Rapid Identification of Resistance Loci Effective Against *Puccinia graminis* f. sp. *tritici* Race TTKSK in 33 Spring Wheat Landraces

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Abstract

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Wheat breeders worldwide are seeking new sources of resistance to *Puccinia graminis* f. sp. *tritici* race TTKSK. To prioritize field-resistant landraces for follow-up genetic studies to test for the presence of new resistance genes, seedling response to *P. graminis* f. sp. *tritici* race TTKSK, molecular markers linked to specific *Sr* genes, segregation ratios among progeny from crosses, and bulked segregant analyses (BSA) were used. In total, 33 spring wheat landraces with seedling resistance to *P. graminis* f. sp. *tritici* race TTKSK were crossed to a susceptible genotype, LMPG-6. The segregation ratios of stem rust reactions in F_2 seedlings fit a single dominant gene model in 31 populations and progeny from two crosses gave ambiguous results. Using the 90K wheat single-nucleotide polymorphism genotyping platform, BSA showed that the seedling resistance in 29 accessions is probably controlled by loci on chromosome 2BL. For the three remaining accessions, BSA revealed that the seedling resistance is most likely controlled by previously unreported genes. For confirmation, two populations were advanced to the $F_{2:3}$ and screened against *P. graminis* f. sp. *tritici* race TTKSK. Segregation of the $F_{2:3}$ families fit a 1:2:1 ratio for a single dominant gene. Using the $F_{2:3}$ families, BSA located the TTKSK locus on chromosome 6DS to the same location as *Sr42*.

Wheat stem rust caused by the fungus *Puccinia graminis* f. sp. *tritici* Erikss. & Henning is potentially the most devastating disease of wheat worldwide (Roelfs et al. 1992; Singh et al. 2011). Since 1956, stem rust has been controlled in North America through eradication of barberry (*Berberis vulgaris*), the pathogen's alternate host, and deployment of effective *Sr* genes in widely grown cultivars (Jin and Singh 2006; Kolmer et al. 2007). In the past decade, stem rust has reemerged as a threat to global wheat production with the evolution of new pathotypes, known as the Ug99 race group, that overcome the widely used stem rust resistance genes *Sr31*, *Sr24*, and *Sr36* (Pretorius et al. 2000; Jin et al. 2008, 2009). The first occurrence of Ug99 was race TTKSK, collected in Uganda in 1998. *P. graminis* f. sp. *tritici* race TTKSK is significant due to its virulence on *Sr31*, which is widely deployed in spring wheat cultivars in developing countries (Singh et al. 2008). Race TTKSK evolved and gained additional virulence to *Sr24* and *Sr36*, which are common stem rust resistance genes in U.S. hard and soft winter wheat, respectively (Jin et al. 2008, 2009). Another variant of TTKSK with virulence to lines with resistance gene *Sr9h* and designated as race TTKSF+ was detected in South Africa (Pretorius et al. 2012; Rouse et al. 2014). Currently, there are only five designated genes (*Sr9h*, *Sr28*, *Sr42*, *Sr57*, and *SrTmp*) originating from *Triticum aestivum* that are effective against *P. graminis* f. sp. *tritici* race TTKSK (Ghazvini et al. 2012; McVey and Hamilton 1985; Rouse et al. 2012). Recently, race TKTTF with virulence to a line with resistance gene *SrTmp* has been detected in Ethiopia (Olivera et al. 2015).

Due to the ongoing evolution of new *P. graminis* f. sp. *tritici* races and widespread susceptibility in wheat to the Ug99 race group, work is underway to identify new sources of Ug99 resistance. Wheat landraces represent a valuable gene pool for new sources of resistance to wheat pathogens. To discover potentially new resistance to Ug99, 2,509 spring wheat landraces from the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) National Small Grains Collection were screened in Kenya, where *P. graminis* f. sp. *tritici* races TTKST and TTKSK are naturally present, and 246 showed field resistance as adult plants (Newcomb et al. 2013). However, it is not known which of these resistant landraces might have new TTKSK resistance genes. Developing an efficient means to identify the landraces most likely to carry new resistance genes would enable researchers to prioritize the materials for further genetic studies.

Bulked segregant analysis (BSA) has been used extensively to identify molecular markers associated with resistance genes and to assign resistance genes to a chromosomal location (Chantret et al. 2000; Michelmore et al. 1991; Shen et al. 2003). As an alternative to biparental mapping, BSA is both faster and less expensive and, thus, can be used to economically screen many populations. Single-nucleotide polymorphism (SNP) markers for wheat are now available and frequently used in genetic analysis. Compared with other molecular markers, SNP markers are cost effective, provide better genome coverage, and are amenable for genotyping using high-throughput automated scoring platforms (Wang et al. 2014). The purpose of the present study was to (i) characterize the inheritance of resistance to *P. graminis* f. sp. *tritici* race TTKSK in 33 wheat landraces and (ii) determine the likely chromosomal locations of the resistance genes. The results of this study will be used to select the most promising populations for further characterization and mapping as part of our goal of identifying new resistance genes.

Materials and Methods

Plant materials. Of the 246 resistant accessions previously identified, 78 were classified as having a higher level of field-resistance to Ug99 based on median infection responses (Newcomb et al. 2013). Of these, 25 originating from Ethiopia were excluded from the present study because these are likely not landraces (Tesfaye et al. 1991). The remaining 53 accessions were screened for the presence of *Sr22* (Periyannan et al. 2011), *Sr24* (Mago et al. 2005; Olson et al.

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2010), *Sr26* (Liu et al. 2010), *Sr31* (Saal and Wricke 1999), and *Lr19*/*Sr25* (Prins et al. 2001) using linked molecular markers. Five accessions were removed from consideration because of the presence of *Lr19*/*Sr25*. In addition, seven accessions were excluded due to seedling-stage susceptibility to *P. graminis* f. sp. *tritici* race TTKSK. The remaining 41 accessions with seedling resistance to race TTKSK were crossed to the susceptible line LMPG-6 (Knott 1990), from which F_1 seed were obtained from 33 crosses. A single F_1 seed from each cross was sown to generate F_2 populations. To confirm the F_2 results from two populations, LMPG-6/PI 184221 and LMPG-6/PI 625696 were advanced to generate $F_{2:3}$ populations.

Seedling evaluations. Parents and the 33 F_2 populations were screened with *P. graminis* f. sp. *tritici* race TTKSK (isolate 04KEN156/04) at the USDA-ARS Cereal Disease Laboratory in St. Paul, MN following the protocol described by Rouse et al. (2011a). About 100 seedlings from each F_2 population were tested, and seedling infection types (IT) using the 0-to-4 scale developed by Stakman et al. (1962) were recorded. A χ^2 analyses was performed to test for goodness-of-fit to the models of one, two, or four genes. To postulate the presence of one of the known *Sr* genes, the 33 resistant landraces were also tested against the *P. graminis* f. sp. *tritici* races TRTTF (isolate 06YEM34-1), TTTTF (isolate 01MN84A-1-2), RKQQC (isolate 99KS76A), MCCFC (isolate 59KS19), TPMKC (isolate 74MN1409), QFCSC (isolate 06ND76C), QTHJC (isolate 75ND717C), RCRSC (isolate 77ND82A), SCCSC (isolate 09ID73-2), and QCCSM (isolate 75WA165-2A). Based on the initial BSA results, it was necessary to distinguish the gene on chromosome 2BL from *Sr9h*. Therefore, nine of the landraces were screened against *P. graminis* f. sp. *tritici* race TTKSF+ (isolate UVPgt61/1) at the University of Free State, South Africa (Pretorius et al. 2012). Race TTKSF+ is known to be virulent against *Sr9h* (Rouse et al. 2014). Two wheat cultivars with *Sr9h*, 'Webster' and 'Matlabas', and line-37 were included as checks. Based on the BSA results, PI 184221, PI 625696, and PI 625661 were screened against *P. graminis* f. sp. *tritici* race TKTTF (isolate 13ETH18-1) to distinguish the resistance in these landraces from *SrTmp*.

Genetics of seedling resistance to *P. graminis* f. sp. *tritici* race TTKSK. To confirm the F_2 results, 110 and 106 $F_{2:3}$ families were derived from LMPG-6/PI 184221 and LMPG-6/PI 625696, respectively. From each family, 15 to 20 seedlings were screened against *P. graminis* f. sp. *tritici* race TTKSK. Based on the IT, families were classified as homozygous resistant (HR), homozygous susceptible (HS), or segregating for resistance (Seg). A χ^2 analysis was performed to test for goodness-of-fit to the expected ratio of 1:2:1 for a single resistance gene.

BSA. For each of the 33 populations, DNA was extracted from parents, F_1 seedlings, and two susceptible bulks comprising 10 F_2 susceptible seedlings each. To confirm the F_2 results for the PI 184221 and PI 625696 populations, DNA was extracted from parents and three susceptible and three resistant bulks, each comprising 5 to 10 $F_{2:3}$ families. Total genomic DNA was extracted from 4-week-old seedlings using the protocol previously described by Babiker et al. (2015). DNA samples were genotyped with the wheat 90K iSelect SNP genotyping platform following the manufacturer's protocol (Illumina Inc., San Diego, CA) at the USDA-ARS Cereal Crops Research Unit in Fargo, ND. Allele calling for each SNP was performed using Illumina's GenomeStudio v2011.1 and manually inspected for call accuracy. SNP markers were assigned to chromosomes and positioned according to the rescaled hexaploid wheat 90K SNP consensus map (Wang et al. 2014).

Based on the BSA results, it was necessary to distinguish genes in PI 625696 and PI 184221 from *Sr42* and *SrTmp*. Therefore, these two accessions were screened with simple-sequence repeat (SSR) markers *cfd49*, *gpw5182*, and *barc183* to determine the presence of *Sr42* (Hiebert et al. 2011). Markers *cfd49* and *barc183* were previously found to be flanking a gene postulated to be *SrTmp* in two wheat genotypes, 'Ripper' and 'Blouk' (Lopez-Vera et al. 2014).

Results

Inheritance of seedling resistance to *P. graminis* f. sp. *tritici* race TTKSK. Of the 33 selected resistant accessions, 30 accessions exhibited IT of 2 and 2+ when inoculated with *P. graminis* f. sp. *tritici* race TTKSK versus IT of 3+ to 4 for LMPG-6. Of the 33 selected landraces, 29 showed resistance to race RKQQC (Table 1). All of the 33 accessions were susceptible to *P. graminis* f. sp. *tritici* races TRTTF and TPMKC. Except for wheat landrace PI 626409, race TTTTF produced high IT on all tested accessions. When inoculated with *P. graminis* f. sp. *tritici* race TTKSK, wheat landraces PI 184221 and PI 626308 exhibited IT scores of 2–, the lowest observed for this race among the test materials. The F_2 populations segregated for resistance to *P. graminis* f. sp. *tritici* race TTKSK with resistant seedlings exhibiting 2 and 2+ IT and susceptible seedlings exhibiting IT of 3+ and 4. The responses of the F_1 seedlings from all the crosses were resistant, with IT of 2–, 2, and 2+, which were similar to the reactions of the resistant parents, indicating dominant gene action. The F_2 seedlings from 31 populations fit a 3:1 resistant/susceptible segregation ratio, indicating the presence of single dominant genes in each population (Table 2). One accession, PI 192187, exhibited an IT of ;13, and F_1 seedlings from the LMPG-6/PI 192187 cross were resistant, with an IT score similar to the resistant parent. However, many F_2 seedlings from this population displayed intermediate reactions to TTKSK, which made it difficult to classify most of the progeny as clearly resistant or susceptible; therefore, no markers linked to resistance were obtained from the BSA for this cross.

BSA and parents genotyping. For each of the remaining 32 crosses, BSA identified from 8 to 81 SNP markers as polymorphic both between the parents and between the resistant parent and susceptible bulks. BSA showed that the seedling resistance to race TTKSK in 29 accessions is likely controlled by loci on chromosome 2BL (Table 3). The chromosomal location and the IT of 2 displayed by these 29 accessions suggested that the race TTKSK resistance in these landraces could be due to *Sr9h*. To verify these results, nine of these accessions were tested against *P. graminis* f. sp. *tritici* races TTKSF+ and TTKSF, which are known to be virulent and avirulent, respectively, on *Sr9h* (Rouse et al. 2014). The nine tested wheat landraces exhibited low IT of 1+, 2–, and 2 when inoculated with *P. graminis* f. sp. *tritici* race TTKSF, which is avirulent on *Sr9h*. Six of the accessions showed high IT of 3 and 4 when inoculated with *P. graminis* f. sp. *tritici* race TTKSF+, indicating the likely presence of *Sr9h* in these accessions. Three accessions displayed IT of 22+ and 2++ against TTKSF+ (Table 4). The intermediate IT of 22+ and 2++ compared with the low IT of 1+, 2–, and 2 indicate that the three accessions may, indeed, possess *Sr9h*. Further studies would be necessary to determine the genetics of the intermediate reactions in these three accessions.

The BSA from the three remaining populations indicated association with markers on chromosomes other than 2B. In LMPG-6/PI 625661 F_2 population, 11 SNP markers on chromosome 7AS and 4 on 1BL revealed polymorphism between parents as well as between PI 625661 and the susceptible bulks, suggesting that the TTKSK resistance could be controlled by an *Sr* gene on either 7AS or 1BL (Table 3). Due to a limited number of F_2 seed for the LMPG-6/PI 625661 cross, we were not able to validate these results in an advanced population. In the LMPG-6/PI 625696 F_2 population, evidence of association occurred on chromosomes 6AS, 6DS, and 3AS. In the F_2 population derived from LMPG-6/PI 184221, six SNP markers from chromosome 1AS, five from 2BL, one from 5B, and one from 6DS revealed polymorphism between parents as well as between PI 184221 and susceptible bulks (Table 3).

To confirm the BSA results from the F_2 populations and identify markers cosegregating with resistance in populations that could have new *Sr* genes, two populations were advanced to $F_{2:3}$ and screened against *P. graminis* f. sp. *tritici* race TTKSK. Segregation of $F_{2:3}$ families from LMPG-6/PI 184221 and LMPG-6/PI 625696 crosses fit a 1:2:1 ratio for HR/Seg/HS ($\chi^2 = 0.78$, $P = 0.67$ and $\chi^2 = 0.39$, $P = 0.14$, respectively), thus confirming the F_2 results. In the LMPG-6/PI 184221 $F_{2:3}$ families, BSA identified one marker on 5BL and four on 6DS associated with resistance (Table 5). Only one of the markers detected in the F_2 BSA on 6DS was also detected in the $F_{2:3}$ BSA. PI 184221 displayed an IT reaction similar to that of *Sr42* and *SrTmp* to *P. graminis* f. sp. *tritici* race TTKSK (Jin and

Singh 2006). Susceptibility of PI 184221 to *P. graminis* f. sp. *tritici* races TPMKC, MCCFC, TTTTF, TRTTF, and TKTTF and resistance to QCCSM, QTHJC, RCRSC, and TTKSK suggested the presence of either *Sr42* or *SrTmp* in PI 184221 (Olivera et al. 2012a; Rouse and Jin 2011; Rouse et al. 2011b). To further test for the possible presence of *Sr42* and *SrTmp*, two SSR markers previously found to be associated with *Sr42* and *SrTmp* were tested in PI 184221. SSR markers *gpw5182* amplified a 158-bp fragment, while *barc183* amplified 149- and 166-bp fragments from PI 184221, indicating that the TTKSK resistance in PI 184221 is likely due to *Sr42*. Using $F_{2:3}$ families from the LMPG-6/PI 625696 cross, BSA clearly identified 10 SNP markers located on the short arm of chromosome 6D associated with the TTKSK resistance (Table 5). Of the 10 SNPs detected, 2 were also found in the BSA of the F_2 population. These markers localized in a region covering 10.4 centimorgans on chromosome 6DS. To test for possible redundancy with *Sr42* or *SrTmp*, the two SSR markers linked to *Sr42* were used to genotype PI 625696 (Ghazvini et al. 2012). SSR marker *gpw5182* did not amplify a fragment from PI 625696 while *barc183* amplified 149- and 166-bp fragments. Consequently, we were unable to confirm that the TTKSK resistance in PI 625696 was due to the presence of *Sr42* or *SrTmp*.

Discussion

As part of an effort to identify new genes for field resistance to the Ug99 race group of *P. graminis* f. sp. *tritici*, a diverse collection of 2,509 wheat landraces were evaluated in Kenya and 246 resistant accessions were identified (Newcomb et al. 2013). To select accessions for follow-up, we identified landraces with the lowest disease levels in the field and eliminated those unlikely to be true landraces. The assumption behind this approach is that landraces would be more likely to carry new resistance genes than would germplasm that had been previously used in wheat breeding. The process resulted in 41 selected accessions, from which 33 successful crosses were made. These 33 landraces were screened against an array of *P. graminis* f. sp. *tritici* races to postulate the presence of known resistance genes. *P. graminis* f. sp. *tritici* races TPMKC, TTTTF, and TRTTF were virulent on all of the tested landraces, except for PI 626409 in the case of the reaction to race TTTTF (resistant IT of 2+). *P. graminis* f. sp. *tritici* race TRTTF was detected in 2006 from stem rust samples from Yemen and Ethiopia (Olivera et al. 2012b). Race TTTTF was detected in the United States in 2000 and 2001 from stem rust collections from Texas and Minnesota, respectively (Jin 2005). *Sr24* is effective against race TTTTF; however, results from tests with diagnostic markers for *Sr24* showed that the TTTTF resistance in PI 626409 was not due to *Sr24*. The resistance to TTTTF in PI 626409 is likely due to an unknown gene, because Jin (2005) attributed the TTTTF resistance in most spring wheat cultivars to unknown *Sr* genes. The F_1 seedlings from all crosses were resistant, with IT similar to the parents, indicating that the TTKSK resistance is characterized by dominant inheritance from each accession. The F_2 segregation ratios of 3:1 resistant/susceptible showed that seedling resistance to TTKSK in 31 accessions is controlled by single dominant genes. Crosses with PI 192187 and PI 623164 gave ambiguous segregation ratios. In LMPG-6/PI 192187, this ambiguity may be attributed to the reaction of the resistant parent, which exhibited an IT of ;13, with larger pustules toward the leaf base. This IT makes it difficult to classify each F_2 seedling as either clearly resistant or susceptible. Therefore, this population will be advanced for analyses in fixed recombinant inbred lines.

Of the 33 tested landraces, 29 showed evidence of loci for resistance to race TTKSK on chromosome 2BL. Most of these landraces showed similar IT scores when tested against races TTKSK and

Table 1. Accession number, origin, and seedling infection types of wheat landraces tested against 11 physiologic races of *Puccinia graminis* f. sp. *tritici*[a]

Accession	Origin	TTKSK	MCCFC	QCCSM	QFCSC	QTHJC	SCCSC	RCRSC	RKQQC	TPMKC	TTTTF	TRTTF
CItr 15035	Afghanistan	2	3+	ND	4	3+	ND	3+	2–	4	3+	4
PI 165193	Turkey	2	;1	2+	2	1	3–	2–	2–	3	3	4
PI 165700	Afghanistan	2	4	4	4	4	4	4	2	4	4	4
PI 166675	Turkey	2	2	3+	2+	2+	2+	2	2	3–	3	4
PI 167531	Turkey	2	3+	3+	2+	3+	3+	3+	2	3+	4	4
PI 178188	Turkey	2	4	4	3+	3–	4	3–	2	4	4	4
PI 181433	Afghanistan	2	4	4	3+	4	4	4	2	3	4	4
PI 184221	Serbia	2–	3+	;1	2–	2+	3+	3	4	4	4	4
PI 192187	Portugal	;13	;	2+	0	3–	4	3+	4	4	4	4
PI 220127	Afghanistan	2	4	4	3+	4	4	4	2	3+	3+	3+
PI 243779	Iran	2	4	4	3	4	4	4	;1	4	4	4
PI 24484	Uzbekistan	2	3+	4	4	3+	4	4	2	4	4	4
PI 347169	Afghanistan	2	4	4	4	4	4	4	2	4	4	4
PI 429407	Iran	2	4	3	3–	ND	3+	3–	3–	4	3	4
PI 623118	Iran	2+	3	ND	3–	ND	3+	ND	2	4	3+	4
PI 623162	Iran	2+	4	4	4	4	4	4	2+	4	4	4
PI 623164	Iran	2	4	4	4	4	4	4	3–	4	4	4
PI 623355	Iran	2	4	4	4	3+	4	3	2–	4	4	4
PI 623582	Iran	2	3+	4	3+	4	ND	4	2+	3+	4	4
PI 623785	Iran	2	3	4	4	3+	3+	3–	2	3+	3–	3+
PI 624149	Iran	2+	3	4	3+	3+	3+	2+	2–	4	4	4
PI 625315	Iran	2+	3	ND	2+	2+	ND	2	2–	4	3	4
PI 625348	Iran	2+	3+	ND	2	2	ND	2	2	4	4	4
PI 625661	Iran	2+	4	1	3	3	3+	4	2	4	4	4
PI 625673	Iran	2	4	ND	3	ND	ND	ND	2	4	4	4
PI 625696	Iran	2	2+	4	2+	2+	4	2+	2+	3+	4	4
PI 626074	Iran	2	4	4	4	4	4	3	2	4	4	4
PI 626252	Iran	2	4	3–	4	4	4	4	2	4	3–	4
PI 626255	Iran	2	4	4	3+	4	4	4	2–	4	3+	4
PI 626308	Iran	2–	3–	4	4	ND	4	3–	2	4	3+	4
PI 626409	Iran	2	3	4	3–	3–	0	2+	2–	4	2+	4
PI 626491	Iran	2	4	4	4	4	4	3+	2	4	4	4
PI 626634	Iran	2	4	4	3+	4	3+	3	2	4	4	4

[a] ND = no data.

RKQQC, suggesting that the resistance in these landraces may be controlled by the same *Sr* gene. Five Ug99-effective *Sr* genes (*Sr9h*, *Sr28*, *Sr40*, *Sr39*, and *Sr47*) have been mapped to chromosome 2B. *Sr39* and *Sr47* are translocations from *Aegilops speltoides* (Klindworth et al. 2012; Niu et al. 2011) and *Sr40* is a translocation from *T. timopheevii* (Dyck 1992); *Sr39* and *Sr40* are, both located on the short arm of chromosome 2B. *Sr9h* and *Sr28* originated from *T. aestivum* and are located on the long arm of chromosome 2B (Rouse et al. 2012, 2014). Many of the markers associated with the resistance in these 29 accessions mapped to chromosome 2BL. The *Sr9h*, *Sr28*, *Sr39*, *Sr40*, and *Sr47* monogenic lines displayed IT of 2, ;13, ;1, 1, and 2 −, respectively, to *P. graminis* f. sp. *tritici* race TTKSK (Jin et al. 2007; Niu et al. 2011; Rouse et al. 2014). The IT associated with the various genes on chromosome 2B versus race TTKSK are consistent with the presence of *Sr9h* in many of the accessions tested in this study. The presence of *Sr9h* in the landraces is further supported by the susceptibility of six of these landraces to *P. graminis* f. sp. *tritici* race TTKSF+, which is known to be virulent on *Sr9h* (Rouse et al. 2014). Further studies are needed, though, to verify the presence of *Sr9h* in the other three tested landraces with intermediate reaction to race TTKSK. BSA in the LMPG-6/PI 625661 F_2 population revealed two regions on chromosome arms 7AS and 1BL associated with race TTKSK resistance. Chromosome arm 7AS is known to harbor *Sr44*, which confers resistance to *P. graminis* f. sp. *tritici* race TTKSK with an IT of 1+ (Jin et al. 2007). Only *Sr14* has been mapped to the long arm of chromosome 1B (McIntosh et al. 1995). The reaction of a monogenic line for *Sr14* to *P. graminis* f. sp. *tritici* race TTKSK was reported to be inconclusive (Jin et al. 2007). Because the IT of PI 625661 differs from that of both *Sr44* and *Sr14*, and because *Sr44* and *Sr14* are derived from species related to *T. aestivum*, PI 625661 may carry a new *Sr* gene. This population is being advanced for further study. Two other populations showed potential for new resistance based on the F_2 BSA results. The BSA results from $F_{2:3}$ families from PI 625696 indicated that the resistance is most likely due to a dominant gene located on chromosome 6DS. The stem rust resistance gene *Sr42* is the only TTKSK-resistant gene that has

Table 2. Segregation of resistance to *Puccinia graminis* f. sp. *tritici* race TTKSK in F_2 progeny derived from 33 crosses between the susceptible wheat line LMPG-6 and resistant wheat landrace accessions

	Number of individuals		3:1 ratio	
Population	**Resistant**	**Susceptible**	χ^2	***P* value**[a]
LMPG-6/CItr 15035	70	16	1.80	0.190
LMPG-6/PI 165193	49	18	0.12	0.720
LMPG-6/PI 165700	64	27	1.05	0.305
LMPG-6/PI 166675	43	15	0.02	0.879
LMPG-6/PI 167531	55	15	0.48	0.490
LMPG-6/PI 178188	68	22	0.02	0.900
LMPG-6/PI 181433	83	21	1.28	0.250
LMPG-6/PI 184221	64	26	0.73	0.394
LMPG-6/PI 192187	51	34	10.20	**0.001**
LMPG-6/PI 220127	71	20	0.44	0.507
LMPG-6/PI 243779	71	23	0.01	0.906
LMPG-6/PI 24484	85	20	1.98	0.159
LMPG-6/PI 347169	69	21	0.13	0.715
LMPG-6/PI 429407	82	17	3.20	0.070
LMPG-6/PI 623118	78	17	2.55	0.110
LMPG-6/PI 623162	73	24	0.01	0.950
LMPG-6/PI 623164	41	24	4.90	**0.026**
LMPG-6/PI 623355	81	30	0.24	0.624
LMPG-6/PI 623582	91	21	2.33	0.130
LMPG-6/PI 623785	89	20	2.57	0.110
LMPG-6/PI 624149	86	27	0.07	0.787
LMPG-6/PI 625315	67	20	0.19	0.665
LMPG-6/PI 625348	86	19	2.67	0.102
LMPG-6/PI 625661	96	22	2.54	0.110
LMPG-6/PI 625673	91	20	2.88	0.089
LMPG-6/PI 625696	81	21	1.06	0.303
LMPG-6/PI 626074	75	36	3.27	0.070
LMPG-6/PI 626252	74	19	1.03	0.310
LMPG-6/PI 626255	75	34	2.22	0.136
LMPG-6/PI 626308	80	33	1.06	0.303
LMPG-6/PI 626409	78	23	0.27	0.605
LMPG-6/PI 626491	95	27	0.53	0.460
LMPG-6/PI 626634	79	22	0.55	0.458

[a] Values in bold indicate significantly different from the expected 3:1 ratio at $P = 0.05$.

Table 3. Chromosomal location of markers associated with resistance to *Puccinia graminis* f. sp. *tritici* race TTKSK as detected by bulk segregant analysis of 33 F_2 populations

Population	**Chromosome locations of SNP markers**[a]
LMPG-6/CItr 15035	(24) 2B, (3) 2D, (2) 2A, (6) 3AL
LMPG-6/PI 165193	(25) 2BL, (5) 4A, (3) 7B
LMPG-6/PI 165700	(9) 2B, (3) 2AL
LMPG-6/PI 166675	(34) 2B
LMPG-6/PI 167531	(10) 2BL, (2) 1DS, (2) 7A
LMPG-6/PI 178188	(46) 2B, (9) 5D, (4) 7A
LMPG-6/PI 181433	(9) 2BL, (3) 1B, (2) 6A
LMPG-6/PI 184221	(6) 1AS, (5) 2BL, (1) 5BL, (1) 6DS
LMPG-6/PI 192187	(4) 2A, (3) 6B, (2) 7A, (2) 3B, (2) 1A
LMPG-6/PI 220127	(47) 2BL, (4) 2D, (4) 7A
LMPG-6/PI 243779	(33) 2B, (4) 2D, (8) 4A
LMPG-6/PI 24484	(12) 2BL
LMPG-6/PI 347169	(25) 2BL
LMPG-6/PI 429407	(9) 2BL, (1) 2A
LMPG-6/PI 623118	(28) 2BL, (5) 4B, (5) 6B
LMPG-6/PI 623162	(47) 2B, (7) 2A, (5) 7A
LMPG-6/PI 623164	(33) 2B, (6) 6A, (3) 4A
LMPG-6/PI 623355	(13) 2BL, (2) 4A
LMPG-6/PI 623582	(28) 2BL, (5) 2A, (2) 6A, (2) 6B
LMPG-6/PI 623785	(19) 2B, (2) 5A, (2) 6B
LMPG-6/PI 624149	(49) 2B, (32) 3BS
LMPG-6/PI 625315	(31) 2BL, (9) 6A
LMPG-6/PI 625348	(20) 2BL, (2) 6B
LMPG-6/PI 625661	(11) 7AS, (4) 1BL
LMPG-6/PI 625673	(19) 2BL
LMPG-6/PI 625696	(9) 6AS, (3) 6DS, (3) 3AS
LMPG-6/PI 626074	(30) 2B, (3) 6A
LMPG-6/PI 626252	(7) 2B, (2) 2A
LMPG-6/PI 626255	(31) 2BL
LMPG-6/PI 626308	(19) 2BL, (7) 1B, (9) 3AL, (4) 3B, (10) 6A
LMPG-6/PI 626409	(18) 2BL, (2) 6B, (2) 7B
LMPG-6/PI 626491	(16) 2BL, (5) 7BL
LMPG-6/PI 626634	(34) 2B, (2) 6B, (2) 6D

[a] Chromosome locations of single-nucleotide polymorphism (SNP) markers associated with resistance. Number of associated markers for each chromosome is in parentheses.

Table 4. Reactions of spring wheat landraces tested against *Puccinia graminis* f. sp. *tritici* races TTKSF and TTKSF+ and *Sr* gene postulation

Accession	**TTKSF**	**TTKSF+**	***Sr* gene postulation**[a]
PI 178188	1+	4	*Sr9h*
PI 623118	2–	3	*Sr9h*
PI 623582	2	4	*Sr9h*
PI 623785	2	4	*Sr9h*
PI 625315	2	2++	*Sr9h*+
PI 625348	2	2++	*Sr9h*+
PI 626255	2–	4	*Sr9h*
PI 626409	2	22+	*Sr9h*+
PI 626491	2	3	*Sr9h*
Matlabas	2–	4	*Sr9h*
Webster	2	4	*Sr9h*
Line 37	4	4	no

[a] *Sr9h*+ indicated the presence of *Sr9h* plus an additional *Sr* gene.

been mapped to 6DS (Ghazvini et al. 2012). PI 625696 displayed an IT similar to that described for *Sr42* when tested against *P. graminis* f. sp. *tritici* race TTKSK but, when tested against *P. graminis* f. sp. *tritici* races QFCSC, QTHJC, and MCCFC, PI 625696 displayed an IT of 2+, which differs from the IT of 33+ described for Norin 40 (*Sr42*) in response to these races (Ghazvini et al. 2012). The resistance of PI 625696 to *P. graminis* f. sp. *tritici* races QFCSC, QTHJC, MCCFC, and RKQQC may be due to an additional *Sr* gene.

Although χ^2 analysis revealed single dominant genes in 31 of the landraces, several markers from different chromosomes showed polymorphism between parents as well as between the resistant parent and susceptible bulks. These polymorphisms from multiple chromosomes could be attributed to the presence of paralogous loci as a consequence the hexaploid nature of common wheat (Wang et al. 2014). BSA in the F_2 population derived from LMPG-6/PI 184221 detected four potential locations for the TTKSK resistance. Using the $F_{2:3}$ families, BSA detected only two potential locations on chromosome 5BL and 6DS. Based on the susceptibility of PI 184221 to *P. graminis* f. sp. *tritici* races TPMKC, MCCFC, TTTTF, TKTTF, and TRTTF and resistance to QCCSM, QTHJC, RCRSC, and TTKSK, the TTKSK resistance in PI 184221 may be due to *Sr42*, *SrTmp*, or a new gene. The stem rust resistance gene *SrTmp* originally was derived from the hard red winter wheat 'Triumph 64' (McVey and Hamilton 1985) and it has been postulated to be present in several wheat cultivars from Eastern Europe and in a small group of wheat cultivars from the United States (Jin and Singh 2006; McVey and Hamilton 1985). Recently, a gene for TTKSK resistance from two wheat cultivars, Blouk and Ripper, was mapped to chromosome 6DS at a similar location compared with that of *Sr42* and postulated to be *SrTmp* (Lopez-Versa et al. 2014). Based on the IT, race specificity, and marker results, the TTKSK resistance in PI 184221 can be attributed to *SrTmp* or *Sr42*, similar to Blouk and Ripper. Because the TTKSK-resistant landraces were susceptible to TRTTF and several domestic races, resistance from these landraces should be combined with genes effective against TRTTF and domestic races. In addition, landrace accessions may contain deleterious traits compared with most modern wheat germplasm, and some degree of prebreeding via backcrossing to adapted germplasm may be necessary before the resistance genes from these accessions could be deployed in a breeding program. In this study, seedling response to *P. graminis* f. sp. *tritici* race TTKSK, molecular markers linked to specific *Sr* genes, population segregation ratios, and BSA results using genome-wide SNPs were used to identify three wheat landraces with higher priority for follow-up studies. Resistance in PI 625696 and PI 184221 may be controlled by either *Sr42* or *SrTmp* or a novel gene whereas, in PI 625661, the TTKSK resistance may be due to either *Sr14* or a novel gene. Similar utilization of the 90K iSelect SNP genotyping assay for BSA to rapidly prioritize germplasm may be applicable to studies of diverse traits in wheat.

Table 5. Single-nucleotide polymorphism (SNP) markers associated with *Puccinia graminis* f. sp. *tritici* race TTKSK resistance in PI 625696 and PI 184221 as detected by bulked segregant analysis of $F_{2:3}$ families

Accession, SNP ID[a]	Chromosome	Arm	Distance (cM)[b]
PI 625696			
IWB55116	6D	S	14.347
IWB20798	6D	S	16.243
IWB28838	6D	S	18.996
IWB262	6D	S	21.827
IWA984	6D	S	22.925
IWB61233	6D	S	22.925
IWB59282	6D	S	22.925
IWB47567	6D	S	22.925
IWB6902.2	6D	S	23.841
IWB48184	6D	S	24.766
PI 184221			
IWB7108	6D	.	9.471
IWB10804	6D	.	9.471
IWB6072.2	6D	S	23.841
IWB11787	6D	S	24.766
IWB3275	5B	L	143.548

[a] Markers in bold also detected by bulk segregant analysis of the F_2 population.

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

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