Association mapping of resistance to emerging stem rust pathogen races in spring wheat using genotyping-by-sequencing

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Abstract
The identification and characterization of resistance genes should outpace the rapid emergence of new P. graminis f. sp. tritici races, such as TTRTF and TTKTT, to mitigate stem rust damage to wheat. The objective of the current study was to identify and characterize P. graminis f. sp. tritici race resistance association signals. A total of 250 North American spring wheat lines were evaluated at the seedling stage with a total of seven isolates including TKKTP, TKTTF, TKTTF, TTRTF, TTKSK, and TTKTT. The lines were genotyped by a GBS platform and 9,042 SNPs were used for identification of chromosome regions associated with resistance against the seven isolates. Strong association signals were detected on chromosomes 6BL (Sr11 gene region) and 4AL, likely Sr7a, for resistance against both TKKTP and TKTTF. Similarly, association signals were also detected on chromosomes 4AL (race TTRTF resistance) and 4BS (race TTKSK and TTKTT resistance). Association analysis based on mean phenotypic differences between closely related isolates identified QTL that were not elucidated by direct association mapping of the responses, individually. Overall, with the exception of race TRTTF, each race shared at least one association signal with another race. However, the number of race-specific association signals are larger than that of association signals common among races suggesting the need for identifying and characterizing QTL/genes for newly emerging stem rust pathogen races. There was also high concordance between PCA-based GWAS association signals and association signals from that of both single and multi-locus mixed models.

1 | INTRODUCTION

Wheat stem rust, caused by the fungus Puccinia graminis f. sp. tritici (Pgt), has been a major threat to the production of wheat at the global scale. Although stem rust damage to wheat production can be controlled by chemicals, this...
approach can be difficult to implement in countries with limited access to agricultural chemicals. Alternately, wheat stem rust can be controlled effectively through developing stem rust resistant varieties.

In the past 50 years several stem rust resistance (Sr) genes have been discovered and effectively utilized to control stem rust. For instance, the deployment of gene Sr31 into modern wheat cultivars starting in 1960 successfully and sustainably controlled the disease until the emergence of a new Pgt race named Ug99 (TTKSK; Pretorius, Singh, Wagoire, & Payne, 2000) in Uganda. Stem rust pathogen races in the Ug99 race group threaten global wheat production because they are virulent on the majority of deployed Sr genes that had been effectively utilized for many years (Singh et al., 2006). The Ug99 race group threatens more than 80% of worldwide wheat production (Singh et al., 2011). Moreover, subsequent monitoring of Pgt races in East Africa indicated that members of the Ug99 race group are evolving rapidly, and there are now 13 reported variants, most of which are virulent to genes that were resistant to the original race TTKSK (Jin et al., 2008; Jin et al., 2009; Newcomb et al., 2016). The Ug99 race group has also already spread to countries outside Africa such as Yemen, and Iran, where it is now poised to threaten wheat production in South Asia. Because of the constant evolution and mutation in Pgt, new virulent races that are not in the Ug99 race group have been observed in recent years. Recently, Li et al. (2019) reported that somatic hybridization, and multiple nuclear exchange events between Pgt strains have contributed to the genetic diversity in global Pgt populations.

Race TTKTF that caused a severe epidemic in the South-eastern part of Ethiopia in 2013–2014 is not a member of the Ug99 race group (Olivera et al., 2015). This race was recently reported in several countries including in European countries such as Sweden, Denmark, and Germany (Olivera et al., 2017; Rahmatov et al., 2016). Genes that provide resistance to race TTKTF include Sr7a, Sr11, and Sr31. Other races were reported from Germany including a race TTKKT with virulence to several Ug99-effective genes including SrTmp, Sr24, and Sr1RSamigo (Olivera et al., 2017). Pgt race TRTTF was detected in Yemen in 2006 and subsequently in Ethiopia. It is virulent to wheat stem rust resistance genes such as Sr1RSamigo, Sr36, and SrTmp (Olivera et al., 2012). However, genes such as Sr8a, Sr22, Sr24, Sr26, Sr27, Sr31, Sr32, Sr33, Sr35, Sr39, Sr40, Sr46, Sr47, and Sr50 confer resistance to race TRTTF (Olivera et al., 2012). A race TTRTF (initially reported as TTTTF) caused an epidemic of stem rust on wheat in Sicily in 2016 (Bhat- tacharya et al., 2017). Race TTRTF possesses virulence to Sr13b, a gene common in durum wheat (Zhang et al., 2017), and several other resistance genes (Bhattacharya et al., 2017). The virulence of this race on conventional wheat germplasm has not been reported.

Since the discovery of Ug99, the search for resistance genes has examined both domesticated wheat and its wild relatives. There are 32 numerically designated stem rust resistance genes that are effective to the Ug99 race group (Rouse, Talbert, Singh, & Sherman, 2014b; Singh et al., 2015); Sr2, Sr12, Sr13, Sr14, Sr15, Sr22, Sr25, Sr26, Sr27, Sr28, Sr29, Sr32, Sr33, Sr35, Sr37, Sr39, Sr40, Sr42, Sr43, Sr44, Sr45, Sr46, Sr47, Sr48, Sr50, Sr51, Sr52, Sr53, Sr55, Sr56, Sr57, and Sr58. Of these genes, relatively few are available in conventional North American spring wheat germplasm including Sr2, Sr13, Sr25, Sr26, Sr55, and Sr57 (Bajgain et al., 2015a; Edae, Pumphrey, & Rouse, 2018). Many QTL have also been reported using bi-parental populations (Haile, Nachit, Hammer, Badebo, & Roder, 2012; Hiebert, Fetch, & Zegeye, 2010; Kumsa et al., 2015; Njau, Bhavani, Huerta-Espino, Keller, & Singh, 2013; Zurn et al., 2014; Bajgain et al., 2015b), and association mapping (Bajgain et al., 2015a; Edae et al., 2018; Letta et al., 2013; Yu et al., 2011; Zhang, Bowden, Jin, Carver, & Bai, 2014). This effort of identifying new resistance genes needs to continue in order to mitigate the effects of the constantly evolving pathogen. Therefore, in the current work, we identified chromosome regions associated with emerging races of Pgt using GBS SNP markers.

2 | MATERIALS AND METHODS

2.1 | Plant materials and phenotyping

The spring wheat association mapping panel used in this study was assembled by the Triticeae Coordinated Agricultural Project (TCAP) and consisted of 250 accessions from spring wheat breeding programs in the United States, Canada, and the International Maize and Wheat Improvement Center (CIMMYT) (Edae et al., 2018). We screened
the panel with five non-Ug99 Pgt races including TKKTP (isolate 13GER16-1; Olivera et al., 2015), TKTTF (13ETH18-1; Olivera et al., 2015), TKTTF (13GER10-5; Olivera et al., 2015), TTKTF (06YEM34-1; Olivera et al., 2012), TTRTF (14GEO189-1; Olivera et al., 2019), and Ug99 variants TTKSK (04KEN156/04; Jin & Singh, 2006), and TTKTT (14KEN58-1; Newcomb et al., 2016). In this manuscript we distinguish the two isolates of race TKTTF by an abbreviation for each: TKTTF-ETH (13ETH18-1), and TKTTF-GER (13GER10-5). The races were evaluated in a greenhouse for seedling responses at the USDA-ARS Cereal Disease Laboratory, St. Paul, MN according to methods described by Hundie et al. (2019). Race TTKSK was screened both in the greenhouse under a normal temperature (19–22 °C), and a cold temperature regime (15 °C to 18 °C, night/day, with a 16 h photoperiod) in a growth chamber. Infection types (ITs) were recorded according to the 0–4 scale (Stakman, Stewart, & Loegering, 1962), where “0”, “−”, “1”, and “2” indicate resistance and “3” and “4” indicate susceptibility. Relatively smaller and larger pustule sizes for each IT class were noted with “−” and “+” symbols, respectively. When two or more ITs were present on the same leaf, all ITs were recorded; with the most frequent IT listed first. When a line was heterogeneous for IT, a “/” symbol was used to separate ITs recorded for different plants. The 0–4 scale data were converted to a 0–9 linear scale for subsequent analysis (Gao, Turner, Chao, Kolmer, & Anderson, 2016). For the linear scale, values of 6 or lower indicated resistance, corresponding to 2+ or lower on the 0–4 scale. Each race was evaluated under two replications. The races were assessed on the germplasm between the year 2013 and 2019. The seedling data of each race were subjected to analysis of variance using SAS v. 9.3 software (SAS Institute, 2011). Best linear unbiased Estimators (BLUEs) were used for GWAS analysis.

### 2.3 Population structure and kinship analyses

The pattern of population structure was previously reported for this population (Bajgain et al., 2015a; Edae et al., 2018) using Illumina Infinium iSelect SNP chip for wheat (Illumina Inc., Hayward, CA, USA; Wang et al., 2014). Population structure was also assessed with GBS-based SNPs in the current work using principal component analysis using prcomp command in R (R Core Team, 2016).

### 2.4 Linkage disequilibrium (LD) analysis

Pairwise LD was calculated among 9,042 SNP markers as squared allele frequency correlation (r²) in Synbreed R package (http://synbreed.r-forge.r-project.org/). LD decay curves were also computed for chromosome arms 6BL and 7BL on which strong association signals were detected for resistance against three Pgt races (TTKSK, TKKTP and TKTTF).

### 2.5 Genome-wide association analysis

To identify chromosome regions associated with stem rust resistance, a single-locus mixed linear model (SLMM) (Yu et al., 2006) that was implemented in GAPIT R package (Lipka et al., 2012) was used with a compressed mixed linear model that involved clustering of individuals into groups using clustering algorithms (CMLM) (Li et al., 2014; Tang et al., 2016). The multi-locus mixed model (MLMM) developed by Segura et al. (2012) was used on the same data set to identify the most significant marker-trait associations. The extended Bayesian information criterion (EBIC) was used for model selection criteria in the MLMM. Both SLMM and MLMM analyses were included as a form of validation of the results from both methods as has been effectively used in other studies of wheat stem rust resistance association mapping (Edae et al., 2018; Mihalyov, Nichols, Bulli, Rouse, & Pumphrey, 2017). In addition to validation of results from SLMM, the MLMM proved useful for detecting QTL that would not have been
detected without taking into account the most significant QTL (Mihalyov et al., 2017). Population structure using the first 10 principal components and genetic relatedness among individuals (K) were fitted as covariates in both mixed models except for races TKTTF-GER and TTKSK evaluated under cold temperature for which only population structure was included in the models. This decision was based on how well the model used in GWAS accounts for population structure and familial relatedness using quantile-quantile (Q-Q) plot of p-values. A marker-wise p-value of .0001 was used to declare significant QTL in the SLMM and MLMM. The GWAS analysis was also conducted for mean difference of infection type for race TTKSK under normal temperature and cold temperature, mean difference in infection type between race TTKSK and TTKTT, and mean difference in infection type for TKTTF from Ethiopia (TKTTF-ETH) and TKTTF from Germany (TKTTF-GER). Mean difference in infection type was calculated by determining the difference in BLUE values of the pairs of races for each line.

Principal component analysis was applied to identify loci that are effective against all Pgt races. Phenotypic responses of the test materials for all Pgt races were subjected to Principal component analysis and the scores of each PC were used as a trait in GWAS. The loadings from PCA which represent the correlation between variables and PCs were extracted to understand the relationship between Marker-trait associations (MTAs) detected with each PC and response to the individual Pgt races.

3 | RESULTS

3.1 | Stem rust response

The percentage of resistant lines varied from 4.84% to 8.07% for Ug99 race group variants TTKTT and TTKSK. The percentage of resistant lines for the remaining Pgt races varied from 19.76% (race TTRTF) to 74.19% (TKKTP) suggesting Ug99 race variants are more virulent than non-Ug99 races on conventional North American germplasm (Figure 1). The largest phenotypic correlation was between TTKSK data under normal and cold temperature conditions ($r = 0.81$) followed by the correlation coefficient of race TTKTP with races TKTTF-GER and TKTTF-ETH (Figure 2).

3.2 | Genome-wide marker distribution, population structure and LD analysis

The 9,042 GBS markers were not evenly distributed across the 21 chromosomes of hexaploid wheat. Chromosomes 2B and 7A included the largest number of markers while chromosome 4D had the least number (Supplemental Figure S1). Generally, the D genome chromosomes comprised less markers compared to the A and B genomes. Two groups within the population were previously reported based on 90K SNP data for this mapping panel (Edae et al., 2018). Similarly with GBS markers, there were also two major groups using principal component analysis (PCA; Supplemental Figure S2) representing germplasm from either the northern Great Plains or from western North America including CIMMYT lines. The LD curve crossed the $r^2 = 0.2$ line at a physical distance of 3.8 Mbp for chromosome arm 6BL (Supplemental Figure S3) while it crossed the $r^2 = 0.2$ line at a physical distance of 4.5 Mbp for 7BL (Supplemental Figure S4). A majority of the pair-wise comparisons were weak (less than 0.1) for both chromosomes.
6DL and 7BL as indicated by LD heat maps in Supplemental Figures S5 and S6, respectively.

MTAs identified with the SLMM and MLMM models are given in Tables 1 and 2, respectively. Overall, 64 and 23 MTAs were detected with the SLMM and MLMM, respectively. The MTAs detected by SLMM were visualized comparatively in Figure 3. A majority of the MTAs identified corresponded to four genomic regions: chromosome 1B, likely conferred by Sr31; chromosome 4AL, likely conferred by Sr7a; chromosome arm 6AS, likely conferred by Sr8a; and chromosome arm 6BL, likely conferred by Sr11. These genomic regions conferred race-specific resistance to the expected races. Each of the four regions were detected in both the SLMM and MLMM, providing some confidence in the results. In addition to these four known resistance genes, several QTL were identified to various races including seven QTL validated by both the SLMM and MLMM: chromosomes 2A and 2B, effective to TTRTF; chromosome 7B, effective to TKTTF-GER; chromosome 4B, effective to TTKSK and TTKT; chromosome 7D, effective to TTKT; chromosome 3A, effective to TTKSK and TTKT; and chromosome 5A, effective to TTRTF. The positions and p-values of these QTL can be found in Tables 1 and 2.

3.2.1 Mean infection type difference-based MTAs

For the mean infection type difference between TKTTF isolates from Ethiopia and Germany, strong association signals were detected on chromosome 4AL both with SLMM and MLMM models (Table 3 and 4; Figure 4 track 1). This result is expected since the two isolates differ for virulence to Sr7a, which is located on chromosome arm 4AL. For the difference between TTKSK and TTKTT races, association signals were detected on chromosomes 5AL and 7BL (Table 3; Figure 4 track 2), but only the latter MTA was picked by MLMM (Table 4). A single MTA on chromosome on chromosome 3DL was obtained for the mean difference of infection responses of race TTKSK under normal and cold temperature (Table 3; Figure 4 track 3) but an MTA on chromosome 3AL was found significant with the MLMM (Table 4).

3.2.2 Principal component analysis-based GWAS

We examined whether common stem rust resistance chromosome regions can be identified for the stem rust pathogen races via a PCA-based GWAS approach. The 1st, 2nd and 3rd principal components explained 35.92, 27.87 and 13.45% of the phenotypic variation, respectively (Supplemental Table S1). The remaining principal components each explained < 8% of the phenotypic variation, and the 1st five principal components explained 90.73% of the phenotypic variation. Loading of PCs are given in Supplemental Table S1. The 1st PC showed high loading values in the same direction for all Pgt races except for TRTTF indicating variation in this PC explained resistance against the majority of the races. Similarly, in the 2nd PC with the exception of both TTRTF and TTTRTF all Pgt races had high loadings, but the TKTTF-ETH and TKKTP loadings were in the opposite direction from the other races. In this 2nd PC, loadings of TKTTF isolates from Ethiopia and Germany were high but opposite in direction. This means PC2 captures variation in a composite phenotype that represents lines resistant to the TKTTF isolate from Ethiopia but susceptible to the TKTTF isolate from Germany or, vice versa. PC3 was characterized by high loadings of TTRTF whereas PC4 was characterized by high loadings for race TTKT. PC5 was characterized predominantly by high TTRTF loadings whereas PC7 discriminated the TTKSK normal temperature from that of the TTKSK low temperature as loadings for both temperature conditions were high but in opposite directions (represents variation of lines resistant to TTKSK under normal temperature but susceptible to TTKSK under low temperature, or vice versa).

All significant marker-trait associations (P < .0001) for the total of eight principal components (PCs) are shown in Table 5. Three SNPs on chromosome 1BS, in addition to one SNP on 1DS and 3BL each were significantly associated (P < .0001) with stem rust response PC1 (Figure 5 track 8, track counting is from inside outward). The association signals on chromosomes 1BS (1BS/S1B_PART1_156598900), 1DS for race TTRTF, and 3BL for race TTKSK (under cold temperature) were detected with single-trait GWAS. Similarly, four SNPs on chromosome arm 6BL, and one SNP on chromosome arms 4AL and 7BL each were associated with PC2 (Figure 5 track 7). Association signals on chromosomes 6BL (S6B_PART2_257935127, for race TKTTF-ETH and TTKTP), 7BL (S7B_PART2_293794262 for race TKTTF-GER) and 4AL (for race TTRTF) were also detected with single trait GWAS. A total of 10 and 4 SNPs on chromosomes 1BS and 6AS showed association with PC3 (Figure 5 track 6), respectively. The association signal on chromosome arm 6AS (S6A_PART1_3015737/S6A_PART1_3206675) was detected with single trait GWAS for stem rust resistance against race TRTTF in the region of Sr8a. For PC4, there was only one association signal with SNP SIB_PART2_187509081 (1BL) and it was not detected with single trait GWAS for any race (Figure 5 track 5). Two SNPs on chromosome arm 6AS (SNPs S6A_PART1_3015737 and S6A_PART1_6842176)
**TABLE 1** Marker-trait associations (MTAs) detected for seven *Puccinia graminis* f. sp. *tritici* isolates corresponding to races TRTTF, TTKSK, TKTTF, TRTTF, TKTTF and TTRTF

| Race   | SNP              | Chrom | map pos (Mbp) | P-value     | MAF  | FDR_p-value | R²   |
|--------|------------------|-------|---------------|-------------|------|-------------|------|
| TRTTF  | S1B_PART1_15659890 | 1B    | 15.66         | 1.73×10⁻⁹   | 0.06 | 7.81×10⁻⁹   | 0.127|
| TRTTF  | S1B_PART1_28041285 | 1B    | 28.04         | 7.24×10⁻⁶   | 0.14 | 5.456×10⁻⁵  | 0.07 |
| TRTTF  | S1B_PART1_39577562 | 1B    | 39.58         | 7.79×10⁻⁸   | 0.06 | 7.83×10⁻⁵   | 0.10 |
| TRTTF  | S1B_PART1_53250247 | 1B    | 53.25         | 5.70×10⁻⁸   | 0.05 | 6.44×10⁻⁵   | 0.10 |
| TRTTF  | S1B_PART1_74249006 | 1B    | 74.25         | 4.15×10⁻⁸   | 0.06 | 5.36×10⁻⁵   | 0.10 |
| TRTTF  | S1B_PART1_135449622| 1B    | 115.45        | 1.11×10⁻⁸   | 0.06 | 2.00×10⁻⁵   | 0.11 |
| TRTTF  | S1B_PART1_136168911| 1B    | 136.17        | 7.60×10⁻⁹   | 0.06 | 2.00×10⁻⁵   | 0.12 |
| TRTTF  | S1B_PART1_146796395| 1B    | 146.80        | 1.11×10⁻⁸   | 0.06 | 2.00×10⁻⁵   | 0.11 |
| TRTTF  | S1B_PART1_158545386| 1B    | 158.55        | 1.73×10⁻⁹   | 0.06 | 7.81×10⁻⁶   | 0.13 |
| TRTTF  | S1B_PART1_180406818| 1B    | 180.41        | 9.39×10⁻⁸   | 0.05 | 8.49×10⁻⁵   | 0.10 |
| TRTTF  | S1B_PART1_209129922| 1B    | 209.13        | 2.84×10⁻⁸   | 0.054| 4.28×10⁻⁵   | 0.11 |
| TRTTF  | S1D_PART1_37956518 | 1D    | 37.96         | 7.19×10⁻⁵   | 0.05 | 0.069       | 0.05 |
| TRTTF  | S1D_PART1_105157874| 1D    | 105.16        | 1.20×10⁻⁷   | 0.05 | 9.84×10⁻⁵   | 0.10 |
| TRTTF  | S2A_PART1_29263799 | 2A    | 29.26         | 7.55×10⁻⁵   | 0.34 | 0.045388    | 0.05 |
| TRTTF  | S2B_PART1_23965311 | 2B    | 23.97         | 2.14×10⁻⁵   | 0.09 | 0.01489     | 0.06 |
| TTKSK (NT)| S2B_PART2_324160151| 2B    | 777.38        | 9.81×10⁻³   | 0.08 | 0.102       | 0.06 |
| TTKSK (NT)| S3A_PART2_116010925| 3A    | 570.12        | 5.35×10⁻⁵   | 0.14 | 0.096       | 0.06 |
| TTKSK (NT)| S3B_PART2_188289477| 3B    | 636.44        | 2.64×10⁻⁵   | 0.47 | 0.06        | 0.07 |
| TTKSK (CT)| S3B_PART2_251114407| 3B    | 699.27        | 1.33×10⁻⁶   | 0.48 | 0.012       | 0.09 |
| TKTTF-ETH| S4A_PART2_280264506| 4A    | 732.82        | 1.17×10⁻⁵   | 0.48 | 0.035       | 0.05 |
| TKTTF-ETH| S4A_PART2_280270591| 4A    | 732.83        | 4.26×10⁻⁵   | 0.48 | 0.064       | 0.04 |
| TKTTF-ETH| S4A_PART2_286220305| 4A    | 738.78        | 1.74×10⁻⁵   | 0.27 | 0.036       | 0.05 |
| TKTTF-ETH| S4A_PART2_290053290| 4A    | 742.61        | 2.01×10⁻⁵   | 0.29 | 0.036       | 0.04 |
| TKTTF-ETH| S4A_PART2_290196354| 4A    | 742.75        | 7.85×10⁻⁵   | 0.46 | 0.079       | 0.04 |
| TKTTF-ETH| S4A_PART2_290628489| 4A    | 743.18        | 1.03×10⁻⁵   | 0.28 | 0.035       | 0.05 |
| TTKSK (CT)| S4B_PART1_20568400 | 4B    | 20.57         | 1.28×10⁻⁵   | 0.45 | 0.058       | 0.07 |
| TRTTF  | S4D_PART2_4614810  | 4D    | 4.614         | 5.92×10⁻⁵   | 0.13 | 0.038247    | 0.05 |
| TRTTF  | S5A_PART2_133066686| 5A    | 586.3         | 6.18×10⁻⁵   | 0.06 | 0.069       | 0.05 |
| TRTTF  | S5A_PART2_135170900| 5A    | 588.4         | 6.62×10⁻⁵   | 0.06 | 0.069       | 0.05 |
| TKTTF-ETH| S5A_PART2_138745963| 5A    | 591.98        | 4.98×10⁻⁵   | 0.22 | 0.064       | 0.04 |
| TKTTF-ETH| S5B_PART2_150047392| 5B    | 601.42        | 4.68×10⁻⁵   | 0.44 | 0.339       | 0.06 |
| TTKSK (NT)| S5B_PART2_166247712| 5B    | 617.62        | 2.49×10⁻⁵   | 0.18 | 0.06        | 0.07 |

(Continues)
| Race               | SNP         | Chrom     | map pos (Mbp) | P-value | MAF     | FDR_p-value | R²  |
|--------------------|-------------|-----------|---------------|---------|---------|-------------|-----|
| TTKSK (NT)         | S5B_PART2_194543855 | 5B       | 645.92        | 1.88×10⁻⁵ | 0.45    | 0.06        | 0.07 |
| TTKSK (CT)         | S5B_PART2_227153820 | 5B       | 678.53        | 3.76×10⁻⁵ | 0.48    | 0.113       | 0.06 |
| TRTTF              | S6A_PART1_3015737 | 6A       | 3.02          | 9.73×10⁻¹² | 0.41    | 0           | 0.15 |
| TRTTF              | S6A_PART1_3206675 | 6A       | 3.21          | 1.66×10⁻¹³ | 0.49    | 0           | 0.18 |
| TRTTF              | S6A_PART1_3691352 | 6A       | 3.7           | 9.48×10⁻⁹  | 0.30    | 0           | 0.10 |
| TRTTF              | S6A_PART1_3705925 | 6A       | 3.71          | 7.62×10⁻⁵  | 0.40    | 0.069       | 0.05 |
| TRTTF              | S6A_PART1_5569284 | 6A       | 5.57          | 1.46×10⁻⁷  | 0.33    | 0           | 0.08 |
| TRTTF              | S6A_PART1_6842176 | 6A       | 6.82          | 1.46×10⁻⁹  | 0.46    | 0           | 0.11 |
| TTKSK (NT)         | S6B_PART1_201789705 | 6B      | 201.79        | 9.9×10⁻⁵  | 0.38    | 0.102       | 0.06 |
| TTKSK (NT)         | S6B_PART1_203291041 | 6B      | 203.29        | 1.81×10⁻⁷  | 0.33    | 0.002       | 0.10 |
| TTKSK (NT)         | S6B_PART2_229827112 | 6B      | 681.9         | 8.27×10⁻⁵  | 0.48    | 0.102       | 0.06 |
| TTKSK (NT)         | S6B_PART2_257935127 | 6B      | 710.01        | 7.24×10⁻⁶  | 0.23    | 0.035       | 0.05 |
| TTKTF-ETH          | S6B_PART2_257935127 | 6B      | 710.01        | 1.11×10⁻⁶  | 0.23    | 0           | 0.12 |
| TTKTF-GER          | S6B_PART2_257935127 | 6B      | 710.01        | 2.18×10⁻⁹  | 0.23    | 0           | 0.11 |
| TTKTF-GER          | S6B_PART2_257987924 | 6B      | 710.07        | 2.06×10⁻⁷  | 0.38    | 0           | 0.09 |
| TTKTF              | S6B_PART2_260047154 | 6B      | 712.12        | 7.98×10⁻⁸  | 0.48    | 0           | 0.09 |
| TTKTF-ETH          | S6B_PART2_260047154 | 6B      | 712.13        | 5.01×10⁻⁷  | 0.48    | 0.002       | 0.08 |
| TTKTF-GER          | S6B_PART2_260245119 | 6B      | 712.32        | 6.29×10⁻⁵  | 0.32    | 0.063       | 0.05 |
| TTKTF-GER          | S6B_PART2_262414407 | 6B      | 714.49        | 4.56×10⁻⁶  | 0.43    | 0.006       | 0.07 |
| TTKTF              | S6B_PART2_262414407 | 6B      | 714.49        | 8.97×10⁻⁷  | 0.43    | 0.002       | 0.07 |
| TTKTF-GER          | S6B_PART2_265342488 | 6B      | 717.42        | 2.95×10⁻⁵  | 0.46    | 0.033       | 0.06 |
| TTKTF-GER          | S6B_PART2_265342731 | 6B      | 717.42        | 4.18×10⁻⁶  | 0.29    | 0.006       | 0.07 |
| TTKTF              | S6B_PART2_265342731 | 6B      | 717.42        | 7.69×10⁻⁶  | 0.29    | 0.014       | 0.06 |
| TTKTF-GER          | S6B_PART2_267826895 | 6B      | 719.9         | 2.92×10⁻⁸  | 0.28    | 0           | 0.10 |
| TTKTF              | S6B_PART2_267826895 | 6B      | 719.9         | 3.45×10⁻⁵  | 0.28    | 0.045       | 0.05 |
| TTKTF-ETH          | S7B_PART2_293794262 | 7B      | 745.87        | 6.74×10⁻⁵  | 0.36    | 0.076       | 0.04 |
| TTKTF-GER          | S7B_PART2_293794262 | 7B      | 745.87        | 1.51×10⁻¹⁰ | 0.36    | 0           | 0.14 |
| TTKTF              | S7B_PART2_293794262 | 7B      | 745.87        | 1.39×10⁻⁷  | 0.36    | 0.001       | 0.09 |
| TTKTF              | S7D_PART1_450678111 | 7D      | 45.07         | 8.32×10⁻⁵  | 0.05    | 0.339       | 0.05 |
| TRTTF              | SUN_231626593       | UN       | -             | 8.25×10⁻¹¹ | 0.47    | 0           | 0.13 |

*NT, Normal temperature, CT, Cold temperature; MAF, Minor allele frequency.
| Race       | SNP                   | Chrom | Position (Mbp) | p-value          |
|------------|-----------------------|-------|----------------|------------------|
| TKKTP      | S6B_PART2_257935127   | 6B    | 710.01         | 2.41×10⁻¹⁰      |
| TKKTP      | S6B_PART2_265342731   | 6B    | 710.01         | 6.93×10⁻⁵       |
| TKKTP      | S2B_PART2_133689108   | 2B    | 586.91         | 4.11×10⁻⁶       |
| TKKTP      | S4A_PART2_280264506   | 4A    | 732.82         | 1.84×10⁻⁵       |
| TKTTF-GER  | S7B_PART2_293794262   | 7B    | 745.87         | 3.32×10⁻⁷       |
| TKTTF-GER  | S6B_PART2_257935127   | 6B    | 710.01         | 4.46×10⁻⁶       |
| TKTTF-GER  | S2B_PART2_239396449   | 2B    | 692.62         | 0.00016         |
| TTKSK-CT   | S7A_PART1_28119447    | 7A    | 28.12          | 8.88×10⁻⁸       |
| TTKSK-CT   | S4B_PART1_20568400    | 4B    | 20.57          | 4.23×10⁻⁷       |
| TTKTT      | S4B_PART1_20568400    | 4B    | 20.57          | 4.43×10⁻⁷       |
| TKTTF      | S7D_PART1_42519839    | 7D    | 42.52          | 3.29×10⁻⁸       |
| TKTTF      | S3A_PART2_237279779   | 3A    | 691.38         | 2.65×10⁻⁶       |
| TKTTF_ETH  | S6B_PART2_257935127   | 6B    | 710.01         | 1.34×10⁻⁹       |
| TKTTF_ETH  | S4A_PART2_290628489   | 4A    | 743.18         | 6.39×10⁻⁸       |
| TKTTF_ETH  | S4B_PART2_210617604   | 4B    | 661.63         | 5.69×10⁻⁷       |
| TRTRF      | S6A_PART1_3206675     | 6A    | 3.21           | 7.95×10⁻¹⁰      |
| TRTRF      | S6A_PART1_2408343     | 6A    | 2.41           | 2.39×10⁻¹¹      |
| TRTRF      | S5A_PART2_13570900    | 5A    | 588.4          | 7.22×10⁻⁷       |
| TRTRF      | S6A_PART1_6842176     | 6A    | 6.842          | 1.14×10⁻⁵       |
| TRTRF      | S1B_PART1_15659890    | 1B    | 15.66          | 1.12×10⁻⁸       |
| TRTRF      | S2B_PART1_23965311    | 2B    | 23.97          | 8.15×10⁻⁶       |
| TRTRF      | S2A_PART1_30330014    | 2A    | 30.33          | 1.44×10⁻⁴       |
| TRTRF      | S4A_PART2_268332600   | 4A    | 720.89         | 7.98×10⁻⁵       |
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**Figure 3** Association signals detected for *Puccinia graminis* f. sp. *tritici* races TKKTP, TRTTF, TKTTF-ETH, TKTTF-GER, TTKSK-CT, TTKSK-NT, TTKTT and TTRTF. The track number starts from inside outward with TKKTP assigned to track 1 whereas the last track (track 8) was assigned to TTRTF, respectively. The threshold p-value ($p = .0001$) was indicated by dotted red line for each track showing significant associations with PC$_6$ (Figure 5 track 3) and both associations were shared with PC$_5$. Similarly, PC$_1$ and PC$_3$ shared association signals on chromosome arm 1BS with SNPs 1B_PART1_15659890, S1B_PART1_13616891 and SIB_PART1_158545386. There were three significant associations (two on chromosome arm 3AL and one on chromosome arm 6AL) for PC$_7$ (Figure 5 track 2) while there was only one association signal detected for PC$_8$ (Figure 5 track 1).

**4 | DISCUSSION**

We report that 80% of North American spring wheat advanced breeding lines and cultivars are susceptible as seedlings to emerging *Pgt* race TTRTF. Though this level of susceptibility is lower than that for the Ug99 race group, the broad susceptibility to TTRTF suggests that this race could cause devastating wheat yield losses if it were to arrive in North America. However, we detected modest levels of susceptibility (25.81%) to the new race TKKTP from Germany suggesting that North American spring wheat is not as vulnerable to this race. The abundant resistance to race TKKTP in North American spring wheat is likely conferred by *Sr11* and *Sr7a*.

Race TTRTF is a relatively newly emerged stem rust pathogen race. A key objective of this study was to identify QTL effective to this emerging race. Association signals were consistently detected on chromosome 1BS (15.66 Mbp), 2BS (23.97 Mbp), 2A (30.33 Mbp) and 4AS (268.33 Mbp). Out of these associations, the strongest signal was on chromosome 1BS ($P$-value of $1.12 \times 10^{-11}$). The resistance gene *Sr31* that has been widely used in several breeding programs is located on a 1RS translocation that replaces 1BS. Given that *Sr31* is effective against TTRTF as confirmed by the wheat differential set (Olivera et al., 2019; Wanore, Chala, Shikur, Hodson, & Szabo, 2019), and that lines with *Sr31* are present in the panel (Bajgain et al., 2015a), the resistance against race TTRTF on chromosome 1BS is most likely due to the effect of *Sr31*. Significant
| Response-difference | SNP                         | Chrom | Map position (Mbp) | P-value       | MAF  | FDR_P-value | R² |
|---------------------|-----------------------------|-------|-------------------|---------------|------|-------------|----|
| TKTTF-ETH-GER       | S4A_PART2_290628489         | 4A    | 743.18            | 1.17×10⁻⁷     | 0.47 | 0.00        | 0.07 |
| TKTTF-ETH-GER       | S4A_PART2_290053290         | 4A    | 742.61            | 3.19×10⁻⁷     | 0.47 | 0.00        | 0.06 |
| TKTTF-ETH-GER       | S4A_PART2_281141168         | 4A    | 733.70            | 5.40×10⁻⁷     | 0.50 | 0.00        | 0.06 |
| TKTTF-ETH-GER       | S4A_PART2_280264506         | 4A    | 732.82            | 1.02×10⁻⁶     | 0.48 | 0.00        | 0.06 |
| TKTTF-ETH-GER       | S4A_PART2_280212255         | 4A    | 732.77            | 3.14×10⁻⁶     | 0.50 | 0.01        | 0.05 |
| TKTTF-ETH-GER       | S4A_PART2_280270591         | 4A    | 732.83            | 4.21×10⁻⁶     | 0.48 | 0.01        | 0.054 |
| TKTTF-ETH-GER       | S4A_PART2_289908604         | 4A    | 742.46            | 5.50×10⁻⁶     | 0.47 | 0.01        | 0.05 |
| TKTTF-ETH-GER       | S4A_PART2_290196354         | 4A    | 742.75            | 6.18×10⁻⁶     | 0.46 | 0.01        | 0.05 |
| TKTTF-ETH-GER       | S4A_PART2_290050764         | 4A    | 742.59            | 1.67×10⁻⁵     | 0.46 | 0.02        | 0.04 |
| TKTTF-ETH-GER       | S4A_PART2_286223035         | 4A    | 738.78            | 3.72×10⁻⁵     | 0.50 | 0.03        | 0.04 |
| TKTTF-ETH-GER       | S4A_PART2_271952636         | 4A    | 724.51            | 6.93×10⁻⁵     | 0.37 | 0.07        | 0.04 |
| TTKSK-NT-TTKTT      | S5A_PART2_1531133271        | 5A    | 409.68            | 3.51×10⁻⁵     | 0.08 | 0.27        | 0.07 |
| TTKSK-NT-TTKTT      | S7B_PART2_276983635         | 7B    | 730.81            | 5.68×10⁻⁵     | 0.08 | 0.26        | 0.07 |
| TTKSK-NT-CT         | S3D_PART2_97432482          | 3D    | 573.67            | 3.67×10⁻⁵     | 0.05 | 0.33        | 0.07 |

¹ TKTTF-ETH-GER, difference between TKTTF-ETH and TKTTF-GER; TTKSK-NT-TTKTT, TTKSK under normal temperature and TTKTT; TTKSK-NT-CT, TTKSK under normal and cold temperatures.
Association signals were detected on the short arm of chromosome 1DS both for races TTRTF and TRTTF at different locations. Major known stem rust resistance genes on chromosome arm 1DS are Sr33, Sr45, Sr50 and SrTAI662 (Yu et al., 2014). From cluster analysis of stem rust pathogen isolates using a custom SNP chip by Olivera et al. (2019), race TTRTF is genetically related to race TRTTF as both are in Clade III with TTRTF in sub-clade III-B, and with TRTTF in sub-clade III-A. Although the two isolates had been collected from different regions (TTRTF from Georgia and TRTTF from Yemen), a recent study by Li et al. (2019) indicated that Pgt lineages originated from distinct geographic areas can share a large portion of their genomes. Previously, association signals were reported for resistance against race TRTTF on chromosome 1B (43.9–64.9 cM) by Bajgain et al. (2015a). However, we found no QTL in common between responses to TRTTF and TTRTF that suggests that, even for related isolates, resistance can be quite specific. At the time of writing this manuscript, our result was the first report of chromosome regions associated with resistance to race TTRTF based on association mapping. However, of the QTL found effective to race TTRTF in the current study, QTL on chromosomes 2BS, 2A, and 4AS do not correspond to previously characterized QTL in this germplasm. Thus, these QTL need to be validated before utilization in wheat breeding.

Another objective of this study was to identify chromosome regions associated with resistance against other Pgt races. Resistance against the two closely related races TKTTF and TKKTP was identified on chromosome arm 6BL (~93.32 cM). The major known gene that confers stem rust resistance on chromosome arm 6BL is Sr11 (Nirmala et al., 2016; Sears, 1966), and is effective to both races. These two races, TKTTF and TKKTP, are genetically different from members of the Ug99 race group. Race TKTTF is avirulent to stem rust resistance genes Sr11, Sr24, and Sr31 (Olivera et al., 2015). However, it is virulent on gene SrTmp that is carried by the widely adopted bread wheat variety Digalu whose susceptibility to TKTTF resulted in stem rust epidemics in Ethiopia in 2013–2014. Although the race TKTTF isolate that was obtained from Germany was phenotypically different from the Ethiopian TKTTF isolate (the former is virulent to Sr7a, Sr45 and SrTt-3 genes; Olivera et al., 2017), the association signals detected on chromosome arms 6BL and 7BL were common for both German and Ethiopian isolates of race TKTTF. Previously a significant QTL was reported on chromosome arm 6BL for resistance against the Ethiopian TKTTF isolate in both with GWAS and biparental analysis (Bajgain et al., 2015a; Nirmala et al., 2016). Therefore, markers linked to Sr11 can be used to track resistance against both German and Ethiopian TKTTF isolates in addition to race TKKTP. However, there were association signals specific to

| Marker-trait associations (MTAs) detected for the mean difference in infection type between TKTTF-ETH and TKTTF-GER, TTKSK (NT) and TTKSK (CT) and TTKSK (NT) and TTKSK (CT) using MLMM | SNP Chrom Position (Mb) | P-value | FDR_P-value |
|---|---|---|---|
| TKTTF-ETH-GER S4A PART2 290628489 4A 743.18 | 1.74×10^-8 | 0.00 | |
| TKTTF-ETH-GER S7A PART1 62187944 7A 62.19 | 7.94×10^-5 | 0.36 | |
| TTKSK-NT-TTKTT S7B PART2 276986365 7B 730.81 | 1.29446×10^-5 | 0.12 | |
| TTKSK-NT-CT S3A PART2 249803574 3A 703.91 | 9.80682×10^-5 | 0.64 | |

a TKTTF-ETH-GER, difference between TKTTF-ETH and TKTTF-GER; TTKSK-NT-TTKTT, TTKSK under normal temperature and TTKTT; TTKSK-NT-CT, TTKSK under normal and cold temperatures.
particular isolates. For instance, the association signal on chromosome arm 4AL was consistently detected for response against the Ethiopian isolate of race TKTTF whereas an association signal was detected on chromosome arm 2BL for response to the German isolate. There was also an association signal for race TKTTP (586.91 Mbp) on chromosome 2BL at a different position from that of TKTTF (692.62 Mbp) from Germany. There are at least seven stem rust resistance QTL linked to stem rust resistance on chromosome arm 2BL in addition to major stem rust resistance genes such as Sr9h, Sr28 and Sr47 (Rouse et al., 2014a; Yu et al., 2014). Although no known major gene has been reported on chromosome arm 7BL, stem rust resistance QTLs have previously been reported (Bansal et al., 2008; Yu et al., 2014).

There are chromosome regions associated with resistance against races TKKTP, TKTTF (from Ethiopia) and TTRTF on chromosome 4AL. Although TKKTP and TKTTF (from Ethiopia) QTL shared the same region, the SNP associated with TTRTF resistance is located a minimum of 465 Mbp away from this position. There are at least four stem rust resistance QTL on chromosome 4AL, and Sr7a has been reported on 4AL (Turner, Jin, Rouse, & Anderson, 2016). Sr7a is effective to TKTTF from Ethiopia but not TKTTF from Germany.

Previous mapping work with SNPs and SSR markers located TTRTF resistance on chromosome arm 6AS postulated as Sr8a (Bajgain et al., 2015a; Guerrero-Chavez, Glover, Rouse, & Gonzalez-Hernandez, 2015; Hiebert, Rouse, Nirmala, & Fetch, 2016; Nirmala et al., 2017). Similarly, in the current study, a strong association signal was detected on chromosome 6AS suggesting the involvement of Sr8a as it is the only major stem rust resistance gene present in bread wheat effective to TTRTF on chromosome arm 6AS. There may be other genes that confer TTRTF resistance, but their allele frequency in this mapping panel could be lower than the necessary frequency (>5%) to enable detection. It should be noted that the exclusion

**Figure 4** Association signals detected for *Puccinia graminis* f. sp. *tritici* races with mean difference between TKTTF isolates from Ethiopia and Germany (track 1), mean difference between TTKSK and TTKTF (track 2), and mean difference between TTKSK under cold and normal temperatures (track 3). Track numbering is from inside outward. The threshold p-value (p = .0001) was indicated by a dotted red line for each track.
| SNP/Position | Chrom | Position | FDR_p-value | R² | SNP/Position | Chrom | Position | FDR_p-value | R² |
|-------------|-------|----------|-------------|----|-------------|-------|----------|-------------|----|
| S6B_PART2_257935127 | 1B | 710.01 | 1.35×10⁻⁵ | 0.08 | S6B_PART2_257987924 | 6B | 710.07 | 1.11×10⁻⁵ | 0.08 |
| S6B_PART2_257994262 | 6B | 710.42 | 2.03×10⁻⁵ | 0.06 | S6B_PART1_74249006 | 1B | 608.33 | 2.63×10⁻⁵ | 0.05 |
| S6B_PART2_256342731 | 7B | 710.62 | 3.02×10⁻⁵ | 0.06 | S6B_PART1_3015737 | 6A | 3.02 | 2.63×10⁻⁵ | 0.05 |
| S6B_PART2_256708883 | 1B | 710.07 | 1.11×10⁻⁵ | 0.08 | S6B_PART1_74249006 | 1B | 608.33 | 2.63×10⁻⁵ | 0.05 |
| S6A_PART1_3015737 | 6A | 3.02 | 2.63×10⁻⁵ | 0.05 | S6B_PART2_257935127 | 1B | 710.01 | 1.35×10⁻⁵ | 0.08 |
| S6A_PART2_3015737 | 6A | 3.02 | 2.63×10⁻⁵ | 0.05 | S6B_PART2_257987924 | 6B | 710.07 | 1.11×10⁻⁵ | 0.08 |
| S4A_PART2_269860502 | 4A | 722.42 | 3.55×10⁻⁵ | 0.05 | S6B_PART2_257987924 | 6B | 710.07 | 1.11×10⁻⁵ | 0.08 |
| S4A_PART1_3206675 | 3B | 722.42 | 3.55×10⁻⁵ | 0.05 | S6B_PART2_257994262 | 6B | 710.42 | 2.03×10⁻⁵ | 0.06 |
| S4A_PART2_269860502 | 4A | 722.42 | 3.55×10⁻⁵ | 0.05 | S6B_PART2_257994262 | 6B | 710.42 | 2.03×10⁻⁵ | 0.06 |
| S3B_PART2_94939208 | 3B | 543.09 | 6.65 | 1.43 | S6B_PART2_257994262 | 6B | 710.42 | 2.03×10⁻⁵ | 0.06 |
| S3B_PART2_212174230 | 3B | 660.33 | 1.88×10⁻⁵ | 0.05 | S6B_PART2_257935127 | 1B | 710.01 | 1.35×10⁻⁵ | 0.08 |
| S3B_PART2_94939208 | 3B | 543.09 | 6.65 | 1.43 | S6B_PART2_257935127 | 1B | 710.01 | 1.35×10⁻⁵ | 0.08 |
| S6B_PART2_256342731 | 7B | 710.62 | 3.02×10⁻⁵ | 0.06 | S6B_PART2_256342731 | 7B | 710.62 | 3.02×10⁻⁵ | 0.06 |
| S6B_PART1_74249006 | 1B | 608.33 | 2.63×10⁻⁵ | 0.05 | S6B_PART2_256342731 | 7B | 710.62 | 3.02×10⁻⁵ | 0.06 |
| S3A_PART2_249803574 | 4A | 209.13 | 7.24 | 1.43 | S6B_PART2_256342731 | 7B | 710.62 | 3.02×10⁻⁵ | 0.06 |
| S3A_PART2_249803574 | 4A | 209.13 | 7.24 | 1.43 | S6B_PART2_256342731 | 7B | 710.62 | 3.02×10⁻⁵ | 0.06 |

**TABLE 5**: Marker-trait associations (MTAs) detected for wheat response to seven stem rust pathogen isolates using PCA-GWAS.
of low frequency alleles from GWAS analysis is mainly due to lack of resolution to detect association signals for functional alleles present in low frequency (usually they are ignored in analysis). Otherwise, rare alleles could significantly explain genetic variations in phenotypic traits (Alqudah, Sallam, Baenziger, & Börner, 2020). These rare variants that are unique to a specific group of individuals and would only be detected in GWAS if the frequency was greater (Brachi et al., 2011).

The unique technical components of this study were the association mapping of differences between phenotypic mean responses to pairs of Pgt races (e.g., the mapping of difference between race TTKSK responses at two temperature regimes) and the PCA-based association analysis. The identified difference in between race TKTTF isolates from Germany and Ethiopia on 4AL was expected because of the previously characterized difference in avirulence to Sr7a between these two isolates. However, we also detected a QTL on chromosome arm 7AS based on phenotypic mean difference. The 7AS QTL was not detected using the data from either isolate of TKTF, individually.

Similarly, for the difference between races TTKSK and TTKTT, we identified a QTL on chromosome arm 7BL that was not detected when mapping the responses of these two races individually. This implies that mapping using phenotypic mean difference can be a useful way to identify cryptic virulence changes between closely related isolates. Finally, when mapping the mean difference in responses to race TTKSK at normal and low temperature, we identified a QTL on chromosome arm 3AL that was not detected with either temperature regimes. Only a single association signal (3B, genetic map position 58.7–60.30 cM) co-localized in both the normal and cold temperatures. Previous studies showed that there are both major stem rust resistant genes and QTL on chromosome 3BS. For instance, one of the previously known genes, Sr12 is a seedling stem rust resistance gene that resides in the centromeric region of chromosome 3B (flanked by SNP markers IWA6086 at consensus genetic position 82.85 cM and IWA4613 at consensus genetic position 85.83 cM; Hiebert et al., 2016). Bajgain et al. (2015a) also reported SNPs that were significantly associated with resistance against TTKSK on
chromosome 3B (67.5–76.9 cM) near the Sr12 locus. The 3AL QTL detected in mapping the difference between low and normal temperature was also detected with PCA-GWAS for PC1 which is characterized by individuals resistant for TTKSK under normal temperature condition but susceptible under low temperature, or vice versa. This QTL was not identified in the linearized infection type data for TTKSK at low temperature, likely because the response was masked by the QTL on 3B (Sr12) and other QTL detected at this temperature. Therefore, PCA-GWAS proved useful for identifying QTL that would not have been detected using mixed model mapping and phenotyping at normal temperatures. It is worthwhile to mention that the known major genes (Sr7a, Sr8a, Sr11, Sr12) detected in this study are common in the current mapping panel with the exception of Sr3I, which is present in the panel at a lower frequency (Edae et al., 2018). Going forward, association mapping of phenotypic differences may be valuable for elucidating the genetics of genotype by environment interactions including those mediated by temperature. We also validated the use of PCA-GWAS approach to confirm MTAs identified with other approaches especially when highly correlated traits are used for GWAS (Ried et al., 2016). Interestingly, all association signals in the region of major genes (Sr7a, Sr8a, Sr11, Sr12, and Sr3I) and other strong association signals that were detected with standard GWAS were also detected with the PCA-GWAS with the 1st two PCs. This implies that there is high concordance between PCA-GWAS and standard GWAS methods, and the PCA-GWAS can be used especially to identify strong association signals.

In conclusion, for all Pgt isolates evaluated in this study two or more association signals were detected implying resistance against these Pgt races is multigenic. Although there are some QTL regions shared across the races, none of the association signals were detected in response to all races. We also found that there was a case where a major gene that was ineffective to an older virulent race but effective to newly emerged virulent race (e.g., Sr3I is effective to the new race TTRTF but not to TTKSK). This suggests pyramiding major genes is still a useful strategy to mitigate dangerous stem rust pathogen races including recently-emerged races.

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**DATA AVAILABILITY STATEMENT**

The GBS raw sequence data can be accessed with BioProject ID PRJNA641969 from NCBI (National Center for Biotechnology Information) website at [http://www.ncbi.nlm.nih.gov/bioproject/641969](http://www.ncbi.nlm.nih.gov/bioproject/641969). However, both raw phenotypic and genotypic data of the SNPs that showed significant associations for all races evaluated in this study were given in Supplemental Table S2.

**AUTHOR CONTRIBUTIONS**

EE and MR conceived the experiment. MR generated greenhouse phenotypic data and obtained the funding. EE conducted SNP calling and drafted the manuscript.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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