted for each of the five traits with four statistical models: naïve, P, K, and PK models. A PC analysis was performed using the 1699 markers with missing values < 20%. Based on the Scree plot, the first five PCs were chosen to incorporate in the models as covariates to capture population structure. The best model was determined based on MSD values for each trait. For all traits, the K and PK models were better than naïve and P models (data not shown). The PK model showed slightly smaller MSD value for semolina protein content, while no MSD difference between the K and PK models was found for any of the other four traits. Significant SNPs identified from the PK model are reported here. Significant marker-trait association was determined by FDR as a q-value smaller than 0.01. SNPs significantly associated with test weight, sedimentation volume, and semolina color were identified, but no SNPs were significantly associated with semolina extraction rate or semolina protein content. The Manhattan plots for test weight, sedimentation volume, and semolina color are shown in Fig. 1. Supplementary Table S4 lists the significant SNPs identified for each trait.

In total, 32 SNPs significantly associated with test weight were identified on chromosomes 1AL, 1BL, and 5AS (Fig. 1 and Supplementary Table S4). The most significant markers (SIB_289976153 and SIB_289976156) within the QTL on chromosome 1BL explained only 3.2% of the total phenotypic variation (Supplementary Table S4). The other two QTL explained 1.9% and 2.2% of the total variation (Supplementary Table S4). For semolina color, there were 96 SNPs with significant association detected on chromosomes 1BS, 2BS, 5BL, 7AL, and 7BL (Fig. 1). The most significant markers within the five QTL explained 1.6, 1.6, 2.5, 2.6, and 4.0% of the total variation, respectively (Supplementary Table S4). The most significant markers from the QTL on chromosome 5BL were close to candidate gene ABA2 (Supplementary Table S4 and S5), and the QTL on chromosome 7AL was near candidate gene AA03 (Supplementary Table S4 and S5). In total 192 SNPs for sedimentation volume were identified on chromosomes 1AS, 1BS, 2AL, 2BL, 3AL, and 4BL (Fig. 1). The most significant markers from each of the six QTL explained 5.0, 4.1, 2.1, 2.1, 1.6, and 3.6% of the total variation, respectively (Supplementary Table S4). Of the six QTL, multiple peaks

<table>
<thead>
<tr>
<th>Trait†</th>
<th>Mean</th>
<th>Range</th>
<th>Heritability ($h^2$)</th>
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</thead>
<tbody>
<tr>
<td>TW, kg/L</td>
<td>47.8</td>
<td>45.1 – 50.3</td>
<td>0.67</td>
</tr>
<tr>
<td>SedV, mm</td>
<td>59.3</td>
<td>30.9 – 87.0</td>
<td>0.57</td>
</tr>
<tr>
<td>SE, %</td>
<td>59.1</td>
<td>51.4 – 66.6</td>
<td>0.54</td>
</tr>
<tr>
<td>SP, %</td>
<td>12.9</td>
<td>11.1 – 15.3</td>
<td>0.46</td>
</tr>
<tr>
<td>SC, score</td>
<td>9.5</td>
<td>6.6 – 11.8</td>
<td>0.61</td>
</tr>
</tbody>
</table>

† TW, test weight; SedV, sedimentation volume; SE, semolina extract rate; SP, semolina protein content; SC, semolina color.

Table 2. Pearson correlation coefficients ($r$) for the five grain and semolina quality traits.

<table>
<thead>
<tr>
<th>Trait†</th>
<th>SedV</th>
<th>SE</th>
<th>SP</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TW</td>
<td>0.07</td>
<td>0.31</td>
<td>$-0.24$</td>
<td>$-0.04$</td>
</tr>
<tr>
<td>SedV</td>
<td>0.11</td>
<td>0.09</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>$-0.25$</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP</td>
<td>$-0.02$</td>
<td></td>
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</table>

† TW, test weight; SedV, sedimentation volume; SE, semolina extract rate; SP, semolina protein content; SC, semolina color.

Table 1. Summary information for phenotypic data of the five quality traits. Heritability, means, and ranges of estimated BLUEs of the 1184 lines across all trials. Heritability of the traits was estimated using the R package rrBLUP.
of significant SNP markers were found within the QTL regions on chromosomes 1AS and 1BS (Supplementary Table S4). This suggested that multiple genes could exist within each of the two QTL. Candidate gene analysis suggested that the two QTL were located in homeologous regions of the A and B subgenome that include the Glu-3 and Gli-1 gene families (Supplementary Tables S4 and S5).

Fig. 1. Manhattan plots for test weight, semolina color, and sedimentation volume.
Haplotype-Based GWAS for QTL Identification

We grouped the SNPs into haplotype blocks using four-gamete method and a total of 3568 haplotype blocks were obtained (Supplementary Table S6). The haplotype blocks contain 2 to 70 SNPs (Supplementary Table S6), with the number of haplotype alleles ranging from two to eight. Haplotype-based association analysis identified nine haplotype blocks significantly associated with semolina color (Supplementary Table S7). Of the nine significant haplotype blocks, 5B.Block_88, 6B.Block_68, and 7B.Block_82 contained markers that were not identified in SNP-based association analysis (Supplementary Table S4 and S7). The 96 significant semolina color SNPs identified from SNP-based analysis were grouped into 22 haplotype blocks (Supplementary Table S4). Of those 22 haplotype blocks, 16 blocks on chromosomes 1BS, 2BS, and 7AL were not detected from haplotype-based association analysis. For sedimentation volume, eight haplotype blocks were identified from haplotype-based association analysis and SNPs in block 1B.Block_6 were not identified as significant associations via SNP-based association analysis (Supplementary Table S4 and S7). Alternatively, the 192 significant sedimentation volume SNPs identified from SNP-based association analysis were grouped into 20 haplotype blocks, of which 13 blocks were not detected from haplotype-based association analysis (Supplementary Tables S4 and S7).

Genomic Prediction

Genomic prediction accuracies for the five traits were evaluated with rrBLUP method. Prediction accuracies using five-fold cross validation were 0.55 to 0.69 for the five traits (Table 3). The highest prediction accuracy was found for sedimentation volume and semolina color and the lowest prediction accuracy observed for semolina protein content (Table 3). To evaluate forward prediction accuracy, we treated lines from 2015 or 2016 AYTs as the testing population, and lines from previous years’ AYTs as the training population. In the forward prediction method, five statistical models (rrBLUP, BL, BRR, RKHS, and GBLUP) were evaluated. The five models conferred similar prediction accuracies (Supplementary Table S8). Forward prediction accuracies generally increased as training population size increased (Table 3). The highest forward prediction accuracy approached 0.45 for test weight, 0.66 for sedimentation volume, 0.53 for semolina extraction rate, 0.44 for semolina protein content, and 0.51 for semolina color (Table 3). For test weight, forward prediction accuracy decreased when 2016 AYT was used as testing population compared to 2015 AYT as testing population (Table 3). A total of 170 lines from 2016 AYT were tested at two locations, Minot and Williston (Supplementary Tables S2 and S3). The estimated $h^2$ of TW was 0.48. Of the 173 lines from 2015 AYT, 50 were tested at over four environments across three locations in 2 yr 2015 and 2016 (Supplementary Tables S2 and S3). The estimated $h^2$ was 0.60. Lower quality of the phenotypic data for 2016 AYT might partially explain the observed lower prediction accuracy.

Currently in the NDSU durum wheat breeding program, the first test of grain and semolina quality traits is conducted in AYT on an average of ~200 lines. From the AYT, ~60 lines are selected for further testing in multiple years and locations. In the current phenotypic selection strategy for quality traits, the selected proportion is 30% and selection intensity ($i_X$) is 1.14. Comparatively, GS could be conducted on 3000 F$_5$ lines of each breeding cycle, for example, conferring 300 lines in preselection into subsequent yield trial, whereby the selected proportion from GS is 10% and selection intensity ($i_Y$) is 1.76. Given the selection intensities and $h^2$ estimated for each trait, the relative genetic gain of GS compared with phenotypic selection was calculated using the forward prediction accuracy with 2016 AYT lines as testing population. GS showed a greater response to selection than phenotypic selection for all traits except for test weight (Table 4). Increased gain ranged from 7% for semolina extraction rate to 78% for sedimentation volume (Table 4).

Discussion

Major QTL Identified for Grain and Semolina Quality Traits

In durum and bread wheat, previous biparental QTL mapping studies found QTL involved in test weight on every chromosomes (Huang et al., 2006; Sun et al., 2009; Zhang et al., 2008). Recently, GWAS were commonly used for QTL discovery in wheat diverse collections or breeding populations. A major test weight QTL was found on chromosome 6B in a soft wheat breeding population of 207 elite

Table 3. Genomic prediction accuracies using rrBLUP for grain and semolina traits validated with cross-validation and forward prediction methods.

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<tbody>
<tr>
<td>Size</td>
<td>352</td>
<td>552</td>
<td>841</td>
<td>1041</td>
<td>1184</td>
</tr>
<tr>
<td>Cross-validation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TW†</td>
<td>0.73</td>
<td>0.70</td>
<td>0.65</td>
<td>0.64</td>
<td>0.63</td>
</tr>
<tr>
<td>SedV</td>
<td>0.73</td>
<td>0.78</td>
<td>0.70</td>
<td>0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>SE</td>
<td>0.51</td>
<td>0.55</td>
<td>0.61</td>
<td>0.62</td>
<td>0.61</td>
</tr>
<tr>
<td>SP</td>
<td>0.56</td>
<td>0.55</td>
<td>0.55</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>SC</td>
<td>0.74</td>
<td>0.69</td>
<td>0.71</td>
<td>0.69</td>
<td>0.69</td>
</tr>
</tbody>
</table>

2015 (173 lines)  
| TW       | 0.30 | 0.33     | 0.45       |
| SedV     | 0.44 | 0.56     | 0.56       |
| SE       | 0.36 | 0.50     | 0.53       |
| SP       | 0.26 | 0.21     | 0.32       |
| SC       | 0.33 | 0.46     | 0.51       |

2016 (170 lines)  
| TW       | -0.01| 0.02     | 0.13       | 0.27      |
| SedV     | 0.64 | 0.71     | 0.69       | 0.66      |
| SE       | 0.38 | 0.38     | 0.34       | 0.38      |
| SP       | 0.32 | 0.18     | 0.33       | 0.44      |
| SC       | 0.36 | 0.42     | 0.39       | 0.46      |

† TW, test weight; SedV, sedimentation volume; SE, semolina extract rate; SP, semolina protein content; SC, semolina color.
lines using GWAS, which explained 34% of the total variation (Reif et al., 2011). In this study, we performed GWAS using 1184 elite durum lines from the NDSU breeding program and found one major QTL related to test weight on chromosome 1BL. However, the QTL only explained about 3.4% of the total variation. The larger mapping population used in this study could provide higher power to detect QTL with relatively small effects and also reduce overestimation due to small population size.

Semolina color mainly results from carotenoid pigment content in grain. Linkage and association mapping studies have found numerous QTL on all chromosomes for carotenoid pigment content in durum wheat grain (Ficco et al., 2014). Among them, large-effect QTL on the long arms of the homeologous chromosome 7A (Patil et al., 2008; Zhang et al., 2008; Blanco et al., 2011) and 7B (Elouafi et al., 2001; Kuchel et al., 2006; Pozniak et al., 2007; Zhang et al., 2008) were commonly found in mapping studies. Carotenoid biosynthesis has been well elucidated in model species like Arabidopsis thaliana and rice (Oryza sativa L.; Moise et al., 2014). The first step of the carotenoid pathway is the formation of phytoene from geranylgeranyl diphosphate, catalyzed by phytoene synthase (PSY). Co-localization analysis demonstrated that PSY-1 was the candidate gene for the carotenoid pigment QTL identified on chromosome 7 (Pozniak et al., 2007; He et al., 2008). Phytoene is the precursor to several physiological reactions leading to various secondary metabolites, including carotenoids. Those reactions are mediated by numerous enzymes, including carotene desaturase (PDS) and zetacarotene desaturase (ZDS), lycopene ε-cyclase (LYCE), lycopene β-cyclase (LYCB), carotenoid β–hydroxylase (BCH), and abscisic aldehyde oxidase (AAO3; Colasuonno et al., 2017). Candidate gene regression analysis using diverse tetraploid wheat accessions including durum wheat found that 10 of the 19 genes in carotenoid biosynthesis pathway were significantly associated with carotenoid pigment content (Colasuonno et al., 2017). In this study, we found 96 SNPs significantly associated with semolina color, with the most significant SNPs near candidate genes ABA2 (chromosome 5BL) and AAO3 (chromosome 7AL). Other color-associated genes either have no polymorphism or have been fixed in the NDSU breeding populations through previous phenotypic selection. Regression analysis for markers designed for those candidate genes is needed to elucidate their functional relationship with semolina color.

High gluten strength is a desirable grain quality that results in pasta with better firmness and stability after cooking (AbuHammad et al., 2012; Payne, 1987). Gluten strength is a function of the quality and quantity of gluten proteins, including glutenins and gliadins. Glutenins are classified into high-molecular-weight (HMW) subunits and low-molecular-weight (LMW) glutenin subunits based on differential mobility in sodium dodecyl sulfate polyacrylamide gel electrophoresis. All glutenin subunits are bound together in glutenin polymers. Genes encoding HMW glutenin subunits (Glu-1) and LMW glutenin subunits (Glu-3) have been well studied and reside on long and short arms of chromosome 1, respectively (D’Ovidio and Masci, 2004). Most Glu-1 loci in diverse wheat accessions encode only two functional HWM glutenin subunits. The simple protein pattern makes identification and selection of the favorable allele much easier and is widely adopted worldwide by wheat breeding programs (Payne, 1987; D’Ovidio and Masci, 2004). Compared with HMW glutenin subunits, LMW glutenin subunits are more abundant and encoded by a larger multigene family (D’Ovidio and Masci, 2004). Gliadins are heterogeneous monomeric proteins and are encoded by two large gene families: Gli-1 on chromosome 1 and Glu-2 on chromosome 6 (Troccoli et al., 2000). Moreover, studies have shown that Glu-3 is tightly linked with Gli-1 (Troccoli et al., 2000). Recently, association analysis in European winter wheat (Triticum aestivum L.) breeding populations found that Glu-B1 and Gli-B1 have significant effect on gluten strength measured as sedimentation volume and explained 24.6 and 19.5% of the total phenotypic variation, respectively (Würschum et al., 2016). In this study, we found 95 SNPs on chromosome 1AS and 84 SNPs on chromosome 1BS significantly associated with sedimentation volume. In addition, multiple peaks of significant SNPs and haplotype blocks were found within each of the QTL (Supplementary Tables S4 and S7). Clusters of Glu-3 and Gli-1 family genes encoding different groups of LMW glutenins and gliadins were interspersed in this region in the diploid grass Aegilops tauschii, the D-genome donor of bread wheat (Dong et al., 2016). Candidate gene analysis suggests that multiple copies of both Glu-3 and Gli-1 genes are located in these QTL regions (Supplementary Tables S4 and S5). Taken together, multiple Glu-3 and Gli-1 genes in both the A and B subgenomes likely contribute to the sedimentation volume variation in the NDSU durum breeding populations. In addition, multiple disease resistance genes, including nine genes from the nucleotide-binding domain leucine-rich repeat protein family and 16 genes from the receptor-like kinase family, were also found in the same region (Dong et al., 2016). Further studies are needed to elucidate causal

Table 4. Relative genetic gain of GS compared with phenotypic selection. In phenotypic selection, selected proportion is 30% and selection intensity (iX) is 1.14. In GS, selected proportion is 10% and selection intensity (iY) is 1.76.

<table>
<thead>
<tr>
<th>Trait†</th>
<th>r‡</th>
<th>Heritability (h²)</th>
<th>CR/R§</th>
</tr>
</thead>
<tbody>
<tr>
<td>TW</td>
<td>0.27</td>
<td>0.67</td>
<td>0.63</td>
</tr>
<tr>
<td>SedV</td>
<td>0.66</td>
<td>0.57</td>
<td>1.78</td>
</tr>
<tr>
<td>SE</td>
<td>0.38</td>
<td>0.54</td>
<td>1.07</td>
</tr>
<tr>
<td>SP</td>
<td>0.44</td>
<td>0.46</td>
<td>1.48</td>
</tr>
<tr>
<td>SC</td>
<td>0.46</td>
<td>0.61</td>
<td>1.17</td>
</tr>
</tbody>
</table>

† TW, test weight; SedV, sedimentation volume; SE, semolina extract rate; SP, semolina protein content; SC, semolina color.
‡ r is the forward prediction accuracy using 2012–2015 advanced yield trial (AYT) lines as training population and 2016 AYT lines as testing population.
§ CR/R is the relative genetic gain of GS compared with phenotypic selection.
SNPs, haplotypes, and/or potential contributions of structural variation. This information will be crucial for developing diagnostic markers to facilitate MAS for gluten strength and disease resistance in durum wheat.

Grain and semolina quality traits have been major selection targets in the NDSU durum wheat breeding program. Phenotypic selection imposes primary selection for large-effect QTL. Major QTL likely are fixed in the breeding populations following many cycles of phenotypic selection. However, we found that there were still QTL for the quality traits segregating in the current NDSU durum wheat breeding populations. Several reasons might explain this observation. First, it could be due to negative correlations between important traits. For example, the gluten strength QTL identified on chromosomes 1AS and 1BS contain multiple disease resistance genes. Repulsion linkage between genes for gluten and disease resistance might have forced breeders to balance those traits, leading to segregation of the QTL. Second, target traits like grain yield and protein content have required more focus by breeders. High yielding lines with poor performance for traits like semolina color could have been selected as parents, resulting in variation for major QTL of those other traits. Third, owing to the large mapping population, QTL with intermediate effects could be detected. For example, most significant QTL or SNPs identified in this study explained no more than 6% of the total variation. Phenotypic selection to fix such QTL might be difficult, as only a couple hundred lines were evaluated.

**Comparison of SNP- and Haplotype-Based Association Analysis**

Previous simulation and empirical studies indicated that haplotype-based association increased power and accuracy of QTL detection (Contreras-Soto et al., 2017; Hamblin and Jannink, 2011; N’Diaye et al., 2017). However, our association analysis results of end-use quality traits in durum breeding population are consistent with findings from other studies (Lorenz et al., 2010; Zhao et al., 2007), where no advantage of haplotype-based association was observed. A simulation study indicated that increased power from haplotype-based association analysis were limited under certain conditions such low marker density, ascertained markers, etc. (Hamblin and Jannink, 2011). Genetic architecture of QTL also plays a critical role on the increased power of haplotype-based association. For example, multiallelic QTL have mismatched allele frequencies with biallelic SNPs, while haplotype alleles are in stronger LD with QTL and provide more accurate association analysis. Properties of QTL are varied across traits and populations. Performing both single marker association and haplotype-based association analysis may capture more QTL (Hamblin and Jannink, 2011).

**Genomic Prediction of the Grain and Semolina Quality Traits**

Predicting untested genotypes from new breeding cycles in untested years is important for the implementation of GS in breeding programs. GS could shorten the breeding cycle and increase selection intensity, thereby enhancing genetic gain per unit time and cost. For grain and semolina quality traits, preselection based on predicted breeding values allows the elimination of poor lines prior to costly replicated-plot yield trials. With this in mind, cross-validation overestimates prediction ability of the GS model; full-sib lines were assigned to a training population as well as to the testing population. This leads to a closer relationship between the training and testing population in cross-validations compared with that between the training and testing population of new breeding cycles. The relationship between training and testing population has an effect on estimated prediction accuracy, with a closer relationship resulting in higher prediction accuracy (Habier et al., 2013; Crossa et al., 2014). Forward prediction reflects the predictive ability of a GS model toward predicting lines for new breeding cycles. In this study, forward prediction accuracies of 0.27 to 0.66 were obtained for the grain and semolina quality traits of durum wheat. For semolina protein content, forward prediction accuracy reached as high as 0.44. Similar forward prediction accuracies of 0.3 to 0.6 were also observed by others for grain yield and end-use quality traits in bread wheat breeding populations using GS models constructed with historical data and large training populations (Battenfield et al., 2016; He et al., 2016; Michel et al., 2016). Given low to medium $h^2$ generally observed for grain yield and some quality traits like protein content, GS with such prediction accuracy could enhance genetic gain compared with phenotypic selection (Battenfield et al., 2016; He et al., 2016; Michel et al., 2016). In this study, genetic gain from GS for the quality traits was estimated to be between 1.07 to 1.78 times that of phenotypic selection in durum wheat. In light of high phenotypic testing costs for grain yield and end-use quality traits, utilization of GS in preselection could reduce the number of poor lines advanced to yield trials for grain yield and/or end-use quality evaluations, therefore allowing more efficient genetic selection and improvement for those traits (Guzman et al., 2016).

Improvement in forward prediction accuracy as training population size increases has been observed for end-use quality traits in spring wheat (Battenfield et al., 2016; He et al., 2016). In dairy cattle, implementation of GS leads to better prediction ability as the model is updated with a larger training population and increased genetic gain for milk production, fertility, longevity, and other traits (Meuwissen et al., 2016). We also found that forward prediction accuracy increased as the training population size increased for quality traits in durum wheat. By implementing GS in a durum wheat breeding program, one could expect higher prediction ability in the updated GS model as the training population increases.

**Application of MAS and GS to Breeding**

Selection based on pedigree has been the breeding methodology utilized in the NDSU durum wheat breeding
program to date. Currently, ~3,000 F3 lines are single-row tested for agronomic traits like plant height and days to heading in each breeding cycle. About 1500 lines are selected for grain yield testing in preliminary yield trial (PYT, F6 generation) with four-row plots and two replications per line. Then, ~200 lines are selected for grain yield and quality trait testing in AYT (F7 generation), when quality traits are tested for the first time. The identified QTL and SNPs for test weight, semolina color, and sedimentation volume from this study could be directly used for MAS in the NDSU durum wheat breeding program. This will allow breeders to select the most suitable parents to make crosses and therefore quickly fix the favorable alleles or increase their frequency. When high yield parents with poor alleles for the identified quality trait QTL are included in crossing blocks, MAS in early generations (e.g., F3 or F4) will allow breeders to eliminate lines with poor grain quality.

The enhanced genetic gain of GS relative to phenotypic selection for grain and semolina quality traits suggest that initiation of GS is practical in the NDSU durum wheat breeding program. GS for the quality traits could be applied on the F4 generation prior to PYT. Further, phenotypic selection on grain yield combined with GS for quality traits could be applied on F4 lines from PYT. This would allow a breeder to select promising lines for inclusion in the preliminary yield trials including PYT and AYT, therefore increasing selection efficiency for both grain yield and quality traits. Increased genetic gain could be obtained by applying GS on larger selection populations; say, 10,000 F3 lines. One major limiting factor is genotyping cost. In this study, GS analysis was performed using 19,863 SNPs with a cost of about $15 per line. Given this cost and available resources, applying GS on F4 generation (~10,000 lines) in the NDSU durum wheat breeding program is not feasible now. It was reported that a panel of low-density markers evenly distributed across the genome or selected based on estimated additive effects could generate similar high prediction accuracy (Heffner et al., 2011; Spindel et al., 2015). Such a panel of markers could be genotyped with a SNP array or amplicon GBS at lower cost, potentially enabling the utility of GS on larger populations (Campbell et al., 2015; Pemberton et al., 2016). As genotyping technologies advance, genotyping costs for both low-density and high-density markers is expected to decrease. GS will be a cost-effective means to enhance genetic gain for complex traits in durum wheat. In addition to grain and semolina quality traits, other agronomic traits like grain yield, plant height, and disease resistance are also available for the training population utilized in the current study and are being used to develop GS models. In GS, the same marker data for the selection population could be used predictively for all traits. Compared with phenotypic selection, multiple-trait GS without extra genotyping cost will be more effective in terms of genetic gain per unit cost per trait.

Conclusions
The goal of this study was to identify major QTL to facilitate MAS and explore the utility of GS for grain and semolina quality traits in the NDSU durum wheat breeding program. Grain and semolina quality traits are essential selection criteria in durum wheat breeding, and are required for new cultivar release. However, phenotypic testing is so costly that evaluation of these traits is performed at late stages and is limited to a small number of breeding lines. This indirectly restrains selection efficiency for grain yield and cultivar development. We performed GWAS and evaluated GS for five quality traits by utilizing historical data collected for 1184 lines from the NDSU durum wheat breeding program. We found several major QTL for test weight, semolina color, and gluten strength. High forward prediction accuracies and increased genetic gain of GS relative to phenotypic selection were observed for the quality traits from the GS models. Given the promising results, implementation of MAS and GS will enable breeders to make selection for those quality traits in a larger population at earlier stages, thereby enhancing selection efficiency. Opportunity exists for the exploration of the genetics underlying other traits including grain yield and agronomic characteristics, and this will be addressed in future investigations.

Conflict of Interest Disclosure
The authors declare that there is no conflict of interest.

Supplemental Information Available
Supplemental information is included with this article.

Supplementary Table S1. Characteristics of the 19,863 SNP markers genotyped for the 1184 durum wheat breeding lines.
Supplementary Table S2. Description of the NDSU durum wheat breeding trials used in this study.
Supplementary Table S3. Number of environments in which the 1184 breeding lines were tested.
Supplementary Table S4. List of SNPs markers with significant association identified for test weight, semolina color, and sedimentation volume.
Supplementary Table S5. Candidate genes for carotenoid pigments and gluten subunits biosynthesis.
Supplementary Table S6. Characteristics of the 3568 haplotype blocks.
Supplementary Table S7. List of haplotype blocks with significant association identified for semolina color and sedimentation volume.
Supplementary Table S8. Forward prediction accuracies for the five quality traits using GS models rBGLUP, BL, BRR, RKHS, and GBLUP.
Supplementary Fig. S1. Scree plot of the first 30 PCs derived from a principal component analysis.
Supplementary Fig. S2. Scatter plots of PC1 and PC2 derived from a principal component analysis for the 1184 durum wheat breeding lines.
Supplementary Fig. S3. Plots of average linkage disequilibrium measure $r^2$ values against distance between pairs of SNP markers.
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