Evaluation of Genetic Diversity and Host Resistance to Stem Rust in USDA NSGC Durum Wheat Accessions

Shiaoman Chao,* Matthew N. Rouse, Maricelis Acevedo, Agnes Szabo-Hever, Harold Bockelman, J. Michael Bonman, Elias Elias, Daryl Klindworth, and Steven Xu

Abstract

The USDA–ARS National Small Grains Collection (NSGC) maintains germplasm representing global diversity of small grains and their wild relatives. To evaluate the utility of the NSGC durum wheat (Triticum turgidum L. ssp. durum) accessions, we assessed genetic diversity and linkage disequilibrium (LD) patterns in a durum core subset containing 429 lines with spring growth habit originating from 64 countries worldwide. Genetic diversity estimated using wheat single-nucleotide polymorphism (SNP) markers showed considerable diversity captured in this collection. Average LD decayed over a genetic distance to within 3 cM at $\theta^2 = 0.2$, with a fast LD decay for markers linked at >5 cM. We evaluated accessions for resistance to wheat stem rust, caused by a fungal pathogen, Puccinia graminis Pers. Pers. f. sp. tritici and E. Henk (Pgt), using races from both eastern Africa and North America, at seedling and adult plant stages. Five accessions were identified as resistant to all stem rust pathogen races evaluated. Genome-wide association analysis detected 17 significant associations at the seedling stage with nine likely corresponding to Sr7, Sr12, and Sr13 and the remaining potentially being novel genes located on six chromosomes. A higher frequency of resistant accessions was found at the adult plant stage than at the seedling stage. However, few significant associations were detected possibly a result of strong G x E interactions not properly accounted for in the mixed model. Nonetheless, the resistant accessions identified in this study should provide wheat breeders with valuable resources for improving stem rust resistance.

Core Ideas

• Characterized the utility of a core subset of USDA–NSGC worldwide durum wheat accessions
• The durum core subset captured a considerable amount of genetic diversity
• Identified accessions’ resistance to wheat stem rust pathogen races
• Assessed genome-wide LD present in the durum core subset
• Revealed novel genes or QTL associated with stem rust resistance in durum wheat

Wheat stem rust caused by a fungal pathogen, P. graminis (Pgt), poses a significant threat to production of both common wheat (T. aestivum L.) and durum wheat throughout the world. Ug99, formally characterized as race TTKSK (Jin et al., 2008), from...
eastern Africa and its rapidly evolving lineage are particularly alarming because of their broad virulence to many stem rust resistance (Sr) genes commonly found in wheat cultivars and impacting >90% of the wheat cultivars grown worldwide (Jin et al., 2007; Singh et al., 2011). Compared with common wheat, a greater percentage of durum wheat germplasm is resistant to race TTksK, particularly those developed in North America (Jin et al., 2007; Pozniak et al., 2008). This more frequent resistance is mostly provided by the Sr13 gene originating from Khapli, an emmer wheat (T. turgidum, ssp. dicoccum) (Jin et al., 2007; Klindworth et al., 2007). However, races JRCQC and TrtTtF, identified in Ethiopia and Yemen, respectively, were characterized as virulent to Sr13 and Sr9e (Olivera et al., 2012b), thus rendering many durum cultivars vulnerable to stem rust.

Currently, more than 70 Sr genes have been characterized in wheat (McIntosh et al., 2013, 2014; Rahmatov et al., 2016), ~34 of which remain effective against races of the Ug99 lineage (Singh et al., 2015). Molecular markers linked to many of these genes are readily available (http://maswheat.ucdavis.edu). Genetic studies to identify and map new sources of resistance genes are underway worldwide through a concerted effort organized by the Borlaug Global Rust Initiative (http://www.globalrust.org). However, most of these studies have focused on the stem rust resistance in common wheat and wild relatives of wheat, and the genetics of stem rust resistance in durum wheat is less studied. The molecular markers linked to Sr genes developed for common wheat may not always be informative for durum wheat (Haile et al., 2013). Efforts to discover novel Sr genes or alleles in durum wheat have been reported using either biparental mapping populations (Haile et al., 2012; Singh et al., 2013), or association mapping approaches based on a diverse panel of cultivars and breeding lines (Pozniak et al., 2008), or elite accessions (Letta et al., 2013, 2014). Evaluations of the cultivated emmer wheat accessions in the field and at the seedling stage have also identified lines with novel resistance to TTksK and other races with broad virulence (Olivera et al., 2012a).

The USDA–ARS NSGC located in Aberdeen, ID, is a genebank that maintains collections representing global diversity of small grains and their wild relatives (http://www.ars.usda.gov/main/docs.htm?docid=2884). Since 1897, over 142,000 accessions have been acquired and deposited. Resistance to diseases and insects have been extensively explored in both wheat (Porter et al., 1993; Bonman et al., 2006, 2007; Olivera et al., 2012a; Newcomb et al., 2013; Maccferri et al., 2015b) and barley (Hordeum vulgare L.) (Bonman et al., 2005; Dahleen et al., 2012) accessions, providing valuable resources for crop improvement. A core subset of the small grains collection was established in 1995 containing ~10% of the entire collection randomly selected with broad representation of geographic regions. Using high-density SNP markers genetic diversity present in the core set for barley (Muñoz-Amatrian et al., 2014) and common wheat (Bonman et al., 2015) has been assessed, thus permitting genetic characterization and exploitation of the diverse core set using methods such as association mapping. Of the NSGC accessions, 8325 are durum collected from 80 different countries and regions, with 5700 being landraces and the remaining being cultivars and breeding lines. The durum core set is comprised of 782 accessions, and their extent of genetic diversity is not yet investigated.

Genome-wide association studies (GWAS) provide a mapping approach that exploits historical recombination events accumulated over many generations in a mapping panel and allows associations of DNA markers and trait gene and quantitative trait loci (QTL) to be detected when they are in LD. In self-pollinated crops, such as barley and wheat, as demonstrated in previous studies, the extent of LD varied on different chromosomes (Chao et al., 2010; Muñoz-Amatrian et al., 2014), presumably a result of selection pressure at regions associated with important agronomic traits. Linkage disequilibrium patterns can also vary greatly depending on the populations used. In general, cultivars have much higher LD than landraces, while rare outcrossing events occurred in self-pollinated wild species, such as wild barley, were enough to maintain linkage equilibrium in a large population (Morrell et al., 2005; Hamblin et al., 2011). Previous studies showed that in durum wheat, LD can extend from within 5 to 20 cM in elite cultivars and advanced lines accessed by simple-sequence repeat (SSR) or diversity arrays technology (DArT) markers (Maccferri et al., 2005, 2011, 2014; Somers et al., 2007). Knowledge of LD patterns can help determine what constitutes adequate genome coverage for detecting marker and trait associations.

The extent and distribution of LD in the NSGC cultivated barley and common wheat accessions have been evaluated (Muñoz-Amatrian et al., 2014; Maccferri et al., 2015b). Novel QTL associated with stripe rust resistance have been identified from the NSGC common wheat accessions using GWAS (Maccferri et al., 2015b). In this study, we assessed genetic diversity and LD patterns in a subset of 429 NSGC durum core accessions with spring growth habit. We further evaluated the set for resistance to stem rust using multiple North American Pgt races, and more recently emerged eastern African races, both at the seedling stage and under field conditions and conducted GWAS to identify potentially novel genes and QTL associated with stem rust resistance in durum wheat.

Materials and Methods

Plant Materials

A total of 497 NSGC durum wheat lines from the durum core subset selected as part of the Triticeae Coordinated Agricultural Project were obtained from USDA–ARS NSGC at Aberdeen, ID, for this study. This sample represents 80% of the spring habit accessions in the durum core subset. Limited information indicated that these accessions were acquired between 1900 and 2004 (Supplemental Table S1). Seed increase was performed in the field
Table 1. Avirulence and virulence responses on the North American differentials for the five races of *Puccinia graminis* f. sp. *tritici* (*Pgt*) evaluated at the seedling stage.

<table>
<thead>
<tr>
<th>Pgt race (isolate)</th>
<th>Avirulent</th>
<th>Virulent</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCCBC(09CA15-2)</td>
<td>Sr5 6 7b 8a 9a 9d 9e 9b 10 11 21 24 30 31 36 38 Tmp</td>
<td>Sr9g 17 McN</td>
</tr>
<tr>
<td>TRTTF(06YEM34-1)</td>
<td>Sr8a 24 31</td>
<td>Sr5 6 7b 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 MnN Tmp</td>
</tr>
<tr>
<td>TTTTF(01MN84A-1-2)</td>
<td>Sr24 31</td>
<td>Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 MnN Tmp</td>
</tr>
<tr>
<td>TTKSK(04KEN156/04)</td>
<td>S24 36 Tmp</td>
<td>Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 MnN Tmp</td>
</tr>
<tr>
<td>JRCQC(09ETH08-3)</td>
<td>S5 7b 8a 36 9b 30 10 Tmp 24 31 38 Sr21 9e 1 1 6 9g 17 9a 9d McN</td>
<td></td>
</tr>
</tbody>
</table>

at Prosper, ND, in 2012 and in 2013 from heads harvested in the greenhouse. Sixty-four duplicate lines with identical SNP genotypes were identified and merged, and the line with the most complete data was retained. Two hexaploid wheat accessions (PI 477895 and PI 585023) misclassified as durum were identified and eliminated based on cytogenetics analysis. After removing two lines (PI 68288 and PI 78810) with winter habit, the final set containing 429 unique durum wheat accessions with spring habit, representing 69% of the spring habit accessions in the core subset, was used for the analyses performed in this study. The improvement status recorded in the passport data indicated that 75 accessions were breeding materials, 49 were cultivars, 113 were of uncertain improvement status, and 192 were landraces (Supplemental Table S1).

Except for five accessions with unknown country of origin, the other 424, representing 64 countries and regions, were grouped based on geographic origin for data analysis. The groups included Africa containing 78 accessions from 13 countries, the Americas containing 80 accessions from 11 countries, Asia including 150 accessions from 22 countries, and Europe including 116 accessions from 18 countries (Supplemental Table S2).

**Phenotype Data**

The entire 497 accessions were evaluated for stem rust resistance both at the seedling stage in the greenhouse and at the adult plant stage in the field. Phenotypic evaluation results can be accessed from The Triticeae Toolbox Wheat database (https://triticeaetoolbox.org/wheat/).

**Seedling Evaluations**

Seedling tests were performed using five *Pgt* races, BCCBC, TCCBT, TTKSK, TRTTF, and JRCQC (Table 1), and a bulk of six races, MCCFC, QFCSC, QTHJC, RCRSC, RKQQC, and TPMKC (isolates 59KS19, 06ND76C, 75ND717C, 77ND82A, 99KS76A, and 74MN1409, respectively). Except for three races, TTKSK, TRTTF, and JRCQC, originating from Kenya, Yemen, and Ethiopia, respectively, the other races were collected from the United States. The evaluation experiments were conducted at the USDA–ARS Cereal Disease Laboratory, St. Paul, MN, following the procedure described by Rouse et al. (2011). Briefly, six seeds per accession were planted in trays filled with vermiculite. The 8- to 9-d-old seedling plants were inoculated with *Pgt* urediniospores. After inoculation, the plants were grown in a greenhouse at 22 ± 2°C and 18 ± 2°C (day and night temperature, respectively) with supplemental lighting for a photoperiod of 16 h. Plants were scored at 14 d after inoculation using the infection type (IT) scale (0, , 1, 2, 3, and 4) described by Stakman et al. (1962). Two symbols, “−” and “+”, were used to indicate smaller and larger pustules, respectively, for each of ITs 1, 2, or 3 (Roelfs and Martens, 1988). To estimate the frequency of the accessions resistant to different races at the seedling stage, lines were grouped in three categories based on IT scores. Those with IT ≤ 2 were considered as resistant (R), those with IT between 2+ and 3− were intermediate (I), and those with IT ≥ 3 were susceptible (S) (Supplemental Table S3).

**Field Evaluations**

Field experiments for evaluating resistance at the adult plant stage were performed in St. Paul, MN, from April to July in three seasons from 2012 to 2014 and in Debre Zeit, Ethiopia from June to October in 2014 following the experiment procedures described by Olivera et al. (2012a). At the St. Paul location, the accessions were planted in one-row (1 m long) plots with one replicate in each of the three seasons, and the inoculum contained a mixture of six US races (MCCFC, QFCSC, QTHJC, RCRSC, RKQQC, and TPMKC). At Debre Zeit, the accessions were planted in two-row (1 m long) plots in a random complete block design with two replicates, and the inoculum was a bulk of urediniospores (including races TTKSK and JRCQC) collected from durum wheat. In both nurseries, a mixture of susceptible wheat cultivars were planted perpendicular to all entries as stem rust spreaders. The plants in spreader rows were artificially inoculated two to three times (once a week) starting at stem elongation stage. The infection response (pustule type and size) and disease severity (percentage of infected tissue) were generally scored at two different dates at the soft-dough stage. In the St. Paul, MN, trials, higher missing scores were observed for one of the two ratings in Season 1 (season1_2) and Season 3 (season3_1) (Supplemental Table S3). This was mainly because the rating date was not ideal for durum wheat. For example, the second rating for Season 1 (season1_2) was too late when most plants rated in the first rating were already matured by the second rating, while the first rating for Season 3 (season3_1) was too early for most plants. Disease severity was determined based on the modified Cobb Scale (Peterson et al., 1948). The infection response was scored as R, MR, MS,
and S categories for resistant, moderately resistant, moderately susceptible, and susceptible, respectively (Roelfs et al., 1992). Combinations of single categories were also recorded when two different infection responses occurred on a single stem. To calculate the frequency of adult plant resistance in the set, the accessions were grouped in resistant and susceptible categories as described by Newcomb et al. (2013) (Supplemental Table S3).

For GWAS, both seedling scores and field disease ratings were linearized using a custom Perl script (https://github.com/umngao/rust_scores_conversion) (Gao et al., 2016). The seedling IT scores were converted using a scale of 0 to 9 following the method described by Zhang et al. (2014). The adult plant disease ratings from each replicate or season were converted to the coefficient of infection values by multiplying disease severity values on a scale of 0 to 100 by infection response ratings linearized to a scale of 0 to 1 based on the method of Yu et al. (2011). Ethiopia data set was obtained from one field season with two replications, and least-squares means were calculated across replicates to obtain adult plant disease values from the Ethiopia trial by fitting genotype as a fixed effect in a linear model using JMP (v.11; SAS Institute, 2014a) (Supplemental Table S3). The fixed effect model used was

\[ x_{ji} = \mu + a_i + b_j + \epsilon_{ij}, \]

where \( a_i \) is the \( i \)th line fixed effect, \( b_j \) is the \( j \)th year random effect, and \( \epsilon_{ij} \) is residual error. The St. Paul data set was obtained from three field seasons with one replication in each season, and best linear unbiased prediction values were estimated across all three seasons for the St. Paul, MN, field trials by fitting genotype as a random effect in a linear mixed model using the lmer function in the lme4 package (Bates et al., 2015) for R (https://www.r-project.org/) (Supplemental Table S3). The random effect model used was

\[ x_{ij} = \mu + a_i + \beta_{ij} + \epsilon_{ij}, \]

where \( a_i \) is the \( i \)th line fixed effect, \( \beta_{ij} \) is the \( ij \)th line random effect, and \( \epsilon_{ij} \) is residual error.

Genotype Data

The entire set of 497 accessions were genotyped with Illumina’s iSelect wheat 9K array containing 9000 gene-derived SNPs, of which 8632 are functional (Cavanagh et al., 2013), using Illumina’s Infinium method following manufacturer’s protocols. The SNP genotype calls were performed using the genotyping module implemented in the Illumina’s GenomeStudio software v.2011.1. Genotype data were manually inspected for call accuracy before exporting the data file containing 6538 polymorphic SNP markers, and are publicly available from The Triticeae Toolbox Wheat database (https://triticeaetoolbox.org/wheat/). After converting heterozygote calls to missing data, SNPs with a missing data rate of 10% or higher were filtered out. The high-density SNP based consensus map developed for tetraploid wheat (Maccarferri et al., 2015a) was then used to remove SNPs with no map information, and the resulting final data set retained 3806 SNP markers.

Genetic Diversity and Genome-Wide Association Analysis

Using the full data set containing 3806 SNP markers, genetic diversity measured as polymorphic information content (PIC) (Weir, 1996) was calculated for all samples and for samples grouped based on improvement status and geographic origin separately using JMP Genomics 7 (SAS Institute, 2014b).

Linkage disequilibrium between markers measured as \( r^2 \), representing the correlation between alleles at two loci, was calculated by TASSEL4 (Bradbury et al., 2007). To reduce large variances from rare alleles, a subset of 2827 SNPs with minor allele frequency (MAF) \( \geq 0.10 \) was used to calculate LD (Remington et al., 2001). The \( p \) values for the LD estimates were determined based on a two-sided Fisher’s exact test. Linkage disequilibrium decay with genetic distance in centimorgans between markers on the same chromosomes was evaluated by nonlinear regression following the method described by Remington et al. (2001) using nls function in R (https://www.r-project.org/). The 95th percentile of \( r^2 \) distribution for unlinked markers was taken as a population-specific critical value for background LD (Breseghello and Sorrells, 2006).

Population structure was analyzed using both principal component analysis (PCA) in R (https://www.r-project.org/), and a Bayesian clustering method implemented in Structure v.2.3.4 (Pritchard et al., 2000). To minimize overestimation of the number of subpopulation affected by SNPs in strong LD, the Structure analysis was performed using a subset of 694 SNPs with LD at \( r^2 < 0.2 \) by setting the number of subpopulations \( (k) \) from 1 to 8 with each run repeated 10 times. The length of burn-in period was set at 5000 followed by a simulation run length set at 10,000 using admixture model and correlated allele frequency (Falush et al., 2003). To estimate the optimal number of subpopulations, \( \Delta k \) values were calculated using the log probability \( [\text{LnP}(\text{D})] \) values according to Evanno et al. (2005). The graphical display of population structure showing the proportion of the genomes from each of the accessions clustered in the subpopulations was done using the software program Distruct (Rosenberg, 2004) (Supplemental Fig. S1). To investigate the amount of genetic variation and population differentiation for samples from different geographic origin and within the same origin, analysis of molecular variance (AMOVA) was performed using Arlequin v.3.5 (Excoffier et al., 2005).

For GWAS, the data set was first filtered by removing SNPs with MAF below 0.01. Because markers in perfect LD generally provided no additional information, a tag SNP was selected representing SNPs in perfect LD \( (r^2 = 1.0) \) using the LD tag SNP Selection function in JMP Genomics 7 (SAS Institute, 2014b). The resulting data set used for GWAS contained 3268 SNPs distributed on a map spanning a total of 2530 cM on 14 chromosomes. Association analysis for individual traits was performed using the GWAS function implemented in rrBLUP 4 (Endelman, 2011) in R (https://www.r-project.org/) based on the efficient mixed-model association eXpedited
algorithm (Kang et al., 2010). Two models, K-only and $P+K$, were tested and compared. In the K-only model, an identical-in-state kinship (K) matrix was calculated to estimate phenotypic covariance from relatedness. The $P+K$ model contained both the K matrix fitted as a random effect and the first two principle components ($P$) accounting for 12.1 and 6.1%, respectively, of the phenotypic variations as fixed effects. The threshold to declare significant associations was calculated using the qvalue package incorporated in rrBLUP that corresponds to a false discovery rate of 0.05. Chromosome and chromosome arm locations for markers significantly associated with the disease responses to Pgt races were determined by BLASTn searches of the SNP contextual sequences against the Chinese Spring (CS) chromosome survey sequences (http://wheat-urgi.versailles.inra.fr/Seq-Repository/BLAST). The best hits with e-value cutoff at 1 $\times$ 10$^{-30}$ were extracted from the BLASTn output. Genotype data and phenotype data (Supplemental Table S3) used for GWAS are available from the GrainGenes database (http://wheat.pw.usda.gov/GG3/).

Results

Host Resistance to Stem Rust in Durum Accessions

We evaluated a subset of the spring habit accessions from the NSGC durum wheat core for their responses to five Pgt races and a mixture of six races at the seedling stage and observed various frequencies of resistance to different races (Table 2). The highest frequency of resistance was found to race BCCBC (56.1%)—a very avirulent race—and to the bulked North American races (38.5%). The frequencies of resistance to the four races, JRCQC, TTKSK, TTTTF, and TRTTF, were 7, 17, 22.2, and 22.5%, respectively. Although only 29 (7%) accessions were resistant to JRCQC—a highly virulent race from Ethiopia—three were highly resistant exhibiting an immune response (IT 0 and 0) (Table 2; Supplemental Table S3). Accessions highly resistant to TRTTF (18)—a virulent race from Yemen—and to the North American race TTTTF (14) were also observed. The 14 accessions exhibiting an immune or near-immune response to race TTTTF all had low IT ($\leq$2) to race TRTTF, with 12 of them also having low IT to race TTKSK. In contrast to the seedling evaluations, a much higher frequency of resistance was observed under the field conditions in both Ethiopia (63.7%) and the United States (45.9%) (Table 2). Except for the field data obtained from Ethiopia, correlations between the various tests were highly significant ($p < 0.001$) (Fig. 1). The lack of correlation for the Ethiopia test was possibly due to a strong environmental influence on the genotype $\times$ race interactions, although more field experiments are needed to further confirm this hypothesis.

To examine the percentage distributions of the durum accessions from different geographic origin and at different improvement status in terms of disease responses, the accessions were grouped in three categories—resistant, susceptible, and intermediate—from the seedling scores, but in two categories for the field ratings—resistant and susceptible (Fig. 2). In general, resistant accessions to different races were well-represented in countries from all geographic origins. Although ample resistance sources were present among accessions of various improvement statuses, a higher proportion of breeding lines, in particular, were more resistant to TTTTF and three African races, TTKSK, TRTTF, and JRCQC. Except for race JRCQC, accessions resistant to TTKSK, TRTTF and TTTTF were also represented in both uncertain improvement status and landrace categories. Cultivars developed in the Americas generally had a much higher frequency of resistance to these races than those from Africa, where none of the cultivars were resistant to TTTTF and the three African races evaluated. Altogether 12 accession were identified as resistant or intermediate to all races at the seedling stage and in the two field locations, of those, five were resistant to all races evaluated (Supplemental Table S3): Cltr 15769 (a breeding line from the United States), PI 94758 (a line with uncertain improvement status from Ethiopia), and three landraces from Ethiopia, PI 298547, PI 479941, and PI 479983.

Genetic Diversity and Population Structure

Among the durum accessions studied, PIC values ranged from 0.018 to 0.375 (data not shown) with a mean of 0.260, and the B-genome SNPs were slightly more diverse than those on the A genome (Table 3). Mean PIC values were then calculated for the accessions in improvement status and in geographic origin, separately (Table 3). Higher levels of genetic diversity were observed for landrace and accessions of uncertain improvement status, and accessions originating from the African countries tended to be more diverse than those from other regions (Table 3). Population structure among accessions in the four geographic groups was investigated using both a model-based clustering method implemented in the Structure program and PCA. From Structure results, mean log

<table>
<thead>
<tr>
<th>Race</th>
<th>Resistant</th>
<th>Susceptible</th>
<th>Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling evaluations</td>
<td>BCCBC</td>
<td>238 (56.1)</td>
<td>144 (34.0)</td>
</tr>
<tr>
<td></td>
<td>TTTTF</td>
<td>95 (22.2)</td>
<td>264 (61.7)</td>
</tr>
<tr>
<td></td>
<td>TTKSK</td>
<td>73 (17.0)</td>
<td>350 (81.6)</td>
</tr>
<tr>
<td></td>
<td>TRTTF</td>
<td>96 (22.5)</td>
<td>308 (72.1)</td>
</tr>
<tr>
<td></td>
<td>JRCQC</td>
<td>29 (7.0)</td>
<td>378 (90.6)</td>
</tr>
<tr>
<td>Field</td>
<td>163 (38.5)</td>
<td>208 (49.2)</td>
<td>52 (12.3)</td>
</tr>
<tr>
<td>Field evaluations</td>
<td>Ethiopia</td>
<td>267 (63.7)</td>
<td>152 (36.3)</td>
</tr>
<tr>
<td></td>
<td>St. Paul, MN</td>
<td>191 (45.9)</td>
<td>225 (54.1)</td>
</tr>
</tbody>
</table>

Table 2. Number and percentage of National Small Grains Collection accessions sampled from the durum core subset exhibiting resistant, susceptible, and intermediate responses to different P. graminis f. sp. tritici races at the seedling stage and in the field evaluations.
Fig. 1. A biplot from principle components on correlation for stem rust data obtained at both the seedling and adult plant stages.

Fig. 2. Percentage distributions of the durum accessions in the categories of resistant (R), susceptible (S), and intermediate (I) response to *P. graminis* f. sp. *tritici* races among geographic origins the accessions originating from and their improvement status. (1) BCCBC, (2) TTTTF, (3) TTKSK, (4) TRTTF, (5) JRCQC, (6) field bulk races, (7) Ethiopia, and (8) St. Paul, MN.
posterior probability, LnP(D), over 10 runs for each \( k \) between 1 and 8 increased continuously without reaching a plateau (Supplemental Fig. S1). An optimal number of genetic clusters calculated by \( \Delta k \) values showed that the maximum \( \Delta k \) was reached at \( k = 2 \), although a lower peak was also obvious at \( k = 6 \) (Supplemental Fig. S1). A total of 190 (44%) accessions were found clustered in one of the six subpopulations at a posteriori probability >0.70 (data not shown). However, no distinct genetic clusters were formed containing accessions from specific regions even at higher \( k \) (Supplemental Fig. S2), indicating a high degree of admixture present among the accessions from the core subset collected from diverse origins over the past 100 yr. This finding was further confirmed from PCA results, from which the first two principle components accounted for only 18% of the genetic variation (Fig. 3). To further assess genetic diversity within and among accessions from different geographic groups, we performed AMOVA that partitioned 93.6% of the genetic variation \((p < 0.0001)\) to within each group and only 6.4% \((p < 0.0001)\) to among groups (data not shown). The overall Fst value estimated among geographic groups was 0.064, providing further evidence of low population differentiation present in this core subset.

### Genome-Wide Linkage Disequilibrium and Association Mapping Analysis

Knowledge of the extent of LD in a population helps assess the power and resolution of GWAS. We evaluated genome-wide LD in the subset of core accessions through pair-wise comparisons among 2827 SNPs with MAF > 0.10. Of the total 120,040 estimates calculated, 14% \((16,568)\) of the loci pairs were in long-range LD between markers on different chromosomes, with 30% \((4998)\) of them significant at the level of \( p < 0.01 \). Among the 103,472 estimates obtained between loci on the same chromosomes, 84% \((86,401)\) were between linked markers \(<40 \text{ cM}\), of those, 49% \((42,716)\) were in significant LD \((p < 0.01)\). Significant LD \((p < 0.01)\) was also observed for 35% \((5980/17,071)\) of the unlinked loci pairs on the same chromosome \(>40 \text{ cM}\). High levels of LD were generally found for markers closely linked at \(<5 \text{ cM}\) (Table 4; Fig. 4). The \( r^2 \) values declined to 0.2 within 3 cm for the linked loci, and the significant threshold for \( r^2 \) in this set was 0.09 (Fig. 4). When evaluating LD pattern on the A genome separately from the B genome, the rate of LD decay for SNPs for each genome appeared to be very similar to the combined SNP loci on both genomes (Supplemental Fig. S3). Linkage disequilibrium levels were slightly higher on the A genome than the B genome, as reflected by the higher mean \( r^2 \) values for the closely linked marker \(<5 \text{ cM}\) (Table 4) possibly because the A-genome SNPs were slightly less diverse. The LD patterns were then assessed for accessions of different improvement statuses based on loci pairs with \( r^2 \geq 0.1 \), a general cutoff value for estimating genome-wide LD block; as expected, higher level and longer extent of LD were observed in both breeding and cultivar accessions than those in the accessions with uncertain improvement status and landrace accessions (Table 4; Supplemental Fig. S4). Nonetheless, overall LD in the entire set was generally similar to LD in the subsets because of weak population structure. While loci pairs with \( r^2 \geq 0.1 \) were found for 4% of the unlinked markers in the entire set, the fraction was higher in breeding (10%) and cultivar (14%) accessions, probably a result of slightly higher levels of genetic relatedness present within these improvement status categories (Table 4).

To perform association analysis, we compared three models—naive, \( K \), and \( P+K \)—to evaluate the effect and ability of population structure to reduce the number of false positives for each trait. Results showed that both \( P+K \) and \( K \)-only models performed equally well over the simple naive model, and that the \( P+K \) model had the same ability in controlling Type I error rate as the \( K \)-only model (Supplemental Fig. S5), indicating that the \( K \)-only model was sufficient to account for sample relationships and reduce false positives. Therefore, the subsequent analysis was performed based on the \( K \)-only model.

Association analysis detected a total of 17 markers significantly associated with disease responses to five \( Pgt \) races—BCCBC, TTTTTF, TTTTK, TRTTF, and JRCQ—evaluated at the seedling stage (Table 5; Supplemental Fig. S6). Six significant associations to race BCCBC, with the two most significant SNP markers residing on the same CS chromosome survey sequence contig 10401664, were within a 2-cM region on chromosome 3B, and they are likely linked to the Sr12 gene based on the previously mapped location of Sr12 (Rouse et al., 2014b). Three SNP loci on the long arm of chromosome 1B derived from the same CS survey sequence contig 3798174 were significantly associated with race TTTTTF, two of which were also associated with race TRTTF. Therefore, a single novel gene possibly confers resistance to both races TTTTTF and TRTTF, although the possibility of two closely linked resistance genes cannot be ruled out. Significant associations for resistance to race TTTTTF were detected at two additional chromosome locations. One was on the long arm of chromosome 6A, which was also associated
with resistance to race TTKSK, and is likely the Sr13 gene (Simons et al., 2011). The other on the long arm of chromosome 7A is at a similar location to the Sr22 gene (Olson et al., 2010), although Sr22 is derived from einkorn wheat (T. monococcum L.), and unlikely to be present in durum wheat. In addition to the potentially novel gene on chromosome arm 1BL resistant to TRTTF, two more potentially novel genes located on chromosome arms 5AS and 5BL were also characterized as resistant to race TRTTF. For race JRCQC, resistance loci were detected on chromosome arms 4BS, 4AL, and 5AL, with the 4AL loci likely being near or at the Sr7 locus (Turner et al., 2016), and the other two are possibly novel. Significant associations were not detected for the seedling evaluations using the six bulked North American races from the field in St. Paul, MN. The two SNP markers with the smallest p-values at $6.46 \times 10^{-5}$, and $5.54 \times 10^{-5}$, respectively, below the significant threshold were detected on chromosome arms 2BL and 5AS. The one found on 2BL likely corresponds to a gene or an allele near Sr9 or Sr28 (Rouse et al., 2014a), whereas the other on 5AS is possibly a novel gene.

Far fewer significant associations were detected for disease responses evaluated at the adult plant stage compared with those evaluated at the seedling stage (Table 5; Supplemental Fig. S6). A single association on the short arm of chromosome 7A was significant at the threshold level for the field evaluations performed in St. Paul, MN. By lowering the significant threshold, a SNP marker on chromosome arm 1BS with the smallest p-value at $8.32 \times 10^{-5}$ was detected from the Ethiopia field evaluations.

Table 4. Distribution of mean $r^2$ values and the proportion of the loci pairs with $r^2 \geq 0.1$ at different ranges of marker distance in centimorgans (cM) for accessions at four improvement statuses and in the entire set of durum accessions for the entire genome and on the A and the B genome separately.

<table>
<thead>
<tr>
<th>Genetic distance</th>
<th>A genome</th>
<th>B genome</th>
<th>All</th>
<th>Breeding</th>
<th>Cultivar</th>
<th>Uncertain</th>
<th>Landrace</th>
</tr>
</thead>
<tbody>
<tr>
<td>cM</td>
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<td></td>
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<tr>
<td>0</td>
<td>0.56 (83)†</td>
<td>0.52 (75)</td>
<td>0.54 (79)</td>
<td>0.60 (81)</td>
<td>0.60 (81)</td>
<td>0.54 (79)</td>
<td>0.52 (79)</td>
</tr>
<tr>
<td>&gt;0–5</td>
<td>0.13 (34)</td>
<td>0.13 (32)</td>
<td>0.13 (33)</td>
<td>0.19 (42)</td>
<td>0.17 (39)</td>
<td>0.13 (32)</td>
<td>0.13 (33)</td>
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<tr>
<td>&gt;5–10</td>
<td>0.05 (15)</td>
<td>0.04 (11)</td>
<td>0.04 (13)</td>
<td>0.07 (24)</td>
<td>0.07 (21)</td>
<td>0.05 (13)</td>
<td>0.05 (17)</td>
</tr>
<tr>
<td>&gt;10–20</td>
<td>0.04 (10)</td>
<td>0.03 (8)</td>
<td>0.04 (9)</td>
<td>0.06 (17)</td>
<td>0.07 (20)</td>
<td>0.04 (11)</td>
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<tr>
<td>&gt;20–40</td>
<td>0.03 (6)</td>
<td>0.03 (7)</td>
<td>0.03 (6)</td>
<td>0.05 (13)</td>
<td>0.06 (16)</td>
<td>0.04 (9)</td>
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<td>&gt;40</td>
<td>0.03 (5)</td>
<td>0.02 (3)</td>
<td>0.02 (4)</td>
<td>0.04 (10)</td>
<td>0.05 (14)</td>
<td>0.03 (7)</td>
<td>0.03 (8)</td>
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</tbody>
</table>

† Values in parentheses represent percentage $r^2 \geq 0.1$
Fig. 4. Genome-wide linkage disequilibrium (LD) decay among single-nucleotide polymorphism loci pairs as a function of genetic distance in centimorgans (cM) in accessions from the durum core subset. Markers with genetic distance >40 cM were binned as unlinked. The line corresponds to the population-specific threshold for background LD at $r^2 = 0.09$.

Table 5. The $-\log(p)$ values for markers significantly associated with disease responses to stem rust races at the seedling stage and under the field conditions, marker chromosome locations, BLASTn matches to Chinese Spring (CS) chromosome arm survey sequences, and known stem rust resistance gene postulations. Numbers in bold indicate the possible associations with the smallest $p$-value below the experiment-wise threshold (Supplemental Fig. S6).

<table>
<thead>
<tr>
<th>SNP†</th>
<th>Chromosome</th>
<th>Position</th>
<th>CS survey sequence</th>
<th>BCCBC</th>
<th>TTTF</th>
<th>TTSS</th>
<th>TRTF</th>
<th>JRCQC</th>
<th>Field bulk</th>
<th>Ethiopia</th>
<th>St Paul, MN</th>
<th>Near or at the known gene or QTL‡</th>
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<td>86.90</td>
<td>3B_10543761</td>
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<td>3B_10481084</td>
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<td>3B_10401664</td>
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<td>107.50</td>
<td>1BL_3798174</td>
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<td>4BS_4948049</td>
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<td>137.90</td>
<td>2BL_8058911</td>
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† SNP, single-nucleotide polymorphism.
‡ QTL, quantitative trait loci.
Discussion
Genetic Diversity and Linkage Disequilibrium
Pattern of the National Small Grains Collection
Durum Core Accessions

In this study, we evaluated 429 durum accessions with spring habit deposited at USDA–ARS NSGC representing 69% of the spring accessions from the durum core subset and originating from 64 countries worldwide. Genetic diversity estimated using wheat SNP markers showed a mean PIC value of 0.26 for this set (Table 3). While it is not possible to compare SNP marker diversity in this material with SSR marker-based diversity in the diverse and elite durum germplasm reported previously (Maccarelli et al., 2005; Somers et al., 2007), a separate study using SNP markers on a set of 150 worldwide durum wheats, including both landraces and cultivars, showed a mean PIC value of 0.19 (Ren et al., 2013). Similar levels of marker diversity present in both cultivars and landraces in the current set further indicated that a considerable amount of diversity was captured in this historical and worldwide durum collection. Population structure was generally weak among the accessions originating from different geographical origins with Fst values ranging from 0.05 to 0.08, suggesting that either germplasm was frequently shared among researchers from different countries, or limited selection pressure and genetic drift maintained high levels of genetic diversity in this set as evidenced by a large proportion of genetic variance within geographic origins from the AMOVA test.

Assessments of LD patterns have been reported in cultivated durum wheat before, but those studies were mostly based on SSR or DArT markers. Depending on the germplasm evaluated, LD extent varied from within 5 cM in a diverse set of 93 breeding lines (Somers et al., 2007) and 183 accessions adapted to the Mediterranean areas (Maccarelli et al., 2014), to 10 cM in a population of 189 cultivars and advanced breeding lines mostly from the Mediterranean regions (Maccarelli et al., 2006, 2011), and to 20 cM in 134 worldwide accessions (Maccarelli et al., 2005). It might not be prudent to directly compare LD patterns measured using multiallelic markers with that measured with biallelic markers (Hedrick, 1987). In addition, we used far more SNP markers (2827) to assess the LD pattern than any of the previous studies. With these two caveats, it seemed that average LD estimates between loci pairs at different genetic distances were generally lower in the present study than those observed in the study using 183 accessions from the Mediterranean regions evaluated by 957 SSR or sequence-tagged site and DArT markers (Maccarelli et al., 2014). This result implied that LD decayed much faster, particularly after 5 cM, in the NSGC core subset population providing adequate resolution for finding closely linked markers using an association mapping approach.

Identification of Genomic Regions Potentially Harboring Novel Stem Rust Resistance Genes

Except for the lower frequency of resistance to race JRCQC, we observed >15% of the accessions to be resistant to the other four Pgt races (BCCBC, TTTTF, TTKSK, and TRTTF) at the seedling stage (Table 2). Results also showed that a high proportion of breeding materials were resistant to the races at the seedling stage and that accessions originating from the Americas tended to be more resistant than accessions from other geographic areas (Fig. 2). These findings generally agreed with the results of Olivera et al. (2012b), who evaluated 137 NSGC durum accessions, of which 66 accessions were included in the set used in the present study, for their responses to races TTKSK, TRTTF, and JRCQC at the seedling stage. They found that 66% of the susceptible entries were landraces and materials of uncertain improvement status originating from Africa and Asia.

To identify potentially novel resistance genes, we performed association analysis and detected 17 significant associations for resistance to five races at the seedling stage. Nine of these associations likely corresponded to three known Sr genes, Sr7, Sr12, and Sr13, conferring resistance to races BCCBC, TTTTF, TTKSK, and JRCQC. The remaining associations likely corresponded to novel genes located at six chromosome arm regions on 1BL, 4BS, 5AS, 5AL, 5BL, and 7AL conferring resistance to races TTTTF, TRTTF, and JRCQC. None of the novel genes discovered in the present study correspond to those found in a previous association mapping analysis based on a set of diverse durum germplasm screened with the same races, TRTTF, TTTTF, TTKSK and JRCQC (Lettia et al., 2014). In their study, one of the significant associations identified on chromosome 4AL tagged by a SSR marker, barc78, was 12 cM proximal to the two SNP markers we identified according to the consensus map for durum wheat (Maccarelli et al., 2015a). Therefore, the 4AL gene found in their study would be different from the postulated Sr7 allele found in our study. In contrast to the GWAS results from using a single race, evaluation of seedling resistance using a mixture of six races from the field did not yield any significant associations. A possible explanation is that we were in fact mapping a complex disease resistance trait conditioned by at least six or more resistance genes responsive to the six races used. Previous simulation studies have shown that by increasing the samples surveyed the power to detect associations can be improved for traits controlled by QTL accounting for increasing phenotype variation (Long and Langley 1999; Bradbury et al., 2011). Results from the latter study based on diverse barley breeding populations indicated that one would need 1600 lines to achieve the detection power to above 0.80, assuming 10 QTL with additive effects and heritability of 0.75, using the K-only model (Bradbury et al., 2011). Although it is not possible to estimate the genetic effects of resistant genes to the six races evaluated and their interactions, we speculate that sample size (429) used in this study may have not been large enough to provide adequate detection power.
A much higher frequency of resistant accessions was found in the field assays (Table 2). However, association mapping detected only one significant association in the St. Paul, MN, environment, and one below the experiment-wise threshold in the Ethiopia trial. This result is in contrast to the previous field studies performed in Kenya (Pozniak et al., 2008) and in Ethiopia (Letta et al., 2013). Pozniak et al. (2008) used a set of 96 diverse durum wheats from 13 countries and detected >10 loci affecting field resistance, five of which were possibly linked to known resistance genes. Using the 183 elite accessions assembled by Maccarferri et al. (2006, 2011), the study of Letta et al. (2013) measured resistance responses to TTKSK and a mixture of durum-specific races through artificial inoculation. They showed over 30 significant associations expressed in multiple environments, five of which mapped near known resistance genes. Environmental conditions and the presence of pathogen races in different field settings can vary greatly and made comparisons among different field trials difficult. Our Ethiopian study was performed in only one environment with two replications where genetic effects confounded by genotype × environment interactions not properly accounted for in the model could have reduced the power of association analysis, particularly when there may be many genes for the trait each with smaller effects influencing adult plant resistance (Zhu et al., 2008). Smaller sample size would have reduced the detection power even further.

None of the resistant loci detected for the seedling infections affected field response in our study. However, the locus on chromosome 7AL near the mapped location of Sr22 that conditioned resistance to TTTTF in our study was identified previously as being associated with the field resistance by Pozniak et al. (2008) and Letta et al. (2013), although the former study also showed that this locus was associated with seedling resistance to race Ug99. Though Sr22 was indicated as possibly being detected by Pozniak et al. (2008) and Letta et al. (2013), the presence of Sr22 in durum wheat would be unusual since this gene was derived from einkorn wheat. Further studies are needed to determine if the association detected in durum wheat near the Sr22 locus is indeed conferred by Sr22.

Conclusion

We characterized a set of 429 historical durum accessions in this study. The high levels of genetic diversity, weak genetic structure, and <10 cM of average rate of LD decay found in this set make it suitable for association mapping studies. Accessions identified in the present study with resistance to the recently emerged virulent races to durum wheat, such as TRTTF and JRCQC, should provide wheat breeders with valuable resources for improving stem rust resistance. The GWAS results provided preliminary evidence of genomic regions potentially harboring novel stem rust resistance genes in durum wheat. To better understand which resistant accessions carrying particular novel genes, follow up studies are underway to more precisely map the genes and QTL and study their genetic effects with biparental mapping populations and to develop markers for use by breeders.

Supplemental Information

Supplemental Fig. S1. Population structure analysis.
Supplemental Fig. S2. Proportion of the genomes from each accessions sampled from the NSGC durum core subset clustered in the subpopulations from $K = 2$ to $K = 6$. Four populations predefined were accessions grouped in Africa, the Americas, Asia, and Europe.
Supplemental Fig. S3. Genome-wide LD decay estimated for SNPs on the wheat A (a) and B (b) genome, and the combined SNP set. The line corresponds to the population-specific threshold for background LD at $r^2 = 0.09$.
Supplemental Fig. S4. LD decay for samples at different improvement status and in the entire accessions.
Supplemental Fig. S5. Model comparisons to test the effectiveness of including population structure in the mixed model to control type I error rate. The line in diagonal corresponds to expected $p$-value distribution. The cumulative distribution of $p$-values for either $K$ only or $P+K$ model closely followed the expected distribution, indicating both models were equally adequate to reduce spurious associations (Kang et al., 2008).
Supplemental Fig. S6. Quantile–quantile (QQ) plots of the distribution of $p$-values and genome-wide Manhattan plots displaying association analysis findings with respect to their genomic positions for each trait. The dash line in the Manhattan plots showed $p$-value corresponding to a false discovery rate of 0.05.
Supplemental Table S1. Passport data for the NSGC durum accessions used.
Supplemental Table S2. Number of NSGC durum accessions, their country or regions, and the geographic origin each accessions grouped into.
Supplemental Table S3. Original disease score, linearized data, and accessions grouped in the categories of resistant (R), susceptible (S), and intermediate (I). Lines highlighted exhibited resistant response to all races evaluated.

Acknowledgments

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References


