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ORIGINAL PAPER

Mapping resistance to the Ug99 race group of the stem rust pathogen in a spring wheat landrace

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Abstract

Key message **A new gene for Ug99 resistance from wheat landrace PI 374670 was detected on the long arm of chromosome 7A.**

Abstract Wheat landrace PI 374670 has seedling and field resistance to stem rust caused by *Puccinia graminis* f. sp *tritici* Eriks. & E. Henn (*Pgt*) race TTKSK. To elucidate the inheritance of resistance, 216 BC_1F_2 families, 192 double haploid (DH) lines, and 185 recombinant inbred lines (RILs) were developed by crossing PI 374670 and the susceptible line LMPG-6. The parents and progeny were evaluated for seedling resistance to *Pgt* races TTKSK, MCCFC, and TPMKC. The DH lines were tested in field stem rust nurseries in Kenya and Ethiopia. The DH lines were genotyped with the 90K wheat iSelect SNP genotyping platform. Goodness-of-fit tests indicated that a single dominant gene in PI 374670 conditioned seedling resistance to the three *Pgt* races. The seedling resistance locus mapped to the long arm of chromosome 7A and this result was verified in the RIL population screened with the flanking SNP markers using KASP assays. In the same region, a major QTL for field resistance was detected in a 7.7 cM interval and explained 34–54 and 29–36 % of the variation in Kenya and Ethiopia, respectively. Results from tests with specific *Pgt* races and the *csIH81* marker showed that the resistance was not due to *Sr22*. Thus, a new stem rust resistance gene or allele, either closely linked or allelic to *Sr15*, is responsible for the seedling and field resistance of PI 374670 to Ug99.

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Introduction

Stem rust disease caused by *Puccinia graminis* f. sp *tritici* Eriks. & E. Henn (*Pgt*) is one of the most important diseases of wheat worldwide (Roelfs et al. 1992). A new race of *Pgt* with virulence to the widely deployed stem rust resistance gene *Sr31*, originally designated as race TTKS by International *Pgt* race nomenclature (Wanyera et al. 2006; Jin and Singh 2006) and commonly referred to as 'Ug99', was identified in Uganda in 1999 (Pretorius et al. 2000). Variants of race TTKS were found with virulence to *Sr24* in Kenya, South Africa, and Eritrea (Jin et al. 2008; Pretorius et al. 2010; Wolday et al. 2011; Hale et al. 2013). This discovery resulted in addition of a fifth set of genes to the International *Pgt* race nomenclature differential set (Jin et al. 2008). Variants of race TTKSK with combined virulence to *Sr31* and *Sr36* were detected in Kenya and Tanzania (Hale et al. 2013; Jin et al. 2009; Hale et al. 2013). In addition, race TTKSF+ was detected in South Africa with additional virulence to lines with resistance gene *Sr9h* (Pretorius et al. 2012; Rouse et al. 2014). Due to the evolution of the new races and wide spread susceptibility in wheat to the Ug99 race group, work is underway worldwide to identify resistance to Ug99 in both cultivated and wild relatives of wheat. One potential source of new genes for resistance to Ug99 is the many wheat landrace accessions available in the USDA-ARS National Small Grains Collection (NSGC). Previously, 2509 NSGC landrace accessions were evaluated in a field stem rust screening nursery in Njoro, Kenya, where several races of the Ug99 race group appear to be endemic. Among the resistant landrace accessions was PI 374670, originally from Bosnia and Herzegovina. This accession showed adult infection responses in Njoro ranging from resistant (R) to moderately resistant (MR) with a low disease severity (Newcomb et al. 2013). In seedling tests this accession showed infection types (IT) ranging from ';' to ';13' to races TTKSK, MCCFC and TPMKC (*unpublished data*) on the standard IT scale (Stakman et al. 1962).

Identifying molecular markers tightly linked to the Ug99 resistance in new resistant sources is necessary for efficient transfer of the resistance into breeding lines, minimizing linkage drag, and combining such resistance with other effective resistance genes. Recently, the development of SNP platforms that assay 9,000 and 90,000 markers in parallel has resulted in the construction of a high-resolution SNP map of wheat (Cavanagh et al. 2013;Wang et al. 2014), which will facilitate mapping *Pgt* resistance genes to specific chromosomes. The objectives of this study were to determine the inheritance of Ug99 resistance in wheat landrace PI 374670, to ascertain if resistance assayed in seedling tests corresponds to resistance assayed under field conditions, and to map the resistance using SNP markers.

Materials and methods

Population development

To elucidate genetic control of stem rust resistance in PI 374670, a population of 192 double haploid (DH) lines and a population of 185 recombinant inbred lines (RIL) ($F_{5:7}$) were developed from a cross between LMPG-6 and PI 374670. Accession PI 374670 is a common wheat (*Triticum aestivum* subsp. *aestivum*) landrace from the NSGC that was collected from Bosnia and Herzegovina in 1972. LMPG-6 is a highly susceptible wheat line developed by Knott (1990). F_1 plants derived from F_1 seed of the same spike were used to generate 216 BC_1F_2 families by crossing with the recurrent parent LMPG-6.

Seedling evaluation

Pgt race TTKSK, isolate 04KEN156/04, was used to evaluate seedling response to stem rust in PI 374670, LMPG-6, the BC_1F_2 families, 186 DH lines, and 185 RILs at the USDA-ARS Cereal Disease Laboratory (CDL) in St. Paul, MN. The DH and the RIL populations were also screened against *Pgt* races MCCFC and TPMKC (isolates 59KS19 and 74MN1409, respectively). PI 374670 and LMPG-6 were also tested against the *Pgt* race TTKST (isolate 06KEN19v3) (Jin and Singh 2006). For seedling tests, five plants for each line were inoculated with the *Pgt* races according to Rouse et al. (2011a) and assessed for seedling ITs using the 0–4 scale developed by Stakman et al. (1962). For the BC_1F_2 families, at least 15 plants were evaluated in each family. Chi-square (χ^2) analyses was performed to test for goodness-of-fit to the models of one, two, or four genes. Based on the initial mapping results, it was necessary to distinguish the gene in PI 374670 from *Sr22*. Therefore, PI 374670 was screened against *Pgt* races TRTTF (isolate 06YEM34-1), TTTTF (isolate 01MN84A-1-2) and RKQQC (isolate 99KS76A) which are known to be virulent on *Sr22* (Rouse and Jin 2011; Olson et al. 2010).

Field evaluation

In 2013, parents and 153 DH lines were evaluated in the field in Njoro, Kenya (infection of wheat genetic stock lines indicated that race TTKST was predominant in this nursery) and at Debre Zeit, Ethiopia (infection of wheat genetic stock lines indicated that race TTKSK was predominant). Lines were planted in hill plots with three replicates for each line, nine replicates for each of the parents, and cultivar 'Red Bobs' as a susceptible check in the Njoro nursery. In Ethiopia, each line was planted in a one meter row with three replicates and nine replicates for each of the parents. A mixture of Ug99-susceptible cultivars with *Sr31* and

Sr24 were planted adjacent to the plots as spreader rows in Njoro. The spreader rows were inoculated with *Pgt* isolates of the Ug99 race group at the jointing growth stage to produce spores for natural dispersal to the experimental plots. 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 susceptible (S), moderately susceptible (MS), MR (Singleton et al. 1982), and resistant (R) (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).

Molecular marker analysis

Total genomic DNA was extracted from leaves of 2-week-old seedlings using the protocol of Anderson, et al. (1992) with modification including use of a bead grinder FastPrep homogenizer (MP Biomedical) for 5 min at 25 strokes/second to homogenize tissue. The newly developed iSelect SNP genotyping platform containing 81,587 wheat SNP markers that resulted in a successful assay was used to genotype parents and 192 DH lines from the LMPG-6/PI 374670 population as described by the manufacturer (Illumina). Allele calling for each SNP was performed using Illumina's GenomeStudio v2011.1, and results were manually inspected for call accuracy. In addition to the SNP genotyping, PI 374670 was screened with a sequence tagged site marker (*csIH81*) diagnostic for the presence of *Sr22* (Periyannan et al. 2011).

Map construction and QTL analysis

A linkage map was constructed using JMP Genomics 6.0 (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 (Kolodinska Brantestam et al. 2007) threshold of 3.0. Genetic distances between markers were calculated in centiMorgans (cM) using the Kosambi map function and linkage groups were assigned to chromosomes based on comparison to the 90,000 consensus map (Wang et al. 2014)

Development of competitive allele-specific PCR (KASP) assays for SNP validation

KASP assays were designed using the source sequences from the wheat 90K iSelect assay for 11 SNP markers flanking the race TTKSK resistance locus identified based on the seedling resistance test of the DH population. To verify these SNPs in a second population, KASP assays were tested on 185 RILs developed and derived from the same parents as the DH population. Also, to examine the relationship between the stem rust resistance locus in PI 374670 and the resistance gene *Sr15*, the KASP assays were used to genotype CItr 13130 'Norka' containing the resistance gene *Sr15* (Watson and Luig 1966). KASP assays were performed according to manufacturer's instructions (LGC Genomics, Beverly, MA). End-point genotyping was performed on a PHERAstar Plus microplate reader (BMG LABTECH, Inc., Cary, NC) and data were analyzed using KlusterCaller software (LGC Genomics, Beverly, MA).

Results

Inheritance and mapping of seedling resistance

Wheat landrace PI 374670 exhibited a low IT ';13' when inoculated with *Pgt* races TTKSK, TTKST, MCCFC, and TPMKC, while LMPG-6 exhibited high ITs '3+' to '4' to these races. The progeny of the BC_1F_2 families and RIL population segregated for resistance to *Pgt* race TTKSK with resistant seedlings exhibiting ';1' and ';13' ITs and susceptible seedlings exhibiting ITs of '3+' and '4'. Among the BC_1F_2 families, the ratio of susceptible families to segregating families was 112:104 ($\chi^2 = 0.29$, $P = 0.6$) fitting a 1:1 ratio. Within the 104 segregating BC_1F_2 families, the R:S ratio of seedlings was 1,760:598 ($\chi^2 = 0.15$, $P = 0.67$), fitting a 3:1 ratio for a single dominant gene. Segregation of resistant:susceptible lines to race TTKSK was 96:89 in the RIL population, fitting a 1:1 ratio ($\chi^2 = 0.26$, $P = 0.48$) confirming that a single gene controlled seedling resistance to race TTKSK in PI 374670. The phenotype of the DH population was determined after independent inoculations with *Pgt* races TTKSK, MCCFC and TPMKC. Lines classified as resistant or susceptible were consistently classified as such across the three *Pgt* races. The DH population segregated as 98 resistant (IT = ';1' and ';13') and 88 susceptible (IT = '3+' and '4'), which fit a ratio of 1:1 ($\chi^2 = 0.53$, $P = 0.46$) indicating a single gene controlled seedling resistance to all three *Pgt* races.

To map seedling resistance to races TTKSK, MCCFC, and TPMKC, data from the SNP marker assay of the parental lines and 186 DH lines from the LMPG-6/PI 374670 population were used. Of the 81,587 markers tested, 10,667 were polymorphic between the two parents. Among the polymorphic markers, 7,181 had previously been assigned to chromosomes (Wang et al. 2014) and were used to map the resistance gene in PI 374670. The 7,181

SNPs were assigned to 37 linkage groups representing 21 wheat chromosomes with a total length of 1,975.6 cM. CIM analysis revealed that the resistance locus mapped to the terminal region on the long arm of chromosome 7A, was tightly linked to SNP marker *IWB46162* and was 1.6, 3.8, and 7.7 cM proximal to the SNP markers *IWB19694*, *IWB27289*, and *IWB28967*, respectively (Fig. 1a).

The resistance gene *Sr22* is also located on the long arm of chromosome 7A (McIntosh et al. 1995; Olson et al. 2010). We found that PI 374670 displayed high ITs ('3+' to '4') to *Pgt* races TRTTF, TTTTF and RKQQC, whereas these races produced low ITs on *Sr22* monogenic lines (Olson et al. 2010; Rouse and Jin 2011). The race specificity observed in PI 374670 indicated that the resistance gene in PI 374670 is different from *Sr22*. The diagnostic markers for *Sr22* (*csIH81-BM* and *csIH81-AG*) (Periyannan et al. 2011) tested negative for the presence of *Sr22* in PI 374670.

Inheritance and mapping of field resistance

PI 374670 exhibited disease severities and infection responses ranging from 10 R to 10 MR-R and LMPG-6 exhibited 35 MS-S to 40 S in the field stem rust nursery in Njoro in 2013. In the Debre Zeit nursery, severities and infection responses ranged from 25 MR-MS to 35 MR-MS for PI 374670 and 60 S to 70 S for LMPG-6. The severities and infection responses of the 153 DH population ranged from 10 R to 45 S in Njoro and 5 MR to 80 S in Debre Zeit.

QTL analyses were conducted for each of the two field experiments using the mean CI values calculated from the infection response and severity data for each of the 153 DH lines. Using the CIM function, one QTL for resistance to Ug99 was detected on the long arm of chromosome 7A in a 7.7 cM interval (Fig. 1a) flanked by *IWB46162* and *IWB28967*. A detected QTL designated as *QSr.abr-7AL* was significant across four linked SNP markers that explained 34–54 and 29–36 % of the phenotypic variation in Kenya and Ethiopia, respectively (Table 1; Fig. 1a). One additional QTL with four significant markers, designated *QSr.abr-2DS*, was detected on the short arm of chromosome 2D across a 32.5 cM interval using the Ethiopia data. This QTL explained 16–21 % of the phenotypic variation in Ethiopia (Table 1, Supplemental Fig. 1).

Development and testing of KBioscience competitive allele-specific PCR (KASP) assays

To verify mapping results from the DH population, the four Illumina SNP markers mapped to the resistance locus and an additional seven markers distributed around the resistance locus were converted to KASP assays and

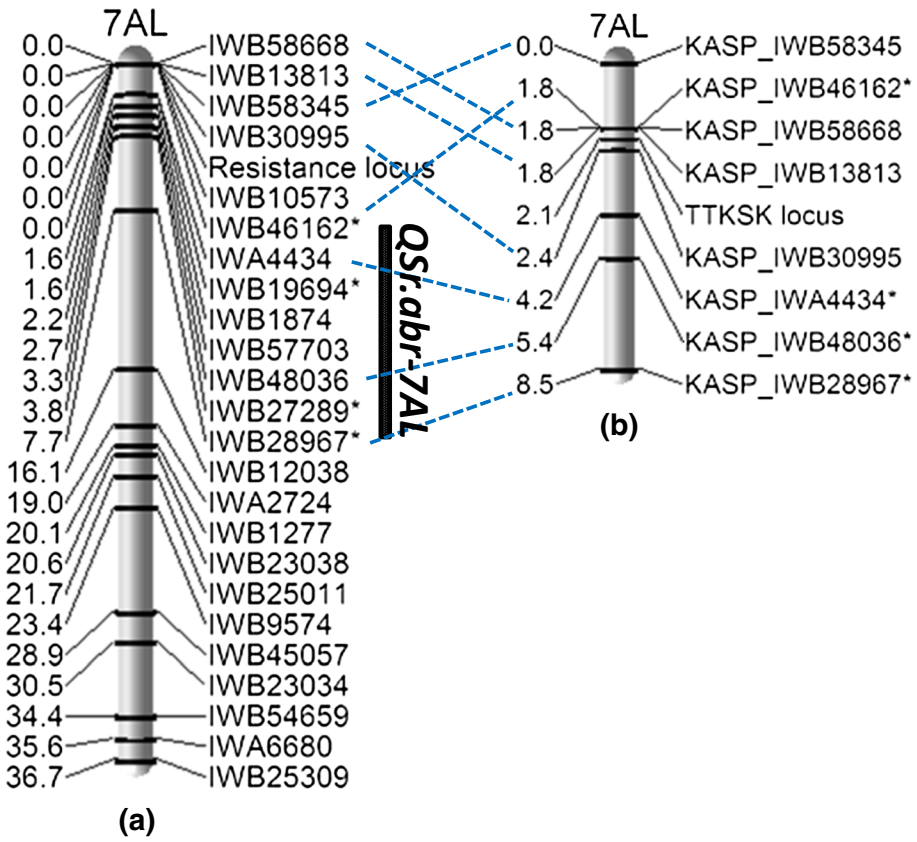

Fig. 1 SNP-based genetic linkage maps of the *Sr* gene in PI 374670 on chromosome 7AL constructed from the **a** LMPG-6/PI 374670 DH population using an iSelect 90K Infinium assay and **b** from the LMPG-6/PI 374670 RIL population using KASP assays. The values next to the marker names are the distances (cM) generated using the Kosambi mapping function. *Asterisks* markers significantly associated with the resistance locus

Table 1 Quantitative trait loci for the field resistance to Ug99 in the LMPG-6/PI 374670 DH population assessed in Kenya and Ethiopia

QTL	Marker	Chromosome	Environment	Position (cM)	LOD	Additive effect	R^2
QSr.abr-7AL	IWB46162	7AL	Kenya	0	21.4	−22	54
	IWB19694	7AL	Kenya	1.6	18.6	−21	49
	IWB27289	7AL	Kenya	3.8	15.9	−20	44
	IWB28967	7AL	Kenya	7.7	11.4	−17	34
	IWB46162	7AL	Ethiopia	0	13.0	−17	36
	IWB19694	7AL	Ethiopia	1.6	12.2	−16	34
	IWB27289	7AL	Ethiopia	3.8	11.3	−16	32
	IWB28967	7AL	Ethiopia	7.7	9.7	−15	29
QSr.abr-2DS	IWA989	2DS	Ethiopia	2.2	6.7	−12	21
	IWB8481	2DS	Ethiopia	27	5.5	−11	17
	IWA1601	2DS	Ethiopia	30.3	5.3	−11	17
	IWB17693	2DS	Ethiopia	32.5	4.9	−10	16

Table 2 KASP assays developed for SNP markers linked to the stem rust resistance locus in PI 374670 on chromosome arm 7AL

SNP ID	Primer name	Primer sequence[†]	Allele	Parent
IWB28967	IWB28967_ALA	AAACAACGGGTTCTTGCAGAGCAT	A	PI374670
	IWB28967_ALG	ACAACGGGTTCTTGCAGAGCAC	G	LMPG-6
	IWB28967_C1	CGATAAAGGAGATATCTTCCTGCAAGTAT		
IWAB8036	IWAB8036_ALA	ATCTCGTTTCCATTCATCTTGTACTTATA	A	PI374670
	IWAB8036_ALG	CTCGTTTCCATTCATCTTGTACTTATG	G	LMPG-6
	IWAB8036_C1	AGCCAGTTGCTCCCACTCTATGTTT		
IWA4434	IWAB8036_ALT	GGCAGCAAGAAGAGAAAGAAAGGATT	T	LMPG-6
	IWAB8036_ALC	GCAGCAAGAAGAGAAAGAAAGGATC	C	PI374670
	IWAB8036_C1	CAGCGGCCTTCACCTGGGCTT		
IWB46162	IWB46162_ALT	GAACTACGACAGCGTCTGGATCA	T	PI374670
	IWB46162_ALC	AACTACGACAGCGTCTGGATCG	C	LMPG-6
	IWB46162_C1	ATCAACCCATGCTTTTGAAGAAGGAAATTA		
IWB13813	IWB13813_ALA	CACGTGAGACCTGCATGAAGTCA	A	LMPG-6
	IWB13813_ALG	ACGTGAGACCTGCATGAAGTCG	G	PI374670
	IWB13813_C1	GGAGGTGATCTTTGGCCAAGACAAT		
IWB58668	IWB58668_ALT	CCCCAAAACTTGAGTTGGACGGA	T	LMPG-6
	IWB58668_ALG	CCCAAAACTTGAGTTGGACGGC	G	PI374670
	IWB58668_C1	TGACTCCAAGGCATTGGTTCCCTTA		
IWB58345	IWB58345_ALA	TTGCCCTAGGTAGAAATACAAGACTA	A	PI374670
	IWB58345_ALG	GCCCTAGGTAGAAATACAAGACTG	G	LMPG-6
	IWB58345_C1	GCTATAAGGCTCTTTTCCCAAAGCTTTA		
IWB30995	IWB30995_ALT	GAATCTACACCTACATGGGAGAACA	T	LMPG-6
	IWB30995_ALC	AATCTACACCTACATGGGAGAACG	C	PI374670
	IWB30995_C1	GAGATCGGGTGGCCGTGCTATA		

[†] Sequences of the allele-specific primers do not include the tail sequences that interact with the fluor-labeled oligos in the KASP reaction mix

used to genotype 185 RILs from the LMPG-6/PI 374670 population. Of the 11 markers used in KASP assays, eight markers produced two distinct clusters (Table 2). CIM analysis revealed that the race TTKSK resistance locus in the RIL population mapped to the terminal region on the long arm of chromosome 7A and confirmed the genetic association of the four markers. The KASP assays designed from the SNP marker *IWB46162* mapped 0.3 cM distal to the race TTKSK resistance locus while three markers *IWA4434*, *IWB48036* and *IWB28967* were mapped 2.1, 3.3 and 6.4 cM proximal to the race TTKSK resistance locus in the RIL population, respectively (Fig. 1b).

In addition, the KASP assays were performed and tested on the cultivar 'Norka' (NSGC accession CItr 13130) which is reported to have *Sr15*. Using KASP assays, the four flanking SNP markers were monomorphic between PI 374670 and CItr 13130 'Norka'.

Discussion

PI 374670 is a landrace that was collected from Bosnia and Herzegovina in 1972 and its resistance to Ug99 was first detected in Kenya in 2007. A previous molecular marker survey of PI 374670 showed that this accession did not have any of the six genes associated with breeding activity (i.e., the 1RS translocation, *Sr24*, *Sr36*, *Sr2*, *Rht-B1d*, and *Rht-D1b*) (Newcomb et al. 2013), indicating that PI 374670 is most likely a landrace and not a product of modern breeding. Based on analyses of three different mapping populations, the seedling resistance in PI 374670 was found to be conditioned by a single dominant gene. Mapping results using the Illumina SNP genotyping platform located the locus on the long arm of chromosome 7A.

To date, one Ug99-effective *Sr* gene, *Sr22*, has been described on chromosome arm 7AL (Olson et al. 2010; Jin et al. 2007). Though *Sr15* has also been described on chromosome arm 7AL (Watson and Luig 1966), *Sr15* present in line Prelude*2/Sr15 did not confer resistance to race TTKSK (Jin et al. 2007). In addition, results from Kenya field tests showed that two lines with *Sr15* (Prelude*2/Sr15 and W2691Sr15NK) were susceptible to Ug99 in 2013 season (*data not shown*). Results from the KASP assay of PI 374670 and *Sr15* cultivar Norka (CItr 13130) showed that the four markers linked to the resistance locus were all monomorphic between the two lines. Though the molecular data are not suggestive of any difference between PI 374670 and *Sr15* cultivar Norka around the resistance locus in PI 374670, the race specificity data available suggest that resistance in PI 374670 is independent of *Sr15*. We also found that the susceptibility of PI 374670 to *Pgt* races classified as avirulent to lines with *Sr22* excludes the resistance gene in PI 374670 from being *Sr22*. In addition, the diagnostic markers for *Sr22* not being detected in PI 374670 and the origin of *Sr22* from *Triticum monococcum* (L.) suggest that PI 374670 does not possess *Sr22*. Though we do not have any evidence to suggest that the resistance gene PI 374670 is not allelic to *Sr15* or *Sr22*, the race specificity data suggest that the resistance gene in PI 374670 is neither *Sr15* nor *Sr22*.

KASP assays were performed to verify the results of the DH population using the RIL population. Four of the flanking markers identified using the DH population also flanked the *Sr* gene in PI 374670 based on the RIL population. If polymorphic in target breeding germplasm, these markers could be used in marker-assisted selection for the *Sr* gene in PI 374670. The order of the SNP markers in the DH population is in agreement with the order of the markers in the RIL population using two different assay systems. Marker *IWB46162* mapped 0.3 cM distally from the race TTKSK locus in the RIL population, whereas it was tightly linked to the resistance locus in the DH population. This difference could be attributed to the increased number of meiotic events allowing for the opportunity for additional recombination in the RIL population compared to the DH population (Somers et al. 2004).

Within the region with the seedling resistance to the three *Pgt* races on chromosome arm 7AL, one major QTL for resistance in the field was detected in Kenya and Ethiopia. The QTL for this field resistance was consistent across both locations. This result suggests that the field resistance to Ug99 in PI 374670 is conditioned primarily by the single major gene that was detected in the seedling assays. An additional QTL was detected in Ethiopia on chromosome arm 2DS. Two stem rust resistance genes, *Sr6*, and *Sr46*, have been mapped to the short arm of chromosome 2D. *Sr6* is temperature-sensitive gene and effective against several *Pgt* races at 18 °C but ineffective against TTKSK (Knott 2001; Jin et al. 2007). *Sr46* was identified in *Aegilopes tauschii* and is effective against *Pgt* race TTKSK (Rouse et al. 2011b). This additional QTL might be explained by a difference between the two environments such as the presence of other *Pgt* races at the Debre Zeit, Ethiopia location, including races JRCQC and TRTTF (Olivera et al. 2012).

The order of markers in our map is in agreement with the previously published SNP map (Wang et al. 2014) with the exception of a few markers where the order was reversed. This deviation in order could be attributed to the fact that the consensus map was built using genetic data from seven mapping populations of wheat.

In conclusion, we described a new source of resistance to the Ug99 race group from an NSGC wheat landrace accession. Recently several genes for resistance to race TTKSK have been overcome by new races in the Ug99 race group. The four KASP assays developed in this study may facilitate rapid introgression of the new gene in PI 374670 into wheat breeding lines and could be also used to pyramid this gene with *Sr22* and other effective resistance genes using marker-assisted selection. As with any novel gene for stem rust resistance, proper stewardship would require that the gene should not be deployed except when pyramided with other effective genes (Schafer and Roelfs 1985).

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, T. C. Gordon and M. Acevedo generated the F_1 seeds, advanced the mapping populations and helped in the domestic races screening. S. Chao genotyped the DH population with the 90K wheat iSelect SNP genotyping platform. M. N. Rouse and Y. Jin involved in the domestic and foreign races screening and contributed to the final manuscript version. R. Wanyera helped in the field screening in Kenya. G. Brown-Guedira and S. Williamson performed

the genotyping of the RIL population using KASP assays. All authors have contributed to the final manuscript.

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Conflict of interest The authors declare no conflict of interest.

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