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Molecular Mapping of Stem Rust Resistance Loci Effective Against the Ug99 Race Group of the Stem Rust Pathogen and Validation of a Single Nucleotide Polymorphism Marker Linked to Stem Rust Resistance Gene *Sr28*

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ABSTRACT

Wheat landrace PI 177906 has seedling resistance to stem rust caused by *Puccinia graminis* f. sp. *tritici* races TTKSK, TTKST, and BCCBC and field resistance to the Ug99 race group. Parents, 140 recombinant inbred lines, and 138 double haploid (DH) lines were evaluated for seedling resistance to races TTKSK and BCCBC. Parents and the DH population were evaluated for field resistance to Ug99 in Kenya. The 90K wheat single nucleotide polymorphism (SNP) genotyping platform was used to genotype the parents and populations. Goodness-of-fit tests indicated that two dominant genes in PI 177906 conditioned seedling resistance to TTKSK. Two major loci for seedling resistance were consistently mapped to the chromosome arms 2BL and 6DS. The BCCBC resistance was mapped to the same location on 2BL as the TTKSK resistance. Using field data from the three seasons, two major QTL were consistently detected at the same regions on 2BL and 6DS. Based on the mapping result, race specificity, and the infection type observed in PI 177906, the TTKSK resistance on 2BL is likely due to *Sr28*. One SNP marker (*KASP_IWB1208*) was found to be predictive for the presence of the TTKSK resistance locus on 2BL and *Sr28*.

The TTKSK race of the wheat stem rust pathogen *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. has evolved to overcome widely deployed resistance genes including, *Sr24*, *Sr36*, *Sr9h*, and *SrTmp* since its detection in Uganda in 1999 (Jin et al. 2008, 2009; Patpour et al. 2016a; Rouse et al. 2014). To date, 13 variants within the Ug99 race group have been detected across east and southern Africa (Fetch et al. 2016; Hale et al. 2013; Jin et al. 2009; Patpour et al. 2016b; Wolday et al. 2011). Of these races, only race TTKSK has been detected outside of Africa, in Yemen and Iran (Singh et al. 2011). Stem rust in the United States has been effectively controlled since the 1950s through eradication of barberry (*Berberis vulgaris*) and deployment of cultivars with effective resistance genes (Kolmer et al. 1991). Because of the continued evolution of new *P. graminis* f. sp. *tritici* races and the widespread susceptibility in U.S. wheat to the Ug99 race group, work is underway to identify new sources of resistance to Ug99. Several genes for resistance to Ug99 have been transferred into wheat from the secondary and tertiary wheat gene pools, but most of these genes have yet to be utilized in breeding due to linkage drag (Klindworth et al. 2012; Periyannan et al. 2011). Presently, only five designated TTKSK-effective genes originate from common wheat (*Triticum aestivum* L.): *Sr9h*, *Sr28*, *Sr42*, *SrCad*, and *SrTmp* (Ghazvini et al. 2012; Hiebert et al. 2011; Jin et al. 2007; Rouse et al. 2012, 2014). Although *Sr28* is not effective against most of the domestic *P. graminis* f. sp. *tritici* races in the United States (Rouse et al. 2012), it is effective against *P. graminis* f. sp. *tritici* race TTKSF+ which overcomes *Sr9h* (Pretorius et al. 2012). Discovery and characterization of new sources of resistance to Ug99 from within the primary gene pool could not only provide US wheat breeders with additional genes, but could also accelerate the deployment of *Sr* genes. Linkage drag from common wheat landraces should be less than that encountered with genes derived from wild relatives of wheat. The 19,812 common wheat landrace accessions available in the USDA-ARS National Small Grains Collection (NSGC) are a valuable resource for discovery of new genes for resistance to Ug99. In previous screening tests, wheat landrace accession PI 177906 from Turkey showed a high level of field resistance to Ug99 (Newcomb et al. 2013). Identifying molecular markers tightly linked to the Ug99 resistance in PI 177906 is necessary for combining this resistance with other effective resistance genes and for indirect selection of the Ug99 resistance genes. The objectives of this study were to determine the inheritance of the field and seedling resistance in PI 177906, to ascertain if resistance assayed in seedling tests corresponds to resistance assayed under field conditions, and to map the resistance using single nucleotide polymorphism (SNP) markers.

MATERIALS AND METHODS

Plant materials. To elucidate genetic control of stem rust resistance in PI 177906, the susceptible wheat line LMPG-6 was crossed to PI 177906. The F_1 seeds were used to generate 138 DH lines and 140 recombinant inbred lines (RILs) ($F_{5:8}$). LMPG-6 (Little club/Prelude*8/Marquis/3/Gabo) is a susceptible wheat line developed by Knott (1990). PI 177906 is hexaploid wheat landrace from the NSGC that was collected by J. Harlan in Corum, Turkey in

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1948. To validate the significant SNP markers in a different genetic background, 160 $F_{2:5}$ RILs were developed from cross between 'Red Bobs' and PI 177906. Red Bobs is a hexaploid wheat variety from Canada (Dyck and Green 1970). In addition to the parents and progeny from these crosses a set of 96 wheat accessions were included in this study. Of the 96 accessions, six (CI 7611, 'Kota', SD 1691, 'Ceres', PI 670015, and W2691/Sr28Kt) carry *Sr28* (Rouse et al. 2012), two (CItr 12636 and CItr 12737) were postulated previously to have *Sr28* (Rouse et al. 2012), and 11 displayed ITs of ;13, ;2, and ;3 similar to *Sr28*. The remaining accessions were chosen based on diversity of geographic origin and IT reactions to TTKSK.

Seedling tests. *P. graminis* f. sp. *tritici* races TTKSK (isolate 04KEN156/04), BCCBC (isolate 09CA115-2), and QFCSC (isolate 06ND76C) were used to evaluate seedling response to stem rust in the parents, 138 DH lines, and 140 RILs at the USDA-ARS Cereal Disease Laboratory (CDL) in St. Paul, MN. Five plants for each line were inoculated with the *P. graminis* f. sp. *tritici* races according to Rouse et al. (2011) and assessed for seedling infection type (IT) using the 0-to-4 scale developed by Stakman et al. (1962). χ^2 analyses were performed to test for goodness-of-fit to the models of one, two, or four genes. *P. graminis* f. sp. *tritici* race BCCBC was also used to evaluate seedling response to stem rust in the parents and 160 RILs from Red Bobs/PI 177906 cross. In addition, a set of 96 wheat accessions were screened against *P. graminis* f. sp. *tritici* race TTKSK.

Based on the mapping results, we speculated that PI 177906 could have *Sr28*. Therefore, PI 177906 was screened against *Sr28*-virulent *P. graminis* f. sp. *tritici* races QFCSC (isolate 06ND76C), MCCFC (isolate 59KS19), RCRSC (isolate 77ND82A), RKQQC (isolate 99KS76A), TPMKC (isolate 74MN1409), TTTTF (isolate 01MN84A-1-2), and TRTTF (isolate 06YEM34-1) (Rouse et al. 2012). PI 177906 was also screened against *Sr28*-avirulent *P. graminis* f. sp. *tritici* races BCCBC (isolate 09CA115-2) and TTKST (isolate 06KEN19v3) (Rouse et al. 2012).

Field test. Parents and 138 DH lines were evaluated for field resistance 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 meter rows with two replicates for each line, six replicates for each of the parents, and cultivar Red Bobs was included as a susceptible check throughout the nursery. A mixture of Ug99-susceptible cultivars with *Sr31* and *Sr24* were planted adjacent to the plots as spreader rows. The spreader rows were inoculated with a bulk of locally collected *P. graminis* f. sp. *tritici* 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 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 each infection response category to obtain the coefficient of infection (CI) as described by Knott (1989).

Map construction and QTL analysis. Genomic DNA was extracted from leaves of 2-week-old seedlings using the modified CTAB protocol as described by Babiker et al. (2015). The 90K iSelect SNP genotyping platform (Wang et al. 2014) was used to genotype parents, F_1 seedlings, 138 DH lines, and 140 RILs ($F_{5:8}$) as described by the manufacturer (Illumina, San Diego, CA). Allele calling for each SNP was performed using Illumina's GenomeStudio v2011.1, and results were manually inspected for call accuracy. A linkage map was constructed using JMP Genomics 7.1 (SAS Institute, Cary, NC). Markers with extreme segregation distortion and more than 9% missing data were excluded from the analysis. The recombination and linkage groups function was used to determine the initial number of linkage groups and to assign SNPs to linkage groups. The linkage map order function was used to determine the most likely marker order. Genetic distances between markers were calculated in centiMorgans (cM) using the Kosambi map function and linkage groups were assigned to chromosomes based on comparison with the 90K wheat consensus maps (Avni et al. 2014; Wang et al. 2014). 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 arbitrary logarithm of odds (LOD) threshold of 5.0.

Relationship of the gene on 2BL to *Sr28* and the gene on 6DS to *Sr42* and *SrCad*. Based on the observed ITs to several *P. graminis* f. sp. *tritici* races and the initial mapping results, we postulated that PI 177906 possesses *Sr28*. Therefore, the *Sr28* markers *wPt-7004-PCR* and *wmc332* were used as described by Rouse et al. (2012) to examine the relationship between the *Sr* gene in PI 177906 and *Sr28*. Two wheat accessions with *Sr28* (CItr 12499 and CItr 5878) were included as checks. In addition, LMPG-6, Red Bobs, PI 177906, Norin 40 (with *Sr42*), and PI 595667 (*SrCad*) were screened with two Kompetitive allele specific PCR (KASP) assays (IWB31561 and IWB36391) diagnostic for the presence of *Sr42* (Gao et al. 2015) and two KASP assays (kwm907 and kwm987) diagnostic for the presence of *SrCad* (Kassa et al. 2016).

Development of KASP assays for SNP validation. Five SNP markers (IWB1208, IWB23232, IWB57292, IWB7292, and IWB5864) closely linked to the race BCCBC resistance locus were used in KASP assays to genotype parents, F_1 seedlings, 53 fixed $F_{2:5}$ RILs derived from the Red Bobs/PI 177906 cross, and a set of 96 hexaploid wheat accessions. KASP assays were designed using the source sequences from the wheat 90K iSelect assay and performed according to the manufacturer's instructions (LGC Genomics, Beverly, MA).

Reassessment of wheat accessions postulated to carry *Sr28* based on *wPt-7004-PCR* and *wmc332* markers. To assess the efficiency of the KASP assay developed in the present study in predicting the presence of *Sr28* compared with previously developed markers linked to *Sr28* (Rouse et al. 2012), a subset of 47 of the 96 wheat accessions used for KASP validation, were genotyped with *Sr28* markers, *wPt-7004-PCR* and *wmc332*. Of these, two accessions (CItr 12499 and CItr 5878 'Kota') carry *Sr28* (Rouse et al. 2012).

RESULTS

Inheritance of seedling resistance in PI 177906. When tested against *P. graminis* f. sp. *tritici* races MCCFC, RCRSC, RKQQC, TPMKC, TTTTF, and TRTTF, which are known to be virulent on *Sr28*, PI 177906 showed high ITs of 3+ and 4. Based on seedling inoculation tests, wheat landrace PI 177906 and the F_1 seedlings from LMPG-6/PI 177906 exhibited low ITs of ; and ;13 when inoculated with *P. graminis* f. sp. *tritici* races TTKSK and BCCBC, whereas LMPG-6 exhibited a high IT of 4 to both races. The progeny of the RIL and DH populations segregated for resistance with resistant seedlings exhibiting ;, ;1, and 2 ITs, and susceptible seedlings exhibiting ITs of 3+ and 4 to *P. graminis* f. sp. *tritici* race TTKSK. Among the 138 DH lines, 108 were resistant and 30 were susceptible. The segregation ratio among the RIL population was 91 homozygous resistant and 30 homozygous susceptible. The segregation of resistance in the DH and the RIL populations fitted the 3:1 ratio ($\chi^2 = 0.78$, $P = 0.38$; $\chi^2 = 0.0027$, $P = 0.95$) for two genes.

When the two populations were inoculated with *P. graminis* f. sp. *tritici* race BCCBC, the DH and the RIL populations segregated for resistance with resistant seedlings exhibiting ITs of ;1 and ;13 and susceptible seedlings exhibiting ITs of 3+ and 4. The DH and the RIL populations segregation fitted the 1:1 ratio ($\chi^2 = 0.029$, $P = 0.86$; $\chi^2 = 1.6$, $P = 0.20$) for a single gene. When inoculated with

P. graminis f. sp. *tritici* race QFCSC, LMPG-6 and PI 177906 exhibited high and low ITs of 4 and 2–, respectively. The DH and the RIL populations segregation fitted the 1:1 ratio ($\chi^2 = 1.6$, $P = 0.2$; $\chi^2 = 0.75$, $P = 0.38$) for a single gene. When the 160 RILs from Red Bobs/PI 177906 cross were tested against *P. graminis* f. sp. *tritici* race BCCBC, Red Bobs showed IT of 2–, the F_1 seedlings exhibited IT ;2 and 53 RILs were fixed for IT and displayed ITs of 2– and ;0 similar to the two parents.

Mapping resistance to *P. graminis* f. sp. *tritici* races TTKSK, BCCBC, and QFCSC. To map seedling resistance to *P. graminis* f. sp. *tritici* races TTKSK, BCCBC and QFCSC, data from the SNP marker assay of the parental lines, 138 DH lines, and 140 RILs from the LMPG-6/PI 177906 populations were used. Among the polymorphic markers, only 8,043 and 4,206 SNP markers from the DH and RIL populations, respectively, produced two distinct clusters in GenomeStudio, had less than 9% missing data and had previously been assigned to chromosomes (Avni et al. 2014; Wang et al. 2014). These markers were assigned to 45 and 41 linkage groups for which previous map locations were used to assign to the 21 chromosomes of wheat. The total lengths of the maps were 2,210.51 and 2,078.66 cM in the DH and RIL populations, respectively. Two major loci for TTKSK resistance were mapped to the long arm of chromosome 2B and the short arm of chromosome 6D in both populations. In the DH population, the TTKSK resistance locus on 2BL was tightly linked to a group of 28 SNP markers (Fig. 1A). In the RIL population, the TTKSK resistance locus was mapped 0.7 cM distal to a group of 15 SNP markers and 0.35 cM proximal to a group of 16 SNP markers on chromosome arm 2BL (Fig. 1B). Linkage analysis showed that the 2BL locus conditioned both the TTKSK and BCCBC resistance. The 6DS resistance locus mapped to the terminal region of chromosome 6DS, 3.7 cM distal to the SNP marker IWA2338 in the DH population (Fig. 2A). In the RIL population, the TTKSK resistance locus mapped 3.1 cM distal to the SNP marker IWB49086 on chromosome arm 6DS (Fig. 2B). Three of the SNP markers, IWB49086, IWB7135, and IWB262, were mapped on chromosome arm 6DS in the two populations. The QFCSC resistance locus was located at the end of the long arm of chromosome 4A and cosegregated with a group of 10 SNP markers that mapped 15.03 cM distal to the resistance locus in the DH population. These markers were mapped to a location 150.7 to 164.1 cM on the consensus map (Wang et al. 2014). This result was further validated in the RIL population, where the QFCSC resistance locus mapped 7.26 cM proximal from a group of 35 SNP markers located to a location of 147.28 to 164.13 cM on the long arm of chromosome 4A (Wang et al. 2014). Of these, 14 SNP markers were detected in both the DH and RIL populations (Supplementary Table S1).

The race specificity observed in PI 177906 suggested that the resistance gene on chromosome arm 2BL of PI 177906 could be *Sr28*. Markers *wmc332* and *wPt-7004-PCR*, previously found to be associated with *Sr28*, were tested in PI 177906 and in two accessions known to have *Sr28*, CItr 12499 and CItr 5878 (Rouse et al. 2012). Based on Rouse et al. (2012), fragments of 194 bp amplified with the *wPt-7004-PCR* were associated with the presence of *Sr28*, whereas fragments of 208 bp or less amplified with the SSR marker *wmc332* were associated with the absence of *Sr28*. SSR marker *wmc332* amplified a 209-bp fragment from PI 177906 and 211-bp fragment from CItr 12499 and CItr 5878, whereas *wPt-7004-PCR* amplified a 192-bp fragment from PI 177906, CItr 12499, and CItr 5878. The marker results support the hypothesis that the TTKSK resistance locus on chromosome arm

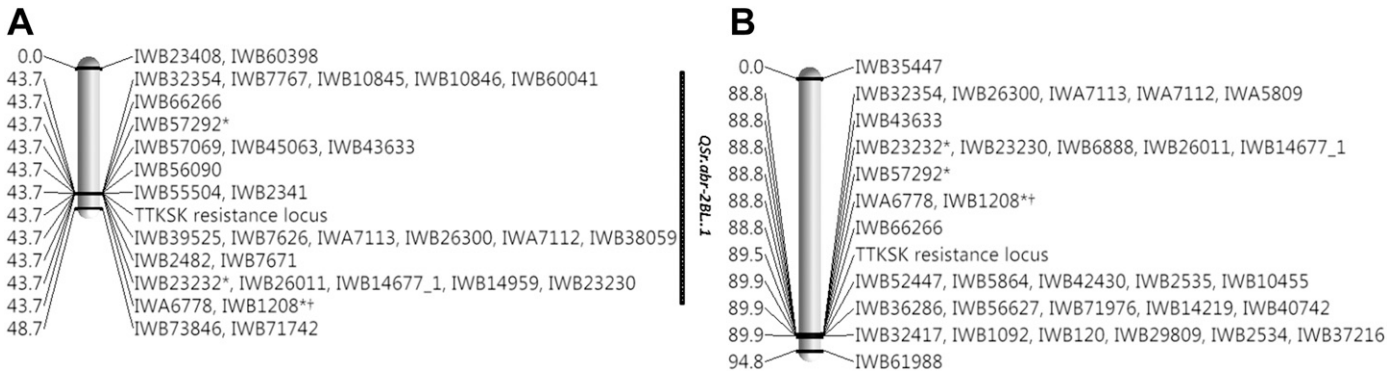

Fig. 1. A portion of the single nucleotide polymorphism (SNP)-based genetic linkage maps of the *Sr* gene in PI 177906 on chromosome arm 2BL constructed using an iSelect 90K Infinium assay from **A,** the double haploid and **B,** the recombinant inbred line populations from the LMPG-6/PI 177906 cross. The values next to the marker names are the distances (cM) generated using the Kosambi mapping function. * Indicates SNP markers converted to Kompetitive allele specific PCR assays.

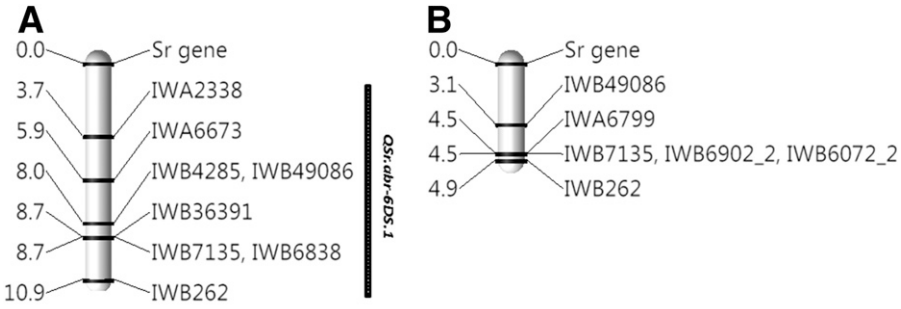

Fig. 2. Single nucleotide polymorphism-based genetic linkage maps of the *Sr* gene in PI 177906 on chromosome arm 6DS constructed using an iSelect 90K Infinium assay from **A,** the double haploid and **B,** the recombinant inbred line populations from the LMPG-6/PI 177906 cross. The values next to the marker names are the distances (cM) generated using the Kosambi mapping function.

2BL in PI 177906 is due to *Sr28*. The diagnostic KASP assays for *Sr42* (IWB31561 and IWB36391) and for *SrCad* (kwm907 and kwm987) tested negative for the presence of *Sr42* and *SrCad* in PI 177906, indicating that the resistance gene in PI 177906 is likely different from *Sr42* and *SrCad*.

Genetic mapping of field resistance. During the three field experiments, PI 177906 exhibited disease severities and infection responses ranging from 10MR to 20MR and LMPG-6 exhibited 35MSS to 60S in the Kenya field stem rust nursery. The severities and infection responses of the 138 DH populations ranged from 5R to 60S. QTL analyses were conducted for each of the three field experiments using the mean CI values calculated from the infection response and severity data. Using the CIM function with LOD of 5, two major QTL for resistance to the Ug99 race group were detected on chromosome arms 2BL and 6DS. The 2BL QTL (*QSr.abr-2BL.1*) was detected in a 3.6 cM interval, spanned by 67 SNP markers that explained 16 to 35% of the phenotypic variation in the three seasons (Table 1, Fig. 1A). The second QTL (*QSr.abr-6DS.1*) was detected in a 7.2 cM interval on chromosome arm 6DS using the data from three seasons in Kenya. The 6DS QTL was spanned by eight SNP markers that explained 29 to 34% of the phenotypic variation in the three seasons (Table 1, Fig. 2A).

Marker validation. To verify mapping results from LMPG-6/PI 177906 RIL and DH populations, five Illumina SNP markers mapped to the resistance locus were converted to KASP assays and used to genotype parents and F_1 seedlings. Using KASP assays, three SNP markers, IWB1208, IWB23232, and IWB57292, produced two distinct clusters between PI 177906 and the two susceptible parents and detected the heterozygosity in F_1 seedlings. These SNP markers were further used to genotype the 53 fixed RILs from the Red Bobs/PI 177906 cross. Using KASP assay, the SNP marker IWB1208 (*KASP_IWB1208*) clearly discriminated between RILs with IT of 2– similar to Red Bobs and IT of ; similar to PI 177906. To examine if the SNP marker predicted the presence of *Sr28* in diverse hexaploid wheat germplasm, a set of 96 wheat accessions were genotyped using the KASP assay *KASP_IWB1208* (Table 2). The SNP marker *KASP_IWB1208* produced the same allele in PI 177906 as in six accessions known to possess *Sr28*, two accessions CItr 12636 and CItr 12737 postulated to have *Sr28* (Rouse et al. 2012), and 11 accessions that displayed ITs similar to *Sr28* against *P. graminis* f. sp. *tritici* race TTKSK (Table 3, Fig. 3).

Reassessment of wheat accession postulated to carry *Sr28* based on *wPt-7004-PCR* and *wmc332* markers. When genotyped with the *Sr28* markers (Rouse et al. 2012), *wPt-7004-PCR* amplified a 192-bp fragment from 19 accessions, out of these, only eight accessions produced fragments of 209 bp or more when genotyped with the SSR marker *wmc332* (Table 4). Out of the eight, three accessions (PI 345474, PI 142509, and PI 207121) produced 211 bp similar in size to lines with *Sr28* (CItr 12499 and CItr 5878). Both PI 142509 and PI 207121 displayed susceptibility to race BCCBC, which was known to be avirulent on *Sr28*, indicating that *wmc332* and *wPt-7004-PCR* may not be predictive for the presence of *Sr28* and amplify false positives from accessions. In addition, two accessions (PI 94439 and PI 623538) produced fragments similar in size to PI 177906, when genotyped with *wmc332* and *wPt-7004-PCR* (Table 4). Both accessions displayed ITs different than the ITs displayed by PI 177906 when tested against either race TTKSK or BCCBC (Table 4). When the five accessions (PI 345474, PI 142509, PI 207121, PI 94439, and PI 623538) were genotyped with SNP marker *KASP_IWB1208*, only PI 345474 produced the same allele as *Sr28* lines (Table 4), indicating that the SNP marker *KASP_IWB1208* is more accurate and could be used to predict the presence of *Sr28* in uncharacterized germplasm.

DISCUSSION

In 3 years of screening, the spring wheat landrace PI 177906 exhibited a high level of resistance to the Ug99 race group in field trials in Kenya. A previous molecular marker survey of PI 177906 showed that this accession did not have any of the genes associated

TABLE 1. Quantitative trait loci for field resistance to Ug99 in the LMPG-6/PI 177906 double haploid population assessed in three seasons in Kenya

			May 2014			October 2014			May 2015		
Single nucleotide polymorphism ID	Chromosome	Distance (cM)[a]	LOD	Additive	R^2	LOD	Additive	R^2	LOD	Additive	R^2
IWB10455, IWB1663_1, IWB120, IWB37216, IWB42430, IWB52447, IWB32416, IWB6334_1, IWB4865, IWB6113, IWB5864, IWB56526, IWB36279, IWB32417, IWB2535, IWB2534, IWB29809, IWB23797, IWB11366, IWB36753, IWB36334, IWB8813, IWB1092, IWB40742, IWB56627, IWB36286, IWB14219, IWB71976, IWB59226	2BL	43.00	7.0	–29.7	21.6	10.9	–32.0	31.3	12.4	–21.2	34.0
IWB32354, IWB7767, IWB38059, IWB39525, IWB60041, IWB7626, IWB10845, IWB26300, IWA7113, IWA7112, IWB10846, IWB57292, IWB43633, IWB45063, IWB57069, IWB23232, IWB14959, IWB26011, IWB23230, IWB14677_1, IWB66266, IWB55504, IWB2341, IWB2482, IWB7671, IWB56090, IWB1208[b], IWA6778	2BL	43.67	7.3	–30.5	22.4	11.3	–32.3	32.3	12.8	–21.5	34.8
IWB4648, IWB69630, IWB57663, IWA8449, IWA8534, IWB25868, IWB69631, IWB69628, IWB23660, IWB55966_1, IWB25869	2BL	46.53	5.1	–24.9	16.3	9.1	–29.5	26.9	9.3	–18.7	26.6
IWA2338	6DS	3.66	10.5	–29.0	30.6	10.8	–29.8	31.0	11.7	–24.9	32.4
IWA6673	6DS	5.85	9.8	–28.5	29.1	11.0	–30.0	31.5	12.5	–25.6	34.2
IWB49086, IWB4285	6DS	8.01	9.9	–28.5	29.2	11.1	–30.0	31.7	12.6	–25.7	34.3
IWB7135, IWB36391, IWB6838	6DS	8.73	9.7	–27.9	28.8	10.1	–28.8	29.4	11.4	–24.5	31.7
IWB262	6DS	10.88	11.5	–29.6	33.1	10.6	–29.5	30.6	11.0	–24.0	30.9

[a] Distance based on linkage map from the recombinant inbred lines population.
[b] Marker used in Kompetitive allele specific PCR (KASP) assay.

with breeding activity (i.e., the 1RS translocation, *Sr2*, *Sr25/Lr19*, *Sr24*, *Sr26*, *Sr36*, *Rht-B1d*, and *Rht-D1b*) (Newcomb et al. 2013), which supports the current classification of PI 177906 within the NSGC as a landrace and not a product of modern breeding. In the present study, χ^2 analyses revealed that the TTKSK resistance in PI 177906 was governed by two genes. Two loci for seedling resistance to TTKSK were mapped to chromosome arms 2BL and 6DS. Four *Sr* genes (*Sr9*, *Sr16*, *Sr28*, and *Sr47*) have been mapped to 2BL (Klindworth et al. 2012; Loegering and Sears 1966; Rouse et al. 2012, 2014). The stem rust gene *Sr9h* was mapped to chromosome arm 2BL, 17.7 cM proximal from *Sr28* (Rouse et al. 2014). In a previous study (Babiker et al. 2016), a gene postulated to be *Sr9h*

TABLE 2. Kompetitive allele specific PCR (KASP) assay developed for single nucleotide polymorphism (SNP) marker IWB1208 linked to the stem rust resistance locus in PI 177906 on chromosome arm 2BL

SNP ID	Primer name	Primer sequence[a]	Allele	Parent
IWB1208	KASP_IWB1208_A	GTGTATAAGATTCCTGCCGTGTGA	A	LMPG-6
IWB1208	KASP_IWB1208_C	GTATAAGATTCCTGCCGTGTGC	C	PI 177906
IWB1208	KASP_IWB1208_C1	GTGTCCACATCAGCAGAATATGTTATCTA		

[a] Sequences do not include a tail sequence that correspond with the FAM and HEX dye.

TABLE 3. Designation, origin, seedling infection types when tested against *Puccinia graminis* f. sp. *tritici* race TTKSK, and allele calls of *KASP_IWB1208* linked to the TTKSK resistance locus on chromosome arm 2BL of PI 177906 and *Sr28*

Accession[a]	Origin	TTKSK[b]	*KASP_IWB1208*	Accession	Origin	TTKSK[b]	*KASP_IWB1208*
LMPG-6	Canada	4	A	PI 623582	Iran	2	A
PI 177906	Turkey	;0	C	PI 623785	Iran	2	A
F1 Red Bobs/PI 177906	ID, USA	;2–	AC	PI 624149	Iran	2+	A
PI 670015*	MN, USA	;3	C	PI 625285	Iran	2	A
CItr 12499* (SD 1691)	SD, USA	;3	C	PI 625315	Iran	2+	A
CItr 7611*	Russia	;3	C	PI 625348	Iran	2+	A
CItr 5878* (Kota)	Russia	;3	C	PI 625661	Iran	2+	A
PI 351188* (Ceres)	ND, USA	3	C	PI 625673	Iran	2	A
W2691/Sr28Kt*	MN, USA	;3	C	PI 625696	Iran	2	A
CItr 12737**	ND, USA	;3	C	PI 626074	Iran	2	A
CItr 12636**	ND, USA	;3	C	PI 626252	Iran	2	A
PI 167530	Turkey	;3	C	PI 626255	Iran	2	A
PI 178221	Turkey	;3	C	PI 626308	Iran	2–	A
PI 268468	Afghanistan	;3	C	PI 626409	Iran	2	A
PI 345474	Serbia	;13	C	PI 626491	Iran	2	A
CItr 6900	USA	;3	C	PI 626634	Iran	2	A
PI 269238	Portugal	03–	C	PI 626703	Iran	2	A
PI 470469	Turkey	;2	C	PI 627093	Iran	3	A
PI 48200	France	0;	C	PI 341431	Turkey	4	A
PI 565387	Uzbekistan	;13	C	PI 374670	Bosnia and Herzegovina	;23	A
PI 572693	Georgia	;	C	PI 374510	Serbia	2	A
CItr 13586	MT, USA	;3	C	Red Bobs	Canada	4	A
CItr 15026	Afghanistan	3	A	Line E	MN, USA	4	A
CItr 15035	Afghanistan	2	A	McNair 701	NC, USA	4	A
Cltr 4311	Iran	2	A	Louise	WA, USA	4	A
Cltr 83248	China	;1	A	Penawawa	WA, USA	4	A
PI 94439	Russian Federation	3+	A	KS05HW14	KS, USA	4	A
PI 165193	Turkey	2	A	Prelude	Canada	4	A
PI 165194	Turkey	2	A	Norin 40 (*Sr42*)	Japan	2	A
PI 165700	Afghanistan	2	A	Ac Cadillac (*SrCad*)	Canada	2–	A
PI 166675	Turkey	2	A	Norka (*Sr15*)	Australia	;13	A
PI 167531	Turkey	2	A	Chinese spring (*Sr9f*)	MO, USA	4	A
PI 178188	Turkey	2	A	CItr 14035 (*Sr9h*)	Mexico	2	A
PI 181433	Afghanistan	2	A	ISr9a-RA	USA	4	A
PI 184221	Serbia	2–	A	W2691Sr9b	USA	4	A
PI 192162	Ethiopia	2+	A	Vernstine (*Sr9e*)	Australia	4	A
PI 212466	Afghanistan	2	A	Excalibur (*Sr15*)	Australia	;3	A
PI 220127	Afghanistan	2	A	Kukri	Australia	3	A
PI 243711	Iran	2–	A	PI 634866	WA, USA	3+	A
PI 24484	Uzbekistan	2	A	PI 601814	WA, USA	4	A
PI 243779	Uzbekistan	2	A	PI632857	WA, USA	3+	A
PI 366076	Egypt	22+	A	CItr 3641	Canada	4	A
PI 347169	Afghanistan	2	A	PI 520003	CIMMYT	3+	A
PI 429407	Iran	2	A	CItr 7635	Russian Federation	4	A
PI 622722	Iran	1+	A	CItr 7599	Russian Federation	3+	A
PI 623118	Iran	2	A	CItr 7641	Russian Federation	3+	A
PI 623162	Iran	2+	A	CItr 7709	Russian Federation	4	A
PI 623164	Iran	2	A	CItr 7725	Russian Federation	4	A
PI 623020	Iran	2–	A	PI 165542	India	4	A
PI 623355	Iran	2+	A				

[a] * Indicates accessions with *Sr28*. ** Indicates accessions postulated to carry *Sr28* according to Rouse et al. (2012).

[b] The infection types were assessed at the seedling stage following the scale developed by Stakman et al. (1962), where + and – indicate variation within a given infection type.

was located at 101.5 to 109.5 cM on the wheat consensus map (Wang et al. 2014). In the present study, the gene on 2BL in PI 177906 mapped to a location 126.3 to 134.7 cM on the consensus map, which corresponds to the location of *Sr28* rather than that of *Sr9h*. In addition, PI 177906 exhibited low IT ;0 to *P. graminis* f. sp. *tritici* race BCCBC, which is known to be avirulent on *Sr28* (Rouse et al. 2012), and this resistance mapped to the same location as the TTKSK resistance at 126.3 to 134.7 cM in the present study. In response to *P. graminis* f. sp. *tritici* race TTKSK, the IT of 2 for *Sr9h* differs from the IT of ;3 of *Sr28* (Rouse et al. 2012, 2014). The IT displayed by PI 177906, fragments size produced by the two markers (*wPt-7004-PCR* and *wmc332*) flanking *Sr28*, and mapping results support the conclusion that the gene on 2BL in PI 177906 is likely to be *Sr28* and is not *Sr9h*. Among the other stem rust resistance genes on chromosome 2B, *Sr16* was characterized as being ineffective against Ug99 (Jin et al. 2007) and therefore is not expected to be the gene conferring resistance to TTKSK in PI 177906. *Sr47* is effective against Ug99 and was introgressed into wheat chromosome 2B from *Aegilops speltoides* (Klindworth et al. 2012) making the presence of this alien chromatin highly unlikely to be present in a wheat landrace. In addition, *Sr47* confers resistance to races MCCFC and TPMKC (Klindworth et al. 2012), whereas these two races are virulent on PI 177906. Also, *Sr47* is effective against race QFCSC with an infection type of ;0 and the resistance in PI 177906 to race QFCSC has an IT of 2– and maps to chromosome 4A in the present study, not to 2BL. This evidence supports the conclusion that *Sr47* is not present in PI 177906. Chromosome arm 4AL is known to harbor *Sr7b*, which confers resistance to *P. graminis* f. sp. *tritici* race QFCSC (Rouse et al. 2011); therefore, the QFCSC resistance in PI 177906 could be due to *Sr7b*. Two of the detected SNP markers IWA2202 and IWA1067 were recently found to be associated with a gene postulated to be *Sr7a* in the hard red winter wheat 'Jagger' (Turner et al. 2016). The SNP markers, IWA2202 and IWA1067, were mapped 18.6 and 29.2 cM, respectively, from the QFCSC resistance in PI 177906. Mapping results using the two populations detected two loci for TTKSK resistance, one each on chromosome arms 2BL and 6DS. Three Ug99-effective *Sr* genes, *SrTmp*, *SrCad*, and *Sr42*, were previously mapped to chromosome arm 6DS (Ghazvini et al. 2012; Hiebert et al. 2011; Lopez-Vera et al. 2014). When tested against *P. graminis* f. sp. *tritici* races TTKSK and BCCBC, 27 RILs and 32 DH lines from LMPG-6/PI 177906 displayed ITs of 2 to TTKSK and ITs of 4 to BCCBC. Of these 59 lines, only two RILs amplified the 2BL resistance allele for two of the flanking markers, indicating that the IT of 2 against TTKSK was not conferred by the 2BL locus but instead by the 6DS locus. Susceptibility of PI 177906 to *P. graminis* f. sp. *tritici* races TPMKC, MCCFC, TTTTF, and TRTTF and resistance to TTKSK suggested the presence of *Sr42*, or *SrCad* or *SrTmp* in PI 177906 (Ghazvini et al. 2012; Hiebert et al. 2011; Jin et al. 2007). Three of the detected SNP markers IWB6902.2, IWB6072.2, and IWB262 were previously found to be associated with a gene postulated to be *Sr42* or *SrTmp* in two spring wheat accessions from Iran and Serbia (Babiker et al. 2016). In addition, two of the detected SNP markers in the present study, IWB36391 and IWB49086, were previously found to be associated with *Sr42* and *SrCad*, respectively (Gao et al. 2015; Kassa et al. 2016). The diagnostic markers for *Sr42* and *SrCad* (Gao et al. 2015; Kassa et al. 2016) suggest that PI 177906 does not possess either *Sr42* or *SrCad*. The SNP marker IWB49086 mapped 3.01 cM from the TTKSK locus on 6DS. This marker was recently mapped 0.8 cM distal to *SrTmp* in LMPG-6/Triumph 64 population (Hiebert et al. 2016), indicating that the TTKSK resistance gene on chromosome 6DS could be either *SrTmp* or a novel gene. Further experimentation, including allelism testing, is needed to determine the

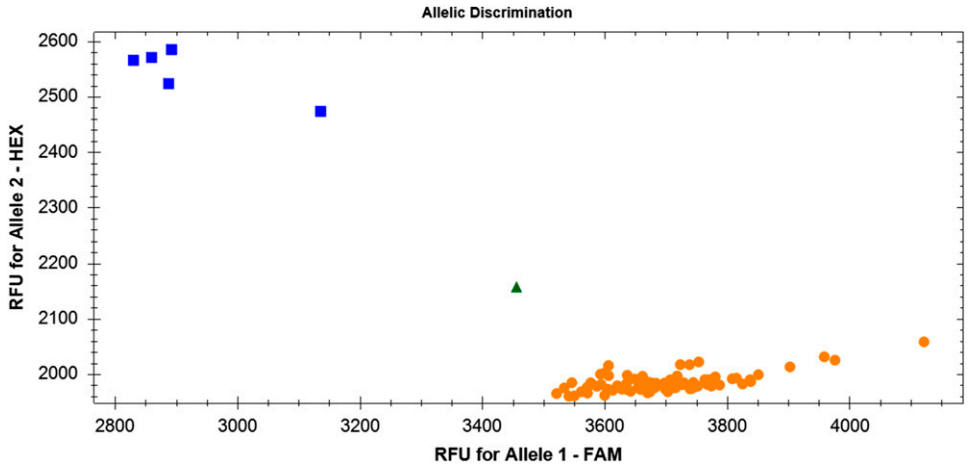

Fig. 3. Genotyping profile of *KASP_IWB1208* marker linked to the TTKSK resistance locus on chromosome arm 2BL of PI 177906 (*Sr28*).

TABLE 4. Seedling infection types of wheat accessions tested against *Puccinia graminis* f. sp. *tritici* races TTKSK and BCCBC, *Sr28* linked markers alleles, and allele calls of single nucleotide polymorphism marker *KASP_IWB1208* linked to the TTKSK resistance locus on chromosome arm 2BL of PI 177906

Accession	TTKSK	BCCBC	*wPt-7004-PCR*	*wPt-7004-PCR*	*wmc332*	*IWB1208*
LMPG-6	3+	3+	164	0	172	A
PI 177906	0	;13	164	192	209	C
CItr 12499[a]	;13	;13	164	192	211	C
CItr 5878[a]	;13	;13	164	192	211	C
PI 470469	;2	ND	163	0	237	C
PI 345474	;13	ND	164	192	211	C
PI 142509	ND	3+	164	192	211	A
PI 207121	2	3+	164	192	211	A
PI 94439	3+	;13	164	192	209	A
PI 623538	;2	2+3	164	192	209	A

[a] Accessions with *Sr28* according to Rouse et al. (2012).

relationship between *SrTmp* and the TTKSK locus on chromosome 6DS in PI 177906. Within the two regions with the seedling resistance to the *P. graminis* f. sp. *tritici* race TTKSK, two QTL contributing to the field resistance were consistently detected on the same regions on chromosome arms 2BL and 6DS across the three seasons. This result indicated that the field resistance to Ug99 in PI 177906 is conditioned primarily by the two major genes that were detected in the seedling assays. In previous field tests a monogenic line with *Sr28* (W2691/Sr28Kt) provided an intermediate level of resistance ranging from 30MRMS to 40MSS to *P. graminis* f. sp. *tritici* race TTKSK in Kenya (Jin et al. 2007). In the three field trials, W2691/Sr28Kt displayed 25MSS disease severity and infection response, whereas PI 177906 exhibited a higher level of resistance ranging from 10MR to 20MR to the Ug99 race group, indicating an additive effect of the two loci for resistance. Further allelism studies are needed to establish the relationship between the TTKSK locus on chromosome arm 2BL of PI 177906 and *Sr28* and the locus on chromosome arm 6DS with *SrTmp*.

Using KASP assay, the SNP marker *KASP_IWB1208* was found to be associated both with the *Sr* gene in PI 177906 and *Sr28*. By comparing the KASP assay developed in the present study with the *Sr28* markers developed by Rouse et al. (2012), we demonstrated the reliability of the SNP marker *KASP_IWB1208* in predicting the presence of *Sr28* gene in diverse wheat germplasm. This marker could be suitable for marker-assisted selection to pyramid *Sr28* with other effective *Sr* genes to improve stem rust resistance in new cultivars and possibly prolong their effectiveness.

In conclusion, two loci on chromosome arms 2BL and 6DS for Ug99 resistance were mapped in DH and RIL populations developed from a cross between the wheat landrace accession PI 177906 and LMPG-6. Mapping results and ITs observed in the present study, suggested that the wheat landrace PI 177906 most likely has *Sr28* on chromosome arm 2BL and *SrTmp* or a new gene on chromosome arm 6DS. One of the SNP markers found in the present study was clearly associated with the presence of *Sr28* in a set of 96 wheat accessions suggesting the utility of this marker for marker-assisted selection of *Sr28*.

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