Euphytica (2012) 186:15–23
DOI 10.1007/s10681-011-0476-0

Seedling resistance to stem rust race Ug99 and marker analysis for *Sr2*, *Sr24* and *Sr31* in South African wheat cultivars and lines

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Received: 15 March 2011 / Accepted: 7 June 2011 / Published online: 17 June 2011

Abstract The appearance and spread of races of *Puccinia graminis* f. sp. *tritici* with virulence for the *Sr31* resistance gene has renewed interest in breeding for durable resistance to stem rust of wheat. Since the occurrence of stem rust has been low in South Africa until the detection of race TTKSF in 2000, breeding for resistance to this disease has not been a primary objective. The aim of this study was to test bread wheat cultivars and lines at the seedling stage for their infection response to stem rust, thus determining their level of resistance or vulnerability. A collection of 65 bread wheat entries was tested with one USA race, two Eastern African races, and three South African races of *P. graminis* f. sp. *tritici*. The Eastern African and South African races all belong to the Ug99 lineage. The cultivars Duzi, Caledon, Elands, PAN 3364, PAN 3191, SST 047, SST 399, and Steenbras produced resistant infection types (IT < 3) to all races. The molecular marker Sr24#50 indicated the presence of *Sr24* in 12 entries. In cultivars resistant to TTTTF, TTKSF, and TTKSP but susceptible to TTKSK and PTKST, the iag95 DNA marker indicated the presence of *Sr31* in five wheat lines. Using the cleaved amplified polymorphic sequence marker csSr2, *Sr2* was detected in PAN 3377, Inia, and Steenbras. Few South African wheat cultivars appear to have a broad-based resistance to stem rust, as 88% of the entries were susceptible as seedlings to at least one of the races tested. Diversification of resistance sources and more directed breeding for stem rust resistance are needed in South Africa.

Keywords *Puccinia graminis* f. sp. *tritici* · Resistance · *Sr* genes · *Triticum aestivum* · Ug99

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Introduction

Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, has been an important constraint in South African wheat production for many years (Pretorius et al. 2007). Only since the integration of knowledge on variation in the pathogen and host during the last three to four decades did breeders progress in developing resistant cultivars (Lombard 1986; Pretorius et al. 2007). However, in many cases resistance incorporated into commercial wheat and triticale cultivars was race-specific and short-lived, resulting in the development of races virulent to *Sr9e*, *Sr24*, *Sr27*, and *Sr36* (Le Roux and Rijkenberg 1987a, b; 1988; 1989; Le Roux 1989; Pretorius et al. 2007).

Palmiet (*Sr2* + *Sr24*), a spring habit cultivar released in 1985 and resistant to stem rust, contributed significantly to the reduction of stem rust levels until its withdrawal from production in 1999 (Smit et al. 2010). Low levels of inoculum and absence of widespread stem rust epidemics resulted in less emphasis on breeding for resistance to this disease.

The appearance of stem rust race Ug99 (TTKSK) in East Africa (Pretorius et al. 2000) and subsequent epidemics in Kenya and Ethiopia, accompanied by the occurrence of three Ug99 variants in South Africa (Visser et al. 2009, 2011), have resulted in a renewed interest in understanding the status of stem rust resistance in South African wheat. Two important virulence adaptations [TTKST and TTTSK with virulence on *Sr24* and *Sr36*, respectively, in addition to virulence for *Sr31* (Jin et al. 2008, 2009)] were also detected in Kenya, further broadening the virulence spectrum of the Ug99 lineage. The detection of race PTKST in South Africa in 2009 (Visser et al. 2011) accentuated the migration of races virulent to *Sr31* and the need for durable resistance. We hypothesized that the genetic base of resistance to the Ug99 lineage in leading commercial cultivars and advanced breeding lines in South Africa is narrow. This hypothesis was tested by evaluating elite germplasm with six stem rust races.

Materials and methods

Infection type tests

A collection of 54 wheat cultivars and 11 breeding lines, obtained from the South African seed companies Afgri, ARC-Small Grain Institute, Sensako, and Pannar, was tested with one USA race, two East African races, and three South African races of *P. graminis* f. sp. *tritici*. Tests with the USA and East African races were done at the USDA-ARS Cereal Disease Laboratory (CDL), St. Paul, MN, whereas tests with the South African races were conducted at the University of the Free State, Bloemfontein. At the CDL, urediniospores of stem rust isolates representing races TTTTF, TTKSK, and TTKST stored at −80°C were heat-shocked at 40°C for 10 min and rehydrated for 2 h in a chamber containing a 23.5% KOH solution (80% RH at 20°C) (Rowell, 1984). Urediniospores were then suspended in light mineral oil (Soltrol 170) and sprayed onto the fully expanded primary leaves of 7 to 9-day-old wheat seedlings raised in vermiculite. Seedlings were incubated at 18°C in a dew chamber in darkness for 14 h. Upon removal from the chamber, plants were exposed to 3 h of fluorescent light. Inoculated plants were then placed on a greenhouse bench at 18 ± 2°C with a photoperiod of 16 h. In the South African tests, seedlings were grown in a soil–peat moss mixture and inoculated with fresh spores of TTKSF, TTKSP, and PTKST. Inoculation and incubation procedures were similar to those at the CDL. The avirulence/virulence profiles of the races are according to the North American nomenclature system described by Jin et al. (2008).

Infection types (ITs), described by Stakman et al. (1962), were assessed 14 days post-inoculation. A set of the 20 stem rust differentiating lines, including lines LCSr24Ag and Sr31/6*LMPG, was included in each inoculation using the USA and Eastern African races. In the South African test, an abbreviated set containing the genes *Sr5*, *Sr8b*, *Sr9e*, *Sr24*, *Sr27*, *Sr31*, *Sr38*, and *SrSatu* were evaluated alongside entries. Infection types 0–2 or combinations thereof were considered low ITs, whereas ITs 3–4 were considered high, indicating that the corresponding resistance gene is not effective against the race tested. Seedling tests with the USA race were repeated once whereas those with East and South African races were conducted 3 times.

Materials also were evaluated for the expression of seedling chlorosis, a trait previously reported to be linked to the adult-plant resistance gene *Sr2* (Brown 1997). Five entries were grown in vermiculite per 10-cm-diameter pot. Plants were inoculated with race TTKSF 12 days after sowing when the second leaves were well developed. After inoculation, plants were incubated at 24 ± 4°C in a greenhouse. Seedling chlorosis on the second and third leaves was rated on a 0–5 scale at 16 days after inoculation. Plants rated as 0 showed no chlorosis, whereas a rating of 5 represented severe mottling and systemic yellowing of uninoculated leaves. Suneca and Chinese Spring (Hope 3B) substitution were included as control entries carrying *Sr2*. All seedling chlorosis tests were repeated.

DNA marker analysis

Genomic DNA was isolated from freeze-dried leaves according to Saghai-Maroof et al. (1984). DNA

samples were quantified using a ND-1000 Spectrophotometer (Nanodrop Technologies) and normalized to 50 ng/µl. PCR assays were performed according to the published protocols for *Sr2* (Hayden et al. 2004; Mago et al. 2011), *Sr24* (Mago et al. 2005), and *Sr31* (Mago et al. 2002, 2005).

To determine the occurrence of the *Sr2* markers in South African backgrounds, cultivars and lines were tested using the gwm533 marker and its derived stm559tgag and stm598tcac markers (Hayden et al. 2004). The reverse primers of these markers were fluorescent dye-labeled with HEX, NED, and FAM respectively (Applied Biosystems). In a follow-up test on all entries a new forward stm559 sequence (5′-GGAGGGAAACTATCAAAATATGCTGGT-3′, referred to in this study as stm559n) was tested. PCR conditions were similar to that used by Hayden et al. (2004) and PCR products were separated on an ABI3130XL instrument using ROX as an internal size standard. Data were analysed using GeneMapper Ver. 4.0 (Applied Biosystems). The occurrence of *Sr2* was validated in a second round of testing using a recently developed cleaved amplified polymorphic sequence (CAPS) marker (csSr2) for the gene (Mago et al. 2011). Reaction conditions were used as prescribed for the KAPA 2G Fast ReadyMix PCR Kit (www.kapabiosystems.com). A final concentration of 1× for the 2× KAPA2G Fast ReadyMix and 0.5 µM for both the forward and reverse primers were used in a reaction volume of 25 µl. PCR was performed in a GeneAmp PCR system 9700 (Applied Biosystems) with the following cycling conditions: 3 min at 95°C, 40 cycles of 15 s at 95°C, 15 s at 60°C and 15 s at 72°C, and a final extension step of 7 min at 72°C. After amplification, 5 µl of the PCR product was run on a 1% (w/v) agarose gel and only those that amplified a product were digested with *Pag*I (*BspHI*) (Fermentas) by adding 2 µl molecular grade water, 2.5 µl 10× Buffer O, and 0.5 µl *Pag*I to each PCR reaction and incubating at 37°C for 1 h. The CAPS product was separated on a 2.5% (w/v) agarose gel and visualized under UV light.

The presence of *Sr24* and *Sr31* was assessed in one plant of each of the South African wheat lines. In cases where the data did not correlate with the phenotypic response, four additional plants were checked with the relevant marker. The STS marker Sr24#50, which is informative for all forms of the *Sr24* segment, was employed according to Mago et al. (2005). PCR products were separated on a 2.5% agarose gel and visualized under UV light. An additional marker for *Sr24* (Sr24#12) was also tested. To clarify the presence of two similar sized bands, the Sr24#12 forward primer was labeled with VIC and its PCR product was separated on a ABI3130XL fragment analyzer using GeneScan™ 500 LIZ® as an internal size standard. To assess the presence of *Sr31*, the marker iag95 (previously shown to be 1.7 cM from the gene, Mago et al. 2002, 2005) was used. PCR products were separated on a 1% agarose gel and visualised under UV light. In the marker analyses, the lines Chinese Spring (Hope 3B), RL6078, and LCSr24Ag served as control entries for *Sr2*, *Sr31*, and *Sr24*, respectively.

Results and discussion

The IT data and marker analysis for *Sr2*, *Sr24*, and *Sr31* were informative in characterizing stem rust resistance in South African wheat lines (Table 1). In cases where slight variation was observed between replicates of the infection studies, the highest IT was used. In all infection studies the differential and control lines confirmed the identities of races used.

The Sr24#50 marker provided more reliable results than Sr24#12 (data not presented) and was detected in 12 entries (Table 1). SST 843 and PAN 3404 were heterogeneous for the *Sr24* marker. In comparison with race TTKSK, a clearly high IT was observed for TTKST on eight of these entries, thus confirming the presence of *Sr24*. The mixed response of SST 843 to TTKSK and TTKST prevented confirmation of *Sr24* based on IT alone. Although the ITs for SST 399 and Duzi were 2+ and 2++ to TTKST, respectively, the presence of *Sr24* is confirmed by the distinct 2− IT to race TTKSK commonly produced on lines carrying this gene. However, Exp. line 4 showed a lower than expected ;1 IT to TTKSK. *Sr24* was not detected by the East African races in SST 347, but TTKSP and PTKST detected susceptible plants within this cultivar in replicated tests. PAN 3492 showed IT 1 to TTKSF, and a 2− IT to TTKSP and PTKST, suggesting that it carries another resistance gene in addition to *Sr24*.

Marker analysis helped to resolve the identification of *Sr24* where IT data were insufficient. Historically, Agent derivatives carrying *Sr24* have been

Table 1 Infection types produced by six races of *Puccinia graminis* f. sp. *tritici* on 65 South African bread wheat cultivars and lines, and marker data for *Sr2*, *Sr24* and *Sr31*

Entry code	Cv./Line	Infection types[a] to stem rust races[b]						Markers					*Sr* gene
		TTTTF	TTKSK	TTKST	TTKSF	TTKSP	PTKST	Sr24#50 (*Sr24*)	iag95 (*Sr31*)	stm559n (*Sr2*)	csSr2 (*Sr2*)	sc[c]	
ZA-2006_3	AFG SPN 3	2	3+	3+	4	3	3	–	–	237	–	3	
ZA-2006_4	AFG SPN 4	;	4	3+	1	1	3++	–	+	237	–	0	*Sr31*
ZA-2006_5	AFG SPN 5	;1	3+	3	1	1	3++	–	+	237	–	1	*Sr31*
ZA-2006_6	AFG SPN 6	X–	4	4	3+	3	3	–	–	237	–	3	
ZA-2006_7	AFG SPN 7	;/3+	4	4	;1	1	3+	–	+	237	–	0	*Sr31*
ZA-2006_8	SST 806	0;/2–/2+	3+	3	3	3	3	–	–	237	–	0/1	
ZA-2006_9	SST 822	2	4	3	3+	3+	3	–	–	237	–	1	
ZA-2006_10	SST 825	;1/4	4	3+	4	3	3	–	–	237	–	0/1	
ZA-2006_11	CRN 826	0;/2/3++	3+	3	3	3	3	–	–	237	–	0/2	
ZA-2006_12	SST 835	2+	4	3	3	3++	3+	–	–	237	–	1	
ZA-2006_13	SST 843	;1/3	2+/3	2+/3	2+	2/3	2	–/+	–	Null	–	0	*Sr24*
ZA-2006_14	SST 876	2/4	4	3	3	3	3++	–	–	Null/237	–	1	
ZA-2006_15	PT04/3	3	4	3	3	3	3	–	–	235	–	0	
ZA-2006_16	SST 308	3	2+	2+	2	2	2	–	–	Null	–	0	
ZA-2006_17	SST 319	2–/3	2+	2+	2+	2+	2+	–	–	Null	–	0	
ZA-2006_18	SST 322	3	3	3	3	2	2	–	–	237	–	2	
ZA-2006_19	SST 334	3	3	3	2+/3	2++	2++	–	–	237	–	3	
ZA-2006_20	SST 347	2–/3	2	2	1	1/4	1/4	+	–	Null	–	2	*Sr24*
ZA-2006_21	SST 356	;1	4	3+	;1	;1	4	–	+	Null	–	2	*Sr31*
ZA-2006_22	SST 399	2	2–	2+	;1	2+	2+	+	–	Null	–	0	*Sr24*
ZA-2006_23	SST 935	3	2+/4	2+/4	3	3–	3–	–	–	235	–	0	
ZA-2006_24	SST 946	3	2+/4	2++	;1/3	3	1+/3	–	–	231	–	0	
ZA-2006_25	SST 966	3	2+/4	3	3+	3	2/3+	–	–	231/235	–	0	
ZA-2006_27	EXP.2	2=	2–	3+	1+	3–	3	+	–	237	–	2	*Sr24*
ZA-2006_28	EXP.3	;1	2–	3+	;1cn	;1	3–	+	+	237	–	3	*Sr24,31*
ZA-2006_29	EXP.4	;1	;1	3–	;1	3	3	+	–	237	–	0	*Sr24*
ZA-2006_30	SST 57	;1	2–	3+	;1	2++	2++	+	–	237	–	1	*Sr24*
ZA-2006_31	SST 65	2–	4	4	3+	3+	3	–	–	237	–	1	
ZA-2006_32	SST 88	2	4	4	3+	4	4	–	–	237	–	0	
ZA-2006_33	SST 94	1	2–	3+	;1	3	3	+	–	237	–	3	*Sr24*

Table 1 continued

Entry code	Cv./Line	Infection types[a] to stem rust races[b]						Markers					*Sr* gene
		TTTTF	TTKSK	TTKST	TTKSF	TTKSP	PTKST	Sr24#50 (*Sr24*)	iag95 (*Sr31*)	stm559n (*Sr2*)	csSr2 (*Sr2*)	sc[c]	
ZA-2006_34	SST 015	2	4	4	;1/3−	3++	3++	–	–	237	–	1	
ZA-2006_35	SST 026	2	3+	3+	3+	3++	3	–	–	237	–	0	
ZA-2006_36	SST 027	2	3+	3+	2/3+	3+	3	–	–	237	–	0/1	
ZA-2006_37	SST 035	2	4	3	3	3++	3+	–	–	237	–	0	
ZA-2006_38	SST 047	1	2+	2+	0;/1	0;/2	0;	–	–	237	–	0	
ZA-2006_39	SST 056	2	;1/4	4	;1/3	3+	1/3++	–	–	237	–	0	
ZA-2006_40	PAN 3118	0;	4	4	3+	X/3++	X/3+	–	–	Null	–	0	
ZA-2006_41	PAN 3120	2	4	3+	3+	3+	3+	–	–	Null	–	0	
ZA-2006_42	PAN 3122	;1	4	4	3	3	3	–	–	Null	–	2	
ZA-2006_43	PAN 3144	3	2++	2++	2	2−	2	–	–	237	–	0	
ZA-2006_44	PAN 3191	2	2++	2++	2+	2++	2	–	–	278	–	0	
ZA-2006_45	PAN 3364	2++	2++	2+	1+	1	1+	–	–	Null	–	0	
ZA-2006_46	PAN 3434	2++	4	4	2+	2+	2	–	–	Null	–	0	
ZA-2006_47	PAN 3408	2−	4	4	3	3	3	–	–	259	–	0/3	
ZA-2006_48	PAN 3490	2−	2	3	1	2+	3	+	–	Null	–	1	*Sr24*
ZA-2006_49	PAN 3349	0;/2	4	4	2+	2+	2++	–	–	Null	–	0	
ZA-2006_50	PAN 3355	3−	3+	4	3+	3	3+	–	–	Null	–	0	
ZA-2006_51	PAN 3377	;/3	2+	3+	2+	1/3+	;1/3	–	–	237	+	4	*Sr2*
ZA-2006_52	PAN 3404	2−	2−	3+	1/3	1/3	1/3	–/+	–	237	–	0/2	*Sr24*
ZA-2006_53	PAN 3492	2	2−	3+	1	2−	2−	+	–	Null	–	0	*Sr24*
ZA-2006_54	Baviaans	3	4	4	3−	3	3	–	–	Null	–	1	
ZA-2006_55	Betta-DN	3	2+	2++	1+	1+/3	;1	–	–	Null	–	0	
ZA-2006_56	Caledon	2++	2+	2+	;1	;1	;1	–	–	Null	–	1	
ZA-2006_57	Duzi	2−	;2−	2++	;1	2	2	+	–	Null/237	–	1	*Sr24*
ZA-2006_58	Elands	2++	2+	2+	1c	;1	;1	–	–	Null	–	0	
ZA-2006_59	Gariep	3+	2+	2+	;1	;1	;1	–	–	Null	–	0	
ZA-2006_60	Inia	2+	3+	4	3+	3+	3	–	–	237	+	4	*Sr2*
ZA-2006_61	Kariega	3	4	4	3+	3	3	–	–	Null	–	0	
ZA-2006_62	Komati	3	2+	2+	;1	;1	;1	–	–	Null	–	0	
ZA-2006_63	Krokodil	3	4	4	3	3++	3	–	–	237	–	2	

Table 1 continued

Entry code	Cv./Line	Infection types[a] to stem rust races[b]						Markers					*Sr* gene
		TTTTF	TTKSK	TTKST	TTKSF	TTKSP	PTKST	Sr24#50 (*Sr24*)	iag95 (*Sr31*)	stm559n (*Sr2*)	csSr2 (*Sr2*)	sc[c]	
ZA-2006_64	Limpopo	2/3	2+	2+	;1	;1	1	–	–	Null	–	0	
ZA-2006_65	Marico	3	4	4	3	3+	3	–	–	237	–	0	
ZA-2006_66	Matlabas	2/3	2+	2+	1	1	1	–	–	Null	–	0	
ZA-2006_67	Olifants	3	4	4	4	4	3	–	–	237	–	0	
ZA-2006_68	Steenbras	2	0	0	0;	0;	0;	–	–	237	+	5	*Sr2*
Control	RL6078[d]	X+	nt	nt	;;1	;;1	12+	–	+	235	–	0	*Sr31*
Control	CS[d]	nt[e]	nt	nt	4	4	4	–	–	247, 252	–	0	
Control	CS Hope3B[d]	nt	nt	nt	4	4	4	–	–	237	+	4	*Sr2*
Control	Suneca[d]	nt	nt	nt	;1	;1	;1	–	–	237	+	5	*Sr2*
Control	LCSr24Ag[d]	2	2	3	2	3	3	+	–	237	–	0	*Sr24*
Control	Sr31/6*LMPG	1	4	3	1	1	4	–	+	Null	–	0	*Sr31*
Control	McNair 701	4	4	4	4	4	4	–	–	237	–	0	

[a] Infection types according to a 0 to 4 scale. Within line variation is indicated by '/'

[b] Races were represented by the following isolates: *TTTTF* 01MN84A-1-2, *TTKSK* 04KEN156/04, *TTKST* 06KEN19V3, *TTKSF* UVPgt55, *TTKSP* UVPgt59, *PTKST* UVPgt60

[c] Seedling chlorosis as a phenotypic marker for *Sr2* (Brown 1997) was measured on a 0–5 scale (0 = no chlorosis and 5 = severe chlorosis)

[d] RL6078 and Sr31/6*LMPG (*Sr31*), LCSr24Ag (*Sr24*), Chinese Spring (CS) (*Sr2* negative), CS Hope3B (*Sr2* positive) and Suneca (*Sr2* positive) were used as marker controls

[e] *nt* not tested

commonly used in wheat breeding in South Africa (McIntosh et al. 1995) and its occurrence in 18.5% of the current collection is not surprising. Using the Sr24#12 marker, separation of a PCR product on a 2.5% gel revealed the presence of 500 bp bands in some individuals that differed significantly in intensity from those detected in the *Sr24* control and other *Sr24*-carriers (data not shown). This band is confounding when scoring on an agarose gel. When the marker data were repeated on an automatic fragment analyzer, it was clear that the band associated with resistance also amplified with a lower intensity in non-*Sr24* carriers. The more reliable marker Sr24#50 is a dominant marker, with only the *Sr24* carriers amplifying a PCR product of approximately 200 bp (Mago et al. 2005).

Similar to a previous report, it was found that the value of gwm533 as a marker for *Sr2* was compromised due to allelic homoplasy (Hayden et al. 2004) as an allele of 115 bp was detected both in *Sr2*-carriers and non-carriers (data not shown). This tendency was repeated when using the original STM derived markers. In many instances the stm559tgag marker generated an 83 bp resistance allele in *Sr2* non-carriers. The same scenario was found for the 56 bp allele generated by stm598tcac (data not shown). Stm559n produced a 237 bp allele in the *Sr2* controls. This allele was detected in 35 entries, with two being heterogeneous for the 237 bp allele (Table 1). However, the improved CAPS marker detected *Sr2* only in PAN 3377, Inia (=Inia 66), Steenbras, and the control entries Suneca and Chinese Spring (Hope 3B). When comparing the seedling chlorosis test with the CAPS marker, it was clear that only scores of 4 and 5 were clearly suggestive of *Sr2*. Lower and inconspicuous seedling chlorosis scores should thus be ignored when this phenotypic marker is used for *Sr2*. Brown (1997) mentioned that the expression of seedling chlorosis varied between cultivars and lines, but indicated that in some cases temperatures as high as 35°C are needed for manifestation of this phenotype. In the current work this maximum was not achieved. The presence of *Sr2* should also not be assumed from pedigree analysis without further validation. For example, the cultivar Marico (='Broadbill', Boshoff 2000) which could have derived *Sr2* from Inia 66 (Payne et al. 2002), displayed a 0 on the chlorosis scale as opposed to the rating of 4 for Inia, and was negative for the CAPS marker.

The *Sr31* iag95 marker amplified an expected PCR product of approximately 1000 bp (Mago et al. 2002, 2005) in the positive control RL6078. It acted as a dominant marker with no product generated in lines not carrying *Sr31*. Five entries, viz. AFG SPN 4, AFG SPN 5, AFG SPN 7, SST 356, and Exp. line 3 were positive for the *Sr31* marker (Table 1). The IT data, in particular the comparison between TTKSF and TTKSP (both avirulent to *Sr31*) and PTKST (virulent to *Sr31*), supported the marker analysis in these five lines. The high and low ITs of AFG SPN7 to race TTTTF showed that this line was mixed for *Sr31*. As expected, most entries lacked *Sr31* due to the strict quality standards set by the South African baking and milling industry, and thus general avoidance of the 1B/1R translocation in cultivar development. The use of *Sr31* in breeding in South Africa should be strongly discouraged as virulence exists in both Eastern Africa and South Africa (Pretorius et al. 2000, 2010).

The cultivars Duzi, Caledon, Elands, PAN 3364, PAN 3191, SST 047, SST 399, and Steenbras were the only entries that produced resistant ITs (<3) to all races tested (Table 1). According to their stem rust phenotypes (including other USA races, data not shown), SST 308, SST 319, and PAN 3144 may have *SrTmp* as they were susceptible to TTTTF. *SrTmp* could also be a component of stem rust resistance in the winter wheat cultivars Betta-DN, Gariep, Komati, and Limpopo as these entries were susceptible or produced high intermediate ITs to at least one of the *SrTmp*-virulent races, yet were resistant to TTKSK and TTKST. The additional resistance genes in Duzi, SST 347, and SST 399 are not clear but *SrTmp* is plausible. In general, pedigree information is not disclosed by wheat breeders in South Africa which precluded informed postulations in most cultivars. Boshoff (2000) and Smit et al. (2010) indicated that Betta-DN, Caledon, Elands, Gariep, and Limpopo were all derived from crosses involving Betta. Previously, Le Roux and Rijkenberg (1989) identified a dominant stem rust resistance gene in Betta which may be the effective source of resistance to the Ug99 races seen in this study. In searching the IWIS^{TM} database (Payne et al. 2002), no obvious relationship between Betta (introduced as the Argentinian cultivar Klein Impacto) and Triumph was found. However, more detailed studies on these entries have to be conducted to determine the identity of the gene(s), including *SrTmp*, remaining effective against the Ug99 lineage.

Steenbras produced ITs of 0–0; to the Ug99 races but showed an IT 2 to TTTTF. This pattern is characteristic for *Sr36*. In addition, *Sr2* was confirmed in Steenbras by both the DNA and phenotypic marker tests. *Sr2* most likely was transferred to Steenbras from Hoopvol, an old South African cultivar known for its adult plant resistance to stem rust. The resistant response to TTTTF indicates an additional, yet unknown resistance in Steenbras.

The broad virulence of Ug99 is of global concern as more than 70% of wheat cultivars worldwide are susceptible to this race group (Jin et al. 2007; Singh et al. 2008; Steffenson et al. 2009). This study showed that 88% of the entries were susceptible in the seedling stage to one or more races, indicating that most do not have a combination of effective genes with an expectancy of durability. Moreover, based on present marker data for *Sr2*, the gene is not common in South African wheat germplasm. However, Mago et al. (2011) mentioned that the marker did not detect *Sr2* in some lines predicted to carry the gene. It is possible that *Sr2* could not be validated in some South African backgrounds. Lines such as AFG SPN 3, AFG SPN 6, and Exp. line 3, and the cultivars SST 94, SST 334, and PAN 3408 (heterogeneous) all displayed a seedling chlorosis score of 3 and may have *Sr2*.

Park (2008) emphasized the importance of creating complex rust resistance by using durable genetic backgrounds, e.g. *Sr2*, *Lr34/Yr18* and *Lr46/Yr29*, into which other effective genes are introduced. Furthermore, an over-reliance on certain resistance genes is not advisable and the search for new genes and gene combinations should be a continuing priority in wheat breeding (Park 2007). The current basis of stem rust resistance in South African wheat seems to lack such diversity and complexity, and dedicated efforts will be necessary to strengthen this aspect of cultivar development.

Acknowledgments The authors thank Debbie Snyman and Elsabet Wessels for technical assistance, Afgri, ARC-Small Grain Institute, Sensako and Pannar for seed, and the Winter Cereal Trust and National Research Foundation for funding.

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