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ORIGINAL ARTICLE

Genetic mapping of resistance to the Ug99 race group of *Puccinia graminis* f. sp. *tritici* in a spring wheat landrace CItr 4311

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Abstract

Key message **A gene for Ug99 resistance from wheat landrace CItr 4311 was detected on the long arm of chromosome 2B.**

Abstract Wheat landrace CItr 4311 has seedling resistance to stem rust caused by *Puccinia graminis* f. sp. *tritici* race TTKSK and field resistance to the Ug99 race group. Parents, F_1 seedlings, 121 doubled haploid (DH) lines, and 124 recombinant inbred lines (RILs) developed from a cross between CItr 4311 and the susceptible line LMPG-6 were evaluated for seedling resistance to race TTKSK. Goodness-of-fit tests indicated that a single dominant gene in CItr 4311 conditioned the TTKSK resistance. The 90 K wheat iSelect SNP platform was used to genotype parents and the DH population. The seedling resistance locus was mapped to the chromosome arm 2BL. Parents and the DH population were evaluated for field resistance in Kenya. One major QTL for the field resistance was consistently detected in the same region on 2BL as the seedling resistance. Using KASP assays, five linked SNP markers were used to verify the result in the 124 RIL, 35 wheat accessions, 46 DH lines from the LMPG-6/PI 165194 cross and F_1 seedlings, and susceptible bulks derived from crosses between six resistant landraces with LMPG-6. Race specificity, mapping results, and haplotype similarity with lines with *Sr9h* (Gabo 56, Timstein, and PI 670015), support the hypothesis that the *Sr* gene in CItr 4311 and the landraces is *Sr9h*. The KASP assays developed in this study will be useful for pyramiding the TTKSK resistance from CItr 4311 with other *Sr* genes effective against Ug99.

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Introduction

A variant of the stem rust pathogen *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn (*Pgt*) was reported in Uganda in 1999 (Wanyera et al. 2006; Jin and Singh 2006). This variant was designated as race TTKSK and informally known as Ug99 (Jin and Singh 2006). Race TTKSK is virulent on the previously undefeated stem rust resistance gene *Sr31*, which had been deployed widely for decades (Wanyera et al. 2006). The pathogen has continued to evolve and new *Pgt* races have emerged and overcome other widely deployed resistance genes, including *Sr24*, *Sr36*, and *SrTmp* (Jin et al. 2008, 2009; Olivera et al. 2015). These new *Pgt* races have spread not only within East Africa, but also to Southern Africa, Egypt, Yemen, and Iran, and thus Ug99 and its variants have become important

concerns of wheat breeders and pathologists worldwide (Nazari et al. 2009; Singh et al. 2008, 2011; Patpour et al. 2016). Due to the continued evolution of new races within the Ug99 race group and the widespread susceptibility in wheat to TTKSK, work is underway to explore the genetic variability present in the wheat landraces from the USDA-ARS National Small Grains Collection (NSGC) and identify resistance to Ug99 (Babiker et al. 2016; Bonman et al. 2007; Newcomb et al. 2013). In a previous study, 2509 wheat landraces from the NSGC were evaluated for field response to Ug99 in field plots in Kenya. The spring wheat landrace accession CItr 4311, from Iran, showed field resistance to Ug99 in field tests in Kenya (Newcomb et al. 2013). The objectives of this study were to (1) determine the inheritance of TTKSK resistance in wheat landrace CItr 4311, (2) map the seedling and field resistance using SNP markers, and (3) using KASP assays, validate the ability of closely linked SNP markers to predict the presence of the *Sr* gene in a set of wheat accessions and mapping populations.

Materials and methods

Plant materials

To elucidate the genetic of resistance to race TTKSK in CItr 4311, the stem rust susceptible wheat line LMPG-6 was crossed to CItr 4311. The F_1 seeds were used to generate two populations composed of 121 DH lines and 124 RILs ($F_{5:8}$). LMPG-6 (Little club/Prelude*8/Marquis/3/Gabo) is a susceptible wheat line developed by Knott (1990). Accession CItr 4311 is a hexaploid wheat landrace from the NSGC that was collected in 1914 from Fars province, Iran. Another 46 DH lines were developed from a cross between LMPG-6 and PI 165194. Accession PI 165194 is a hexaploid wheat landrace from the NSGC that was collected from Turkey and was suspected to possess the same *Sr* gene as CItr 4311 based on infection type (IT) to race TTKSK and bulked segregant analysis results using the 90 K iSelect SNP genotyping platform (unpublished data). To verify the CItr 4311 mapping result in this study, 46 DH lines from the LMPG-6/PI 165194 population and a set of 35 hexaploid wheat accessions were used. The 35 accessions were selected as follows: 17 wheat landraces were suspected to possess *Sr9h* based on ITs, response to diverse *Pgt* races and bulked segregant analyses showing the location of resistance on chromosome arm 2BL (Babiker et al. 2016), three accessions (CItr 14035 'Gabo 56', CItr 12347 'Timstein', and PI 670015) with *Sr9h* (Rouse et al. 2014), *Sr9h* genetic stock RL6203 (Hiebert et al. 2010; Rouse et al. 2014), three accessions (ISr9a-RA, W2691Sr9b, and Vernstine) with *Sr9a*, *Sr9b*, and *Sr9e*, respectively (Jin et al. 2008), and the remaining accessions were chosen based on diversity of geographic origin and IT reactions to TTKSK. In addition to 35 wheat accessions, PI 626573 carrying *SrWLR* was included. Of the 17 wheat landraces suspected to carry *Sr9h*, six accessions (PI 178188, PI 623118, PI 623582, PI 623785, PI 626255, and PI 626491) were crossed to LMPG-6 and F_1 seedlings and susceptible F_2 seedlings were used for SNP validation in this study.

Seedling evaluation

Pgt race TTKSK, isolate 04KEN156/04, was used to evaluate seedling response to stem rust in parents, F_1 seedlings, 121 DH lines, 124 RILs, and 46 DH lines at the USDA-ARS Cereal Disease Laboratory (CDL) in St. Paul, MN. CItr 4311, PI 165194 and LMPG-6 were also tested against the *Pgt* race TTKST (isolate 06KEN19v3) (Jin and Singh 2006). Based on the infection type (IT) and the initial mapping results, we suspected that CItr 4311 possessed *Sr9h*. Therefore, CItr 4311 was inoculated with the *Pgt* races TTKSF (isolate UVPgt55) and TTKSF + *Sr9h* (isolate UVPgt61/1) at the University of Free State, South Africa (Pretorius et al. 2012). Races TTKSF and TTKSF + *Sr9h* are avirulent and virulent on *Sr9h*, respectively (Rouse et al. 2014). For seedling tests, five plants for each line were inoculated with the *Pgt* races according to Rouse et al. (2011) and assessed for seedling ITs using the 0–4 scale developed by Stakman et al. (1962). Chi-square (χ^2) test was performed to test for goodness-of-fit to models for one, two, or four genes.

Field evaluation

The parents and 121 DH lines from the LMPG-6/CItr 4311 population were evaluated for field response to the Ug99 race group at the adult plant stage in nurseries at Njoro, Kenya, during three growing seasons in 2014 and 2015. Lines were planted in 1-m rows with three replicates for each line, nine replicates for each of the parents, and cultivar 'Red Bobs' as a susceptible check. A mixture of cultivars with *Sr31* (susceptible to TTKSK) and *Sr24* (susceptible to TTKST) were planted adjacent to the plots as spreader rows. The spreader rows were inoculated with a bulk collection of *Pgt* isolates of the Ug99 race group at the jointing growth stage to produce spores for natural dispersal to the experimental plots (Newcomb et al. 2013). Stem rust severity was assessed at the soft dough stage using the modified Cobb scale (Peterson et al. 1948) and the infection response was rated as S (susceptible), MS (moderately susceptible), MR (moderately resistant), and R (resistant) (Roelfs et al. 1992). For each line, the stem rust severity was multiplied by a constant value for infection response

to obtain the coefficient of infection (CI) as described by Knott (1989). Analysis of variance was carried out using the General Linear Model Procedure in SAS (SAS Institute, Cary, NC, USA). The significance of genotype and replicate was examined. Mean CI value for each genotyped DH line across replication within each experiment was calculated in SAS and used in QTL analysis.

Map construction and QTL analysis

Total genomic DNA was extracted from leaves of two-week-old seedlings using a modified CTAB protocol (Babiker et al. 2015). The recently developed 90 K iSelect SNP genotyping platform was used to genotype parents and 121 DH lines from the LMPG-6/CItr 4311 population (Wang et al. 2014). Allele calling for each SNP was performed using Illumina's GenomeStudio v2011.1, and results were manually inspected for call accuracy. Based on the observed IT and the initial mapping results, we suspected that CItr 4311 possessed *Sr9h*. Therefore, the flanking SSR markers *gwm47* and *wmc332* for *SrWeb* (Hiebert et al. 2010; Rouse et al. 2014), later designated as *Sr9h* by Rouse et al. (2014), were used to genotype parents, 121 DH lines from LMPG-6/CItr 4311, and *Sr9h* genetic stock RL6203 (Hiebert et al. 2010; Rouse et al. 2014). A linkage map was constructed using JMP Genomics 7.1 (SAS Institute, Cary, NC, USA). The recombination and linkage groups function was used to determine the initial number of linkage groups and the linkage map order function was used to determine the most likely marker order. The single marker analysis function was used to identify markers with significant effects, while the composite interval mapping (CIM) analysis function was used to generate a composite interval map of QTL with a minimum logarithm of odds threshold of 5.0. Genetic distances between markers were calculated in centiMorgans (cM) using the Kosambi map function and linkage groups were assigned to chromosomes based on the consensus map (Wang et al. 2014).

Relationship of the gene on 2BL to *Sr9h* and development of KBioscience competitive allele specific PCR (KASP) assays for SNP validation

To verify mapping results from the DH population, seven SNP markers flanking the resistance locus in CItr 4311 were converted to KASP assays and used to genotype 124 RILs from the LMPG-6/CItr 4311 population, 46 DH lines from the LMPG-6/PI 165194 population, a set of 35 hexaploid wheat accessions, PI 626573 (*SrWLR*), and RL6203 (*Sr9h*). In addition, F_1 seedlings, and susceptible bulks of F_2 seedlings derived from cross between each of PI 178188, PI 623118, PI 623582, PI 623785, PI 626255, and PI 626491 with LMPG-6 were genotyped using KASP assays. KASP assays were designed using the source sequences from the wheat 90 K iSelect assay (Table 2) and performed according to manufacturer's instructions (LGC Genomics, Beverly, MA).

Results

Inheritance of seedling resistance in CItr 4311

Based on seedling inoculation tests, wheat landrace CItr 4311 exhibited low IT of '2' when inoculated with *Pgt* race TTKSK, whereas LMPG-6 exhibited high ITs of '3+' to '4'. The progeny of the RIL and DH populations segregated for resistance to *Pgt* race TTKSK with resistant seedlings exhibiting ITs of '2' and '2+' and susceptible seedlings exhibiting ITs of '3+' and '4'. Segregation ratios in both populations fitted the 1:1 expectation for a single gene ($\chi^2 = 3.03$, $P = 0.08$ for the DH; $\chi^2 = 3.7$, $P = 0.054$ for the RIL). The F_1 seedlings were all resistant to *Pgt* race TTKSK at the seedling test, indicating dominant gene action. CItr 4311 exhibited a low IT of '2' when inoculated with *Pgt* race TTKSF, which is avirulent on *Sr9h*, and high IT of '4' when inoculated with *Pgt* race TTKSF + *Sr9h*, indicating the likely presence of *Sr9h* in CItr 4311.

Mapping seedling resistance to *Puccinia graminis* race TTKSK

To map seedling resistance to race TTKSK, data from the SNP markers analysis of the parental lines and 121 DH lines from the LMPG-6/CItr 4311 population were used. Of the 81587 wheat SNP markers tested, 9449 were polymorphic between the two parents. Among the polymorphic markers, 7701 had missing data for less than 9 % and had previously been assigned to chromosomes (Wang et al. 2014). These markers were assigned to 47 linkage groups representing the 21 chromosomes of wheat. Linkage analysis revealed that the TTKSK resistance locus was tightly linked to a group of six SNP markers. Four of the SNP markers (IWB56162, IWB65547, IWB54116, and IWA7850) were located at 109.53 cM on the long arm of chromosome 2B on the wheat consensus map (Fig. 1a).

SSR marker *gwm47* produced fragments of 206, 176, and 186 bp from LMPG-6, CItr 4311, and RL6203, respectively. Marker *wmc332* produced a 169 bp fragment from CItr 4311 and RL6203 and a 195 bp fragment from LMPG-6. When used to genotype the DH lines from the LMPG-6/CItr 4311 population, *gwm47* was tightly linked to a group of nine SNP markers and was mapped 3.7 cM proximal to the TTKSK locus. Marker *wmc332* mapped 39.3 cM proximal to the TTKSK resistance locus (Fig. 1a).

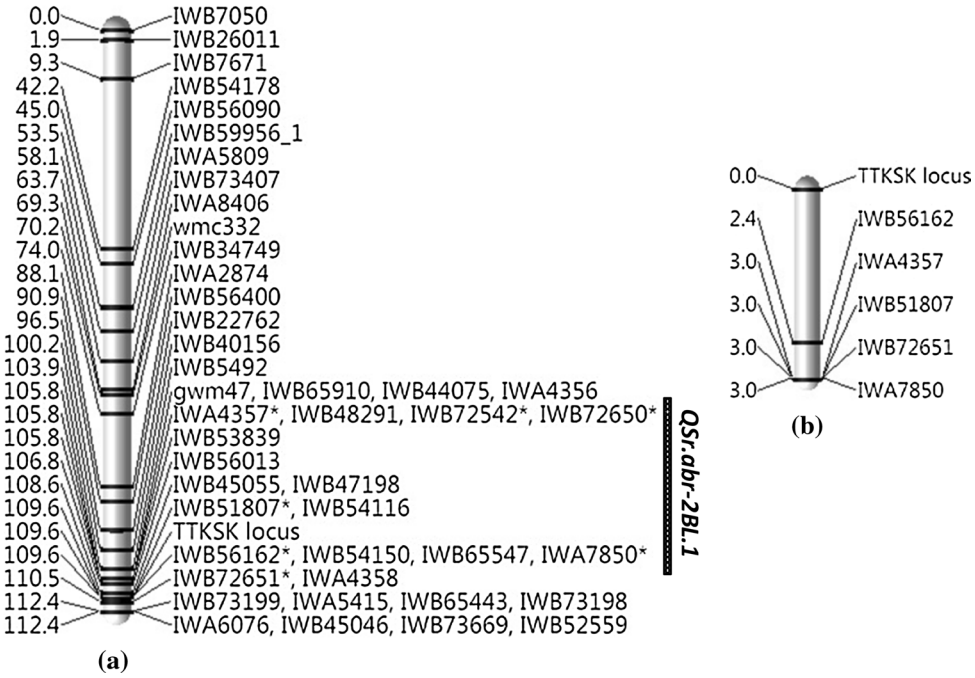

Fig. 1 Genetic map of TTKSK resistance locus on the long arm of chromosome 2B constructed using the **a** LMPG-6/CItr 4311 DH population using the 90 K SNP iSelect Infinium assay and **b** LMPG-6/CItr 4311 RIL population using KASP assays. Distance are reported in cM. *Markers significantly associated with the TTKSK seedling resistance

Genetic mapping of field resistance

In the field stem rust nurseries in Njoro, Kenya, CItr 4311 exhibited disease severities and infection responses ranging from 15MR to 30 M and LMPG-6 exhibited responses ranging from 35MS-S to 60S. The severities and infection responses of the DH population ranged from 5MR to 60S. QTL analyses were conducted for each of the three field experiments using the mean CI values. Using the CIM function, one QTL for resistance to Ug99 was detected on the long arm of chromosome 2B in a 6.1 cM interval in each of the three field experiments. This detected QTL designated as *QSr.abr-2BL.1* and explained 33–46 % of the phenotypic variation (Table 1; Fig. 1a).

Development of KBioscience competitive allele specific PCR (KASP) assays

To select the best SNP markers for KASP assays, the SNP markers tightly linked or flanking the TTKSK resistance locus were inspected in GenomeStudio. Five of the linked SNP markers from QTL mapping showed three distinct clusters discriminating between the LMPG-6, CItr 4311 and the F_1 seedlings. These five SNP markers were selected for marker validation in KASP assays (Table 2). When tested in the RIL population, the KASP assays designed from the SNP marker *IWB56162* mapped 2.4 cM distal to the TTKSK resistance locus, whereas markers *IWA4357*, *IWB51807*, *IWB72651*, and *IWA7850* mapped 2.96 cM distal to the resistance locus (Fig. 1b). When used in KASP assays to genotype the F_1 seedlings and bulks of seedlings susceptible to F_2 progenies developed from crossing each of PI 178188, PI 623118, PI 623582, PI 623785, PI 626255, and PI 626491 with LMPG-6, the five SNP markers were monomorphic between LMPG-6 and the susceptible bulks, polymorphic between LMPG-6 and the resistant parents, and detected the heterozygosity in F_1 seedlings (Table 3). Using KASP assays, the five SNP markers were monomorphic between CItr 4311, the 17 wheat landraces suspected to possess *Sr9h*, and accessions CItr 14035 'Gabo 56', CItr 12347 'Timstein', and PI 670015, which are known to have *Sr9h*, indicating that the TTKSK resistance in the 17 landraces is likely due to the presence of *Sr9h* (Table 4). When the KASP assays were performed on 46 DH lines from the LMPG-6/PI 165194 cross, the five SNP markers were polymorphic between LMPG-6 and PI 165194 and tightly linked with the TTKSK resistance locus in PI 165194 (Table 5), indicating that the TTKSK resistance in PI 165194 is likely controlled by the same gene as in CItr 4311. In contrast, results from the KASP assays on RL6203 and PI 626573, which are reported to carry *Sr9h* and *SrWLR*, respectively, showed that four SNP markers were monomorphic between LMPG-6 and PI 626573 and all the five SNP markers were monomorphic between RL6203 and LMPG-6.

Discussion

New races of *Pgt* have been detected in East Africa since 1998 (Jin et al. 2008, 2009; Olivera et al. 2015). These races overcome several key genes for resistance to stem

Table 1 Quantitative trait loci for field resistance to Ug99 in the LMPG-6/CItr 4311 DH population assessed in three seasons in Kenya

		May-14			Oct-14			May-15		
SNP_ID	Position (cM)[a]	LOD	Additive	R2	LOD	Additive	R2	LOD	Additive	R2
gwm47		11.27	−17.92	0.45	11.25	−30.34	0.41	11.62	−39.09	0.46
IWB65910, IWB44075, IWA4356, IWA4357, IWB50803, IWB53839, IWB48291, IWB72542, IWB72650	109.53	11.27	−17.92	0.45	11.25	−30.34	0.41	11.62	−39.09	0.46
IWB56013	109.53	10.05	−16.97	0.42	10.02	−28.54	0.38	10.38	−37.03	0.42
IWB45055	109.53	10.05	−16.97	0.42	9.79	−27.86	0.37	10.38	−37.03	0.42
IWB47198	110.22	10.05	−16.97	0.42	9.79	−27.86	0.37	10.38	−37.03	0.42
IWB51807	109.14	11.27	−17.92	0.45	11.25	−30.34	0.41	11.62	−39.09	0.46
IWB54150	110.48	11.27	−17.92	0.45	11.25	−30.34	0.41	11.62	−39.09	0.46
IWB56162, IWB65547, IWB54116, IWA7850, IWB72651, IWA4358	109.53	11.27	−17.92	0.45	11.25	−30.34	0.41	11.62	−39.09	0.46
IWB68982, IWB73669	114.09	10.23	−17.74	0.42	8.41	−28.11	0.33	11.27	−38.78	0.45
IWB67609, IWB68993, IWB45046, IWA6076	107.49	10.23	−17.74	0.42	8.41	−28.11	0.33	11.27	−38.78	0.45
IWB73199, IWB73198	108.04	10.23	−17.74	0.42	8.41	−28.11	0.33	11.27	−38.78	0.45
IWB73241	109.53	10.23	−17.74	0.42	8.41	−28.11	0.33	11.27	−38.78	0.45
IWB67744, IWB65526, IWB67608, IWB47224, IWB62876, IWB70307, IWB69070, IWA2131, IWA2903, IWA2903, IWA5789, IWA2130	108.45	10.23	−17.74	0.42	8.41	−28.11	0.33	11.27	−38.78	0.45
IWA5415, IWB52559, IWB65443	107.40	10.23	−17.74	0.42	8.41	−28.11	0.33	11.27	−38.78	0.45

[a] Distance based on a reference consensus map (Wang et al. 2014)

Table 2 KASP assay markers on chromosome arm 2BL used to validate the DH result in the LMPG-6 × CItr 4311 RIL population

SNP ID	Primer name	Primer sequence[a]	Allele	Parent
IWB51807	IWB51807_ALT	CAATGGACGGGACTAGCTAGATT	T	CItr 4311
	IWB51807_ALC	TCAATGGACGGGACTAGCTAGATC	C	LMPG-6
	IWB51807_C1	CTGTGCTATTGCATTACATGGCTTTGATA		
IWA4357	IWA4357_ALA	CTAAATGACGGTCTTCTGAAGTTGA	A	CItr 4311
	IWA4357_ALG	TCTAAATGACGGTCTTCTGAAGTTGG	G	LMPG-6
	IWA4357_C1	CTGCGCAATTTCCAAACTACCATTATGTA		
IWB72651	IWB72651_ALT	TTCTATCTATCAATAAGGCTACTGCAACTT	T	CItr 4311
	IWB72651_ALG	TCTATCAATAAGGCTACTGCAACTG	G	LMPG-6
	IWB72651_C1	AGGTGTGATGGCTGATGAAG		
IWB56162	IWB56162_ALT	CTTCGTCTACAACTGTGCAATAAT	T	CItr 4311
	IWB56162_ALC	TCTTCGTCTACAACTGTGCAATAAC	C	LMPG-6
	IWB56162_C1	TGGGTTTTGCTAAACAAGTGGT		
IWA7850	IWA7850_ALT	CTCCGCGAGTACTCCTTGAT	T	CItr 4311
	IWA7850_ALC	CTCCGCGAGTACTCCTTGAC	C	LMPG-6
	IWA7850_C1	GAGGACGAGAACTCTGAATTCTCA		

[a] Sequences do not include a tail

rust in CIMMYT germplasm and in US wheat cultivars and breeding lines. In this study, seedling and field resistance to Ug99 in CItr 4311 was mapped to chromosome arm 2BL. Four designated *Sr* genes, *Sr9*, *Sr16*, *Sr28* and *Sr47*, were previously mapped to 2BL. *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, and *Sr16* were characterized as ineffective against Ug99 (Jin et al. 2007). *Sr47* is effective against Ug99 and was introgressed into wheat chromosome arm 2BL from *Aegilops speltoides* (Klindworth et al. 2012). However, the gene in CItr 4311 cannot be *Sr47* because *Sr47* confers resistance to *Pgt* races MCCFC, QFCSC and TPMKC (Klindworth et al. 2012) and these three races are virulent on CItr 4311. Similarly, the gene in CItr 4311 is unlikely to be *Sr28* because (1) CItr 4311 shows a different IT of '2'

Table 3 Haplotype analysis of accessions suspected to possess *Sr9h*, F_1 seedlings, and bulks from susceptible F_2 seedlings (BS) generated from crossing these accessions with LMPG-6, genotyped with five SNP markers associated with TTKSK resistance in CItr 4311

Name	IWB51807[a]	IWA7850[a]	IWB56162[a]	IWA4357[a]	IWB72651[a]
LMPG-6	AA	AA	AA	AA	AA
PI 178188	BB	BB	BB	BB	BB
PI 178188 F1	AB	AB	AB	AB	AB
PI 178188 BS1	AA	AA	AA	AA	AA
PI 623118	BB	BB	BB	BB	BB
PI 623118 F1	AB	AB	AB	AB	AB
PI 623118 BS1	AA	AA	AA	AA	AA
PI 623118 BS2	AA	AA	AA	AA	AA
PI 623582	BB	BB	BB	BB	BB
PI 623582 F1	AB	AB	AB	AB	AB
PI 623582 BS1	AA	AA	AA	AA	AA
PI 623582 BS2	AA	AA	AA	AA	AA
PI 623785	BB	BB	BB	BB	BB
PI 623785 F1	AB	AB	AB	AB	AB
PI 623785 BS1	AA	AA	AA	AA	AA
PI 623785 BS2	AA	AA	AA	AA	AA
PI 626255	BB	BB	BB	BB	BB
PI 626255 F1	AB	AB	AB	AB	AB
PI 626255 BS1	AA	AA	AA	AA	AA
PI 626255 BS2	AA	AA	AA	AA	AA
PI 626491	BB	BB	BB	BB	BB
PI 626491 F1	AB	AB	AB	AB	AB
PI 626491 BS1	AA	AA	AA	AA	AA
PI 626491 BS2	AA	AA	AA	AA	AA

[a] SNP markers used in KASP assays

to race TTKSK compared to *Sr28* ';13' (Rouse et al. 2012), (2) CItr 4311 is resistant to race RKQQC and *Sr28* is ineffective against race RKQQC (data not shown), and (3) the gene in CItr 4311 is more loosely linked to SSR marker *wmc332* (39.3 cM) versus values reported for *Sr28* (2.4, 4.7, and 5.6 cM) (Rouse et al. 2012; 2014). In addition to the four designated *Sr* genes, two temporarily designated genes, *SrWeb* and *SrWLR*, were previously mapped to chromosome arm 2BL (Hiebert et al. 2010; Zurn et al. 2014). *SrWeb*, later designated to be *Sr9h* (Rouse et al. 2014), was mapped 1.4 cM distal and 12.4 cM proximal to markers *gwm47* and *wmc332*, respectively, on chromosome arm 2BL near the *Sr9* locus (Hiebert et al. 2010). The stem rust resistance gene *SrWLR* segregated with five SNP markers, IWA6121, IWA6122, IWA7620, IWA8295, and IWA8362 (Zurn et al. 2014) and of these, only IWA6122 and IWA8295 were included in the 90 K wheat iSelect SNP genotyping platform but were monomorphic between LMPG and CItr4311. However, the consensus map locations of IWA6122 and IWA8295 on 2BL is 112.94 cM compared to 109.53 cM for the five co-segregating SNP markers found in this study (Wang et al. 2014). In addition, *SrWLR* was mapped to the same region as *SrWeb* (*Sr9h*), but Zurn et al. (2014), suggested that *SrWLR* could be a different gene than *SrWeb* because of variability among 12 SNPs flanking the *SrWLR* locus. Although in this study the SSR marker *gwm47* produced a fragment size that differed between RL6203 and CItr 4311 (186 versus 176 bp), *gwm47* was nonetheless linked to resistance, mapping 3.7 cM proximal to the TTKSK locus. Marker *wmc332* produced a 169 bp fragment from CItr 4311 and RL6203 and a 195 bp fragment from LMPG-6. Marker *wmc332* mapped 39.3 cM proximal to the TTKSK resistance locus in the LMPG-6/CItr 4311 DH population, whereas in RL6071 X Webster map it was mapped 12.4 cM from *SrWeb* (Hiebert et al. 2010). This inconsistency of the location could be attributed to the increased number of meiotic events allowing for the opportunity for additional recombination in the F_2 population compared to the DH population (Somers et al. 2004). In a previous study, a gene postulated to be *Sr9h* was located at 101.5–109.5 cM on the wheat consensus map (Babiker et al. 2016). In this study, the field resistance QTL on 2BL in CItr 4311 mapped to a location of 109.5–114.1 cM on the wheat consensus map, and the corresponding seedling resistance gene mapped to 109.53 cM, which corresponds to the location of *Sr9h*. To verify these results, CItr 4311 was tested against *Pgt* races TTKSF + *Sr9h*, and TTKSF which are known to be virulent and avirulent on *Sr9h*, respectively

Table 4 Origin and seedling infection type against *Puccinia graminis* f. sp. *tritici* race TTKSK of various wheat accessions genotyped with five SNP markers associated with TTKSK resistance in CItr 4311 using KASP assays

Accession	Origin	TTKSK (IT)	IWB51807[a]	IWA7850[a]	IWB56162[a]	IWA4357[a]	IWB72651[a]
LMPG-6	Canada	4	AA	AA	AA	AA	AA
CItr 4311	Iran	2	BB	BB	BB	BB	BB
PI 165193[c]	Turkey	2	BB	BB	BB	BB	BB
PI 166675[c]	Turkey	2	BB	BB	BB	BB	BB
PI 167531[c]	Turkey	2	BB	BB	BB	BB	BB
PI 178188[c]	Turkey	2	BB	BB	BB	BB	BB
PI 165194	Turkey	2	BB	BB	BB	BB	BB
PI 24484[c]	Uzbekistan	2	BB	BB	BB	BB	BB
PI 429407[c]	Iran	2	BB	BB	BB	BB	BB
PI 623118[c]	Iran	2	BB	BB	BB	BB	BB
PI 623582[c]	Iran	2	BB	BB	BB	BB	BB
PI 623785[c]	Iran	2	BB	BB	BB	BB	BB
PI 625315[c]	Iran	2	BB	BB	BB	BB	BB
PI 625348[c]	Iran	2	BB	BB	BB	BB	BB
PI 626074[c]	Iran	2	BB	BB	BB	BB	BB
PI 626255[c]	Iran	2	BB	BB	BB	BB	BB
PI 626409[c]	Iran	2	BB	BB	BB	BB	BB
PI 626491[c]	Iran	2	BB	BB	BB	BB	BB
PI 626634[c]	Iran	2	BB	BB	BB	BB	BB
PI 243779[c]	Iran	2	BB	BB	BB	BB	BB
CItr 14035[b] (Gabo 56)	Mexico	2	BB	BB	BB	BB	BB
PI 670015[b]	USA	;3	BB	BB	BB	BB	BB
CItr 12347[b]	USA	2	BB	BB	BB	BB	BB
PI 625285	Iran	2	BB	BB	AA	BB	BB
CItr 15035	Afghanistan	2	BB	BB	AA	BB	BB
PI 347169	Afghanistan	2	BB	BB	AA	BB	BB
ISr9a-RA	USA	4	BB	BB	AA	AA	AA
W2691Sr9b	USA	4	BB	BB	AA	BB	BB
Vernstine (*Sr9e*)	Australia	4	AA	AA	AA	BB	BB
PI 625661	Iran	2	AA	AA	BB	AA	AA
Chinese spring	USA	4	AA	AA	AA	AA	AA
CItr 15026	Afghanistan	3	AA	AA	AA	AA	AA
PI 177906	Turkey	;	AA	AA	BB	AA	AA
PI 184221	Serbia	2	AA	AA	AA	AA	AA
PI 374670	Bosnia and Herzegovina	;13	AA	AA	AA	AA	AA
McNair 701	NC, USA	4	AA	AA	AA	BB	AB
PI 626573 (*SrWLR*)	Iran	2	AA	AA	BB	AA	AA
RL6203[b]	Canada	2	AA	AA	AA	AA	AA
PI 341431	Turkey	4	AA	AA	AA	AA	AA

[a] SNP markers used in KASP assays

[b] Accessions known to carry *Sr9 h* (Rouse et al. 2014)

[c] Accessions suspected to possess *Sr9 h* (Babiker et al. 2016)

(Rouse et al. 2014). CItr 4311 exhibited a low IT to TTKSF and a high IT to TTKSF + *Sr9h*, indicating the likely presence of *Sr9h* in CItr 4311. Both the IT pattern of CItr 4311 against *Pgt* races TTKSK, TTKSF, and TTKSF + *Sr9h*, and the mapping results support the hypothesis that this wheat landrace carries *Sr9h*.

Using KASP assays, five SNP markers showed linkage to the *Sr* gene in CItr 4311. Identical alleles were observed

Table 5 Seedling infection type against *Puccinia graminis* f. sp. *tritici* race TTKSK of 46 DH lines from LMPG-6/PI 165194 cross genotyped with five SNP markers associated with TTKSK resistance in CItr 4311

Name	TTKSK (IT)	IWB51807[a]	IWA7850[a]	IWB56162[a]	IWA4357[a]	IWB72651[a]
LMPG-6	4	AA	AA	AA	AA	AA
PI 165194	2	BB	BB	BB	BB	BB
DH-1	2	BB	BB	BB	BB	BB
DH-2	2	BB	BB	BB	BB	BB
DH-4	2	BB	BB	BB	BB	BB
DH-5	4	AA	AA	AA	AA	AA
DH-6	4	AA	AA	AA	AA	AA
DH-7	4	AA	AA	AA	AA	AA
DH-8	4	AA	AA	AA	AA	AA
DH-9	2	BB	BB	BB	BB	BB
DH-10	2	BB	BB	BB	BB	BB
DH-12	4	AA	AA	AA	AA	AA
DH-13	4	AA	AA	AA	AA	AA
DH-14	2	BB	BB	BB	BB	BB
DH-15	2	BB	BB	BB	BB	BB
DH-16	2	BB	BB	BB	BB	BB
DH-17	2	BB	BB	BB	BB	BB
DH-18	4	AA	AA	AA	AA	AA
DH-19	4	AA	AA	AA	AA	AA
DH-20	2	BB	BB	BB	BB	BB
DH-21	2	BB	BB	BB	BB	BB
DH-22	4	AA	AA	AA	AA	AA
DH-23	4	AA	AA	AA	AA	AA
DH-24	2	BB	BB	BB	BB	BB
DH-25	4	AA	AA	AA	AA	AA
DH-26	4	AA	AA	AA	AA	AA
DH-27	4	AA	AA	AA	AA	AA
DH-28	2	BB	BB	BB	BB	BB
DH-30	2	BB	BB	BB	BB	BB
DH-31	2	BB	BB	BB	BB	BB
DH-32	2	BB	BB	BB	BB	BB
DH-33	4	AA	AA	AA	AA	AA
DH-34	2	BB	BB	BB	BB	BB
DH-35	2	BB	BB	BB	BB	BB
DH-36	2	BB	BB	BB	BB	BB
DH-37	2	BB	BB	BB	BB	BB
DH-38	2	BB	BB	BB	BB	BB
DH-39	2	BB	BB	BB	BB	BB
DH-40	2	BB	BB	BB	BB	BB
DH-41	2	BB	BB	BB	BB	BB
DH-42	4	AA	AA	AA	AA	AA
DH-43	2	BB	BB	BB	BB	BB
DH-44	2	BB	BB	BB	BB	BB
DH-45	2	BB	BB	BB	BB	BB
DH-46	2	BB	BB	BB	BB	BB
DH-47	2	BB	BB	BB	BB	BB
DH-48	2	BB	BB	BB	BB	BB
DH-49	2	BB	BB	BB	BB	BB

[a] SNP markers used in KASP assays

for the five SNP markers linked to the *Sr* gene in CItr 4311, the *Sr9h* carriers (Gabo 56, Timstein, and PI 670015), and 17 landraces suspected of carrying the *Sr9h*. Previous allelism testing between Gabo 56 and *Sr9e* line Vernstein, Rouse et al. (2014) suggested that Gobo 56 possess an allele at the *Sr9* locus. In the same study, the allelism test between Gabo 56 and Webster indicated that both Gabo 56 and Webster carry the same *Sr* gene (*Sr9h*) (Rouse et al. 2014). In this study, lines with *Sr9h* (Gabo 56, Timstein, and PI 670015) produced the same haplotype with the five KASP assays as CItr 4311 (Table 4), supporting the hypothesis that CItr 4311 carries *Sr9h*. Neither the *SrWLR* source (PI 626573) nor the *Sr9h* genetic stock (RL6203) used in this study produced the same haplotype as the other *Sr9h* sources (Table 4), supporting the observation of Zurn et al. (2014) of SNP variability in this region. However, allelism studies are needed to confirm the relationship between the TTKSK resistance locus on chromosome arm 2BL of CItr 4311 and *Sr* genes in Gabo 56, Webster, and PI 626573. Results from the bulked segregant analysis of F_2 susceptible seedlings from progenies developed from crosses of six wheat landraces with LMPG-6 and the analysis of DH progeny from the cross with PI 165194 suggest that these seven wheat landraces carry the same *Sr* gene as CItr 4311. Results from this study also indicate that many other NSGC landrace accessions are likely to carry the same *Sr* gene as CItr 4311. The markers developed in this study will be useful for surveying the NSGC collection to determine the relative distribution of *Sr9h* among landraces of diverse geographic origin. The order of markers in the DH population was generally in agreement with the previously published map (Wang et al. 2014) with the exception of a few markers where the order was reversed. The QTL for the field resistance was detected at the same locus found for seedling resistance, suggesting that the same gene is responsible for both seedling and field resistance to Ug99. The QTL for this field resistance was consistent across the three seasons. The tightly linked markers for the *Sr* gene in CItr 4311 will provide a useful tool to pyramid this gene with other genes effective against TTKSK on 2BL and with other effective resistances located on other chromosomes.

In conclusion, we have mapped seedling and field resistance to Ug99 in wheat landrace CItr 4311 to chromosome 2BL and hypothesize that this resistance is due to *Sr9h*. KASP assays used in this study may facilitate rapid introgression of this resistance into wheat breeding lines, but only in combination with other effective genes to preserve the effectiveness of this resistance source (Singh et al. 2008).

Author contribution statement E M. Babiker and J. M. Bonman contributed to all the experimental process, carried out the QTL analysis and prepared the manuscript. M. Newcomb generated the F_1 seeds. T. C. Gordon advanced the mapping populations. S. Chao genotyped the DH population with the 90 K SNP genotyping platform. M. N. Rouse and Z. A. Pretorius involved in the domestic and foreign races screening. R. Wanyera helped in the field screening in Kenya. G. Brown-Guedira genotyped the DH population with SSR markers. All authors have contributed to the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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