Mapping and Validation of Stem Rust Resistance Loci in Spring Wheat Line CI 14275

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Stem rust caused by Puccinia graminis f. sp. tritici (Pgt) remains a constraint to wheat production in East Africa. In this study, we characterized the genetics of stem rust resistance, identified QTLs, and described markers associated with stem rust resistance in the spring wheat line CI 14275. The 113 recombinant inbred lines, together with their parents, were evaluated at the seedling stage against Pgt races TTKSK, TRTTF, TPMKC, TTTTF, and RTQQC. Screening for resistance to Pgt races in the field was undertaken in Kenya, Ethiopia, and the United States in 2016, 2017, and 2018. One gene conferred seedling resistance to race TTTTF, likely Sr7a. Three QTL were identified that conferred field resistance. QTL QSr.cdl-2BS.2, that conferred resistance in Kenya and Ethiopia, was validated, and the marker Excalibur_c7963_1722 was shown to have potential to select for this QTL in marker-assisted selection. The QTL QSr.cdl-3B.2 is likely Sr12, and QSr.cdl-6A appears to be a new QTL. This is the first study to both detect and validate an adult plant stem rust resistance QTL on chromosome arm 2BS. The combination of field QTL QSr.cdl-2BS.2, QSr.cdl-3B.2, and QSr.cdl-6A has the potential to be used in wheat breeding to improve stem rust resistance of wheat varieties.

Keywords: Triticum aestivum, Puccinia graminis f. sp. tritici, genetics, QTL, disease resistance, stem rust, Ug99

INTRODUCTION

Wheat accounts for about 20% of global human consumed calories and proteins with hexaploid wheat [Triticum aestivum (2n = 6x = 42; AABBDD)] being the most widely cultivated species (Gupta et al., 2008). The European Union, China, India, Russia, and the United States are the top five wheat producers (Food and Agriculture Organization [FAO], 2018). Production of wheat is challenged by both abiotic and biotic factors. Among biotic constraints, stem rust of wheat caused by Puccinia graminis f. sp. tritici (Pgt) is considered one of the most devastating fungal diseases with the potential to result in a 100% yield loss on susceptible varieties. The Pgt race Ug99, first isolated from Uganda in the year 1999 (Pretorius et al., 2000) and later characterized as race TTKSK (Fin et al., 2008), was the first Pgt race virulent to stem rust resistance gene Sr31 (Pretorius et al., 2000; Wanyera et al., 2006). The unique virulence combination in race TTKSK rendered more
than 90% of global wheat varieties susceptible during testing in 2005 (Singh et al., 2006). Since then, multiple derivatives of TTKSK have been reported, including TTKST, virulent to Sr24 and identified in 2006 (Jin et al., 2008); TTTSK, virulent to Sr36 and identified in 2007 (Jin et al., 2009); and TTKTT, virulent to SrTmp and identified in 2014 (Newcomb et al., 2016; Patpour et al., 2016). Host resistance remains the most effective and economical approach to controlling stem rust, and this can be expressed at the seedling stage and at the adult plant stage (Priyamvada et al., 2011). Plants with major-effect resistance genes (also known as R-genes) may express intermediate to immune levels of resistance when challenged by an avirulent race or strain of the pathogen. Some of the major genes currently utilized in breeding programs to confer resistance to Pgt are Sr13, Sr22, Sr25, Sr26, Sr33, and Sr50 (Helguera et al., 2003; Mago et al., 2005, 2013, 2015; Simons et al., 2011; Periyanan et al., 2013; Singh et al., 2015). Genes with temporary designations, such as SrND643 and SrNing, are also being utilized to provide resistance against Pgt (Lopez-Vera et al., 2014; Basnet et al., 2015). The downfall of deploying major-effect genes individually is that they can be easily overcome by the pathogen, resulting in what is referred to as “boom-and-bust” cycles. The Ug99 race group clearly highlights the boom-and-bust cycle by the defeat of important R-genes, such as Sr24, Sr31, Sr36, and SrTmp that were widely deployed worldwide (Pretorius et al., 2000; Jin et al., 2008, 2009).

The adult plant resistance genes, such as Sr2 (likely pleiotropic with Yr30), Sr55 (pleiotropic with Lr67, Yr46, Pm46), Sr56, Sr57 (pleiotropic with Lr34, Yr18, Pm38), and Sr58 (pleiotropic with Lr46, Yr29, Pm39), confer slow rusting resistance at the adult plant stage with each having a minor resistance effect and, thus, possibly not providing adequate resistance when present alone under high disease pressure (Bansal et al., 2014; Herrera-Foessel et al., 2014; Singh et al., 2015). Combinations of effective major-effect race-specific resistance genes (R-genes) and/or minor-effect race non-specific resistance genes may result in durable resistance, or resistance that remains effective for a longer period of time (Parlevliet and van Ommeren, 1988; Priyamvada et al., 2011). Identification and deployment of new resistance genes into advanced wheat lines is necessary for new resistance gene combinations to be assembled.

CI 14275 is a Canadian breeding line (Q-2331-34) that was derived from single pustules, increased in isolation and stored at 80°C with a 2–4 h shocking for 15 min in a 45°C water bath, rehydrating for approximately 2–4 h in a chamber maintained at 80% relative humidity by a KOH solution, and suspending in lightweight mineral oil (Soltrol 70; ConocoPhillips Inc., Houston). The plants were inoculated by spraying them with a suspension using an atomizer. After inoculation, the plants were then placed in a fume hood for 30 min to allow the oil to evaporate and, thereafter, transferred to a dew chamber at 100% relative humidity for 14–16 h. After the dew chamber incubation, plants were returned to the greenhouse bench and maintained at 18 ± 2°C with a photoperiod of 16 h.
Two weeks postinoculation, seedlings were assessed for seedling infection types (ITs) based on the 0–4 scale developed by Stakman et al. (1962). The description of infection types used to classify the reactions to *Pgt* are as follows: '0' = no uredinia or any other sign of infection, '1' = small uredinia surrounded by light green chlorosis, '2' = small-medium sized uredinia, surrounded by yellow chlorotic or necrotic areas, '3' = medium-sized uredinia in a dark green island surrounded by a chlorotic area, '4' = large uredinia with no or a limited amount of chlorosis. All observed infection types on the same leaf were recorded with the infection type(s) listed in order according to their prevalence. A comma (,) symbol was used to separate multiple ITs observed on the same plant with the most frequent IT recorded first. Whenever multiple infection types were observed on different plants of the same line, a forward slash (/) symbol was used to separate the infection types. A letter 'C' was recorded when extensive chlorosis was associated with the infection. The plus (+) and minus (−) symbols were used for the pustules that were relatively larger or smaller, respectively, than normally associated with a given IT. Plants with ITs ranging from 0 to 2 were categorized as resistant, and those ranging from 3 to 4 were categorized as susceptible. The seedling infection types assigned on a 0–4 scale were then converted to a 0–9 linear scale according to Peterson et al. (1948). Infection response was rated as resistant (R), small uredinia surrounded by necrosis; moderately resistant (MR), medium-sized uredinia surrounded by necrosis or chlorosis; moderately susceptible (MS), medium-sized uredinia without necrosis; susceptible (S), large uredinia without necrosis; or MRMS, an infection response that included both the MR and MS categories (Roelfs et al., 1992). Coefficient of infection (COI) values were generated by multiplying the stem rust severity value for each line by a constant value for each infection response: 0 = 0, R = 0.2, RMR = 0.3, MR = 0.4, M = 0.6, MS = 0.8, S = 1.0 (Knott, 1989). Average coefficient of infection for the two replicates were determined and used for analyses. Raw mean coefficients of infection values across the two replicates were used for QTL analyses.

Phenotyping of the Primary Population in the Field

The experimental lines were evaluated for stem rust severity in two African field locations: Njoro, Kenya, and Debre Zeit, Ethiopia, in 2016, 2017, and 2018. Evaluations in Africa took place during the off-season of approximately January through May. The lines were evaluated in St. Paul, Minnesota, in 2017 and 2018. Across the three locations, approximately 5 g seed was sown in each of two replicates planted in a randomized complete block design. In Njoro, Kenya, the field plots were two 0.7-m-long rows separated by a distance of 0.3 m. Disease spreader rows consisting of a mixture of susceptible cultivars Carupe, Eagle 10, Robin, and six CIMMYT lines carrying Sr24 were planted to surround the entries 10–14 days before the experimental plots were planted. The disease spreader row mixture was also planted as hill plots on one side of the experimental plots to facilitate the buildup and spread of the disease. From the booting to heading growth stages (Zadok’s growth stages 37–60), freshly collected urediniospores of locally predominant Ug99 *Pgt* races TTKSK, TTKST, TTKTK, and TTKTT were bulked and suspended in distilled water, and approximately 1 mL of the suspension was injected into disease spreader row plants using a hypodermic syringe. Inoculations were conducted in the afternoons over 3 occasions with 7-day intervals. In Debre Zeit, Ethiopia, the experimental plots were planted as double 1-m-long rows, and disease spreader rows, including cultivars Arendeto, Digalu, Local Red, Morocco, and PBW343, were planted perpendicular to the plots and were artificially inoculated at Zadok’s growth stage 37–60 with bulked urediniospores from predominant *Pgt* races TTKSK, TKTT, RRTP, and JRCQ to initiate infection on the plots. In St. Paul, the experimental plots were single 1-m-long rows, separated by a distance of 0.3 m. The spreader rows that consisted of cultivars Baart, Mexico, and Thatcher were planted 1–2 weeks earlier than entries and sown perpendicular to the experimental plots. At the heading stage, the plots were spray-inoculated using an Ula+ sprayer (Micron Sprayers Ltd., Bromyard, United Kingdom) with a light mineral oil suspension of bulked urediniospores of North American *Pgt* races QFSC (isolate 06ND76C), TPMKC (isolate 74MN1409), KRQRC (isolate 99KS76A), RCRSC (isolate 77ND82A), QTHIC (isolate 75ND717C), and MCGFC (isolate 59K519). Disease on the inoculated spreader rows initiated stem rust infection on the experimental plots.

When the spreaders had attained 50% severity in the three locations, stem rust severity was visually scored in the experimental plots based on the modified Cobb scale of 0–100, where 0 = immunity (no uredinia or any other sign of infection) and 100% = completely susceptible (Peterson et al., 1948). Infection response was rated as resistant (R), small uredinia surrounded by necrosis; moderately resistant (MR), medium-sized uredinia surrounded by necrosis or chlorosis; moderately susceptible (MS), medium-sized uredinia without necrosis; susceptible (S), large uredinia without necrosis; or MRMS, an infection response that included both the MR and MS categories (Roelfs et al., 1992). Coefficient of infection (COI) values were generated by multiplying the stem rust severity value for each line by a constant value for each infection response: 0 = 0, R = 0.2, RMR = 0.3, MR = 0.4, M = 0.6, MS = 0.8, S = 1.0 (Knott, 1989). Average coefficient of infection for the two replicates were determined and used for analyses. Raw mean coefficients of infection values across the two replicates were used for QTL analyses.

Phenotyping of the Validation Population in the Field

Twice-replicated plots of the 180 *F₃*₄ validation lines were evaluated for field response to the Ug99 race group in Njoro, Kenya, in 2018. Planting; inoculation with *Pgt* races that included TTKSK, TTKST, TTKTK, and TTKTT; and evaluation was conducted as previously described.

DNA Extraction

Tissues were harvested from three-leaf-stage single plants of the RIL population (*F₇*₄), from single *F₃* plants of the Kwale/line #162 population, and their parents into Eppendorf tubes (1.5 mL). The harvested leaves were dried and ground for 1–2 min using a Genogrinder (SPEX Sample Prep). Extraction buffer (300 µl: 200 mM Tris–HCL pH 8.0, 250 mM NaCl, 25 mM EDTA, 0.5% SDS, ddH₂O) was added to each well, the tubes were shaken gently before incubating at room temperature
for 15 min, followed by centrifuging completed at 2500 rpm. Working under a fume hood, 300 µl of chloroform:isoamyl alcohol (24:1) was added to each well, the suspension was mixed, plates were centrifuged for 20 min at 2500 rpm, and the supernatant (200–300 µl) was transferred to separate tubes. Cold isopropanol (300 µl) was then added to each tube. Plates were shaken gently and placed in a refrigerator for 10–20 min. After decanting the supernatant, 300 µl of 70% ethanol was added to each tube, the tubes were centrifuged for 20 min at 2,500 rpm, the ethanol was poured out, and the DNA pellets were air-dried. When the pellets were completely dry, 100 µl of distilled H2O was added to each tube to resuspend the pellet and the tubes were left in a refrigerator overnight. DNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher, Waltham, MA, United States), and concentrations were diluted to 50 ng/µl.

Genotyping of the Primary Population

Genotyping of the F7;9 RILs and their parents was conducted at the USDA-ARS small grains genotyping lab in Fargo, North Dakota, using the iSelect 90k SNP assay developed by Wang et al. (2014). The 90k SNP assay contains 81,587 SNPs and was developed for allohexaploid and allotetraploid wheat populations. The raw 90K SNP data together with the consensus map were uploaded onto Illumina GenomeStudio genotyping software version 2.0. The SNPs were manually called and the genotypes grouped as AA, AB, or BB. The missing genotypes were indicated as no call (NC). Following calculation of allele frequencies, a total of 12,243 polymorphic markers with both AA and BB allele frequency greater than 0.25 were selected.

QTL Mapping

The allele calls from GenomeStudio were used to create genetic linkage maps with the MSTMap algorithm (Wu et al., 2008) implemented in ASMap R package (Taylor and Butler, 2017) with the following parameters: distance function Kosambi, cut_off_p_value set at 1e-08, no_map_dist set at 15, no_map_size set at 0, missing_threshold set at 0.10, detect_bad_data set at “yes” and objective function set at “COUNT.” A total of 33 linkage groups, each with greater than five markers were generated. The 33 linkage groups included a total of 819 markers (Supplementary Table 1). The genotype calls were manually curated by coding single-marker, double-crossover events as NC. In addition, unusual heterozygous calls were manually curated by replacing these calls with NC. These 33 linkage groups were used for QTL analysis using the R/qtl software (Broman and Sen, 2009). The QTL that conferred resistance to races RTQQC, TTTTF, and TPMKC at the seedling stage and QTL that conferred resistance at the adult plant stage in the Kenya, Ethiopia, and United States environments were identified separately. Interval mapping was conducted using the scanone function, and single-QTL genome scan via Haley-Knott regression was performed. The statistical significance thresholds of the genome scan results were determined by running 1000 permutation tests with a 0.05 significance level. QTL were also reported when LOD values were greater than 3.0, and the corresponding markers were detected in multiple environments. Markers flanking the QTL were identified using the find.flanking function. Linkage groups were assigned to chromosomes based on the 90K consensus map. The physical positions of the QTL were identified by searching positions of the flanking markers in the T3/Wheat website: https://triticaceatoolbox.org/wheat/ (Blake et al., 2016). The probe sequence of the markers was determined by searching the GrainGenes website, https://wheat.pw.usda.gov/GG3/, when marker information was missing in the T3/Wheat website, and the sequences were blasted to the reference genome at the BLAST website: https://wheat-urgi.versailles.inra.fr/Seq-Repository/BLAST to find the physical position of the markers (Alaux et al., 2018).

Confirmation of Presence/Absence of Lr34/Sr57 in CI 14275

The marker csLV34 (Lagudah et al., 2006) linked to the pleiotropic rust resistance gene Lr34/Sr57/Yr18 was genotyped on the parents (LMPG-6 and CI 14275). Wheat cultivar Chinese Spring was used as the positive control for the gene.

Data Analyses

Chi-squared analyses were performed on the linearized seedling data to determine goodness-of-fit to the expected segregation ratios for different inheritance models. Using R software, analysis of variance (ANOVA) was performed using the average COI values with lines and environments both as fixed variables and replication at random. Correlation of stem rust severity between the environments was determined using Pearson correlation coefficients.

RESULTS

Phenotyping in the Greenhouse

One hundred thirteen F7;9 RILs and their parents showed susceptible responses when tested against Pgt races TTKSK and TRTTF in two replicates each (Supplementary Table 2). A 33+ IT was observed on the resistant parent, CI 14275, and an IT of 3/33+ was observed on the susceptible parent, LMPG-6, when tested with race TTKSK (Table 1 and Supplementary Table 2). ITs of 33+ and 3+/33+ were observed on the parents CI 14275 and LMPG-6, respectively, when tested with race TRTTF (Table 1 and Supplementary Table 2). On the other hand, ITs of 1/1+ 3C and 3+ were observed on the resistant and susceptible parents, respectively, when tested with race TTTTF, and the observed frequency of the population fit into the expected ratio of 1:1 (one gene) with a χ² value of 0.704 (p = 0.40) (Table 1). Screening for race RTQQC resulted in 3/33+ and 3+/33+ ITs on CI 14275 and LMPG-6, respectively. Nearly all of the RILs were susceptible to RTQQC in at least one replication, and the rare low responses to RTQQC were usually not consistent across the two replications (Supplementary Table 2 and Table 1). For race TPMKC, ITs of 23−/23+ and 3+/33+ were observed on the resistant and susceptible parents, respectively, and the observed frequency of the population did not fit any simple expected ratio (Supplementary Table 2 and Table 1).
TABLE 1 | Segregation of stem rust resistance in 113 recombinant inbred lines (RILs) along with response of parents of the cross LMPG-6/Cl 14275 against five Puccinia graminis f. sp. tritici races.

| Races | Number of Lines | Seeding Infection Typesa | Chi-square | P-value | Number of segregating genesb |
|-------|----------------|--------------------------|------------|---------|-----------------------------|
|       |     | Susceptible parent – LMPG-6 | 3+/33+     | 3/33+   | –              | None                  |
| RTQQC | 0   | 3+/33+ | –           | –       | None                  |
| TTTTF | 57  | 3+     | 1:1 + 3C    | 0.704   | 0.40 One            |
| TPMKC | 16  | 3+/3+  | 23–23+      | –       | Unknown              |
| TRTTF | 0   | 3+/33+ | –           | –       | None                  |
| TTKSK | 113 | 33+    | –           | –       | None                  |

aInfection types (ITs) follow the Stakman et al. (1962) scale. The plus (+) and minus (−) symbols are used for the pustules that were relatively larger and smaller, respectively, than normal; a forward slash (/) symbol separates multiple infection types observed on different plants of the same line; fleck (:) indicates no uredinia but presence of hypersensitive necrotic flecks; ITs ranging from 0 to 2 were categorized as resistant, and ITs ranging from 3 to 4 were categorized as susceptible; the dash (−) symbol indicates no generated data. bUnknown, segregation of RILs did not fit any expected ratio; none, no resistant plants.

Phenotyping in the Field
For the primary population, stem rust ratings from 0 to 90S were observed in Kenya 2016 (KEN16), 0 to 80S in Kenya 2017 (KEN17), and TR (trace R) to 60S in Kenya 2018 (KEN18) (Supplementary Table 3). Ratings from TR to 80S were observed in Ethiopia 2016 (ETH16), TMS (trace MS) to 50S in Ethiopia 2017 (ETH17), and TMR (trace MR) to 70S in Ethiopia 2018 (ETH18) (Supplementary Table 4). Ratings from 5RMR to 100S were observed in St. Paul 2017 (STP17) and TR to 80S in St. Paul 2018 (STP18) (Supplementary Table 5). Stem rust ratings from 0 to 70S were observed in the validation population. In the three Kenyan environments: KEN16, KEN17, and KEN18, the distribution of stem rust severity was somewhat skewed toward resistance (Figure 1). A symmetric distribution was observed in the ETH16, ETH17, and ETH18 environments (Figure 1). A somewhat symmetric distribution was observed in the STP17 and STP18 environments (Figure 1). ANOVA indicated significant effects for lines and environment, a significant interaction between the RILs and the environments, and a non-significant effect for replication (Table 2). Stem rust severity over the different environments had statistically significant correlation coefficients, ranging between 0.55 and 0.85 for correlations among all environments except with ETH17, with which the correlations were low but still statistically significant, ranging between 0.21 and 0.47 (Table 3).

Confirmation of Presence/Absence of Lr34/Sr57 in Cl 14275
Both parents were negative for the positive allele of the Lr34 gene-based marker, indicating that the resistance in CI 14275 did not involve Lr34/Sr57. This result was confirmed by the lack of significant QTL on chromosome 7D, where Lr34 is located.

LMPG-6/Cl 14275 Genetic Map
The genetic map of LMPG-6/Cl 14275 is listed in Supplementary Table 1. A total of 819 markers mapped to 33 linkage groups. The 33 linkage groups corresponded to 15 wheat chromosomes. Six wheat chromosomes were missing from the genetic map: 2D, 3A, 4B, 4D, 6D, and 7D. The remaining wheat chromosomes were represented in the map from anywhere between one and four linkage groups.

Identified QTLS at Seedling and Adult Plant Stages
The 0.05 LOD significance thresholds for each trait ranged from 3.63 to 3.81. We did not detect any significant QTL for response of the primary population to RTQQC, TPMKC, or ETH17. All significant QTL are reported in Table 4 in addition to QTL with an LOD of 3.0 or greater that were detected in multiple environments.

QTL on Chromosome Arm 2BS
One QTL mapped to chromosome 2BS in the ETH16 and ETH18 environments and was designated QSr.cdl-2BS.2 (Table 4). This QTL also reached an LOD of 3.1 in KEN16. The QTL is flanked by the markers Tdurum_contig54704_176 and BS00038820_51 (Table 4). We designated this QTL with the suffix 2BS.2 because QSr.cdl-2BS was already designated (Rouse et al., 2014b). The phenotypic variance explained by the QTL ranged from 10.5 to 17.6% across the three environments (Table 4).

QTL on Chromosome 3B
One QTL mapped to chromosome 3B in the KEN17, KEN18, STP17, and STP18 environments and was designated QSr.cdl-3B.2 (Table 4). This QTL also reached an LOD of 3.6 in KEN16. We designated this QTL with the suffix 3B because QSr.cdl-3B was already designated (Rouse et al., 2014b). The markers Excilbur_c57658_54, IAAV3838, RAC875_c10595_473, and RAC875_c69_499 were linked to QSr.cdl-3B.2 (Table 4). The phenotypic variance explained by the QTL in the fifth environment ranged from 14.0 to 21.5% (Table 4).

QTL on Chromosome Arm 4AL
One QTL mapped to chromosome arm 4AL for race TTTTF and was designated QSr.cdl-4AL (Table 4). The QTL QSr.cdl-4AL was flanked by markers...
FIGURE 1 | Frequency distribution for stem rust severity (%) in RILs developed from the cross LMPG-6/CI 14275 evaluated in Kenya (KEN), Ethiopia (ETH), and St. Paul (STP) in 2016, 2017, and 2018.

Tdurum_contig42019_1714 and BS00009680_51 (Table 4). This QTL explained a large proportion (42.3%) of the phenotypic variance observed (Table 4). Interestingly, this QTL was observed in the field in ETH18 with an LOD of 3.0 (Table 4).

QTL on Chromosome Arm 6A

One QTL mapped to chromosome arm 6A in the ETH16, ETH18, STP16, and STP17 environments with LOD values of 3.8, 3.5, 3.0, and 3.1, respectively. This QTL did not pass the 0.05 LOD threshold in any of these environments, but it was detected above LOD 3.0 in four environments, which adds confidence to the validity of this QTL. We designated the QTL as QSr.cdl-6A (Table 4). The markers BS00023627_51, IAAV3806, and Excalibur_c60006_452 were linked to QSr.cdl-6A (Table 4). The phenotypic variance explained by the QTL in the four environments tested ranged from 6.4 to 12.6% (Table 4).

TABLE 2 | Analysis of variance for stem rust severity of 113 recombinant inbred lines (RILs) and the parents of the cross LMPG-6/CI 14275 tested in eight environments (Kenya – 2016, 2017, and 2018; Ethiopia – 2016, 2017, and 2018; St. Paul – 2017 and 2018).

| Source          | Degrees of freedom | Mean square | F-value<sup>a</sup> |
|-----------------|--------------------|-------------|---------------------|
| Lines           | 114                | 3653.2      | 30.7***             |
| Environments    | 7                  | 24,129.2    | 202.6***            |
| Replication     | 1                  | 215.2       | 1.8                 |
| Lines × Environments | 786             | 486.0       | 4.1***              |
| Error           | 876                | 119.1       |                     |

<sup>a</sup>***Significant at 0.001 probability level.
TABLE 3 | Correlation coefficients among stem rust coefficient of infection (COI) values for 113 recombinant inbred lines (RILs) and the parents of the cross LMPG-6/CI 14275 in eight environments (Kenya – 2016, 2017, and 2018; Ethiopia – 2016, 2017, and 2018; St. Paul – 2017 and 2018).a

| Environment | KEN16 | ETH16 | STP17 | KEN17 | ETH17 | STP18 | KEN18 | ETH18 |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|
| KEN16       | 1.00  |       |       |       |       |       |       |       |
| ETH16       | 0.82  | 1.00  |       |       |       |       |       |       |
| STP17       | 0.57  | 0.63  | 1.00  |       |       |       |       |       |
| KEN17       | 0.73  | 0.73  | 0.59  | 1.00  |       |       |       |       |
| ETH17       | 0.25  | 0.21  | 0.30  | 0.32  | 1.00  |       |       |       |
| STP18       | 0.64  | 0.69  | 0.85  | 0.65  | 0.25  | 1.00  |       |       |
| KEN18       | 0.72  | 0.74  | 0.55  | 0.78  | 0.42  | 0.62  | 1.00  |       |
| ETH18       | 0.67  | 0.73  | 0.57  | 0.67  | 0.47  | 0.64  | 0.73  | 1.00  |

aAbbreviations of experimental locations: KEN16, Njoro, Kenya 2016; KEN17, Njoro, Kenya 2017; KEN18, Njoro, Kenya 2018; ETH16, Debre Zeit, Ethiopia 2016; ETH17, Debre Zeit, Ethiopia 2017; ETH18, Debre Zeit, Ethiopia 2018; STP17, St. Paul, MN 2017; STP18, St. Paul, MN 2018.

TABLE 4 | Quantitative trait loci (QTL) identified in the recombinant inbred lines (RILs) population derived from the cross LMPG-6/CI 14275 for seedling resistance to Puccinia graminis f. sp. tritici races RQFC, TPMKC, and TTTTF and for field resistance in Njoro, Kenya (2016, 2017, and 2018); Debre-Zeit, Ethiopia (2016, 2017, and 2018); and St. Paul, Minnesota (2017 and 2018).

| Race/environmenta | QTLb | Donor of resistant allele | Linkage Groupc | LODd Position (cMe) | Left flanking markerf | Right flanking markerg | Phenotypic variance (%)h |
|-------------------|------|---------------------------|----------------|---------------------|-----------------------|------------------------|-------------------------|
| Seedling stage    |      |                           |                |                     |                       |                        |                         |
| TTTTF             | QSr.cdl-4AL | CI 14275 | 4AL_2 | 18.4 | 1 | Tdurum_contig42019_1714 | BS00009680_51 | 42.3 |
| Adult plant stage |      |                           |                |                     |                       |                        |                         |
| KEN16             | QSr.cdl-2BS.2 | CI 14275 | 2B   | 3.1  | 0 | Tdurum_contig54704_176 | BS00038820_51 | 14.7 |
| KEN17             | QSr.cdl-3B.2 | CI 14275 | 3B   | 3.6  | 177 | Excalibur_c57658_54 | IAAV3838 | 18.3 |
| KEN18             | QSr.cdl-3B.2 | CI 14275 | 3B   | 6.7  | 177 | Excalibur_c57658_54 | IAAV3838 | 21.5 |
| ETH16             | QSr.cdl-6A | CI 14275 | 6A   | 3.8  | 103 | Excalibur_c60006_452 | BS00023627_51 | 12.6 |
| STP16             | QSr.cdl-6A | CI 14275 | 6A   | 3.9  | 106 | Tdurum_contig54704_176 | BS00038820_51 | 17.6 |
| STP17             | QSr.cdl-3B.2 | CI 14275 | 3B   | 5.9  | 177 | Excalibur_c57658_54 | IAAV3838 | 10.6 |
| STP18             | QSr.cdl-4AL | CI 14275 | 4AL_2 | 4.73 | 0 | Tdurum_contig54704_176 | BS00023627_51 | 8.7 |
| STP16             | QSr.cdl-6A | CI 14275 | 6A   | 3.0  | 103 | Excalibur_c60006_452 | BS00023627_51 | 6.4 |
| STP17             | QSr.cdl-3B.2 | CI 14275 | 3B   | 5.1  | 194 | RAC875_c10595_473 | RAC875_c69_499 | 14.0 |
| STP18             | QSr.cdl-3B.2 | CI 14275 | 3B   | 3.1  | 104 | Excalibur_c60006_452 | BS00023627_51 | 7.1 |

aAbbreviations of experimental locations: KEN16, Njoro, Kenya 2016; KEN17, Njoro, Kenya 2017; KEN18, Njoro, Kenya 2018; ETH16, Debre Zeit, Ethiopia 2016; ETH17, Debre Zeit, Ethiopia 2017; ETH18, Debre Zeit, Ethiopia 2018; STP17, St. Paul, MN 2017; STP18, St. Paul, MN 2018. bNaming of QTL: Q, QTL; Sr, stem rust; cdl, cereal disease lab. cLinkage groups as listed in Supplementary Table 1. dLOD, logarithm of odds value. eCm, centiMorgan, position of the peak LOD. fLeft flanking marker = markers flanking the identified QTL, on the left side. gRight flanking marker = markers flanking the identified QTL, on the right side. hPercent of variance explained by the identified QTL.

Combined 2BS, 3B, and 6A QTLs
Three QTLs, QSr.cdl-2BS.2, QSr.cdl-3B.2, and QSr.cdl-6A, were identified only at the adult plant stage in seven of the eight field environments. No QTL were reported in ETH17 because disease pressure was low although the line responses in ETH17 were significantly correlated with those in other environments. Based on the genotype data of the QTL peak markers, seven lines were found to have all three QTLs combined, and 11 lines lacked the three QTLs. The t-test results from the two groups (with and without the three QTLs) showed statistically significant differences (p < 0.01; 0.001) in seven environments (all environments except ETH17) (Table 5). The combined QTL provided large reductions in stem rust severity in the observed environments and appeared to be a highly effective combination even when the disease pressure was high (Table 5).

Kompetitive Allele-Specific PCR (KASP) Markers
KASP assay primers were designed corresponding to 15 90K SNP markers linked to QTL, but only six of these KASP assays produced clear polymorphism in the validation population (Table 6). Simple ANOVAs for each marker were independently run to determine whether any of the KASP markers predicted the phenotype in the validation population. One marker linked to QSr.cdl-2BS.2, Excalibur_c7963_1722_C1, was associated with reduced stem rust severity in Njoro, Kenya, in 2018 (p-value = 0.003). This validated QSr.cdl-2BS.2, which was identified...
TABLE 5 | Coefficient of infection (COI) (%) for LMPG-6/CI 14275 lines with and without three adult plant resistance (APR) quantitative trait loci (QTL): QSr.cdli-2BS.2, QSr.cdli-3B.2, and QSr.cdli-6A detected in six environments tested in this study, excluding ETH17.

| Environment | KEN16 | KEN17 | KEN18 | KEN mean | ETH16 | ETH18 | ETH mean | STP17 | STP18 | STP mean | Overall mean |
|-------------|-------|-------|-------|----------|-------|-------|----------|-------|-------|----------|--------------|
| Mean value of seven lines without QTL | 60.2  | 36.6  | 35.5  | 43.5     | 54.5  | 51.8  | 52.3     | 65.3  | 92.9  | 79.1     | 56.7         |
| Average Mean value for combination of three QTL detected | 6.7   | 3.7   | 6.9   | 5.9      | 8.8   | 14.7  | 12.4     | 23.8  | 19.1  | 21.4     | 12.3         |

P-value<sup>b</sup>

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**Significant at p < 0.01 probability level, ***significant at p < 0.001 probability level.**

TABLE 6 | Primer sequences of polymorphic Kompetitive allele-specific PCR (KASP) assays in the line #162/Kwale population derived from 90K single nucleotide polymorphism (SNP) markers linked to identified quantitative trait loci (QTL)<sup>a</sup>.

| SNP | A1       | A2       | C1       | SNP1 | SNP2 |
|-----|----------|----------|----------|------|------|
| Tdurum_contig32277_121 | GAAAGTGAGCAAGCTTACGTCGG | TGGCATATGAAATGATATGACTTCG | CGCGTGTACCTCA | A    | C    |
| Excalibur_c7963_1722 | GAAAGTGAGCAAGCTTACGTCGG | TGGCATATGAAATGATATGACTTCG | CGCGTGTACCTCA | A    | C    |
| Ra_c6672_1679 | GAAAGTGAGCAAGCTTACGTCGG | TGGCATATGAAATGATATGACTTCG | CGCGTGTACCTCA | A    | C    |
| Jagger_c8310_70 | GAAAGTGAGCAAGCTTACGTCGG | TGGCATATGAAATGATATGACTTCG | CGCGTGTACCTCA | A    | C    |
| IAAV3806 | GAAAGTGAGCAAGCTTACGTCGG | TGGCATATGAAATGATATGACTTCG | CGCGTGTACCTCA | A    | C    |
| BS00331178_51 | GAAAGTGAGCAAGCTTACGTCGG | TGGCATATGAAATGATATGACTTCG | CGCGTGTACCTCA | A    | C    |

<sup>a</sup>SNP markers linked to the identified QTL: A1, A2, and C1 are the primers.

DISCUSSION

The RIL population, together with the parents, was susceptible at the seedling stage when tested with *Pgt* races TTKSK, TRTTF, and RTQQC. This is an indication that the resistant parent, CI 14275, lacked an effective major gene conferring resistance to these races. This observation is different from that observed by Rouse et al. (2011) for the seedling response of CI 14275 to race TTKSK. Different environmental conditions between the two studies, particularly temperature (Zhang et al., 2017; Chen et al., 2018; Gao et al., 2019), may explain the variable responses although the differences in these findings may warrant further investigation. Similarly, the observed inconsistencies of infection types between replicates in some lines could have been due to the temperature effect on segregating stem rust resistance genes. The seedling resistance to race TTTTF was conferred by a single gene. Based on map location and race specificity, this gene is likely *Sr7a* (Turner et al., 2016; Saini et al., 2018). The distribution of disease severities for the RILs at the adult plant stage across all the environments was mostly continuous but somewhat skewed toward resistance in the Kenyan environments, suggesting a quantitative type of resistance. Lower correlation coefficients (0.21–0.32) were observed between ETH17 and the ETH16, KEN16, KEN17, STP17, and STP18 environments, suggesting genotype by environment interactions as indicated by the ANOVA results.
(Table 2). Stem rust severities of the entire population were relatively low in the ETH17 environment. The differences in environmental conditions, different races used for inoculation, and the amount of inoculum used in different environments could have also contributed to the observed significant genotype by environment interactions.

The identified QTL QSr.cdl-2BS.2 on chromosome arm 2BS with the explained phenotypic variance ranging from 10.5% to 17.6%, conferred adult plant resistance in the KEN16, ETH16, and ETH18 environments. The QTL was also identified in the validation population in Kenya in 2018. Several stem rust resistance genes have been mapped on chromosome arms 2BS and 2BL. The stem rust resistance genes effective to race TTKSK that mapped to chromosome arm 2BS are Sr36, Sr39, and Sr40 (Wu et al., 2009; Niu et al., 2011; Rouse et al., 2012). The gene Sr36 was identified in Triticum timopheevi, Sr39 was identified in Aegilops speltoides, and Sr40 was identified in Triticum araraticum. The resistance provided by the Sr36 gene was overcome by race TTKSK (virulent to Sr31 and Sr36) (Jin et al., 2009), whereas Sr39 and Sr40 genes appear effective against the Ug99 race group but have not been widely utilized in breeding programs due to their potential linkage drag. The alien species origin of genes Sr39 and Sr40 as well as susceptible ITs observed in the seedlings of the entire RIL population along with parents against race TTKSK ruled out the possibility of Sr39 or Sr40 conferring the observed resistance in this study.

The mapped genes on chromosome arm 2BL are Sr9h, Sr16, Sr28, and Sr47 (Tsilos et al., 2007; Hiebert et al., 2011; Rouse et al., 2014a). The genes Sr9h, Sr16, and Sr28 were identified in Triticum aestivum, whereas the gene Sr47 was identified in A. speltoides. The Sr16 gene was shown to be ineffective against Ug99 races (Jin et al., 2007). Because Sr16 and QSr.cdl-2BS.2 are on different chromosome arms, they cannot be the same. The Sr28 and Sr47 genes have been found effective against the Ug99 race group (Jin et al., 2007; Rouse et al., 2012), but the QTL QSr.cdl-2BS.2 identified in this study is unlikely either the Sr28 or Sr47 gene because of the observed susceptibility of CI 14275 at the seedling stage in response to race TTKSK. The Sr9 alleles Sr9a, Sr9b, Sr9d, Sr9e, Sr9f, and Sr9g are ineffective against Ug99 isolates, but the Sr9h allele has been found effective against race TTKSK and other races in the Ug99 race group with virulence to Sr31 (Rouse et al., 2014a). Based on the physical position of the peak marker, the QSr.cdl-2BS.2 found in this study is located around 58.3 Mb on the 90K consensus map (Wang et al., 2014). This map position excludes the possibility that QSr.cdl-2BS.2 could be conferred by stem rust resistance genes on the long arm of chromosome 2B. The QSr.umn-2B.2 reported by Bajgain et al. (2015) in a RB07/MN06113-8 population is between 22.7 and 40.7 Mb on 2B, and QSr.cdl-2BS reported by Rouse et al. (2014b) in the Thatcher/McNeal population is located between 12.0 and 31.7 Mb on 2B. The physical location of the reported QSr.cdl-2BS.2 is near these two previously reported QTL. Rouse et al. (2014b) found that the resistant allele of the reported QSr.cdl-2BS was contributed by Thatcher. Because Thatcher is a major component of the pedigree of CI 14275, it is likely that QSr.cdl-2BS from Rouse et al. (2014b) and QSr.cdl-2BS.2 from this study are conferred by the same gene(s) derived from Thatcher. Tdurum_contig54704_176, the peak marker associated with QSr.cdl-2BS.2, is located at 61 cm in the popseq consensus map, whereas the Excalibur_c7963_1722 marker that was picked up in the validation population is located at 60 cm in the popseq consensus map. A KASP marker was developed for Excalibur_c7963_1722 because it was found to be a flanking marker of QSr.cdl-2BS.2 in a preliminary QTL analysis. Several other studies have reported stem rust resistance QTLs on chromosome 2B (association mapping study QTL at 79.6 Mb on 2BL: Letta et al., 2013; GWAS study QTL located between 11.3 and 79.9 Mb: Prins et al., 2016; Gate and Cacuke/Yaye population between 65.8 and 73.9 Mb on 2BL: Randhawa et al., 2018). Our study is the first to both detect and validate an adult plant stem rust resistance QTL on chromosome arm 2BS.

QSr.cdl-3B.2 explains between 14.0 and 21.5% of the phenotypic variance and confers adult plant resistance in the KEN16, KEN17, KEN18, STP17, and STP18 environments. QSr.cdl-3B.2 is flanked by markers Excalibur_c57658_34, IAVV3838, RAC875_c10595_473, and RAC875_c69_499 in the various environments. The Sr2 gene is located on chromosome arm 3BS, whereas Sr12 is located near the centromere on chromosome 3B. Both were derived from Triticum turgidum. Sr2 is an adult plant resistance gene used in breeding as a source of durable resistance to stem rust although the gene may not provide adequate resistance under high disease pressure. Sr12 is present in all of the parents of CI 14275, cultivars Thatcher, Kenya Farmer, and Lee; therefore, Sr12 is almost certainly present in CI 14275 (unless there is a mistake in the pedigree or the gene postulations of the parents). The identified QSr.cdl-3B.2 in this study is located between 58.6 and 75.9 Mb on 3B and overlaps other reported QTLs on 3B (Thatcher/McNeal population located between 15.4 and 85.8 Mb, Rouse et al., 2014b; association mapping population located between 13.8 and 59.5 Mb, Bajgain et al., 2015; Spark/Ribalto DH population located between 20.1 and 73.8 Mb, Getie et al., 2016; GWAS study, located between 68.9 and 71.0 Mb, Prins et al., 2016; LMPG-6/P1362698-i population located between 62.2 and 72.9 Mb, Zurn et al., 2018) and is likely the gene Sr12.

The Sr12 gene (QSr.cdl-3B.2) alone did not provide strong adult plant resistance in three environments, suggesting the necessity of combining Sr12 and other QTL(s). The gene Lr34/Sr57 has been reported to enhance the effectiveness of Thatcher resistance in North America and Kenya (Kolmer et al., 2011), but the parents (LMPG-6 and CI 14275) were both negative for the Lr34 gene-based marker (CsLV34), indicating that the resistance in CI 14275 did not involve Lr34/Sr57. Lack of significant QTL on chromosome 7D in this study, where Lr34 is located, also is a strong indication that Lr34/Sr57 was not segregating in this population. The KASP markers linked to QSr.cdl-3B.2 were not associated with stem rust response in the validation population. To look into this further, we genotyped CI 14275, LMPG-6, line #162, and Kwale with the Sr12-associated marker from Hiebert et al. (2016): NB-LRR3. We found that LMPG-6 did not possess the Sr12 gene, but CI 14275 and both parents of the validation population (Line #162 and Kwale).
Kwale) did possess Sr12. Therefore, Sr12 is likely fixed in the validation population.

QSr.cdl-4AL, which explained a large proportion of the phenotypic variance (42.3%) for response to race TTTTF, is located at 73.3 Mb on chromosome arm 4AL. QSr.cdl-4AL is flanked by the markers Tdurum_contig42019_1714 and BS00099680_51, SrND643, a temporarily designated gene that is also located on chromosome arm 4AL, is reported to provide inadequate resistance under high disease pressure (Basnet et al., 2015). Even though SrND643 is located between 72.3 and 73.7 Mb at a similar location to identified QSr.cdl-4AL, SrND643 shows a low infection type of 2 to 22+ at the seedling stage against Ug99 races TTKSK and TTKST (Basnet et al., 2015). In contrast, line CI 14275 shows a high infection type of 3 to 33+ against Ug99 race TTKSK. These data suggest that QSr.cdl-4AL is not SrND643. Notably, CI 14275 and SrND643 were tested against the same isolate under the same temperature regime and in the same greenhouse but not in the same experiment. The stem rust resistance gene Sr7 with two characterized alleles Sr7a and Sr7b is located on chromosome arm 4AL, and these alleles confer resistance to some North American Pgt races (McIntosh et al., 1995; Turner et al., 2016). In this study, race TTTTF was virulent on Sr7b in the North American stem rust differential set with infection types ranging from 3− to 3+, ruling out the possibility of Sr7b being involved in the observed resistance. The QTL QSr.rwg-4A (believed to be Sr7a), is located between 59.1 and 73.9 Mb and expresses resistance to race TTTTF (Nirmala et al., 2017) in the durum wheat ‘Lesbock.’ The location of QSr.cdl-4AL and QSr.rwg-4A overlaps, and its contribution of three QTL: QSr.cdl-4AL-(the resistant parent in the validation population) possesses the susceptible alleles of the QSr.cdl-6A-linked markers. Selecting line #162 as a parent was undertaken based on the phenotypic data alone. Therefore, the absence of QSr.cdl-6A from line #162 precludes our laboratory indicate that TTTTF is avirulent to Sr7a although race TTKSK, which is predominant in Kenyan and Ethiopian environments, is virulent to Sr7b (Olivera et al., 2012; Hailu et al., 2015). The susceptibility of the RIL population to TTTTF rules out the possibility of QSr.cdl-6A being Sr7b. Nirmala et al. (2017) mapped Sr8155B1, a gene conferring resistance to race TTKST on chromosome 6A. Sr8155B1 is located between 6.7 and 10.9 Mb, a different location from the QTL QSr.cdl-6A identified in this study. In addition, data from our laboratory indicate that TTTTF is avirulent to Sr8155B1, ruling out the possibility of CI 14275 possessing Sr8155B1 because of its susceptibility to TTTTF (Table 1). The KASP markers linked to QSr.cdl-6A are not associated with stem rust resistance in the validation population. Unfortunately, line #162 (the resistant parent in the validation population) possesses the susceptible alleles of the QSr.cdl-6A-linked markers. Selecting line #162 as a parent was undertaken based on the phenotypic data alone. Therefore, the absence of QSr.cdl-6A from line #162 precludes our laboratory availability to validate this QTL in the validation population. Additional experiments are needed to validate the effectiveness of QSr.cdl-6A.

CONCLUSION

This study reports QTL for wheat stem rust resistance on chromosomes 2BS, 3B, 4AL, and 6A. The identified QTL QSr.cdl-2BS, which conferred adult plant resistance in the KEN16, ETH16, and ETH18 environments, was validated in a second population in KEN18 and can be selected for by the validated linked marker Excalibur_c7963_1722. This QTL, therefore, has the potential of being used in marker-assisted selection. Our study is the first to both detect and validate an adult plant stem rust resistance QTL on chromosome arm 2BS, QSr.cdl-3B.2, which confers resistance in the KEN16, KEN17, KEN18, STP17, and STP18 environments is likely Sr12. QSr.cdl-4AL, which confers resistance to race TTTTF, is postulated to be Sr7a. QSr.cdl-6A, which confers resistance in the ETH16, ETH18, STP16, and STP17 environments, is potentially a new QTL, but the QTL requires validation in another population before it is recommended for use in breeding. The adult plant resistance of wheat line CI 14275 in Africa is characterized as the cumulative contribution of three QTL: QSr.cdl-2BS, QSr.cdl-3B.2, and QSr.cdl-6A.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in online repositories. The names of the repository/repositories

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and accession number(s) can be found in the article/
Supplementary Material.

AUTHOR CONTRIBUTIONS

ZK, RD-M, and MNR contributed to writing of the initial drafts of the manuscript and provided insights on data analysis. YJ developed the mapping population and contributed to manuscript writing. EE helped with genotypic data analysis. WB, AG, GM, MSR, and SB facilitated the field studies in Africa. All authors contributed to the submitted manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.609659/full#supplementary-material

Supplementary Table 1 | Genetic map of the LMPG-6/Ci14275 population.

Supplementary Table 2 | Seedling infection types of 113 recombinant inbred lines (RILs) of the cross LMPG-6/Ci 14275 and the parents against five Puccinia graminis f. sp. triticici races: RTQTC, TTTF, TPMKc, TRTTF, and TTKSK.

Supplementary Table 3 | Stem rust severity and response of 113 recombinant inbred lines (RILs) of the cross LMPG-6/Ci 14275 and the parents to predominant Ug99 races in Kenya in 2016 (KEN16), 2017 (KEN17), and 2018 (KEN18). Data for two replicates [Rep] are displayed.

Supplementary Table 4 | Stem rust severity and response of 113 recombinant inbred lines (RILs) of the cross LMPG-6/Ci 14275 and the parents to predominant Ug99 races in Ethiopia in 2016 (ETH16), 2017 (ETH17), and 2018 (ETH18). Data for two replicates [Rep] are displayed.

Supplementary Table 5 | Stem rust severity and response of 113 recombinant inbred lines (RILs) of the cross LMPG-6/Ci 14275 and the parents to predominant Ug99 races in Ethiopia in 2016 (ETH16), 2017 (ETH17), and 2018 (ETH18). Data for two replicates [Rep] are displayed.

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