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Haplotype diversity of stem rust resistance loci in uncharacterized wheat lines

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Genotyping of U.S. Wheat Germplasm for Presence of Stem Rust Resistance Genes *Sr24*, *Sr36* and *Sr1RS*^{Amigo}

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ABSTRACT

The stem rust resistance genes *Sr24*, *Sr26*, *Sr36*, and *Sr1RS*^{Amigo} confer resistance to race TTKSK (= Ug99) of *Puccinia graminis* f. sp. *tritici* Pers. (*Pgt*). A collection of 776 cultivars and breeding lines of wheat (*Triticum aestivum* L.) from all growing regions of the United States were screened with simple sequence repeat and sequence tagged site markers linked to *Sr24*, *Sr26*, *Sr36*, and *Sr1RS*^{Amigo} to determine frequencies of these genes in U.S. wheat germplasm. Marker efficacy in predicting the presence of these genes was evaluated via comparison with assayed seedling infection type. Among the lines evaluated, the most predominant gene is *Sr24*, present in hard winter, hard spring, and soft winter wheat lines. Resistance in soft winter wheat is primarily due to *Sr36*. The 1RS·1AL rye translocation carrying *Sr1RS*^{Amigo} is present at equal frequencies in hard winter and soft winter wheat. Utilization of marker-assisted selection for stem rust resistance genes can hasten the development of wheat cultivars resistant to TTKSK and its variants and allow for the development of resistance gene pyramids for more durable stem rust resistance.

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Abbreviations: IT, infection type; PCR, polymerase chain reaction; *Pgt*, *Puccinia graminis* f. sp. *tritici*; SSR, simple sequence repeat; STS, sequence tagged site.

THE STEM RUST FUNGUS *Puccinia graminis* f. sp. *tritici* Pers. (*Pgt*) has historically caused dramatic yield losses in cultivated wheat (*Triticum aestivum* L.). Stem rust posed a major threat to wheat production in the United States in the early 20th century, with major epidemics occurring between 1900 and the 1950s (Kolmer et al., 2007). Efforts to remove the alternate host, common barberry (*Berberis vulgaris* L.), and the development of lines possessing genes for resistance led to a decrease in the number and magnitude of stem rust epiphytotics (Kolmer et al., 2007). The diversity of *Pgt* was also greatly reduced during this time, as evidenced by the limited number of races found in recent surveys in the United States (Jin, 2005). Without stem rust as a limiting

Table 1. Number of U.S. wheat lines from different regions and market classes having stem rust resistance genes *Sr24*, *Sr36*, *Sr1R^Amigo* and *Sr31* identified with molecular markers. Resistance gene *Sr26* was not detected in any of the lines tested.

Growing region	Market class	Gene				No. of lines
		Sr24	*Sr36*	*Sr1R^Amigo*	*Sr31*	
Eastern	Soft Winter	20	65	28	57	290
Great Plains	Hard Spring	14	1	0	8	119
	Hard Winter	48	3	29	25	194
Western	Soft Spring	0	0	0	0	20
	Soft Winter	0	0	0	3	40
	Hard Spring	0	0	0	5	83
	Hard Winter	0	0	0	0	30
Totals		82	69	57	101	776

factor in production, there has been less need for breeding programs to focus on incorporating a diversity of stem rust resistance genes into new cultivars.

Worldwide, most wheat germplasm remained resistant to contemporary races of stem rust over the same time period of stem rust quiescence in the United States. A widely distributed source of stem rust resistance is the gene *Sr31*, present on the translocation 1RS·1BL, a chromosome having the short arm of chromosome 1R of Petkus rye (*Secale cereale* L.) translocated onto the long arm of wheat chromosome 1B (Schlegel and Korzun, 1997). This source of resistance was disseminated worldwide through CIMMYT germplasm, and has remained effective for >30 yr. In 1999, however, a novel stem rust race, reported as Ug99, was identified in Uganda with virulence on *Sr31* (Pretorius et al., 2000). This race was later designated as TTKS (Wanyera et al., 2006), based on the North American race nomenclature system (Roelfs and Martens, 1988). This new race combined *Sr31* virulence with virulence to the majority of *T. aestivum*–derived stem rust resistance genes. Since its identification, new variants with additional virulence, most notably on *Sr24* (Jin et al., 2008a) and *Sr36* (Jin et al., 2008b), have been identified in Kenya. TTKS is now divided into three races: TTKSK (original Ug99 race avirulent on *Sr24* and *Sr36*), TTKST (with virulence on *Sr24*; Jin et al., 2008b), and TTTSK (with virulence on *Sr36*; Jin et al., 2008a). The expanded virulence profiles of these races has increased the genetic vulnerability of wheat, and the potential global dispersal of this uniquely virulent group makes the development of resistant cultivars quite urgent (Singh et al., 2006).

Field evaluations in Kenya and greenhouse evaluations at the USDA Cereal Disease Lab have led to the characterization of the following *Sr* genes effective against the original TTKSK race: *Sr2* (partial, adult plant), *Sr13, 22, 24, 25, 26, 27, 28, 32, 33, 35, 36, 37, 39, 40, 44, Sr Tmp, Tt-3,* and *Sr1RS^Amigo* (Jin et al., 2007). Of these effective resistance genes, *Sr24, Sr26, Sr36,* and *Sr1RS^Amigo* have been deployed in wheat cultivars in some countries. Molecular markers linked to TTKSK resistance genes are available for these four genes,

each of which was transferred from wheat relatives possessing nonhomologous or partially homologous genomes (Mago et al., 2005; Tsilo et al., 2008; Saal and Wricke, 1999). The ability to detect the presence of specific stem rust resistance genes using molecular markers presents a viable means of identifying TTKSK resistance in the absence of the pathogen itself. The objective of this study was to determine the frequency of DNA markers associated with resistance genes *Sr24, Sr26, Sr36,* and *Sr1RS^Amigo* in wheat cultivars and breeding lines from breeding programs throughout the United States and to assess the reliability of these markers in predicting the presence of the resistance genes among such diverse germplasm.

MATERIALS AND METHODS
Plant Materials

Elite cultivars and breeding lines representing all market classes of wheat grown in the United States were evaluated for the presence of resistance genes *Sr24, Sr26, Sr36,* and *Sr1RS^Amigo* (Table 1). Seeds of advanced lines and check cultivars from the 2006 and 2007 Uniform Eastern and Southern Soft Winter Wheat Nurseries were obtained from Dr. Harold Bockelman, USDA-ARS, Aberdeen, ID, as representative germplasm from the eastern winter wheat growing region of the United States. Seeds of entries from the 2006 Southern and Northern Regional Performance Nurseries were obtained from Dr. Robert Graybosch, USDA-ARS, Lincoln, NB, as representative of hard winter wheat lines from the Great Plains. Seed of entries from the 2006 Western Regional Soft Winter Wheat, Hard Winter Wheat, Soft Spring Wheat, and Soft White Wheat Nurseries were obtained from Dr. Kim Garland Campbell, USDA-ARS, Pullman, WA, as representative of lines adapted to the western region of the United States. Additional winter wheat lines were obtained directly from breeding programs and from collaborative nurseries, including the 2007 Gulf Atlantic Wheat Nursery and the Mason-Dixon Wheat Nursery. Seeds of hard spring wheat cultivars and breeding lines adapted to California and to the Northern Plains were also obtained directly from breeding programs. The following cultivars with known resistance genes were included as controls: 'NC-Neuse' (*Sr36*; PI633037) (Murphy et al., 2004), 'McCormick' (PI632691; *Sr24* and *Sr1RS^Amigo*) (Griffey et al., 2005), 'AGS 2000' (*Sr31*; PI692596) (Johnson et al., 2002), and 'Eagle' (PI365582; *Sr26*) (Martin 1971). The origin of the translocation chromosome segments including these five genes is summarized in Table 2.

Marker Analyses

Genomic DNA was extracted from fresh tissue from up to five seedlings of each line harvested into 96 well plates and stored at −80°C. Frozen tissue was macerated using steel beads with a GenoGrinder 2000 (SPEX CertiPrep, Metuchen, NJ), and extractions were performed using a QIAGEN DNeasy 96 Plant kit (QIAGEN, Valencia, CA), according to the manufacturer's instructions.

Four simple sequence repeat (SSR) loci were evaluated for efficacy in detecting the *Sr36* gene: *Xgwm319, Xwmc477, Xgwm271,* and *Xgwm501* (Tsilo et al., 2008; Lehmensiek et al., 2004; Bariana et al., 2001). The *Lophopyrum elongatum*–derived segment carrying *Sr24* was detected using the SSR marker for the *Xbarc71* locus and the sequence tagged site (STS) marker *Sr24#12* (Mago et al.,

Table 2. Species of origin, chromosomal location, translocation, diagnostic markers and expected size of amplified fragments for selection of *Sr24*, *Sr36*, *Sr1R^Amigo^*, and *Sr26*.

Gene	Origin	Locus	Fragment sizes	Chromosome	Translocation
			bp		
Sr24	Lophopyrum elongatum	Xbarc71	83, 88, 101	3EL	3DS·3DL-3EL
Sr26	Lophopyrum elongatum	Sr26#43	207	6EL	6AS·6AL-6EL
Sr31	Secale cereale	Xscm9	208	1RS	1RS·1BL
Sr36	Triticum timopheevi	Xwmc477	187	2GS	2BS·2GS-2BS·2BL
*Sr1R^Amigo^*	Secale cereale	Xscm9	224	1RS	1RS·1AL

2005). The STS marker *Sr26#43* was used for detection of resistance gene *Sr26* (Mago et al., 2005). The presence of the short arm of rye chromosome 1 (1RS) was assayed with the rye-specific SSR for the *Xscm9* locus (Saal and Wricke, 1999).

The polymerase chain reaction (PCR) master mix for STS and SSR primers consisted of 2 μL of 20 ng μL^{-1} genomic DNA template, 0.40 μL of a 10 μM mixture of forward and reverse primers, 0.18 μL (0.9 U) of *Taq* polymerase, 1.20 μL of 10× buffer (10 mM Tris-HCL, 50 mM KCl, 1.5 mM MgCl2, pH 8.3), 0.96 μL of a 100 μM mixture of dNTPs, and 7.26 μL of water, bringing the total reaction volume to 12 μL. A touchdown profile was used that consisted of an initial denaturation at 95°C followed by 15 cycles of 95°C (45 s), 65°C (45 s) decreasing by 1°C each cycle, and 72°C (60 s), followed by 25 cycles of 50°C annealing temperature. The cycling conditions for the SSR marker for locus *Xwmc477* included an initial denaturation of 95°C followed by 35 cycles of 95°C (45 s), 61°C (45 s), and 72°C (60 s), followed by a final extension at 72°C (4 min). Cycling conditions for the STS markers involved an initial step of 94°C for 3 min followed by 30 cycles of 94°C (30 s), 56°C (30 s), and 72°C (40 s).

The forward primers for all SSR markers were 5'-modified to include the fluorescent dye 6-FAM. Amplifications were performed using an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). Sizing of PCR products was performed by capillary electrophoresis using an ABI3130xl Genetic Analyzer (Applied BioSystems, Foster City, CA). Analysis of PCR fragments was performed using GeneMarker 1.60 software (SoftGenetics, State College, PA). The STS markers were resolved in 2.0% agarose gels. To produce figures, amplified fragments were resolved in 2.0% agarose gels, stained with ethidium bromide, and photographed.

Phenotypic Analysis

Results of the above marker analyses were compared with phenotypic evaluations of these same lines using data from the USDA Cooperative Regional testing program and made available through the USDA–ARS Cereal Disease Laboratory (http://www.ars.usda.gov/Main/docs.htm?docid=9987 [verified 27 Nov. 2009]). Disease evaluations were conducted in St. Paul, MN, during the winters of 2005 and 2006. Races TTKSK and TTKST were used for evaluating wheat lines following inoculation and disease assessment procedures described previously (Jin et al., 2007). Microsoft Office Excel 2003 (Microsoft Corp., Redmond, WA) spreadsheets containing seedling stem rust reactions and/or results of marker analyses for all cultivars and breeding lines can be found in Supplementary Tables S1 and S2.

RESULTS
Amplification Products for the Different Markers

Control lines amplified the appropriate size fragment for each of the markers assayed. The STS marker *Sr24#12* (Mago et al., 2005) was used to detect the *L. elongatum*–derived segment containing *Sr24* and amplified a 500-bp fragment in the control McCormick (Fig. 1). The primers for the SSR locus *Xbarc71* amplified fragments of 83, 88, 101, and 105 bp in lines possessing this segment (Fig. 2). Four SSR markers reportedly linked to resistance gene *Sr36* (*Xgwm271, Xgwm319, Xwmc477,* and *Xgwm501*) were assayed and amplified fragment lengths of 171, 168, 187, and 107 bp, respectively, from the resistant check NC–Neuse.

The rye-specific SSR marker *Xscm9* acts as dominant marker to detect the presence of the short arm of rye chromosome 1 (1RS) in wheat. This marker amplified a 208-bp fragment in lines with the 1RS·1BL translocated chromosome and resistance gene *Sr31,* and a 224-bp fragment in lines with the 1RS·1AL translocation and its associated resistance gene *Sr1RS^Amigo^* (Fig. 1).

A subset of 250 of the 776 lines was evaluated with the STS marker *Sr26#43*. Of these, which included cultivars of all market classes, none amplified the diagnostic 207-bp fragment that is present in the Australian control cultivar Eagle (*Sr26*). No further evaluation of lines for the presence of *Sr26* was done.
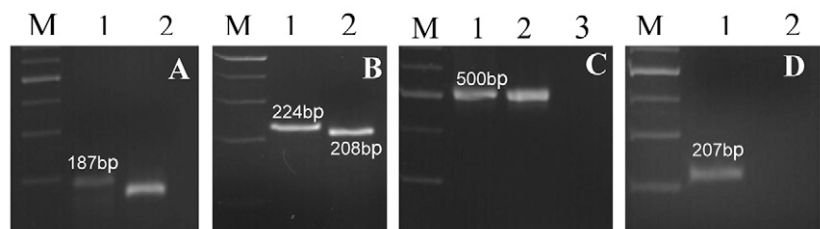

Figure 1. Polymerase chain reaction (PCR) amplification of markers for selection of *Sr36,* and *Sr26*. (A) Fragments amplified by *Xwmc477* for the detection of *Sr36*; 1: 'NC-Neuse' (*Sr36*); 2: 'Yecoro Rojo'. (B) Fragments amplified by *Xscm9* for the detection of 1: *Sr1RAmigo,* 'TAM107' (1RS·1AL translocation); and 2: *Sr31,* 'AGS2000' (1RS·1BL translocation). (C) Fragments amplified by *Sr24#12* for the detection of *Sr24*; 1: 'McCormick' (*Sr24*); 2: 'MN031604' (*Sr24*); 3: 'Lakin'. (D) Fragments amplified by *Sr26#43* for the detection of *Sr26*; 1: 'Eagle' (*Sr26*); 2: 'Millennium'. M = molecular size standard. *Sr24#12* and *Sr26#43* are dominant markers and yield no PCR product in lines without *Sr24* and *Sr26*.
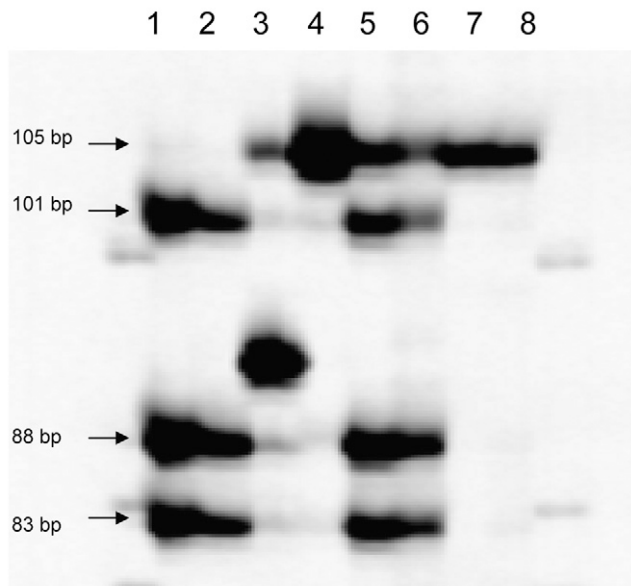
Figure 2. Polymerase chain reaction amplification of BARC71 for the identification of *Sr24* and differentiation between the 3DL/3*Ae* translocation derived from 'Agent' and the 1BL·1BS-3*Ae* translocation derived from 'Amigo' of *Sr24.* Lane 1 'Ernest', *Sr24*+ (3DL); 2 'Keene', *Sr24*+ (3DL); 3 'Amidon', *Sr24*–; 4 'Choteau' *Sr24*–; 5 'McCormick', *Sr24*+ (1BS); 6 'Millennium', *Sr24*+ (1BS); 7 'Roane', *Sr24*–; 8 'Lakin', *Sr24*–.

Phenotypic Data and Selection of the Best Predictive Markers

Phenotypic data of seedling reactions to the TTKS races were available for 366 of the 776 lines evaluated with markers. Of these, 116 lines were predicted by marker analyses to possess *Sr24, Sr36,* or *Sr1RS^{Amigo}*, either singly or in combinations. Overall, a high degree of correspondence was observed between the marker data and seedling infection types. Of the 366 lines for which both genotypic and phenotypic data were available, only 11 lines that lacked marker alleles associated with *Sr24, Sr36,* or *Sr1RS^{Amigo}* exhibited resistant phenotypes, a result potentially due to the presence of other effective resistance genes (Table 3).

Table 3. Lines resistant to TTKSK/T without marker alleles for *Sr24*, *Sr36*, or *Sr1R^{Amigo}*.

Line	Growing region	Postulated gene(s)	TTKSK	TTKST
AGS 2020	Eastern	†	2	–
GA991371–6E12	Eastern	†	2+	2+
GA001435–6E23	Eastern	†	2+	2+
GA011636–6E22	Eastern	†	2	2+
GA991209–6E33	Eastern	†	S/2+‡	2++
GA98401–5E45	Eastern	†	S/2+‡	23
BZ9W02–2051	Great Plains	(SrTmp)	2	2
CO03W269	Great Plains	(SrTmp)	2+	2
Millennium27, ALS1	Great Plains	(SrTmp)	2+	2+
MT0495	Great Plains	(SrTmp)	2+	2++
IDO655	Western	(SrTmp)	2+	2+

† indicates unknown source of resistance.

‡"/" indicates heterogeneity in IT, predominant type given first.

In total, only six lines predicted to have one of the resistance genes had susceptible phenotypes.

Sr24

Of the two markers evaluated for the *Sr24* translocation segment, *Xbarc71* proved to be more accurate. Although the STS marker *Sr24#*12 was generally predictive of the presence of *Sr24,* it also generated false positives. Multiple lines yielding a faint 500-bp fragment (expected for *Sr24#*12) were susceptible to TTKSK and lacked the alleles associated with *Sr24* at *Xbarc71.* A higher level of correspondence between the genotypic and phenotypic data was observed for the marker *Xbarc71,* with all 38 lines having the 83-, 88-, and 101-bp *Xbarc71* fragments exhibiting a resistant infection type (IT) when inoculated with race TTKSK. When inoculated with *Pgt* race TTKST (*Sr24* virulence), lines possessing *Sr24* singly were susceptible (IT 3). However, 10 lines possessing *Sr24* in combination with effective resistance gene *Sr1RS^{Amigo}* were resistant (ITs 2, 2+) to TTKST (Table 4).

Mago et al. (2005) reported that the marker for the *Xbarc71* locus is codominant and maps to the distal region of the long arm of chromosome 3D. However, *Sr24* was also introgressed into a number of U.S. wheat lines through the 1BS–*Lophopyrum* translocation present in 'Amigo' (Jiang et al., 1994; The et al., 1992). The marker for the *Xbarc71* locus is dominant in lines with this non-compensating translocation, amplifying fragments from both the wheat *Xbarc71* locus on 3DL and the *L. elongatum*–derived locus on 1BS. Mago et al. (2005) observed the 83-, 88-, and 101-bp fragments amplified from the *L. elongatum*–derived segment on 1BS (Fig. 2) and observed various wheat alleles at the 3DL locus, including one with a 107-bp fragment. Of the 82 lines identified to carry *Sr24* in our study, 42 amplified only the fragments from the *L. elongatum*-derived segment, indicating the presence of the 3DL translocation. Forty lines amplified fragments from both wheat and *L. elongatum*, indicating that they are heterogeneous or heterozygous for the 3DL translocation or possess the Amigo type 1BS translocation.

Sr1RS^{Amigo}

Data of seedling reactions of both TTKS races was available for 33 lines predicted by marker analyses to have the 1RS·1AL translocation from Amigo and, therefore, the *Sr1RS^{Amigo}* resistance gene either singly or in combination with *Sr24* or *Sr36*. Of these 33 lines, three (VA02W-713, CO02W280, and KY00C269703) were classified as susceptible to TTKSK. Data were available for comparison of the marker results with previous evaluation for the presence of the 1RS·1AL translocation for lines VA02W-713 and CO02W280 (the 1RS Compendium; http://www.ars.usda.gov/Research/docs.htm?docid=11932 [verified 27 Nov. 2009]). Line CO02W280 was previously reported

to the positive for the 1RS·1AL translocation when evaluated with marker *Xscm09*. However, neither line was determined to carry the translocation when the secalin profiles were examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. This suggests that marker *Xscm09* may amplify from nontranslocation lines in some instances. Alternatively, the breeding lines may be a mixture of translocation and nontranslocation types.

Sr36

The four SSR loci *Xwmc477, Xgwm319, Xgwm271,* and *Xgwm501* located near the centromere of chromosome arm 2BL, and reported to be linked to resistance gene *Sr36* (Lehmensiek et al., 2004; Tsilo et al., 2008), were not equally predictive of stem rust resistance in the lines tested, a result likely due to recombination between the SSR loci and *Sr36* in some lines. Tsilo et al. (2008) reported that the 187-bp fragment amplified from locus *Xwmc477* was tightly linked to *Sr36* and was a good predictor for the presence of this gene in diverse germplasm. In this study, low infection types characteristic of *Sr36* to race TTKSK (IT 0 or ;) were observed on 54 of 57 lines carrying the *Xwmc477* 187-bp allele after inoculation with *Pgt* race TTKSK. The remaining three lines were susceptible to TTKSK, either due to recombination between *Xwmc477* and *Sr36* or to heterogeneity of the seed sources.

The 168-bp fragment amplified by *Xgwm319* in the *Sr36* control NC–Neuse was found to be less reliable as a predictor of *Sr36,* being present in only 46 of the 54 lines carrying *Sr36*. Even weaker linkage with *Sr36* was observed for *Xgwm501* and *Xgwm271*. The 108-bp *Xgwm501* allele linked to *Sr36* was detected in only 33 of the 54 *Sr36* lines, whereas the 171-bp *Xgwm271* allele was present in only 21 of the 54 lines. All four markers linked to *Sr36* were found in only 16 lines. Phenotypic data were available for 12 of these 16 lines, all of which exhibited the *Sr36* phenotype. Overall, recombination between markers *Xgwm319, Xgwm271,* and/or *Xgwm501* was identified in 41 of the 54 lines having both the *Sr36* phenotype and the 187-bp *Xwmc477* allele.

Distribution of Resistance Genes among Market Classes and Geographical Regions

Approximately 24% of all 776 lines assayed with markers in this study were predicted to possess *Sr24, Sr36,* or *Sr1RS^Amigo* (Table 1). Based on marker analyses, the most prevalent gene in U.S. germplasm is *Sr24* (found in approximately 11% of all lines), followed by *Sr36* and *Sr1RS^Amigo* (present in 9 and 7% of all lines, respectively). Soft winter germplasm from the eastern growing region exhibits the highest diversity of stem rust resistance genes, with *Sr24, Sr36,* and *Sr1RS^Amigo* each present at frequencies ranging from 8 to 23%. Forty percent of all evaluated hard winter lines from the Great Plains possess resistance to TTKS races based largely on *Sr24* and *Sr1RS^Amigo*. The resistance among

Table 4. Effective *Sr1R^Amigo* resistance in the presence of *Sr24* virulence. A '+' indicates the *Xbarc71* (*Sr24*) genotype or the *Xscm9* (*Sr1R^Amigo*) genotype.

Line	Class	Sr24	Sr1R^Amigo	TTKSK	TTKST
Arapahoe	HRW	+	–	2[†]	S[‡]
Trego	HRW	+	–	2	S
Nuplains	HRW	+	–	2	S
TX03A0563	HRW	+	–	2	S
AR9800151	SRW	+	–	2	S
McCormick	SRW	+	+	2	2
VA05W-168	SRW	+	+	2	2
IL01–11934	SRW	+	+	2-	2
MD01W233068	SRW	+	+	2-	2
TX99A01531	HRW	+	+	2	2+

[†]An IT of 2 or 2+ indicates a resistant phenotype.
[‡]S indicates a susceptible phenotype.

hard spring germplasm of the Northern Great Plains is also primarily from *Sr24,* with *Sr36* found in much lower frequencies compared with other regions (Table 1).

Sr24

The *Xbarc71* allele associated to *Sr24* is more frequent among the lines from the Great Plains (25% of the winter lines and 12% of the spring lines), followed by the soft winter lines (7%). No entries evaluated from the Western region in this study tested positive for the *Sr24* markers.

Sr36

The marker analyses indicate that *Sr36* is widespread in soft winter wheat germplasm (Table 1), with 22% of the tested soft winter lines found to possess the 187-bp fragment at the *Xwmc477* locus. In contrast, only three hard winter lines and one hard spring line from the Great Plains were determined by *Xwmc477* to have *Sr36*. No entries in the nurseries from the Western region were positive for the *Sr36* markers.

Sr1RS^Amigo

Marker analyses indicate that *Sr1RS^Amigo* is present in approximately 15% of hard winter lines from the Great Plains and 10% of soft winter lines from the eastern growing region (Table 1). This translocation was not detected in any U.S. spring wheat germplasm or any winter wheat lines from the western region. Amplification of a 224-bp fragment by the rye-specific SSR marker *Xscm9* indicates the presence of the 1RS·1AL translocation from Amigo, carrying the *Sr1RS^Amigo* resistance gene. Because a 208-bp fragment may also be amplified by *Xscm9,* indicating the presence of the 1RS·1BL translocation carrying *Sr31,* we were able to assess the frequency of this gene in the germplasm tested. The 1RS·1BL translocation was common in U.S. winter wheat germplasm, particularly in eastern soft lines (20%) and hard wheat of the Great Plains (13%). These data were consistent with that reported in the 1RS Compendium (http://www.ars.usda.gov/Research/docs.htm?docid=11932 [verified 27

Table 5. Stem rust resistance genes present in combinations. A '+' indicates possession of the marker alleles associated with the given *Sr* gene.

Line	Class	*Sr24*	*Sr1R^{Amigo}*	*Sr36*	TTKSK	TTKST
VA05W-65	SRW	+	+	+	2/0‡	2/0‡
SD00W024	HRW	+	+	+	NA†	NA
VA05W-376	SRW	+	+	–	S/2	S/2
TRIBUTE	SRW	+	+	–	2	2+
TX99A01531	HRW	+	+	–	2	2+
McCormick	SRW	+	+	–	2	2
MD01W233–06–1	SRW	+	+	–	2	2
VA05W-168	SRW	+	+	–	2	2
T157	SRW	+	+	–	2+/S	2+/S
IL01–11934	SRW	+	+	–	2-	2
MD01W233068	SRW	+	+	–	2-	2
OK102	HRW	+	+	–	2-	2
KS980386–6-3-#1	HRW	+	+	–	NA	NA
KS970274–14-*9	HRW	+	+	–	NA	NA
NuHills	HRW	+	+	–	NA	NA
TN604	SRW	+	–	+	0/S	NA
VA05W-313	SRW	–	+	+	0;/2	0

†"NA" indicates that phenotypic data is unavailable

‡Two scores separated by "/" indicates heterogeneity in IT, predominant type given first.

Nov. 2009]). The translocation was also present in three hard spring wheat lines from the western region.

Lines carrying multiple stem rust resistance genes were identified (Table 5). The combination of *Sr24* and *Sr1RS^{Amigo}* was the most frequent, being present in 13 lines. Marker analyses indicate that *Sr24* and *Sr36* are present together in two lines. One experimental line was found to have the *Sr36* and *Sr1RS^{Amigo}* combination, and two others were found to have *Sr24, Sr36,* and *Sr1RS^{Amigo}* (VA05W-65 and SD00W024). Phenotypically, VA05W-65 was heterogeneous for infection types, exhibiting both IT 0 (indicative of *Sr36*) and IT 2 (indicative of *Sr24* and *Sr1RS^{Amigo}* without *Sr36*) when inoculated with TTKSK. Phenotypic data were not available for SD00W024.

DISCUSSION

The year-round cultivation of susceptible wheat varieties under conditions conducive for disease development can hasten the spread of TTKSK and its variants. After emerging from the East African countries, the range of TTKS-derived races of *Pgt* has since expanded north to Sudan, crossed over to the Arabian peninsula, and pushed as far east as Iran (Nazari et al., 2009), following a similar path as the *Yr9*-virulent race of stripe rust (*Puccinia striiformis* Westend f. sp. *tritici*) that originated in the East African highlands and migrated across the Middle East through West Asia to East Asia (Singh et al., 2007). The majority of current cultivars grown in the migration path are susceptible to TTKSK (Singh et al., 2006).

This study demonstrates that molecular markers can be used to aid in the effort of germplasm characterization by identifying wheat lines possessing the *Sr24, Sr26, Sr36,* and *Sr1RS^{Amigo}* resistance genes effective against *Pgt* race TTKSK. The ability to screen for the presence of such genes in the absence of the pathogen greatly facilitates the timely identification of lines carrying effective resistance, and facilitates its deployment in breeding programs. Molecular markers are particularly useful for identifying lines with multiple genes and for pyramiding multiple resistance genes, which is difficult and sometimes impossible to do using only phenotypic data. In this study, we identified 17 lines in which TTKSK resistance was due to the presence of multiple genes.

Stem rust resistance genes *Sr24, Sr26,* and *Sr1RS^{Amigo}* are present within translocated segments that do not readily recombine with homeologous regions of wheat. Thus the markers detecting the translocations cosegregate with resistance and are considered predictive. By comparing genotypic and phenotypic data for 366 U.S. wheat lines in this report, we determined that the markers for both *Sr24* and *Sr1RS^{Amigo}* predict with high confidence the presence of these resistance genes. The few anomalies present in our screening were found only in experimental lines yet to be released as cultivars, and it is possible that heterogeneity in the seed sources may underlie these discrepancies. Since the 2G chromosome of *T. timopheevii* Zhuk. pairs, albeit poorly, with chromosome 2B (Dvorak and Zhang 1990), the identification of several historical recombination events among the molecular markers in the *Sr36* region is not an unexpected result.

Resistance gene *Sr36* was introgressed into wheat from the tetraploid species *T. timopheevii*, having the A^t and G genomes that are closely related to the A and B genomes, respectively, of *T. aestivum*. The *Sr36* gene and the linked powdery mildew resistance gene *Pm6* were transferred to wheat chromosome 2B (Allard and Shands, 1954) through homologous recombination with chromosome 2G (Gill and Chen, 1987). In this study, marker loci *Xgwm319, Xgwm271,* and *Xgwm501* showed more recombination with *Sr36* than the *Xwmc477* locus, which is therefore the best predictor for *Sr36* (Tsilo et al., 2008). The *Sr36* gene was introduced into U.S. wheat germplasm during the 1950s and, therefore, had sufficient time to go through several generations of intercrossing. The close association between *Xwmc477* and *Sr36* after all these years suggests that these two loci are closely linked.

The results of our marker analyses are largely consistent with the phenotypic evaluations of U.S. wheat cultivars by Jin and Singh (2006) that also determined the most prevalent gene in U.S. cultivars is *Sr24* and observed similar frequencies of the resistance genes in the different market classes. Although selection for stem rust resistance has received less emphasis over recent decades, *Sr24* has likely been maintained due to its complete association with the leaf rust resistance gene *Lr24*.

The 1RS·1AL translocation identified in both hard and soft winter lines has likely been selected for other desirable traits as well, such as the effective powdery mildew resistance conferred by gene *Pm17* (Parks et al., 2008).

Similarly, prevalence of *Sr36* in eastern U.S. germplasm could be due in part to linkage with the resistance gene *Pm6*, though these genes are not completely linked and *Pm6* no longer provides effective resistance in the eastern United States (Niewoehner and Leath, 1998). A number of authors have reported preferential transmission of the *T. timopheevii* 2B chromosome segment carrying *Sr36*, which may have contributed to the maintenance of this gene (Nyquist, 1962; Tsilo et al., 2008).

The *L. elongatum*–derived gene *Sr26* was not present in any of the 250 lines tested, indicating an absence of this gene in U.S. wheat germplasm. This might be related to the reported yield penalty associated with the long *L. elongatum* segment introgressed with this gene (The et al., 1988).

In this germplasm collection, seven soft winter and four hard winter lines exhibited resistance to both TTKS races in the absence of *Sr24, Sr26, Sr36,* or *Sr1RS^{Amigo}* marker genotypes (Table 3). Pedigree information, along with reaction type data, suggests the presence of known resistance gene *SrTmp* in five of the lines, a result which underscores the fact that resistance among U.S. wheat germplasm is based on a limited number of genes. Of six soft red winter wheat lines from the breeding program at the University of Georgia resistant to TTKSK at the seedling stage, only one (GA991371-6E12) exhibited a high level of resistance under conditions of natural infection in the field in Kenya (data not shown). This result suggests that GA991371-6E12 has a resistance gene effective against TTKS different from the ones tested in this study. Further examination of this line could prove worthwhile in the search for novel genes effective against highly virulent strains of stem rust.

Given the identification of *Pgt* races of the TTKS lineage having virulence to *Sr24* and *Sr36* (Jin et al., 2008a, 2008b), incorporating more diverse genes for stem rust resistance into U.S. wheat cultivars is a necessity. Adultplant resistance gene *Sr2* and associated minor genes continue to provide partial resistance to infection from the TTKS lineage. However, their frequency in current U.S. wheat cultivars and breeding lines remains unknown and diagnostic markers for these durable genes are needed. Many of the other known stem rust resistance genes effective against the TTKS races are contained within large introgressions from wild wheat relatives and are associated with linkage drag which has prevented their deployment in modern agriculture (Singh et al., 2008). Reducing the linkage drag associated with these major resistance genes and identification of effective markers for both major and minor genes should allow for the incorporation of new pyramids of effective genes in U.S. wheat cultivars.

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