Genetic Characterization of Stem Rust Resistance in a Global Spring Wheat Germplasm Collection

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ABSTRACT
Stem rust, caused by the fungus Puccinia graminis Pers. f. sp. tritici Ericks, is one of the most damaging diseases of wheat (Triticum aestivum L.). The recent emergence of the stem rust Ug99 race group poses a serious threat to world wheat production. Utilization of genetic resistance in cultivar development is the optimal way to control stem rust. Here, we report association mapping of stem rust resistance in a global spring wheat germplasm collection (2152 accessions) genotyped with the wheat iSelect 9K single-nucleotide polymorphism array. Using a unified mixed model method (or QK method), we identified a total of 47 loci that were significantly associated with various stem rust resistance traits including field disease resistance and seedling resistance against multiple stem rust pathogen races including BCCBC, TRTTF, TTKSK (Ug99), and TTTTF. The 47 loci could be further condensed into 11 quantitative trait locus (QTL) regions according to linkage disequilibrium information among adjacent markers. We postulate that these QTLs represent known stem rust resistance genes including Sr2, Sr6, Sr7a, Sr8a, Sr9h, Sr13, Sr28, and Sr36. We further employed a multilocus mixed model to explore marker-trait associations and identified two additional QTLs (one potentially represents Sr31) that were significantly associated with stem rust resistance against various races. Combinations of the most significant loci for each trait explained up to 38.6% of the phenotypic variance. Markers identified through this study could be used to track the genes or QTLs. Accessions with high numbers of resistance-associated alleles may serve as important breeding materials for stem rust resistance.

STEM RUST, caused by the fungus Puccinia graminis Pers. f. sp. tritici Ericks (Pgt), is one of the most damaging diseases of wheat (Triticum aestivum L.), dating back at least 3300 yr (Kislev, 1982; Chaves et al., 2013), and can cause disease epidemics in all wheat growing regions of the world (Roelfs, 1985; McIntosh and Brown, 1997). Rust pathogens have the ability to evolve within a short period of time to overcome plant resistance. A recent example is the emergence of the highly virulent Pgt race TTKSK (also

Abbreviations: AM, association mapping; BLAST, Basic Local Alignment Search Tool; BLUE, best linear unbiased estimate; COI, coefficient of infection; coi.BLUE, best linear unbiased estimate of stem rust field coefficient of infection trait; GWAS, genomewide association study; IT, infection type; it.BLUE, best linear unbiased estimate of stem rust field infection response trait; IWGSC, International Wheat Genome Sequencing Consortium; KASP, Kompetitive allele specific polymerase chain reaction; LD, linkage disequilibrium; LOESS, locally weighted polynomial regression; MAS, marker assisted selection; MLMM, multilocus mixed model; PCA, principal component analysis; Pgt, wheat stem rust fungus Puccinia graminis; QGLM, generalized linear model was investigated with a population structure adjustment; QK, a genomewide association study method accounting for both population structure and kinship; QQ, quantile-quantile; QTL, quantitative trait locus; sev.BLUE, best linear unbiased estimate of stem rust field severity; SNP, single-nucleotide polymorphism.
known as Ug99) and related races in East Africa (Jin et al., 2008; Newcomb et al., 2016). The Ug99 race group can infect ~90% of the world’s currently grown wheat acres and poses a serious threat to world wheat production (Singh et al., 2011; Singh et al., 2013). The most economical and environmentally sustainable way to control stem rust is through the development of resistant wheat varieties. So far, >60 genes conferring resistance to stem rust have been characterized and named (McIntosh et al., 1995; McIntosh et al., 2013; Yu et al., 2014). Many of these genes are race specific, meaning that the resistance they confer could become ineffective within a short period of time as a result of the frequent emergence of more virulent races of the pathogen. Gene pyramiding of multiple resistance genes might prove to be more durable (Mago et al., 2010).

In recent years, quantitative trait locus (QTL) mapping and tagging major genes with DNA markers have resulted in new tools for rust resistance breeding. Single-nucleotide polymorphisms (SNPs) have become a reliable choice for enriching genetic maps and developing markers for marker assisted selection (MAS) (Semagn et al., 2014). The use of high-density SNP markers to characterize resistance alleles in breeding populations provides breeders with a set of tools to conduct MAS and enhance the development of resistant varieties. One way to efficiently characterize resistance alleles in diverse germplasm is through association mapping (AM), also known as linkage disequilibrium (LD) mapping (Zhu et al., 2008). The rapid advancement of high-density marker platforms as well as AM model improvements have made genomewide association studies (GWAS) a powerful tool for characterizing marker-trait relationships. The resulting SNPs from GWAS analysis could potentially be used to develop markers with diagnostic haplotypes for target genes. Accessions with a high number of favorable alleles could be directly used as potential sources of disease resistance in breeding programs. Moreover, with recent advances in molecular breeding, the SNPs discovered with GWAS could potentially be incorporated into genomic selection models to accelerate crop trait prediction and selection (Rutkoski et al., 2011, 2012; Spindel et al., 2015, 2016).

Various successful GWAS analyses on wheat rust resistance have been conducted (Bajgain et al., 2015b; Maccaferri et al., 2015; Bulli et al., 2016; Gao et al., 2016; Turner et al., 2016b). Multiple GWAS studies have assessed stem rust resistance in the International Maize and Wheat Improvement Center germplasm (Crossa et al., 2007; Yu et al., 2011, 2012). Zhang et al. (2014) employed mostly simple sequence repeat markers to detect stem rust resistance alleles in a collection of US winter wheat and identified loci potentially representing stem rust resistance genes Sr6, Sr24, Sr31, Sr36, Sr38, and Sr1RS Time. Bajgain et al. (2015b) employed the recently available wheat 90K SNP chip to identify resistance loci in a collection of North American spring wheat breeding lines and cultivars and detected genes Sr2, Sr7a, Sr8a, and Sr11, as well as other potentially novel loci. Similarly, GWAS of wheat stem rust resistance has been completed for Canadian wheat germplasm (Singh et al., 2013). However, to the best of our knowledge, no GWAS study has been conducted for stem rust resistance in a global spring wheat germplasm collection with entries from all major wheat growing regions. In addition, most of the AM studies for rust resistance primarily employed the QK method, a mixed model that accounts for both population structure (Q) and kinship (K) within populations (Zhang et al., 2014; Bajgain et al., 2015b; Maccaferri et al., 2015). All associations in QK analysis are based on single marker tests. Segura et al. (2012) suggest that the multilocus mixed model (MLMM) could outperform the QK method, especially for traits controlled by several large effect loci. Compared with single marker analysis, MLMM analysis could lead to higher power and lower false discovery rates by factoring (causative) genomewide loci into stepwise regression analysis (Segura et al., 2012).

The current study includes an approximately 10-fold greater number of breeding lines than previous stem rust AM studies (Yu et al., 2011, 2012; Zhang et al., 2014; Bajgain et al., 2015b). This larger population studied could potentially boost our power to detect rare resistance alleles. The goals of this study were to characterize the stem rust resistance alleles present in a global spring wheat collection, and to identify novel stem rust resistance loci that could potentially be used in molecular breeding efforts. We also explored the use of the more recent MLMM model for detecting marker-trait associations. This study is expected to broaden our understanding of stem rust resistance and assist breeders in developing wheat cultivars with effective and durable stem rust resistance.

**MATERIALS AND METHODS**

**Plant Materials**

As part of the Triticeae Coordinated Agricultural Project, 3042 spring wheat accessions were assembled by the USDA-ARS Small Grains and Germplasm Research Unit. Of these accessions, 2188 were genotyped using the wheat iSelect 9K SNP array (Cavanagh et al., 2013). After quality filtering on the basis of 9K genotyping results (see genotyping section for details), a total of 2152 accessions were retained for subsequent analysis. The 2152 accessions represented a worldwide collection of spring wheat from 108 countries located in four continents: Europe (22.1%), Asia (33.5%), Africa (20.1%), and the Americas (21.8%). The GWAS panel was composed of 489 breeding lines, 422 cultivars, 383 cultivated materials, 9 genetic stocks, and 849 landraces (Supplemental Table S1).

**Stem Rust Phenotyping**

The 3042 accessions were grown under greenhouse conditions and inoculated after full emergence of the primary leaf 7 to 10 d
after, planting according to Rouse et al. (2011), in five independent tests of *Pgt* races BCCBC (isolate 09CA115-2), TRTTF (06YEM34-1), TTKSK (Ug99, 04KEN156/04), TTTTF (01MN84A-1-2), and a race mixture using six US *Pgt* races also used in field inoculations: MCCFC (59KS19), QFCSC (06ND76C), QTHJC (75ND717C), RCRSC (77ND82A), RKQQC (99KS76A), and TPMKC (74MN1409). Readers are referred to a list of publications discussing the virulence and avirulence formulae of these isolates, including recently emerged *Pgt* races and their variants (Rouse et al., 2011; Olivera et al., 2012; Singh et al., 2015; Bhattacharya 2017).

Seedling infection types (IT) were scored 12 to 14 d after inoculation according to the 0–to-4 scale developed by Stakman et al. (1962). The phenotype data were converted to a linearized 0–to-9 scale using a custom Perl script (Gao et al., 2016).

For the field studies, the accessions were planted in single rows in the field and inoculated with the *Pgt* race mixture (see above) ~1 mo after planting when the entries started to tiller (Feeke’s Growth Stage 2–3) (Large 1954). Phenotyping was conducted ~1 mo after inoculation. Disease severity and infection response were recorded according to Rouse et al. (2011) in four different years (2011–2014) at one location (St Paul, MN). For the first and fourth year, only about half of the 2152 accessions (982 for Year 1 and 1170 for Year 4) were evaluated, since the Triticeae Coordinated Agriculture Project panel was expanded in 2012. The phenotypic data were converted to three measures (severity, infection response, and coefficient of infection [COI]) using a separate custom Perl script designed for field data conversion (Gao et al., 2016). The field phenotypic values were further adjusted based on a mixed linear model, with environments as random effects, and genotypes as having fixed effects. Best linear unbiased estimates (BLUEs), calculated using the “lme4” package of the open source statistical language or software environment R (R Core Team 2015) were used to represent genotype performance across different environments. Estimated stem rust disease phenotypes for 2152 accessions are listed in Supplementary Table S1.

**DNA Extraction, Genotyping Platform, and Reference Linkage Maps**

Genomic DNA was extracted from seedling plants using the cetyl trimethylammonium bromide (CTAB) method as described in Maccarèrì et al. (2015) and genotyped with the Illumina’s iSelect 9K SNP array at the USDA-ARS Biosciences Research Laboratory in Fargo, ND. Out of the 2188 accessions, 2152 were retained for GWAS after removing accessions with >10% missing data. A total of 6223 SNP markers were retained for GWAS after filtering based on minor allele frequency (≥0.05) and missing data percentage (≤10%). For map positions of the filtered SNPs, we used the published consensus map for the wheat 9K SNP array (Cavanagh et al., 2013) with reversed orientations of chromosomes 4A, 5A, and 5B, as reported in Maccarèrì et al. (2015). Additionally, based on comparative alignment to the published wheat 90K SNP map (Wang et al., 2014) and Basic Local Alignment Search Tool (BLAST+ command line version 2.2.28) hit information on International Wheat Genome Sequencing Consortium (IWGSC) reference genome contigs (Consortium 2014), we joined fragmented linkage groups with a spacing of 20 cM for linkage groups 3D (3D1, 3D2, and 3D3), 5D (5D1cult [reversed], 5D2cult, and 5D3cult) and 6D (6D1 and 6D2 [reversed]). A final version of the modified 9K consensus map used in this study, as well as comparisons with the wheat 90K map and IWGSC contigs based on BLAST hits, are listed in Supplemental Table S2.

We also genotyped our GWAS panel using known markers linked to *Sr2*, *Sr36*, and *Sr28*. Two Kompetitive allelic specific polymerase chain reaction (KASP) assays wMAS000005 (Sr2) (Mago et al., 2011) and wMAS0000015 (Sr36) (MAS Wheat, 2016), along with an agarose gel based marker WSUSr28 for *Sr28* (forward: ACCCCATTTTGCAAGGTGAAA, reverse: TTTCCGAGAATCCACAGGCA, with resistant allele product of 500 bp and susceptible allele product of 400 bp; Bagijn et al., 2015b), were used in the genotyping. The approximate positions of *Sr36* and *Sr28* were estimated at 70 and 240 cM on chromosome 2B based on published studies (Tsilo et al., 2008; Babiker et al., 2017). To illustrate, IWB55504, a 90K SNP marker (not included in 9K consensus map) associated with *Sr28* (Babiker et al., 2017), is located at 134.5 cM on the 90K map (Wang et al., 2014). We can estimate the equivalent 9K position to be around 240 cM based on the collinear 9K and 90K relationships established in Supplemental Table S2. The differences of 9K and 90K positions are due to scaling differences. This study adopted the modified 9K consensus map positions (Supplemental Table S2; Cavanagh et al., 2013) instead of the 90K map positions, since the published 90K map (Wang et al., 2014) did not incorporate all of the 9K loci into consensus order. The recently developed KASP assay for *Sr28* (Babiker et al., 2017) was not available at the time when our experiments were performed. Gene *Sr2* is located at ~15 cM on 3B in the 9K map. The markers wMAS0000005, wMAS0000015, and WSUSr28 were used as positive controls in our GWAS analysis and were not subject to minor allele frequency filtering steps.

**Population Structure, Kinship, and Linkage Disequilibrium Analysis**

To obtain highly informative and nonredundant SNPs for population structure analysis, the SNP marker data were pruned using PLINK software v1.9 (Purcell et al., 2007). The command line option for pruning was “--indep-pairwise 100 5 0.2.” A total of 1532 markers were retained after pruning. The pruned set of SNPs were used for population structure (Q) analysis using a model-based clustering method in STRUCTURE v2.3.4 (Pritchard et al., 2000) and a principal component analysis (PCA) method in R. The PCA plots were color coded according to STRUCTURE results of membership assignments. For the model-based structure analysis, a total of 10 independent runs were conducted for each specified K (number of subpopulations), with 25,000 burn-in length and 50,000 Markov chain Monte Carlo iterations. The most likely number of clusters (K) was chosen based on the ΔK method (Evanno et al., 2005), implemented in a web-based informatics tool “Structure Harvester” (Earl and vonHoldt, 2012). CLUMPP (Jakobsson and Rosenberg, 2007) software v1.1.2 was used to consolidate STRUCTURE runs to derive the Q matrix used in AM. A kinship matrix (K) for line relatedness was calculated in TASSEL (Bradbury et al., 2007) according to the scaled (or centered) identity–by-state method using the whole set of SNP markers that passed quality filtering. Pairwise marker LD was
also calculated using TASSEL v4.3.13. The LD decay curves were fitted for each subgenome (A, B, or D) separately using a locally weighted polynomial regression (LOESS) function in R. Similar to previous studies (Pasam et al., 2012, Gao et al., 2016), here we used a LD $r^2$ value of 0.2 as a critical level for defining LD decay.

**Marker-Trait Association Analysis Based on Mixed Linear Models, Generalized Linear Models with Population Structure as a Covariate, and a Multilocus Mixed Model Approach**  
To examine the genetic basis of stem rust resistance in this panel, a QK-based unified mixed linear model was first applied with population structure estimates (Q) included as fixed effects and kinship (K) as random effects (Yu et al., 2006). Additionally, the use of a generalized linear model was investigated with a population structure adjustment (QGLM) method using TASSEL v4.3.13 (Bradbury et al., 2007). The model components for QK analysis were previously described (Bernardo, 2010; Gao et al., 2016). The MLMM (Segura et al., 2012) was then applied using a set of R functions (MLMM software, 2012) open sourced by Segura et al. (2012). This method applies a stepwise MLMM regression in structured populations (kinship and population structure can be accounted simultaneously). The maximum number of steps of MLMM analysis was set at 10. Optimal results of MLMM were determined based on Bonferroni corrections.

Marker-trait association test $p$-values, $R^2$, and marker effects were extracted from TASSEL runs or MLMM analysis. For multiple test correction of QK results, we first explored the use of simpleM method (SimpleM software, 2008) (Gao et al., 2008, 2010, 2016; Hirsch et al., 2014) to denote significance of marker-trait associations. Stepwise regressions (Cantor et al., 2010; Gao et al., 2016) were performed on loci detected based on the simpleM threshold. However, to be consistent in multiple test correction methods between different models (QK vs. MLMM), we later adopted the Bonferroni method (0.05/6226 = 8.03 x 10^{-6}) to denote significance of marker-trait associations. The $R^2$ values from LD analysis were used in conjunction with genetic distances to assign cosegregating or adjacent significant markers into a unique QTL block.

**QTL-Tagging SNPs, Multiple Regressions, and Gene Postulations**  
The most significant marker for each LD block or QTL region was selected to represent each QTL. Resistance-associated allele frequencies in the GWAS population or subpopulations were counted for the most significant markers of each QTL region. The multiple regression method was further employed to calculate combined effects (or the percentage of variation explained) of multiple markers (QTLs). Loci postulations were conducted primarily based on marker positions (including BLAST hit contig arm assignments) and existing knowledge of IT patterns for different $Pgt$ races.

**RESULTS**  
**Population Structure Analysis**  
The 2152 accessions used for GWAS analysis in this study were composed of breeding lines, cultivars, cultivated lines, genetic stocks, and landraces from 108 countries (Fig. 1). The model-based structure analysis revealed that the panel can be divided into two subpopulations based on the Evanno $\Delta K$ method. Whereas most of European and American accessions were grouped into Subpopulation 1, Subpopulation 2 consisted of accessions predominantly from Iran and India, with few accessions from other Asian and African countries. (Fig. 1 and 2). The presence of two subpopulation groups in the panel was also confirmed by PCA, suggesting that the use of a QK model with either PCA or Q as a covariate is expected to produce consistent results, as previously revealed in a similar study (Gao et al., 2016). In this study we used the $Q^2$ matrix from the model-based population structure analysis for correcting population structure in GWAS analysis.

**Seedling and Field Stem Rust Disease Phenotypes**  
The responses of seedling plants to single $Pgt$ races were generally skewed toward susceptible types (Fig. 3). A higher frequency of resistant or immune accessions (>300) were observed for response to race BCCBC (Fig. 3). The field response of adult plants to the $Pgt$ race mixture (as measured by percentage severity and COI) showed near-normal distributions (Fig. 3). The phenotypic correlations among various field and seedling phenotypes are significant, with race TTKSK as the exception (Table 1). Seedling responses to race TTKSK had lower correlations with responses to other races (Table 1). Among the three field traits (BLUEs for infection response [it.BLUE], stem rust field severity [sev.BLUE], and COI [coi.BLUE]), the field infection response type (it.BLUE) has the highest correlations to seedling ITs (R = 0.59 for race.mix-it. BLUE [linearized seedling rust scores in response to race mixture inoculations and adult plant response type in the field] and R = 0.6 for BCCBC-it.BLUE [linearized seedling rust scores in response to race BCCBC and adult plant response type in the field] (Table 1).

Correlation and heritability analysis for field stem rust disease phenotypes revealed that the year-to-year correlations for adult plant stem rust traits were highly significant ($p < 2.2 \times 10^{-16}$) and the heritability values for these traits were fairly high. The Pearson’s correlations are 0.61, 0.68, and 0.71, and the heritability values are 0.82, 0.84, and 0.89 for the three traits (severity, COI, and IT), respectively. These values are comparable with those for stripe rust (caused by $Puccinia striiformis$ Westend.) GWAS studies on global spring or winter wheat germplasm collections (Maccalferri et al., 2015; Bulli et al., 2016).
After quality filtering, a total of 6226 markers were retained for GWAS analysis. These markers spanned a genetic distance of 3603 cM based on the modified 9K consensus map (Supplemental Table S2). The average number of markers per centimorgan was 1.99 and 2.12 for the A and B genomes, respectively. In contrast, the average number of markers for the D genome was 0.42 cM\(^{-1}\) (or one marker every 2.4 cM). Though the D genome

**Genomewide Marker Coverage and Linkage Disequilibrium**

Analysis of variance and multiple test comparison analysis of sev.BLUE revealed that the breeding lines and genetic stocks displayed the highest rust resistance levels (26.4 on a 0–100 scale), followed by cultivars (35.4), landraces (38.6), and cultivated materials (41.4). Within each subpopulation, however, the differences between cultivars and landraces were not statistically significant based on Tukey’s honest significant difference test. Consistent results were also observed for it.BLUE and coi.BLUE traits.

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**Fig. 1.** Country-specific distributions of population structure subgroups of the worldwide panel of 2152 spring wheat accessions. Pie charts show the percentage subpopulation (sub.pop) compositions for given countries.

**Fig. 2.** (a) Four major continental origins of accessions used in this study, visualized in red (Africa), blue (America), green (Asia), and yellow (Europe) colors for each subpopulation (sub.pop). Most of European and American accessions belong to Subpopulation1, whereas more than half of Subpopulation 2 consists of accessions collected from Asia with some from Africa. (b) Two subpopulations derived from Structure v2.3.4 analysis, visualized (red and blue) on principal component (PC) analysis plot.
Fig. 3. Disease distributions for field and seedling traits. (a–c) Field disease distributions measured by percentage severity, coefficient of infection, and linearized (0–1) infection response type (see Gao et al. 2016 for details on conversions). (d) Seedling disease distributions for the race mixture. (e–h) Seedling response distributions for single races: BCCBC, TRTTF, TTTTF, and TTKSK. The phenotype distributions in response to single races are skewed toward susceptibility (with BCCBC as a possible exception, with additional frequencies for immune types). sev.BLUE, best linear unbiased estimate of stem rust field severity; coi.BLUE, best linear unbiased estimate of stem rust field coefficient of infection trait; it.BLUE, best linear unbiased estimate of stem rust field infection response trait.

Table 1. Correlations among field and seedling stem rust phenotypes. Diagonal lower left side shows Pearson’s correlation $R$ values. Diagonal upper right side denotes the $p$-values for each correlation.

<table>
<thead>
<tr>
<th>Traits†</th>
<th>sev.BLUE</th>
<th>coi.BLUE</th>
<th>it.BLUE</th>
<th>Race.mix</th>
<th>BCCBC</th>
<th>TRTTF</th>
<th>TTTTF</th>
<th>TTKSK</th>
</tr>
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<tbody>
<tr>
<td>sev.BLUE</td>
<td>1</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$6.11 \times 10^{-6}$</td>
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</tr>
<tr>
<td>coi.BLUE</td>
<td>0.98</td>
<td>1</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$4.35 \times 10^{-5}$</td>
<td></td>
</tr>
<tr>
<td>it.BLUE</td>
<td>0.77</td>
<td>0.85</td>
<td>1</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$3.10 \times 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>Race.mix</td>
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<td>0.50</td>
<td>0.59</td>
<td>1</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$2.44 \times 10^{-2}$</td>
<td></td>
</tr>
<tr>
<td>BCCBC</td>
<td>0.55</td>
<td>0.59</td>
<td>0.60</td>
<td>0.43</td>
<td>1</td>
<td>$&lt;2.2 \times 10^{-16}$</td>
<td>$1.43 \times 10^{-9}$</td>
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<tr>
<td>TRTTF</td>
<td>0.34</td>
<td>0.36</td>
<td>0.40</td>
<td>0.39</td>
<td>0.41</td>
<td>1</td>
<td>$2.2 \times 10^{-16}$</td>
<td>$1.88 \times 10^{-2}$</td>
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<tr>
<td>TTTTF</td>
<td>0.37</td>
<td>0.40</td>
<td>0.46</td>
<td>0.37</td>
<td>0.39</td>
<td>0.39</td>
<td>1</td>
<td>$3.55 \times 10^{-2}$</td>
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<tr>
<td>TTKSK</td>
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<td>0.13</td>
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<td>1</td>
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† sev.BLUE, best linear unbiased estimate of stem rust field severity; coi.BLUE, best linear unbiased estimate of stem rust field coefficient of infection trait; it.BLUE, best linear unbiased estimate of stem rust field infection response trait; Race.mix, linearized seedling rust scores in response to race mixture inoculations.
had the lowest density coverage, this was expected and consistent with previous reports (Cavanagh et al., 2013; Wang et al., 2014; Maccasferri et al., 2015; Gao et al., 2016). Linkage disequilibrium decayed at 7.4 and 4.3 cM for the A and B genomes, respectively, and at 10 cM for the D genome (Fig. 4). The high LD in the D genome practically reduces the number of markers required for association tests. We also observed a moderate to high LD among SNP markers mapped to the paracentromeric or even distal regions of chromosome 2B. For example, Markers IWA2739 and IWA1114, located at 146.6 and 94.01 cM on 2B, respectively, were in high LD ($r^2 = 0.4$), and the two markers wMAS000015 (Sr36) and WSUSr28 (Sr28) were in LD ($r^2 > 0.1$) despite being located on different chromosome arms.

**Marker-Trait Associations Based on QK, QGLM, and MLMM Analysis**

Inclusion of both Q and K into the GWAS analysis in this study significantly reduced the chance for false marker-trait associations. For QK results, the expected $-\log{10}(p)$ values and observed $-\log{10}(p)$ values are close to the diagonal reference ($X = Y$) line on the quantile-quantile (QQ) plot for larger $p$-values (lower left on QQ plot) (Supplemental Fig. S1). The distribution of $p$-values from QGLM analysis skewed upwards in the QQ plot (results not shown), suggesting the presence of more false positives (Type I error). The QK model is generally favored when compared with the QGLM model; therefore, we adopted the $p$-values from QK to determine significance of loci in single marker tests.

For QK analysis, a total of 47 markers were significantly associated with stem rust resistance ($p$-values passing the Bonferroni corrected threshold) (Supplemental Fig. S2). The $p$-values for the 47 marker associations with various traits are listed in Supplemental Table S3. The 47 loci can be further condensed into 11 regions based on LD values of adjacent markers. The 11 regions with significant marker-trait association $p$-values for various traits are listed in Table 2.

For MLMM analysis, a total of 13 QTLs were significantly associated with stem rust resistance to various Pgt races and under seedling or adult plant developmental stages (Fig. 5, Table 3). The MLMM results and QK results often agreed with each other. For example, all of the 11 QK loci were detected in MLMM analysis (Table 3). For specific traits, the exact loci compositions for QK and MLMM models might be different. For example, for the sev.BLUE trait, the QK model detected two genes, Sr2 and Sr36, whereas the MLMM model also detected these two genes but with an additional QTL 2B.3. For the TTTTF trait, the QK model detected 4A.1 and 4A.2 (Sr7a), whereas the MLMM model detected an additional QTL 1B.1A (likely Sr31). For TTKSK trait, the QK model detected Sr36, Sr28, Sr9h, and Sr13, whereas the MLMM model detected Sr28, Sr9h, and potentially novel loci (2B.2, 2B.3).

**Gene Postulations for Field and Seedling Resistance Loci**

Overall, significant loci were detected for seven traits (sev.BLUE, coi.BLUE, it.BLUE, BCCBC, TRTTF, TTKSK, and TTTTF) based on QK analysis. The percentage variation explained by each locus ($R^2$ values) varied between 0.5 and 14.8% (Table 2). Gene names for these loci were postulated (Table 2) based on the 9K consensus map position information (chromosome arms and previously published map locations) and race specificity.

Based on previously published map locations and race specificity, the QTLs identified in this study—4A.1, 6A.1, 2B.4, 2B.5, 2D.1, and 6A.2—likely correspond to the seedling resistance genes Sr7a (Bajgain et al., 2015b; Turner et al., 2016a), Sr8a (Bajgain et al., 2015b; Dunckel et al., 2015; Guerrero–Chavez et al., 2015), Sr9h (Rousé et al., 2014; Babiker et al., 2016), Sr28 (Rousé et al., 2012; Babiker et al., 2017), Sr6 (Tsiloi et al., 2010), and Sr13 (Simons et al., 2011), respectively. The QTLs 4A.1, and 6A.1 explained 6.0 and 7.5% of the observed phenotypic variance in response to the races TTTTF and TRTTF, respectively. QTLs 2B.4 and 2B.5 accounted for 3.8 and 14.8% of the genetic variance, respectively, in response to TTKSK (Ug99); and 2B.2 and 2D.1 accounted for 3.4 and 2.5% of the observed variations, respectively, in response to BCCBC.

For field resistance, two loci (likely Sr2 and Sr36) were consistently detected for different traits (sev.BLUE, coi.BLUE, and it.BLUE) (Table 2). The presumed Sr2 and Sr36 loci explained 7.6 and 3% of the variation for the sev.BLUE trait, respectively (Table 2). The percentage of the variation explained was fairly similar for coi.BLUE (7.1 and 2.2%).

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**Fig. 4.** Genome wide linkage disequilibrium (LD) decays based on 9K genotyping results. The LD decayed rapidly for the A and B genomes, but slower for D genomes. The orange bar indicates LD decay level, $r^2 = 0.2$. 

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**TABLE 2**

<table>
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<tr>
<th>Trait</th>
<th>QK Loci</th>
<th>MLMM Loci</th>
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<td>Sr28, Sr9h, Sr13</td>
</tr>
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<td>Sr7a</td>
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<tr>
<td>It. BLUE</td>
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<tr>
<td>TTTTF</td>
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**TABLE 3**

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<tr>
<td>TTTTF</td>
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**TABLE S2**

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### Cumulative Effects of Most Significant Loci

Under multiple regression models, the most significant markers altogether (Table 2) explained 12.1, 11.5, 18.5, 16.4, 24.6, 19.2, and 27.4% of the phenotypic variation for the seven traits (sev.BLUE, it.BLUE, coi.BLUE, BCCBC, TRTTF, TTTTF, and TTKSK, respectively), after correcting for population structure.

The number of resistance-associated marker alleles and disease resistance levels were correlated for each trait, with accessions having higher numbers of resistance-associated alleles observed as generally more resistant. For example, the correlation between the number of resistance-associated alleles and sev.BLUE, coi.BLUE, and it.BLUE phenotypic values are $r = 0.41$, 0.40, and 0.36, respectively. The $p$-values for these correlation tests are all highly significant ($p < 0.001$). Accessions with multiple resistance-associated alleles could serve as important breeding material. For example, we identified lines with both of the resistance-associated alleles for TRTTF resistance.
We also identified 49 and 34 lines that have three or more of the resistance-associated alleles for BCCBC and TTKSK resistance (Supplemental Table S4). Considering the 13 QTLs together (regardless of traits), we were able to identify >100 accessions with six to eight resistance-associated alleles (Supplemental Table S5). These accessions could be used as valuable sources of resistance to stem rust, including the TTKSK race, in breeding and/or genetic studies.

For MLMM analysis, loci were identified for each of the traits sev.BLUE, it.BLUE, coi.BLUE, Race.mix (linearized seedling rust scores in response to race mixture inoculations), BCCBC, TRTTF, TTTTF, and TTKSK. These loci explained 18.5, 25.9, 17.4, 4.5, 13.8, 34.8, 29.5, and 38.6% of phenotypic variation for each trait, respectively. The cumulative $R^2$ values were generally higher than the cumulative effects of the loci detected by QK analysis (see above).

**DISCUSSION**

**Population Structure and Its Impact on Stem Rust Resistance**

Here, we classified two primary subpopulations among the global spring wheat diversity panel: Asia and the West. These results coincide with several other studies that document the population genetics of worldwide wheat collections (Maccaferri et al., 2015; Bulli et al., 2016; Mihalyov et al., 2017). The STRUCTURE results possibly reflect the domestication and distribution history of modern wheat. Domestication started in West Asia, followed by spread to Europe, Africa, and the Americas. Extensive selections, mutations, and enrichment of favorable alleles followed the spread. New adapted varieties and breeding lines formed distinct population groups that differed from the Asian group.

The two subpopulations were associated with different stem rust resistance levels ($p < 0.001$). The phenotypic variances explained by population structure ranged from 0.8 to 26.2% for various traits, with TTKSK being the lowest and BCCBC being the highest. The average stem rust ratings for Subpopulation 1 (Europe and America) were lower than those for Subpopulation 2 (Asia), by 10.8 on a 0-to-100 scale for sev.BLUE, and by 1.36 on a 0-to-9 scale for seedling plants against Race.mix. It can be speculated that the higher resistance of Subpopulation 1 might be related with the fact that the races used in the race mixture in this study are North American races, and breeding lines in Subpopulation 2 (Asia and Africa) have not been selected for resistance to these races. Interestingly, for resistance to race TTKSK (Ug99), the Subpopulation 2

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Fig. 5. Manhattan plots based on the multilocus mixed model for (a) race mix traits (including best linear unbiased estimated field severity [sev.BLUE], coefficient of infection [coi.BLUE], and response type [it.BLUE]) and (b) single races BCCBC, TRTTF, TTKSK, and TTTTF.
were measured by rust pustule size, shape, and the degree both field infection response (it.BLUE) and seedling ITs (0.59, 0.60) (Table 1) likely reflects the biology that response type shows the highest correlations to seedling which are more quantitative in nature and likely controlled by many loci with small effects. The observation that infection response type shows the highest correlations to seedling ITs (0.59, 0.60) (Table 1) likely reflects the biology that both field infection response (it.BLUE) and seedling ITs were measured by rust pustule size, shape, and the degree of chlorosis in the surrounding area.

### Extent of Linkage Disequilibrium and QTL Block Delineation

This study fitted LD decay curves using the LOESS function in R. The LOESS function returns fitted \( r^2 \) values and ranged from 0 to 0.32 for the A genome, 0 to 0.26 for the B genome, and 0 to 0.43 for the D genome. The selection of \( r^2 \) of 0.2 as the critical decay level was employed in various studies (Pasam et al., 2012; Gao et al., 2016) and deemed appropriate in the current study.

Interestingly, a cluster of QTLs or genes on chromosome 2B (2B.1 on Sr36; 2B.2, 2B.3, and 2B.4 on Sr9h; and 2B.5 on Sr28) were detected that confer resistance to various traits including sev.BLUE, coi.BLUE, BCCBC, and TTKSK (Table 2 and 3). Huang et al. (2012) discovered that the introgression of Sr36 generates segregation distortion throughout the chromosome. This could be the cause of long range LD observed for QTL 2B.3 (94.01–146.6 cm) (Table 2 and 3). Quantitative trait loci on wheat chromosomes other than 2B generally comprised compact or sharply delineated LD blocks.
**Genomewide Marker Trait Association Analysis**

In this study, the wheat 9K SNP array and 2152 spring wheat accessions were used for GWAS analysis of stem rust resistance. First, we employed single marker tests using the QGLM and QK models, then we applied multiple regressions to examine cumulative effects of loci. We identified a total of 47 markers that were significantly (passing Bonferroni multiple-test correction threshold) associated with stem rust resistance to various races at the seedling stage and to a mixture of races in the field at the adult stage. The 47 markers can be collapsed into 11 QTL regions (Table 2) located on five chromosomes including 2B, 2D, 3B, 4A, and 6A. Interestingly, we were not able to detect loci effective for seedling response to a combination of six mixed races. This could be due to the p-value threshold being too stringent, or the race specificities of the races included in the mixture did not overlap.

Researchers have argued that correcting for marker effects based on both population structure and kinship could be overly conservative and might result in a need for relaxed p-values such as 0.001 or alternative ways to correct for background variations (Pasam et al., 2012; Bernardo, 2013; Zegeye et al., 2014). As an exploratory analysis, we lowered our significance threshold to the level determined by the simpleM method (Gao et al., 2008, 2010, 2016; Hirsch et al., 2014). This resulted in the detection of 24 QTLs in 12 chromosomes, with potentially novel loci on eight chromosomes. The QTLs were for all eight traits including the mixed-race seedling resistance, which was previously undetected using the more stringent Bonferroni threshold. The majority of the QTL regions associated with the field race mixture traits (sev.BLUE, coi.BLUE, it.BLUE, and Race.mix) are overlapping, whereas resistance loci associated with individual races are mostly nonoverlapping (Supplemental Fig. S3).

Using the Bonferroni correction threshold, the MLMM method generally agreed with the QK method for all the traits. All 11 QTLs identified through QK analysis were also identified in MLMM analysis. In general, the MLMM method identified a higher number of significant loci, including two new QTL regions (1B.1A and 4A.m1) (Table 2 and 3) that were not detected by the QK method. The cumulative effects of these significant loci for each trait were higher than those detected by the QK method. Gene Sr2 appeared to be the most significant locus for field resistance both in QK and MLMM analyses. The differences in exact QTL compositions between QK and MLMM models could reflect the key differences between single marker analysis and stepwise regression as implemented in MLMM, where genomewide loci could serve as conditionals to remove false positives.

**Comparison of Pgt Resistance QTLs with Known Sr Genes**

In the current study, three genes—Sr2, Sr28, and Sr36—were tagged by markers wMAS000005, WSUSr28, and wMAS000015. Worldwide, gene Sr2 has provided adult plant resistance to Pgt races for >60 yr (Ellis et al., 2014). Our study reveals that Sr2 was present in ~5% of the worldwide diversity panel accessions. Importantly, our study also reveals that the majority of the Sr2 accessions (108 of them) possess at least one other resistance-associated allele detected in this study (Supplemental Table S3). The joint use of Sr2 together with other R genes or QTLs could potentially provide high levels of field rust resistance that would be useful for agriculture. Babiker et al. (2017) used the wheat 90K SNP platform to identify markers linked to Sr28. The majority of the markers identified were specific to the 90K platform, thus not directly comparable with the loci identified through this study. Nonetheless, based on approximate collinear relationships between 9K and 90K markers (Supplemental Table S2), we were able to project the position of Sr28 (as well as WSUSr28) to be ~240 cM on the 9K consensus map. Most of the Sr28 positive accessions postulated by Babiker et al. (2017) were not included in this panel except CITR7611, PI268468, PI572693, of which two (CITR7611 and PI572693) were also positive for Sr2 based on WSUSr28 (QTL 2B.5, Supplemental Table S5). Gene Sr36 confers resistance to predominant US wheat stem rust fungus races (Tislo et al., 2008). The current study reveals that Sr36 is present at low frequency (1.4%) (Table 2) in the worldwide diversity accessions.

Zurn et al. (2014) positioned the TTKSK resistance gene SrWLR with several SNP markers including IWA6121. The 9K position for those markers is 205 to 206 cM (Supplemental Table S2), which corresponded to the QTL region of 2B.4 of this study (likely Sr9h, Supplemental Table S3, Table 2 and Table 3). Babiker et al. (2016) used the 90K SNP platform to identify a locus derived from CItr4311, and the map position of that locus and infection patterns suggest that it is Sr9h. One of the markers, IWA7850, identified by Babiker et al. (2016), is shared with the current study, with resistance allele T and susceptible allele C, consistent with Babiker et al. (2016). Guerrero-Chavez et al. (2015) identified a TTKSK resistance locus from hard spring wheat line SD4279, which also corresponded to the QTL 2B.4 region of this study (Supplemental Table S3). Frequent detection of the Sr9 locus through various studies likely suggests its role as an important source for TTKSK resistance. Furthermore, Nuruoka et al. (2016) mapped a stripe rust resistance gene, Yr5, using the wheat iSelect 9K assay to approximately the same region as the Sr9 locus, although the genetic relationships between Yr5 and the Sr9 locus remain to be elucidated.

In addition to the TTKSK resistance locus, Guerrero-Chavez et al. (2015) also identified a 6AS locus effective
against race TTTTF, with linked SNPs including the marker IWA7913, which was also identified in this study (Supplemental Table S3, Table 2 and 3). Consistent with previous reports, we postulate that this locus represents Sr8a. Postulations of the 6AS TTRTF resistance locus (as Sr8a) can also be found in an independent GWAS study of stem rust resistance in North American spring wheat breeding lines (Bajgain et al., 2015b). Approximately 17% (>300 lines) of the worldwide diversity accessions possess the resistance associated allele for Sr8a.

Turner et al. (2016a) used the wheat 9K chip to identify markers linked to Sr7a in the winter wheat cultivar Jagger. Two of the markers identified (IWA4084, IWA4858) were also detected in the current study for TTTTF resistance (Supplemental Table S3). The detection of Sr7a in not only biparental mapping populations, but also a large AM panel, suggests its effectiveness against TTTTF within a wide collection of germplasm. This study detected two adjacent loci for the Sr7a region (4A.1 and 4A.2). The detection of two loci instead of one locus could be related with the relatively high LD threshold (e.g., when compared with Zegeye et al., 2014) used to define QTL regions, or it could potentially indicate two adjacent loci that were jointly responsible for TTTTF resistance.

This study also identified loci that potentially represent genes Sr6 (2D.1) and Sr13 (6A.2). These postulations are based on projected position information based on published studies (Tsil et al., 2010; Simons et al., 2011) and IT patterns. The Sr6 and Sr13 alleles were present in ~13 and 6% of the accessions studied (Table 2). The 2D.1 (Sr6) allele frequency in Subpopulation 1 is comparable with what was discovered in a previous AM study (Zhang et al., 2014). Two EST markers, CD926040 and BE471213, flanking Sr13 (Simons et al., 2011) were mapped to chromosome 6A, between 613 and 616 Mb (IWGSC reference genome v1). Marker IWA7495 (6A.2, Sr13) was mapped to 615 Mb of chromosome 6A. These position results support our gene postulations. The current study could serve as a starting point to integrate various Sr genes into wheat consensus maps.

Some of the loci detected in this study, even when deemed independent by multiple or stepwise regression analysis, were mapped to the same or adjacent QTL intervals. For example, multiple 2B.4 loci (Sr9h) were detected through MLM analysis (Table 3) and were tentatively annotated as Sr9h. It is very common for resistance genes to have multiple alleles. For example, the barley (Hordeum vulgare L.) Mla gene encodes >30 different specificities (Seeholzer et al., 2010). The wheat Sr50 gene holds extensive haplotype diversity (Mago et al., 2015). The wheat Sr9 locus encodes at least eight different alleles. Thus, even though loci deemed independent by multiple regression models were mapped to approximately the same position, they could be in LD with different Sr gene haplotypes.

In addition to known Sr genes, our study also revealed potentially novel loci on wheat chromosomes 2B and 4A (and more if using simpleM threshold). The QTL 2B.2 was located on the short arm of chromosome 2B (close to Sr36, but in different LD blocks) and was detected for it.BLUE and BCCBC traits (Tables 2 and 3). This locus is likely novel based on infection pattern and location information. The QTL 2B.3 was located in the proximal region of 2BS, (Supplemental Table S2, Table 2) and has a moderate effect (0.85 out of 1–9) on TTKSK resistance. This locus is >30 cM proximal (supplemental Table S1) from a recently detected 2B QTL effective against TTKSK (Bajgain et al., 2015a) and potentially represents a new gene. The QTL 4A.m1 was detected only when using the MLMM method (Table 3). This locus was significantly (p < 0.001) associated with resistance against race mixtures and likely represents a novel locus based on location information and IT pattern. As stem rust resistance in the field tended to be controlled by many loci with small effects, one way to employ these novel loci (and known Sr genes) is through inclusion of these QTL-tagging loci in genomic selection models to enable more accurate trait predictions (Rutkoski et al., 2011, 2012; Spindel et al., 2013, 2016; Thavamanikumar et al., 2015).

This study employed the (most) strict Bonferroni correction (p = 8.03 × 10⁻⁶) method to control for family wise error rate and a modest number of loci were detected. Indeed, using a relaxed threshold (e.g., the simpleM method) (Gao et al., 2008, 2016) reveals more loci (Supplemental Fig. S3). However, we preferred to use the stricter Bonferroni threshold to ensure that detected loci represent true loci. With the large germplasm collection and numerous potential historic recombination events present in this panel, the marker density provided by the 9K assay is likely still not large enough to capture all resistance loci. Higher-density genotyping assays such as the wheat 90K SNP chip, as well as genotyping by sequencing (Elshire et al., 2011) technologies could potentially fill this gap. The relatively few number of novel loci detected could be attributed to the limitation inherent in any AM study, namely the lack of capacity to detect rare alleles present in the population (Bernardo 2016).

This study was successful in detecting a number of known genes or QTLs (Sr2, Sr7a, Sr8a, Sr9h, Sr28, and Sr36 etc.). The total number of potentially known loci detected (nine) is greater than most previous stem rust GWAS studies (Yu et al., 201; Zhang et al., 2014; Bajgain et al., 2015b). Characterization of known loci within a large collection of germplasm could potentially provide breeders a rich selection of lines with desired combinations of Sr genes to use in stem rust resistance breeding.
CONCLUSIONS

Our GWAS analysis detected multiple stem rust resistance genes, such as Sr2, Sr6, Sr7a, Sr8a, Sr9b, Sr13, Sr28, Sr31, and Sr36 in 2152 accessions of a global spring wheat panel. Potentially novel resistance loci on chromosomes 2B and 4A were also identified. Most of the identified loci were effective against one or few, but not all, races or effective under one developmental stage (seedling vs. field). The cumulative effects of significant loci explained 4.5 to 25.9% of the phenotypic variation for resistance against race mixtures, whereas the proportion of variation explained for resistance against single races ranged from 13.8 to 38.6%. Loci identified in this study could potentially be converted to uniplex KASP assays to assist high-throughput marker assisted breeding. The accessions with a high number of favorable alleles can be used as important sources of stem rust resistance in breeding programs.

Conflict of Interest

The authors declare that there is no conflict of interest.

Supplemental Material Available

Supplemental material for this article is available online.

Author Contribution Statement

L. Gao performed data analysis steps including phenotype adjustment, population structure analysis, and marker trait associations. M.N. Rouse performed greenhouse and field stem rust experiments. L. Gao performed Sr28 and Sr36 genotyping on the population. P.D. Mihalyov and P. Bulli performed Sr2 genotyping and population SNP calling (wheat 9K array). L. Gao drafted the first manuscript, M.N. Rouse, P.D. Mihalyov, P. Bulli, M.O. Pumphrey, and J.A. Anderson all contributed to the revision and writing of this manuscript. M.N. Rouse, M.O. Pumphrey, and J.A. Anderson conceived the project and provided guidance throughout project development.

Acknowledgments

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References


Bernardo, R. 2010. Breeding for quantitative traits in plants. 2nd ed. Stemma Press, Woodbury, MN.


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