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ORIGINAL PAPER

Resistance to recombinant stem rust race TPPKC in hard red spring wheat

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Abstract The wheat (*Triticum aestivum* L.) stem rust (*Puccinia graminis* Pers.:Pers. f.sp. *tritici* Eriks. and Henn.) resistance gene *SrWld1* conditions resistance to all North American stem rust races and is an important gene in hard red spring (HRS) wheat cultivars. A sexually recombined race having virulence to *SrWld1* was isolated in the 1980s. Our objective was to determine the genetics of resistance to the race. The recombinant race was tested with the set of stem rust differentials and with a set of 36 HRS and 6 durum cultivars. Chromosomal location studies in cultivars Len, Coteau, and Stoa were completed using aneuploid analysis, molecular markers, and allelism tests. Stem rust differential tests coded the race as TPPKC, indicating it differed from TPMKC by having added virulence on *Sr30* as well as *SrWld1*. Genes effective against TPPKC were *Sr6*, *Sr9a*, *Sr9b*, *Sr13*, *Sr24*, *Sr31*, and *Sr38*. Genetic studies of resistance to TPPKC indicated that Len, Coteau, and Stoa likely carried *Sr9b*, that Coteau and Stoa carried *Sr6*, and Stoa carried *Sr24*. Tests of HRS and durum cultivars indicated that five HRS and one durum cultivar were susceptible to TPPKC. Susceptible HRS cultivars were postulated to have *SrWld1* as their major stem rust resistance gene. Divide, the susceptible durum cultivar, was postulated to lack *Sr13*. We concluded that although TPPKC does not constitute a threat similar to TTKSK and its variants, some cultivars would be lost from production if TPPKC became established in the field.

Abbreviations

HRS	Hard red spring
IT	Infection type
PCR	Polymerase chain reaction

Introduction

Stem rust (*Puccinia graminis* Pers.:Pers. f.sp. *tritici* Eriks. and Henn., *Pgt*) has re-emerged as a serious threat to wheat (*Triticum aestivum* L.) production worldwide since the identification of *Pgt*-TTKSK (commonly known as Ug99) in Africa. Since the 1950s, the emergence of new highly virulent *Pgt* races has become uncommon in North America due to deployment of stem rust resistance genes in wheat and the partial eradication of barberry (*Berberis vulgaris* L.), the alternate host for stem rust. Over the past 10 years, the most notable new race in North America has been *Pgt*-TTTTF (Jin 2005). Despite the fact that TTTTF is virulent on 18 of the 20 stem rust differentials, the exceptions being *Sr24* and *Sr31*, it is avirulent on 93% of North American HRS cultivars (Jin and Singh 2006). Part of this resistance is due to *Sr24* and *Sr31* which occur in Great Plains HRS cultivars at frequencies of 12 and 7%, respectively (Olson et al. 2010). Other genes conferring resistance to TTTTF are unknown (Y Jin, personal communication 2/2/2010), but may involve *SrWld1* (Jin 2005), an important gene for stem rust resistance in North American HRS wheats (Leonard 2001a, b). *SrWld1* was derived from 'Waldron' HRS wheat and conditions

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resistance to all North American field isolates of *Pgt* (McVey and Roelfs 1978).

Miller and Williams (1985) reported recovery of two recombinant races of *Pgt* derived from random crossing and selfing of pycnia. One of these races, originally identified as isolate 81AC-46(2), or simply isolate 46-2 (Miller et al. 1996), was virulent on 'Olaf', 'Alex', and Waldron, all of which were resistant to the predominant field races of that time. However, the recombinant race was avirulent on seedlings of 'Len' and 'Coteau'. Subsequently, Williams and Miller (1989) reported that 'Stoa' was also resistant to this race. The four-letter code for isolate 46-2 was *Pgt*-TPPK.

The numbers of genes that conditioned resistance to isolate 46-2 in Len, Coteau, and Stoa were reported by Williams and Miller (1989) and allelic relationship of these genes were described by Miller et al. (1996). The main conclusions of those studies were Len differed from Waldron by a single gene conditioning infection type (IT) 2 (small to medium pustules with chlorosis) to isolate 46-2. Coteau had three genes for resistance to isolate 46-2, but was heterogeneous for those genes. Some Coteau plants had a single thermosensitive gene conditioning IT 0; (either no uredia or hypersensitive flecks) that was ineffective at high temperatures, and these plants are referred to here as sensitive Coteau (sCoteau). Other Coteau plants produced thermo-insensitive responses and had two resistance genes, one conditioning IT 0; and the second conditioning IT 2. These latter plants are here referred to as insensitive Coteau (iCoteau). Stoa had one gene conditioning IT 0; and two genes conditioning IT 2. Allelism studies indicated that the IT 2 gene from Len was non-allelic to the IT 2 gene in Coteau, but was allelic to one of the IT 2 genes in Stoa. The IT 0; genes of Coteau and Stoa were allelic. Aneuploid analysis located the IT 2 gene from Len to chromosome 2BL and the IT 0; gene from Coteau to chromosome 2D. In addition, we subsequently found that segregation for stem rust resistance in the Stoa crosses showed significant deviations from a three-gene ratio (unpublished), and we concluded that Stoa may carry additional genes for resistance to isolate 46-2.

The data from the studies described were not published. Subsequent studies resulted in clarification in two of the above conclusions, and permitted identification of the genes in those cultivars. Additional molecular genetics work was completed to supplement the studies of Len, Coteau, and Stoa. Isolate 46-2 does not constitute a threat to wheat production similar to TTKSK. Published reports of isolate 46-2 are limited to abstracts (Miller and Williams 1985; Miller et al. 1996; Williams and Miller (1989), and additional reporting of the characteristics of this race would enhance its usefulness for genetic studies. The objectives of this report are (1) to characterize isolate 46-2 by determining its five-letter *Pgt* code, (2) to test the virulence of isolate 46-2 against newer HRS and durum wheat cultivars, and (3) to report the studies conducted in the 1990s on genetics of resistance to isolate 46-2 in Len, Coteau, and Stoa. The latter objective included resolution of the number of genes conditioning resistance to isolate 46-2 in Coteau and Stoa, aneuploid analyses, allelism tests, and additional more recent molecular genetic studies.

Materials and methods

Stem rust inoculation and differential testing

Seedlings of the set of 20 stem rust differentials were planted and inoculated using the techniques of Williams et al. (1992). Briefly, urediniospores were suspended in non-phytotoxic, paraffinic oil and sprayed on 6- to 8-day-old seedlings. The plants remained in a subdued light mist chamber for 24 h following inoculation. Seedlings were then moved to a greenhouse at 20–23°C with supplemental fluorescent light to maintain a 14/10-h (day/night) photoperiod. Seedlings were classified for stem rust IT 12–14 days post-inoculation by scoring the infected primary leaf from each plant (Stakman et al. 1962; Roelfs and Martens 1988). Briefly, in this system of notation, 0;, 1, or 2, were considered resistant, and 3 or 4 were considered susceptible. For leaves exhibiting combinations of IT on single leaves, order indicated predominant types; hence IT 34 was predominantly IT 3, with decreasing amounts of IT 4. Negative (−) and positive (+) indicated small or large pustules within a class; hence, 2^- indicated small IT 2 and 2^+ indicated large IT 2. Finally, addition of the letter c to the notation indicated that the leaf had more chlorosis than normal for that IT.

Plant materials

Lines carrying single genes from iCoteau and Stoa that conditioned resistance to isolate 46-2 were selected for use in genetic studies (Table 1). To select these lines, data from the crosses Waldron/iCoteau and Waldron/Stoa that were made for the inheritance study of Williams and Miller (1989) were reviewed. For the iCoteau IT 0; gene, an F_2-derived family from Waldron/iCoteau that was segregating

Table 1 Pedigrees and infection types of 28 wheat lines postulated to carry single genes for resistance to isolate *Pgt* 81AC-46(2)

Line	Pedigree	IT to isolate 46-2
CoteauA	Waldron/Coteau	0;
CoteauB	Waldron/Coteau	2
StoaA thru StoaD	Waldron/Stoa	0;
StoaE thru StoaZ	Waldron/Stoa	2

in a 3 resistant: 1 susceptible ratio, but lacking plants with IT 2, was selected. Progeny from this family were grown in the greenhouse and selfed. The progeny were screened for reaction to isolate 46-2 and a homozygous IT 0; F_3-derived family was selected and designated as CoteauA. For the IT 2 gene from iCoteau, an F_2-derived family from Waldron/iCoteau that was homozygous IT 2 to isolate 46-2 was selected and designated CoteauB. For the Stoa IT 0; gene, we used the same procedure as used for selection of CoteauA; however, because it was not clear if Stoa carried more than three genes, four F_3-derived families that were homozygous IT 0; were selected and designated StoaA thru StoaD. Because Stoa carried at least two genes conferring IT 2, we selected 22 F_3 families that segregated in 3:1 (IT 2 : IT 34) ratios. The progeny were screened and resistant plants were transplanted to the greenhouse. The $F_{3:4}$ were again screened for resistance to isolate 46-2, and one homozygous F_3-derived family from each of the original 22 F_2-derived families was selected and designated StoaE thru StoaZ.

In addition to the lines produced through crossing and selection, other lines available for screening were the set of 20 stem rust differentials, monogenic lines BtSrWld1 and W2691Sr13 provided by the USDA-ARS, Cereal Disease Laboratory, St. Paul, MN. Waldron derived monogenic lines WDR-A1, WDR-B1, WDR-C2, WDR-D1, WDR-E4, and WDR-F1 (Riede et al. 1995), 6 durum cultivars and 40 HRS wheat cultivars or experimental lines were also used.

Genetic analysis of putative single gene lines

To test whether the putative single gene lines from iCoteau and Stoa were in fact monogenic, the lines were crossed to Waldron. To test allelic relationships among the lines having the IT 0; gene from iCoteau and Stoa, CoteauA was crossed to StoaA, StoaB, StoaC, and StoaD. To test allelic relationships among lines carrying the IT 2 genes, all IT 2 lines were first crossed to Len and CoteauB. After determining that line StoaJ had a resistance gene different from those in Len and CoteauB, we crossed StoaJ to all lines carrying IT 2 genes. For all of these crosses, five F_1 plants were grown in the greenhouse and approximately 25 F_2 progeny of each F_1 plant were tested with isolate 46-2.

Lines were tested for goodness of fit to postulated ratios by chi-squared analysis. Upon review of the results of the genetic analyses of monogenic lines, we made additional crosses using lines developed in this study and monogenic lines ISr6-Ra, W2691Sr9b, and LcSr24Ag, carrying *Sr6*, *Sr9b*, and *Sr24*, respectively.

Aneuploid analyses

Seeds of the 21 Chinese Spring monosomics were germinated on petri dishes. Somatic chromosome numbers of plants were determined by following previously described procedures (Klindworth et al. 2002), and monosomic plants were transferred to the greenhouse. The four single gene lines Len, CoteauB, CoteauA, and StoaJ were used as parents in crosses to the Chinese Spring monosomics to determine the chromosomal location of genes conditioning resistance to isolate 46-2. Chromosome numbers of F_1 plants were determined and monosomic F_1 plants were grown in the greenhouse. Approximately 25 F_2 seedlings were grown for each F_1 plant and tested for resistance to isolate 46-2. The putative critical chromosomes were identified by a deviation from a single gene ratio with excesses of resistant plants.

Molecular analysis of cultivars and single gene lines

Total genomic DNA was isolated from seedlings in the 2-leaf stage following the procedure of Faris et al. (2000). The DNA concentration was adjusted to 100 ng/µl. SSR markers *Xcfd43*, *Xgwm47*, and *Xbarc71* were used to detect *Sr6* (Tsilo et al. 2009), *Sr9a* (Tsilo et al. 2007), and *Sr24* (Mago et al. 2005), respectively. SSR markers were amplified by polymerase chain reaction (PCR) using 15 µl reaction volumes. Touchdown PCR was performed for *Xcfd43* and *Xgwm47* using an initial annealing temperature of 65°C and decreased by 0.25°C per cycle until reaching the final annealing temperature of 60°C; and, 35 additional cycles were completed at 60°C. For *Xbarc71*, touchdown PCR conditions were the same except that the initial and ending annealing temperatures were 61 and 56°C, respectively. Loading dye was added to the reaction mixture. A 4-µl aliquot was loaded onto an 8% polyacrylamide mini-gel along with molecular weight standards VIII (Roche, Mannhein, Germany), and electrophoresis was conducted at 150 volts for 80–90 min. Gels were stained with GelRed (Biotium, Inc, Hayward, CA).

Tests of thermo-sensitivity

During the experiment, the results suggested that the IT 0; gene derived from iCoteau may not be thermo-insensitive as originally hypothesized. We hypothesized that an interaction of the IT 0: gene with other *Sr* genes was the basis for thermo-insensitivity in iCoteau. To test this hypothesis, the F_1 hybrids of five crosses were tested for thermo-sensitivity by conducting stem rust tests when greenhouse temperatures exceeded 21°C. Crosses used were Waldron/sCoteau, Waldron/iCoteau, CoteauA/CoteauB, W2691Sr9b/ISr6-Ra, and ISr6-Ra/LcSr24Ag.

Results

To determine the five-letter *Pgt*-code, we tested isolate 46-2 against the complete North American set of stem rust

differentials. For 19 of the 20 differentials, we observed clear virulent or avirulent reactions. However, Vernstein (*Sr9e*), had an IT 210; which was higher than the expected low IT, but not clearly susceptible. In prior tests, isolate 46-2 was virulent on Vernstein (J.D. Miller, unpublished). We therefore left Vernstein in the virulent category, and isolate 46-2 had the avirulence/virulence formula of *Sr6*, *Sr9a*, *Sr9b*, *Sr24*, *Sr31*, *Sr38*/*Sr5*, *Sr7b*, *Sr8a*, *Sr9d*, *Sr9e*, *Sr9 g*, *Sr10*, *Sr11*, *Sr17*, *Sr21*, *Sr30*, *Sr36*, *SrMcN*, *SrTmp*, and was coded as TPPKC.

The inheritance and allelic relationships of resistance in CoteauA and four Stoa lines postulated to carry a single gene conditioning IT 0; to TPPKC are shown in Table 2. In crosses to Waldron, the F_2 segregated into resistant, intermediate, and susceptible classes. When the inheritance data were tested by chi-squared analysis, all data fit single gene ratios. No segregation was observed in the F_2 for crosses of CoteauA with the four Stoa lines. These results indicated that the five lines carried only a single gene conditioning IT 0; to TPPKC, and that the genes were allelic. Therefore, Stoa carried a single gene conditioning IT 0; to TPPKC.

The inheritance and allelic relationships of the one Coteau and 22 Stoa lines postulated to carry a single gene conditioning IT 2 to TPPKC are shown in Table 3. For crosses involving StoaH, -M, -N, -O, -P and -R, some F_1 plants had poor fertility, the most severely affected being the crosses to StoaH, where there was insufficient seed from the StoaH/Waldron cross to conduct an inheritance test. For all remaining crosses, the F_2 populations segregated in a 3 resistant:1 susceptible ratio, with resistant plants having ITs of 12, 2 or 21. Results from the crosses to Waldron indicated that segregations in the cross to CoteauB and in 18 of the 21 crosses to Stoa lines fit single gene ratios. Crosses to StoaS and StoaW failed to fit a single gene ratio, but because the deviation was due to an excess of susceptible plants, it was concluded that these lines also carried a single gene for resistance to TPPKC. In the Waldron/StoaT cross, there was an excess of resistant plants. Data from the StoaT cross were tested to a 15:1 ratio, and the probability value of 0.047 indicated that the segregation was not a good fit to a two gene ratio; however, it fit a two gene ratio better than a one gene ratio; therefore, StoaT possibly had two genes conditioning IT 2 to TPPKC.

We initiated allelism tests of the Stoa IT 2 lines by crossing to Len and CoteauB. Failure to observe segregation in the F_2 indicated allelism of the genes and good fits to a 15:1 ratio indicated independence of the genes. The IT 2 genes in Len and CoteauB were allelic (Table 3). Some crosses of Stoa IT 2 lines to Len and CoteauB also supported the conclusion that the genes in Len and CoteauB were allelic. Twelve lines (StoaI, -J, -K, -N, -0, -P, -Q, -R, -S, -W, -X, and -Y) had a gene different from the genes in Len and CoteauB, six lines (StoaF, -G, -L, -M, -U, and -Z) had genes allelic to the Len and CoteauB genes, and four lines (StoaE, -H, -T, and -V) had a small number of susceptible plants (one, two, four, and two, respectively) resulting in conflicting conclusions concerning the Len and CoteauB crosses. To complete allelism tests, we selected StoaJ as a line having good fertility and having a gene not allelic to the Len and CoteauB genes.

The results from crosses of StoaJ to the other 21 Stoa-derived lines resolved the conflicting results of crosses of StoaE, -H, -T, and -V to Len and CoteauB (Table 3). The gene in StoaE was not allelic to the gene in StoaJ, and the gene in StoaT was allelic to the gene in StoaJ. Also, the data for StoaJ in Table 3 is pooled data from five F_1 plants for each cross. When the data were examined by family, it was found that for StoaH, all susceptible plants were in three families with pooled segregation of 57:11 (resistant:susceptible), which fit a single gene ratio ($p = 0.093$) but not a two gene ratio. For StoaV, the susceptible plants were also in only three families with pooled segregation of 72:5, which fits a two-gene ratio ($p = 0.930$), but not a single-gene ratio. Because StoaH and StoaV included both segregating and homozygous susceptible families, it was concluded that contrary to the inheritance data, these two lines were not single gene lines, but were heterozygous for one gene and homozygous for the second. In the case of StoaH, some of these conflicting results may have been due to sterility in the hybrids leading to outcrossing.

If Stoa had more than two genes conditioning IT 2 to race TPPKC, the results should indicate that a line had a gene that was not allelic to genes in Len, CoteauB, and StoaJ. Examination of the pooled data in Table 3 indicated that only StoaR fits this condition. However, like StoaH and -V, examination of individual families showed that in StoaR/StoaJ, all of the susceptible plants were confined to

Table 2 F_2 segregations in crosses among five lines derived from Coteau or Stoa and producing infection type 0; to *Pgt* race TPPKC

Cross	R[a]	Inter.	S	Prob.[b]
CoteauA/Waldron	28	67	27	0.550
StoaA/Waldron	26	39	20	0.491
StoaB/Waldron	31	55	29	0.866
StoaC/Waldron	27	66	27	0.549
StoaD/Waldron	33	55	33	0.607
StoaA/CoteauA	117	0	0	<0.001
StoaB/CoteauA	117	0	0	<0.001
StoaC/CoteauA	121	0	0	<0.001
StoaD/CoteauA	122	0	0	<0.001

[a] R = IT 0; Inter = IT 13c-0; to 3c10; S = IT 34

[b] Probabilities for fits to 1:2:1 ratios as determined by chi-squared analysis

Table 3 F_2 segregations in crosses among 23 lines derived from Coteau or Stoa and having infection type 2 resistance to *Pgt* race TPPKC

Female	Male parent											
	Waldron			Len			CoteauB			StoaJ		
Parent	*R*	*S*	Prob[a]	*R*	*S*	Prob[a]	*R*	*S*	Prob[a]	*R*	*S*	Prob[a]
CoteauB	87	36	0.274	118	0	0.005**						
StoaE	87	32	0.634	117	1	0.015*	120	0	0.005**	111	11	0.207
StoaF	85	35	0.292	124	0	0.004**	113	0	0.006**	130	4	0.118
StoaG	90	31	0.875	99	0	0.010*	120	0	0.005**	111	13	0.051
StoaH				84	2	0.133	90	0	0.014*	106	11	0.159*
StoaI	84	39	0.086	91	7	0.715	110	4	0.227	128	0	0.003**
StoaJ	90	32	0.754	108	9	0.519	116	10	0.434			
StoaK	85	33	0.457	114	6	0.572	111	14	0.022*	130	0	0.003**
StoaL	82	39	0.066	118	0	0.005**	122	0	0.004**	123	6	0.453
StoaM	96	29	0.642	116	0	0.005**	114	0	0.006**	120	8	1.000
StoaN	87	39	0.123	92	7	0.736	38	7	0.010*	126	0	0.004**
StoaO	101	23	0.097	116	10	0.434	115	8	0.907	124	0	0.004**
StoaP	85	37	0.174	109	16	0.002**	88	7	0.652	132	0	0.003**
StoaQ	90	28	0.750	111	9	0.572	109	12	0.096	122	0	0.004**
StoaR	36	12	1.000	109	16	0.002**	106	18	<0.001**	104	15	0.004**
StoaS	80	40	0.035*	109	11	0.187	111	11	0.207	126	0	0.004**
StoaT	99	12	<0.001**	116	4	0.187	120	0	0.005**	118	0	0.005**
StoaU	78	35	0.143	122	0	0.004**	112	0	0.006**	107	14	0.016*
StoaV	82	34	0.284	115	0	0.006**	117	2	0.039*	123	5	0.273
StoaW	77	43	0.006**	107	10	0.305	116	7	0.798	124	0	0.004**
StoaX	80	37	0.098	112	10	0.374	114	10	0.404	125	0	0.004**
StoaY	82	37	0.125	107	13	0.038*	116	5	0.336	125	0	0.004**
StoaZ	85	30	0.788	118	0	0.005**	118	0	0.005**	117	10	0.450

* Significant at $p = 0.05$; ** Significant at $p = 0.01$

[a] Probabilities determined by chi-squared tests to a 3:1 ratio for Waldron crosses, and 15:1 ratio for all other crosses

two of five families. These two families had a pooled segregation of 34:15 which does not fit ($p < 0.001$) a 15:1 ratio expected for independent genes. Because StoaR/StoaJ had three families that did not segregate, it was concluded that the genes in StoaR and StoaJ were allelic. Summarizing the data from all crosses of Stoa lines to Len, CoteauB, and StoaJ, seven lines (StoaE, -F, -G, -L, -M, -U, and -Z) had genes allelic to the genes in Len and CoteauB, twelve lines (StoaI, -J, -K, -N, -0, -P, -Q, -S, -T, -W, -X, and -Y) had a common gene that was different from the genes in Len and CoteauB, and three lines (StoaH, -R, and -V) were homozygous for one of the genes and heterozygous for the second. Therefore, Stoa had only two genes conditioning IT 2 to race TPPKC.

Aneuploid analyses

Monosomic analyses of the IT 2 genes in Len and CoteauB were initiated prior to determining that the two genes were allelic. Segregation data from crosses of each of these lines to the 21 CS monosomics are shown in Table 4. For the Len crosses, segregation fit single gene ratios for all crosses except chromosome 4A and 2B. For the CoteauB crosses, segregation in the 1A, 6A, and 2B crosses did not fit single gene ratios. For CS mono 1A/CoteauB, the deviation was due to an excess of susceptible plants; therefore, 1A could not be the critical chromosome. For both Len and CoteauB, chromosome 2B had the highest deviation towards an excess of resistant plants and had the lowest probability values. Therefore, the IT 2 genes in Len and CoteauB were located in chromosome 2B. Because TPPKC was avirulent on ISr9a-Ra and W2691Sr9b, both of which gave an IT of 2 similar to the Len and CoteauB genes, and because *Sr9* is located on chromosome 2B, we hypothesized that the IT 2 gene in Len and CoteauB was *Sr9a* or *Sr9b*.

Miller et al. (1996) concluded that Coteau had two genes conditioning IT 0; to TPPKC, with one gene being thermosensitive and the second being thermo-insensitive. The IT 0; gene in CoteauA was derived from a cross to thermo-insensitive iCoteau. The first trial of the CoteauA gene was

Table 4 Monosomic analyses of four genotypes showing segregation for resistant (*R*) and susceptible (*S*) plants when tested with *Pgt* race TPPKC

Female	Male parent														
	Len			CoteauB			CoteauA trial 1[a]			CoteauA trial 2[a]			StoaJ		
Parent	*R*	*S*	Prob[b]	*R*	*S*	Prob[b]	*R*	*S*	Prob[b]	*R*	*S*	Prob[b]	*R*	*S*	Prob[b]
CS mono 1A	142	46	0.866	135	61	0.048*	142	45	0.768	87	33	0.527	105	45	0.157
CS mono 2A	75	30	0.398	147	53	0.624	100	61	<0.001**	77	22	0.523	92	28	0.673
CS mono 3A	115	42	0.612	125	38	0.619	121	34	0.378	74	35	0.086	101	51	0.015*
CS mono 4A	122	26	0.037*	137	47	0.865	95	48	0.018*	69	22	0.856	64	27	0.304
CS mono 5A	94	36	0.478	139	36	0.176	135	44	0.897	0	0		126	31	0.128
CS mono 6A	182	50	0.225	160	34	0.016*	129	42	0.895	52	21	0.457	68	20	0.622
CS mono 7A	174	51	0.419	120	47	0.348	108	42	0.396	71	22	0.765	53	16	0.728
CS mono 1B	126	56	0.072	136	50	0.553	106	40	0.504	54	13	0.290	95	36	0.512
CS mono 2B	100	17	0.009**	116	21	0.009**	107	38	0.737	88	32	0.673	84	29	0.871
CS mono 3B	149	54	0.598	100	25	0.197	113	35	0.704	48	21	0.297	46	19	0.431
CS mono 4B	135	38	0.357	82	29	0.784	125	24	0.012*	70	24	0.905	104	41	0.362
CS mono 5B	139	37	0.223	128	45	0.759	104	67	<0.001**	48	18	0.670	89	36	0.327
CS mono 6B	148	46	0.678	122	45	0.561	90	38	0.221	73	22	0.678	102	27	0.286
CS mono 7B	174	50	0.355	93	21	0.105	113	57	0.010*	55	19	0.893	73	20	0.436
CS mono 1D	167	61	0.541	114	37	0.888	57	72	<0.001**	49	16	0.943	107	28	0.253
CS mono 2D	144	52	0.621	129	58	0.057	124	34	0.312	124	5	<0.001**	57	29	0.062
CS mono 3D	118	45	0.442	139	36	0.176	90	70	<0.001**	46	15	0.941	137	15	<0.001**
CS mono 4D	145	51	0.741	102	41	0.311	88	31	0.791	51	20	0.537	131	42	0.826
CS mono 5D	132	51	0.370	76	25	0.954	93	44	0.054	52	16	0.779	74	32	0.217
CS mono 6D	164	40	0.075	117	36	0.674	182	53	0.386	77	38	0.046	117	36	0.674
CS mono 7D	157	38	0.075	142	42	0.496	64	40	0.002**	60	16	0.427	93	38	0.289

* Significant at $p = 0.05$; ** Significant at $p = 0.01$

[a] Trial 1 was planted 4-12-94, and Trial 2 was planted 12-21-95

[b] Probabilities determined by chi-squared tests to a 3:1 ratio

conducted in mid-April when it was difficult to maintain daytime greenhouse temperatures between 18–21°C. Although the cross to CS mono 4B produced an excess of resistant plants (Table 4), subsequent analysis of additional plants from that cross (not shown) did not support the conclusion that the gene was located in chromosome 4B. Segregation in seven additional crosses failed to fit a 3:1 ratio; however, all of those crosses had an excess of susceptible plants and could not be the critical cross. A second trial (Table 4) was conducted where plants were inoculated December 21, 1995, when daytime greenhouse temperatures could be more easily maintained between 18 and 21°C. For this trial, the 5A population was missing. For the remaining 20 chromosomes, only the 2D population had a significant deviation from a 3:1 ratio with an excess of resistant plants. We concluded that contrary to the original hypothesis, that the IT 0: gene in CoteauA, and hence iCoteau, conditioned a thermosensitive response to stem rust, and the gene was located in chromosome 2D. The phenotype, chromosomal location, and thermo-sensitivity of this gene suggested that the gene may be *Sr6*.

In the monosomic analysis of StoaJ, 19 of the 21 crosses segregated in a single gene ratio (Table 4). Segregation in the cross mono 3A/StoaJ had an excess of susceptible plants, indicating that 3A was not the critical chromosome. Only the cross to monosomic 3D had an excess of resistant plants, and all susceptible plants were noted to have dark-green, spindly leaves typical of nullisomic plants. Therefore, the gene in StoaJ was located in chromosome 3D. The only known Sr gene that is located in chromosome 3D is *Sr24* (http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Resistancegenes/wsr_loc.xls), and because TPPKC was avirulent on the differential line carrying *Sr24*, we concluded that the gene in StoaJ may be *Sr24*.

Allelic relationships to known Sr genes and confirmation by molecular markers

Allelism tests were performed using lines CoteauA, StoaB, and Len. CoteauA and StoaB were crossed to ISr6-Ra and 200 F_2 plants were tested with TPPKC for each cross, and no segregation was observed (Table 5). We also tested

Table 5 Allelic relationships of genes in Len, Coteau, and Stoa to *Sr6* and *Sr9* by tests of F_2 plants and F_2 families with *Pgt* race TPPKC

Cross	F_2 plants	F_2 families[a]		
	No./IT	H. Res.	Segregating	Other
CoteauA/ISr6-Ra	200/0;	195	2	1
StoaB/ISr6-Ra	200/0;	199	1	0
W2691Sr9b/Len	409/23, 41/32	449	0	0

[a] *H. Res.* homozygous resistant, *Other* a family with infection types not similar to those for *Sr6*, and therefore likely to be a contaminant

approximately 25 progeny in 398 of the F_2 families from crosses CoteauA/ISr6-Ra and StoaB/ISr6-Ra. There were 394 homozygous 0; families, 3 segregating families, and one family having a homozygous IT 2. The homozygous IT 2 family did not have the phenotype expected for *Sr6* (IT 0), and this indicated that the family was probably a seed mixture. The segregating families did not segregate 3 resistant to 1 susceptible but, instead, had only one or two susceptible plants per family.

As a final test of allelic relationships of the IT 0; genes to *Sr6*, we tested SSR marker *Xcfd43* on the three cultivars in the study plus ISr6-Ra, CoteauB, StoaA, -B, -C, and -D, and additional checks (Fig. 1). Chinese Spring had a 185-bp band that was replaced by 244- and 284-bp bands in ISr6-Ra. Waldron, Len, and other checks not postulated to carry *Sr6* had the 185-bp band; and Coteau, Stoa, and all other lines postulated to carry *Sr6* had the 244- and 284-bp bands. Stoa also had the 185-bp band; however, this band was probably caused by contamination.

To test whether the IT 2 gene that is common in Len, Coteau, and Stoa is an *Sr9* allele, we crossed W2691Sr9b to Len. Four-hundred and fifty F_2 plants were tested with TPPKC (Table 5). There were 409 plants that had a IT 23 reaction and 41 plants had IT 32. Although these are similar reactions, an IT 23 is usually considered resistant,

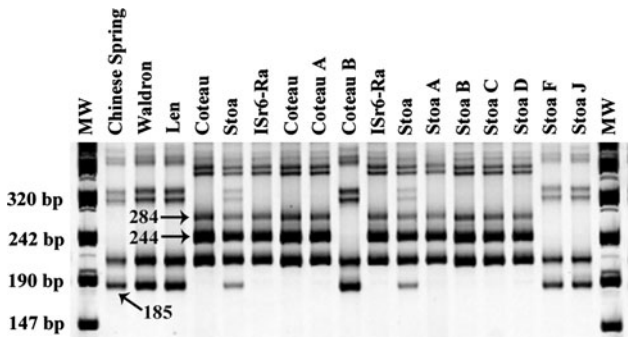

Fig. 1 Tests of *Xcfd43* to detect *Sr6* in cultivars and genetic stocks. Amplicons of 284 and 244 bp were diagnostic for *Sr6*, whereas wheats lacking *Sr6* had a 185 bp amplicon. Amplicons between 317 and 379 bp were also diagnostic for *Sr6*

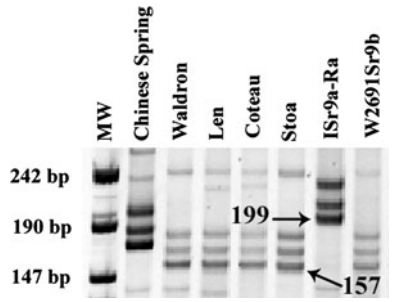

Fig. 2 Tests of *Xgwm47* to detect *Sr9a* in cultivars and genetic stocks. A triplet of amplicons was diagnostic for *Sr9a*. In the wheat cultivars, the smallest amplicon was 157 bp, while in ISr9a-Ra, the smallest amplicon was 199 bp

while an IT 32 may be considered susceptible. Therefore, we tested 25 progeny from 449 of the families. All families were classified as homozygous resistant, indicating that the IT 2 gene common to Len, Coteau, and Stoa was an *Sr9* allele. However, the allelism test could not determine if the gene was *Sr9a* or *Sr9b*.

To determine if the *Sr9* allele of Len, Coteau, and Stoa was *Sr9a*, we tested the parental lines in the study along with the stem rust differentials that carry *Sr9* alleles with *Xgwm47*. Chinese Spring produced bands of 208, 189, and 177 bp, ISr9a-Ra had bands of 234, 214, and 199 bp, and all other lines in the study produced bands of 181, 170, and 157 bp (Fig. 2). The results indicated that Len, Coteau, and Stoa did not carry the marker diagnostic for *Sr9a*. While this was not a positive test for the presence of *Sr9b*, the result indicated that the cultivars likely had *Sr9b*, since it was the only remaining *Sr9* allele that conditioned resistance to TPPKC.

Because *Sr24* was derived from *Thinopyrum ponticum* (Podp.) Z-W. Liu & R.-C. Wang [syn. *Agropyron elongatum* (Host) Beauv., *Elytrigia pontica* (Podp.) Holub., *Lophopyrum ponticum* (Podp.) Á. Löve] (Smith et al. 1968; McIntosh et al. 1976), recombination does not occur between the alien chromosome segment and chromosome 3D. Therefore, identifying the segment in Stoa as coming from *Th. ponticum* could be more accurately determined with molecular markers than with conventional allelism studies. We tested LcSr24Ag, Stoa, and the Stoa-derived lines with marker *Xbarc71* which detects the *Th. ponticum* segment carrying *Sr24* (Fig. 3). Bands of 159 and 111 bp were observed in LcSr24Ag. Genotypes which had the 159- and 111-bp bands replaced by a 104-bp band were postulated to lack the *Th. ponticum* segment carrying *Sr24*. As expected, Stoa was the only cultivar having the amplicons diagnostic for *Sr24*. Lines StoaA thru -D, which were shown to carry *Sr6* produced only a single 104-bp band. StoaE thru -Z, which had an IT 2 similar to *Sr24* were tested, and results from 21 of the 22 lines were consistent with the allelism test. However, the line StoaL,

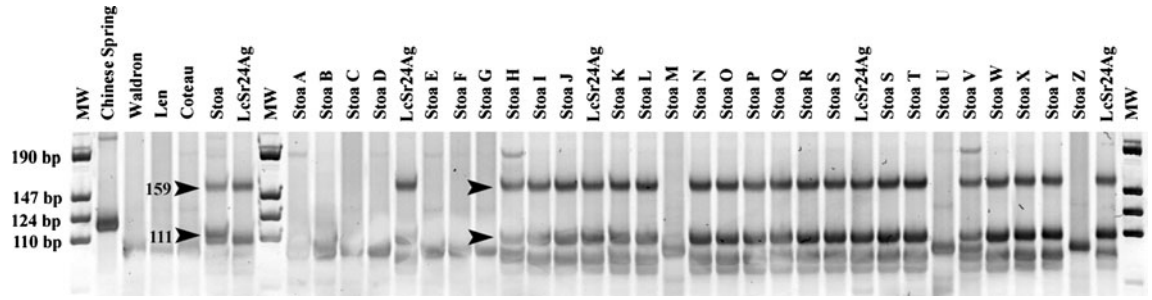

Fig. 3 Tests of *Xbarc71* to detect *Sr24* in cultivars and genetic stocks. Amplicons of 159 and 111 bp (*arrowheads*) were diagnostic for the *Th. ponticum* segment carrying *Sr24*

Table 6 Postulated genotypes and observed infection types on F_1 plants of five crosses heterozygous for *Sr6* and tested with *Pgt* race TPPKC

Cross	Postulated genotype[a]	No of F_1 plants	Range in IT
Waldron/sensitive Coteau	? ?	8	33^{+}c1
Waldron/insensitive Coteau	*Sr9b* ?	8	0;1^{-} to 0;1^{-}1
CoteauA/CoteauB	*Sr9b sr9b*	7	0;1^{-} to 0;1^{-}1
W2691Sr9b/ISr6-Ra	*Sr9b sr9b*	8	0;1^{-} to 0;1^{-}1
ISr6-Ra/LcSr24Ag	*Sr24 sr24*	8	2

[a] ? = The *Sr9* alleles carried by Waldron and sensitive Coteau are unreported

which had previously been determined to carry a single gene that was not allelic to *Sr24*, carried the marker for the *Th. ponticum* segment.

Stem rust testing of thermo-sensitive and insensitive Coteau

The F_1 hybrids of five crosses were inoculated with TPPKC (Table 6). Greenhouse temperatures were near optimum (20–21°C) at night, but daily temperatures were medium to high (22–24°C). All F_1 plants in the test were heterozygous at the *Sr6* locus. For the purposes of the study, CoteauA was considered to be equivalent to sCoteau and ISr6-Ra, having *Sr6* but lacking *Sr9b*. The *Sr9* allele contributed by Waldron and sCoteau was unknown, but was not *Sr9b*. All eight plants of Waldron/sCoteau had ITs of 33^{+}c1, while in Waldron/iCoteau, CoteauA/CoteauB, and W2691Sr9b/ISr6-Ra, the upper range in ITs was 0;1^{-}1. ISr6-Ra/LcSr24Ag plants had IT 2, suggesting that thermo-insensitivity was not conferred to *Sr6* by *Sr24*.

Testing of TPPKC on HRS wheat and durum cultivars

Thirty-six HRS wheat and six durum cultivars were tested with TPPKC and TPMKC (Table 7). Checks included monogenic lines derived from Waldron, BtSrWld1, W2691Sr13, Len, Coteau, CoteauA, Stoa, and StoaA. Two temperatures were used to allow for detection of *Sr6* by differential response at high and low temperatures. Among the Waldron monogenic lines, only WDR-E4 was resistant to TPMKC and had a similar IT to TPMKC as BtSrWld1. Both WDR-E4 and BtSrWld1 were susceptible to TPPKC. The ITs observed on CoteauA and StoaA illustrate the phenotypes produced by the two races on *Sr6* genotypes at the two temperatures. Because CoteauA and StoaA were derived from crosses to Waldron, there is a possibility that they also carry *SrWld1*; and, because CoteauA had an IT 23 to TPMKC at high temperature, CoteauA likely carried *SrWld1*.

Among the 36 HRS cultivars tested, only Ada, Parshall, Granger, ND810, and MN05214-3 were susceptible to TPPKC. Heterogeneity for reaction to TPPKC was observed in Granger and ND810. These cultivars apparently had *SrWld1* as their major stem rust resistance gene. The cultivar '2375' was resistant (IT 0) to both TPMKC and TPPKC at low temperature, which indicated the presence of *Sr6* in '2375'. Like CoteauA, '2375' was resistant (IT 23-) to TPMKC at high temperature, but was susceptible to TPPKC (IT 3), and we therefore postulated that '2375' carried *SrWld1*. Ten cultivars had resistance similar to Coteau and Stoa, with IT 0; at low temperature and IT 1 or 12 at high temperature. These ten cultivars had *Sr6*; but, because resistance did not completely break down at high temperature, they had at least one additional gene for resistance to TPPKC. The additional gene was likely *Sr9a*, *Sr9b*, *Sr24*, or *Sr31*. Twenty cultivars had ITs of 1 or 12 to both races at both temperatures, which suggested that these cultivars had one or more additional genes for resistance to TPPKC that were not *Sr6*. The test of W2691Sr13 indicated that *Sr13* conditioned resistance to TPPKC. Among the six durum cultivars tested, only Divide was susceptible to TPPKC.

Table 7 Infection types (IT) [a] of HRS wheat cultivars, durum cultivars, and check lines when tested with *Pgt* races TPPKC and TPMKC under two growth chamber temperatures

	TPPKC		TPMKC	
	25°C	18–21°C	25°C	18–21°C
LMPG-6	3	32, 34	34	34
WDR-A1	33^{+}	34	34	34
WDR-B1	34	34	34, 23	34
WDR-C2	$3^{+}4$	33^{+}	43	34
WDR-D1	34	33^{-}	43	34
WDR-F1	34	320;	4	34
WDR-E4	43	34	3	2, 23^{-}
BtSrWld1	34	$3^{-}3$	23	23^{-}
Waldron	33^{+}	22^{+}	23	11^{+}
MN05214-3	34	32	23	2
Ada	34	32	33^{+}	213^{-}
Parshall	33^{-}	34	23	0;, 1^{-}
ND810	34	0;, 32	23	12, 0;
Granger	$34c^{-}$	$3^{-}2$, 0;	34, 33^{+}	1^{-}
CoteauA	33^{+}	0;	23	0;
StoaA	33^{+}	0;	32	0;
2375	3	0;	23^{-}	0;
Coteau	33^{+}, 1	0;, 12^{-}	12, 23	0;, 1
Stoa[b]	1	0;	12	0;
Len[c]	1	1^{-}	12	12
W2691Sr13	12	2c	12	21
Divide	3	$3^{-}3$	0;1	0;
Grenora[d]	1^{-}	0;1^{-}	0;	0;

[a] A comma indicates that the cultivar was heterogeneous for stem rust reaction. The predominant IT is listed first, hence 0;, 32 indicates that the 0; phenotype was predominant

[b] Ten HRS cultivars had resistant ITs similar to Stoa, including Amidon, Briggs, Butte86, Ernest, Grandin, Ingot, MN03196, Oklee, RB07, and Sabin

[c] Twenty HRS cultivars had resistant ITs similar to Len, including Alsen, Argent, Barlow, Brick, Dapps, Faller, Glenn, Glupro, Howard, MN00261-4, MN02072-7, Mott, ND808, ND811, Reeder, Russ, Steele-ND, Tom, Traverse, and Ulen

[d] Four durum cultivars had resistant ITs similar to Grenora, including Alkabo, Lebsock, Pierce, and Mountrail

Discussion

Results of this study indicate that resistance to recombinant race TPPKC in North American wheat cultivars was conditioned by *Sr6*, *Sr9a*, *Sr9b, Sr13*, *Sr24*, *Sr31*, and *Sr38*. With the exception of *Sr38*, these are all genes that are commonly found in North American HRS, HRW, and durum cultivars. Although not as widely virulent as TTTTF, TPPKC is a notable race due to its virulence to *SrWld1*, an important gene in northern Great Plains HRS cultivars. TPMKC and TTTTF were reported by Jin and Singh (2006) to be avirulent on 94 and 93% of North American HRS cultivars, respectively. In contrast, TPPKC was avirulent on 86% (31 of 36) of HRS cultivars and advanced lines included in this study. The HRS cultivars susceptible to TPPKC have *SrWld1*, and lack *Sr6*, *Sr9a*, *Sr9b*, and *Sr24*. Among North American durum cultivars, *Sr13* was thought to be very common (Klindworth et al. 2007), and *Sr9e* is quite frequent. Because of the presence of *Sr13*, we did not expect to find that TPPKC was virulent on any durum cultivars and we tested only six durum accessions. We found that Divide had good resistance to TPMKC, but was susceptible to TPPKC, suggesting that Divide does not carry *Sr13*. This result was confirmed by the recent study of Simons et al. (2011) who found that several of the newer North Dakota durum cultivars were susceptible to TTKSK, and therefore likely lack *Sr13*. Furthermore, Divide cannot carry any of the other genes conferring resistance to TPPKC, and because it is a durum, it cannot carry any gene located on a D-genome chromosome, such as *Sr30*. Additional testing is needed to postulate which gene(s) conditioning resistance to TPMKC, but ineffective to TPPKC, is carried by Divide.

Results of the allelism tests of Stoa lines carrying IT 2 genes indicated that 7 of the 22 lines did not carry *Sr24*. Results from the molecular marker analysis indicated that one of the seven lines, StoaL, was positive for *Xbarc71*, a marker for *Sr24*. Possibly StoaL, like StoaH, -R, and -V, was heterozygous for *Sr24*, which could account for the inability to correctly determine its allelic relationships. A second possibility is that StoaL carries either a partial deletion or a translocation of the *Th. ponticum* segment so that *Sr24* has been lost but the *Xbarc71* locus is still present. Crosses involving lines StoaH, -M, -N, -O, -P, and -R were found to have differing severity of male sterility. The sterility in these crosses may also indicate possible chromosomal rearrangements occurring in these lines. The sterility in crosses involving StoaM, which was not postulated to carry *Sr24*, indicated that the sterility was apparently not due to the *Th. ponticum* segment carrying *Sr24*.

Tsilo et al. (2007) reported that marker *Xgwm47* produced a broad-diffuse band associated with *Sr9a*. In contrast, we found that in all lines tested, the broad-diffuse band was replaced by three bands of intermediate staining intensity. The differences in results of our study versus Tsilo et al. (2007) are probably the result of differences in electrophoretic conditions.

We found that when the IT 0; gene from iCoteau was in a background in which the IT 2 gene was absent, the IT 0; gene became thermosensitive. Thermo-sensitivity of stem rust resistance genes has been well documented with the best-known example being *Sr6*, where resistance breaks down at higher temperatures (Mayama et al. 1975). Thermo-sensitivity has also been reported for *Sr9b*

(Gousseau et al. 1985), where resistance to some races increases with temperature, but decreases against other races at very high temperatures (30°C). We hypothesized that *Sr9b* may be conferring thermo-insensitivity to *Sr6*. Enhancement of the expression of a second stem rust resistance gene by *Sr9b* has previously been recognized in crosses involving *Sr7a* (Knott 1957; Roelfs 1988). We found that F_1 plants postulated to be heterozygous for *Sr6* and *Sr9b* were less thermosensitive than F_1 plants postulated to be heterozygous for *Sr6* alone (Table 6). However, this preliminary study of thermo-sensitive versus -insensitive Coteau was only designed to develop a hypothesis for the basis of the genetics of thermo-insensitivity in Coteau. More detailed studies are needed to conclusively prove that *Sr9b* confers thermo-insensitivity to *Sr6*. In a recent stem rust test conducted in a constant 25°C growth chamber, an IT 2 to TPPKC was observed on iCoteau, which is postulated to be homozygous for both *Sr6* and *Sr9b*. Therefore, if *Sr9b* confers thermo-insensitivity to *Sr6*, the effect may be confined to moderate temperatures (21–24°C).

Our conclusions differ from those of Miller et al. (1996) in two respects. Miller et al. (1996) concluded that Len and Stoa had an allelic gene conditioning IT 2 that was not present in Coteau. We concluded that Len, iCoteau, and Stoa all carry an IT 2 gene that was an allele of *Sr9*. This gene was not present in sCoteau. Since *Sr9a* and *Sr9b* were the only *Sr9* alleles conditioning resistance to TPPKC, and because the molecular analysis of *Xgwm47* indicated that the cultivars did not carry *Sr9a*, we concluded that all three cultivars likely carried *Sr9b*. Miller et al. (1996) also concluded that Coteau had two genes conditioning IT 0; to TPPKC. We concluded that Coteau had only a single gene conditioning the fleck phenotype, and that Coteau had two biotypes for stem rust resistance, with iCoteau having *Sr6* and *Sr9b*, and sCoteau having only *Sr6*. The presence of thermo-insensitive phenotype in iCoteau may have been due to an interaction of *Sr6* and *Sr9b*.

The conclusion that Stoa carried *Sr24* may be unexpected, since *Sr24* is thought to be mainly found in winter wheats, and because *Sr24* is linked to *Lr24* (McIntosh et al. 1976), which has so far not been detected in Stoa. Olson et al. (2010) found that marker *Xbarc71* was the most accurate marker for the presence of *Sr24* and found that 12% of Great Plains spring wheat lines carried the *Xbarc71* marker, including Stoa. Stoa is also resistant to TTKSK (Jin et al. 2006) which could be conditioned by *Sr24*. Agent, one of the original cultivars to carry *Sr24* (McIntosh et al. 1976), is in the pedigree of Stoa (http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=germplasm&name=STOA), indicating that it is feasible that Stoa could carry *Sr24*.

TPPKC is a unique *Pgt* race that to the author's knowledge is the only race of North American origin that is virulent on plants with *SrWld1*. TPPKC does not constitute as severe a threat to wheat production as TTKSK or its variants. TPPKC has not been reported in rust surveys of the Great Plains (Roelfs et al. 1995, Jin 2005), and because *Sr6*, *Sr24*. and *Sr31* are major genes in hard red winter wheats (Leonard 2001a, b) it may be difficult for TPPKC to overwinter in the southern US to subsequently infect susceptible northern HRS wheat cultivars. Still, our test of current cultivars indicates that if TPPKC could become established in the field, the loss of a few HRS and durum cultivars would occur, and therefore, additional caution is warranted when working with TPPKC. Our study illustrates that due to its virulence for *SrWld1*, comparisons of the reactions of cultivars with TPPKC and TPMKC are useful to detect cultivars carrying *SrWld1* as their major *Sr* gene. TPPKC may also be useful in genetic studies for the detection of new *Sr* genes.

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