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Adult plant resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) in Pakistani advanced lines and wheat varieties

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Abstract

After decades of effective wheat stem rust control, due mainly to use of the *Sr31* resistance gene in wheat, as of the early 2000s new virulent strains of the stem rust fungus, especially the Ug99 or the TTKSK races, are spreading and overcoming the resistance of commercial varieties worldwide, including the *Sr24* and *Sr36* resistance genes in Kenya. To address this, researchers are working to identify new resistance sources and to develop and release new high-yielding, resistant and adapted varieties. In this study we evaluated 707 advanced spring wheat lines and varieties for adult plant resistance (APR) to stem rust at the Njoro research station of the Kenya Agricultural & Livestock Research Organization, using a modified Cobb's scale, and for seedling resistance at the Cereal Disease Laboratory (CDL), University of Minnesota, using the 0-4 Stakman et al. (1962) scale. We found 101 lines that showed APR and, through molecular marker analysis, identified 18 lines carrying the stem rust resistance marker allele for the *Sr25/Lr19* gene. Of these 18 lines, 11 were resistant to Ug99 at both the seedling and adult stages and 7 were susceptible at the seedling stage, showing infection type (IT) 3 to 4, and moderately susceptible at the adult plant stage. Another 20 lines were resistant at all stages of development, without *Sr25/Lr19* marker allele indicated the possibility of carrying other genes for stem rust resistance. We shared the results with national program breeders and scientists in Pakistan to facilitate the use of resistant lines in crossing programs and enhance stem rust resistance in candidate wheat varieties. As a result number of lines resistant to Ug99/ and its variants (TTKSK, TTKST) have been identified and released as commercial varieties, including NR-397 (Pakistan-2013) and NR-356 (NARC-2011).

Keywords: *Disease severity; Race non-specific resistance; Triticum aestivum; Ug99; wheat rust.*

Abbreviations: APR_ Adult Plant Resistance, **BRS_**Baseline Resistance Study, **CDL_**Cereal Disease Laboratory, University of Minnesota, **CIMMYT_**International Maize and Wheat Improvement Center, Mexico, **IT_**Infection Type, **MR_**Moderately Resistant **MRMS or M_**Moderately Resistant-Moderately Susceptible or Moderate responses. **MS_**Moderately Susceptible, **MSS_**Moderately Susceptible to Susceptible, ***Pgt*_***Puccinia graminis* f. sp. *Tritici,* **RMR_**Resistant to Moderately Resistant, **S_**Susceptible, **USDA-ARS_** United State Department of Agriculture, Agricultural Research Service, **WPEP_**Wheat Production Enhancement Program.

Introduction

Wheat stem rust (*Puccinia graminis* f. sp. *tritici, Pgt*) had largely been controlled from the late 1970s due to the widespread use of host-plant resistance and the elimination of the alternate host *Berberis* spp. in North America and

Europe. In 1999, a new virulent race of *Pgt* was confirmed in Uganda (Pretorius et al., 2000) named isolate Ug99 and later designated as race TTKSK (Jin et al., 2008; Wanyera et al., 2006). Ug99 was unique due to its virulence on the widely deployed stem rust resistance gene, *Sr31.* Variants of Ug99 have spread throughout eastern and southern Africa, Zimbabwe, Sudan, Yemen and Iran (Nazari et al., 2009; Pretorius et al., 2010; Singh et al., 2006) and was most recently reported in Egypt (Patpour et al., 2016). Some Ug99 variants are able to overcome the resistance based on *Sr9h, Sr24*, *Sr36* and *SrTmp* (Jin et al., 2008; Jin et al., 2009; Newcomb et al., 2016; Rouse et al., 2011). Stem rust could cause severe yield losses if the new races migrate to major wheat producing regions in South Asia, especially in India and Pakistan, where it has not yet been reported. The migratory route for variants of the stripe rust pathogen, *Puccinia striformis*, was shown to go from Africa to Asia (Singh et al., 2004, 2011); stem rust may follow same pattern.

The major-effect, seedling resistance gene *Sr31* provided protection from stem rust for almost three decades, but major-effect genes can be overcome by new virulences in the *Pgt* population in as little as 3 to 4 year (Singh and Rajaram, 1992; Singh et al., 2000). Conversely, race-nonspecific adult plant resistance (APR) genes with additive minor effects can be combined to enhance durability for resistance. Genetic studies conducted at International Maize and Wheat Improvement Center (CIMMYT) have shown that APR in its wheat lines derives from combinations from among 12 different genes (Bhavani et al., 2011). By accumulating 4-5 minor resistance genes, resistance levels approaching immunity can be achieved and combinations of 2-3 genes provide moderate levels of resistance (Singh et al., 2005, 2009). Studies have characterized APR genes *Sr55*, *Sr57* and *Sr58*, which confer pleiotropic effects for diseases such as leaf rust, stripe rust and powdery mildew, and *Sr2/Yr30*, which associated with resistance to stem rust and to pseudo black chaff (Hare and McIntosh, 1979; Herrera-Foessel et al., 2014; Rosewarne et al., 2006; Singh, 1992).

International collaborations have quickly and effectively screened wheat germplasm for new sources of stem rust resistance (Jin and Singh, 2006; Njau et al., 2010). To protect and enhance the productivity of wheat in Pakistan, United State Department of Agriculture (USDA) and CIMMYT partnered under the Wheat Production Enhancement Program (WPEP) to characterize Pakistani wheat germplasm, including advanced breeding lines and commercial varieties, for resistance to the Ug99 race group. The objectives of this research were to assess the APR to stem rust against Ug99 resistance in Pakistani wheat germplasm/varieties, identify sources of resistance and promote their use in national wheat breeding programs.

Results and discussion

Spring wheat breeding lines and varieties were evaluated for seedling reactions at the CDL and displayed a range of infection types to the *Pgt* race TTKSK. Lines carrying the marker linked to the gene *Sr25/Lr19* and infection type data against the races TTKSK and TTKST are given in Table 1. Race TTKSK was virulent to 86% of the tested lines. We identified lines that were resistant both as seedlings and in the field. These included both released varieties and advanced lines (Table 2), which confirmed that lines with seedling resistance remained resistant when tested in the field, as previously reported (Kumssa et al., 2015). The low infection responses recorded in the field for seedling resistant lines may be due to the hypersensitive reaction, as reported elsewhere (Rubiales and Nicks, 2000).

Responses of lines carrying marker allele linked with gene Sr25

Based on molecular marker data, the lines postulated to possess the *Sr25* gene are listed in Table 1. Infection type data for the lines with marker detected stem rust resistance gene *Sr25/Lr19* indicated that 7 of the lines were susceptible (IT > 2) to race TTKSK (NR-379, SD-5, NR-421, V-10317, V3007, DN-92 and Imdad-05). We are unsure of the susceptibility of lines carrying *Sr25*, as TTKSK is considered avirulent to *Sr25* (Jin et al., 2008). The disagreement between phenotype and genotype could be attributed to (1) a false-positive, as the linked marker(s) used are probably not diagnostic for the gene(s); (2) false-susceptible infection types, especially for *Sr25*, which has an intermediate infection type; or (3) the heterogeneity of the lines. This also provides information on the effectiveness of using molecular markers to predict phenotypes. Two of these 7 lines (NR-421 and DN-92) were moderately susceptible at the adult stage with a severity of up to 30%, whereas lines V-10317 and V3007 were moderately susceptible to susceptible (MSS), with 15% and 20% severity values respectively. Responses moderately resistant (MR) and resistant to moderately resistant (RMR) were observed for the lines NR-379 and SD-5, with severities of 20% and 10% respectively. Line Imdad-05 showed a moderate response, with 40% rust severity. The rest of the 11 lines carrying *Sr25* gene were resistant to stem rust at both the seedling and adult plant stages. When tested for seedling resistance against TTKST, these lines also showed resistant infection types (Table 1), indicating they are potentially useful sources of resistance.

Field screening for adult-plant resistance (APR) in Kenya

Adult plant resistance is a useful trait for varietal selection. The Njoro field site is considered a hot spot for stem rust infection and artificial epidemics were created to ensure high disease pressure to evaluate wheat lines. Field screening of the baseline resistance study sets revealed a diversity of infection responses and severities. All types of host reactions and their combinations were observed, ranging from R to S types of infection response, whereas severity values in the field ranged from 0 to 100%.

Many of the lines tested were highly susceptible (70S-100S) in the field, showing high disease pressure, which was also evident from the response of susceptible check (Table 3). Similarly, some of the lines tested rusted slowly at the beginning but reached terminal severities of over 70% (data

Table 1. Responses of wheat lines carrying *Sr25* linked-marker allele both at seedling and adult plant stage.

No.	Nomenclature[1]	Parentage	IT[2] to race		APR-Kenya, 2011-13		Other SR gene
			TTKSK	TTKST	*severity (0-100)*	*FR[3]*	
1	NR-356(NARC-11)	OASIS/SKAUZ//4*BC/3/2*PASTOR	2	2-	5	MSS	Sr2
2	NR-379	WHEAR//2*PRL/2*PASTOR	3 LIF	3	20	MR	-
3	NR-378	WHEAR//INQALAB91*2/TUKURU	2	2	15	MS	-
4	NR-392	WHEAR/SOKOLL	2	2-/3	10	RMR	Sr2
5	NR 410	SOKOLL/3/PASTOR//HXL7573/2*BAU	2+	2+	10	MSS	-
6	NR 389	KRICHAUFF/2*PASTOR	2-/4	NT	10	MSS	Sr24/Lr24, Sr2
7	NR 395	OASIS/SKAUZ//4*BCN/3/2*PASTOR	2-;	2	10	R	
8	SD-5	VASCO x TJ-83	3 LIF	3	10	RMR	Sr24/Lr24
9	NR 421	CROC_1/AE.SQUARROSA210)//INQALAB91*2/KUKUNA/3/PBW343*2/KUKUNA	3	NT	30	MS	-
10	B-1 (RF)- 11	CHEWINK	2	2/3	20	MR	-
11	11C022	SOKOLL//SUNCO/2*PASTOR	2-	2+	20	MSS	Sr24/Lr24
12	CCRI-1	OR 9437534/SOKOLL//SOKOLL	0/23-	2+/3	10	M	Sr9a
13	CCRI-2	MTRWA92.161/PRINIA/5/SERI*3//RL6010/4*YR/3/PASTOR/4/….	23-	2+	40	M	-
14	CCRI-4	BERKUT/EXCALIBUR	2	2	30	M	-
15	V-10317	CHRZ//BOW/CROW/3/WBLL1/4/CROC_1/AE.SQUARROSA(213)//PGO	3+	NT	15	MSS	-
16	V3007	CHIR3/4/SIREN/ALTAR84/ - - - - - -	4	NT	20	MSS	-
17	DN-92	CROC_1/AE.SQUARROSA(224)//OPATA/3/KAUZ*2 …	4	NT	10	MS	-
18	Imdad-05	CHIL/2*STAR	3+	NT	40	M	-

[1]**Nomenclature** were based on the breeder coded number
[2]**IT**= Infection types where 0, ; , 1, 2, or combinations were considered low infection types (resistant), and 3 to 4 were considered high infection types (Stakman et al., 1962).
[3]**FR**= Field Response
LIF- Low Infection Frequency.
NT- Not Tested

Table 2. Wheat lines with both seedling and field resistance to stem rust Ug99.

No.	Nomenclature	Parentage	Seedling IT at CDL	Kenya SR Field data
1	Jauhar-78	NAYAB*(Fast Neutron 600 Rads) (Mutant Variety)	2	40MR
2	NR-356	OASIS/SKAUZ//4*BC/3/2*PASTOR	2	20M
3	NR-398	TC870344/GUI//TEMPORALERA87/AGR/3/2*WBLL1	2+3	10M
4	NR-407	UP2338*2/VIVITSI/3/FRET2/TUKURU//FRET2/4/OASIS/SKAUZ//4*BCN/3/2*PASTOR	2-	5M
5	CCRI-10	WON-D 22/HUBARA-13	2/3+	10M
6	CCRI-11	FIDIYA-23	2	20M
7	V-08082	V-87094/2*PAK81//SHAFAQ-06	2+	10M
8	V-08118	MILAN/S 87230//BABAX	2+3	15MS
9	V-10309	SOONOT-10	2-	5MR
10	V3010	BABAX/LRUZ//2/4/SNI/TRAP#1/3/KAUZ*2/ - - -	22+	15M
11	V3019	SW89.5277/BORL//SKAUZ	2+3	10MS
12	V-06BT005	FSD.-85/UFAQ-02/2/SH-02/3/CROW'S	2+3	15MS
13	09FJ21	99FJ03/PAK-81	0;1+	10M
14	09FJ34	ERAF2000/4/FONCHAN#3/TRT'S'//VEE#9/3/COOK/VEE'S'//DOVE'S'/SERI	0/3	5MS
15	99174	-	2+3	10M
16	11B2024	PFAU/MILAN/3/SKAUZ/KS94U215//SKAUZ	0	20RMR
17	11B2057	HUW234+LR34/PRINIA*2//WHEAR	2	40MR
18	B-2 (RF)- 11	SOONOT-10	2/3	15MR
19	B-2 (RF)- 17	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAUZ/6/…	1	30M
20	B-3 (RF)- 19	PFAU/MILAN/3/SKAUZ/KS94U215//SKAUZ	2	20MR

IT= Infection types where 0, ; , 1, 2, or combinations were considered low infection types (resistant), and 3 to 4 were considered high infection types (Stakman et al., 1962).

Table 3. Adult plant resistance in wheat lines for stem rust - Kenya 2011-13.

Nomenclature	Seedling data	APR-Kenya	Nomenclature	Seedling IT	APR-Kenya
Bahawalpur-97	3+	30M	V3027	4	10M
Ghaznavi	3+	30M	V3031	4	15MS
Punjab 81	4	20M	V3032	4	15MS
Barani-70	4	20M	V3033	3+	15MS
Sandal	3	40MR	V-9407	3	10M
SA 75	4	30M	NR-400	4	15M
D-97	4	30M	V-7/2011	4	20M
NR-333	4	20M	NRL-0707	3+	15MS
V-029221	33+	30M	AUP-1052	4	10MS
NR-333	3	30M	AUR-0809	4	10MS
11C018	3+	10M	AUP-1059	3+	10M
11C019	3+	10MS	NR 371	4	15M
DH-31	3+	5MR	NR-394	4	10MS
BARS-09	3+	15MS	NR-402	3+	5M
PR-102	3+	0,5R	NR-404	3+	5M
MPT (N)- 8	4	10M	NR-405	3	5M
MPT (N)- 13	3+	10MS	NR-406	3+	5M
MPT (N)- 29	3	10M	NR-408	3+	5M
MPT (RF)-2	3+	10M	NR-409	4	5M
MPT (RF)-9	4	15M	NR 413	3+	10M
AAS 2011	3	5MS	NR 415	4	5MS
11212	3	5M	NR 417	3+	10MS
11213	3+	10M	NR 418	4	10MS
11214	3+	15M	SUSSUI	3+	15M
11215	3	10M	V-10035	3	10M
11280	3	15M	11B2049	3+	20M
11282	3+	25MS	NR 402	3+	10MS
V-08171	3	5MS	NR 405	3	10RMR
V-08211	3+	5MS	NR 406	3	10M
V-08212	3+	10MS	15-03	3+	20M
V-08314	3+	20MS	AYT (2011-12) 29	3	20M
V-10306	4/;	15M	MPT (N)- 7	3+	20M
09FJ09	3+	20M	PR-98	3+	20MS
09FJ33	3lif	5MS	NIA-MN-8	3+	20MR
06FJS3013	3+	10MS	PR-104	3	30M
08FJ97	3	10MS	V-107 (NR-310)	3	30M
Mairaj-08	4	10M	V-112 (V-11164)	3	30M
10B9913	4	10M	V-117 (10BT002)	3+	15RMR
6422	4	10MS	V-120 (V-10193)	3+	20M
99115	3+	10MS	11C023	3+	20M
76346	3+	10M	V-11168	4	20M
10B-9346	4	10MS	AZRC-5	3+	20MS
10B-9369	3+	10M	CCRI-24	3 LIF	30M
10B-9370	3+	15M	CCRI-28	3+	30M
10B-9381	3+	10MS	AZRC-12	3+	40MR
10B-9383	3+/2	10M	AZRC-15	3+	15M
V3013	4	10M	AZRC-16	3+	20RMR
V3014	4	15MS	AZRC-17	3+	10RMR
V3015	4	15MS	AZRC-30	3+	20M
V3020	4	10MS	V3025	4	20M
V3023	4	10M	Check	4	100S

IT= Infection types where 0, ; , 1, 2, or combinations were considered low infection types (resistant), and 3 to 4 were considered high infection types (Stakman et al., 1962).

Table 4. Wheat lines with seedling resistance to Ug99 and susceptible at adult plant stage.

Nomenclature	Parentage	Seedling IT	Kenya field score
Khyber 87	KVZ/TRM//PTM/ANA	;2-3	50MSS
HB-10	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/…	2+	70M
NRL-0517	PASTOR/3/ALTAR84/AEGILOPS SQUARROSA(TAUS)//OPATA (SOKOLL)	2+	50MSS
TW96007	XIANG 820261x2-KAUZ/MILVUS	2+	40M
NR-383	MUNIA/CHTO/3/PFAU/BOW//VEE#9/4/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/5/BABAX/LR42//BABAX	2-;	90S
NR-389	KRICHAUFF/2*PASTOR	2-	40MSS
11C021	SOKOLL/EXCALIBUR	;2-	30MSS
MPT (N)- 19	PVN//KAUZ/PVN/3/PS-85	2-;	40MSS
11235	WATTAN/FSD//KRITATI	2+3	30MSS
V-09087	V-87094/2*INQ.91/3/SH88/PAK81/MH97	2lif	40MSS
WL-08109	SALEEM 2K / HD-2169	2,3+	25MSS
99108	CHEN/AE.SQ	2-	25MSS
V3008	CHIR3/4/SIREN//ALTAR84/ - - -	;3+lif	40MSS
V3022	HUW234+LR34*2/PASTOR	2/4	25MSS
NR-399	SOKOLL/3/PASTOR//HXL7573/2*BAU	2+	30MSS
V-10014	V-00125 / INQLAB-91	2/3 LIF	70M
11B2058	HUW234+LR34/PRINIA*2//KIRITATI	2	60M
11B2106	CB208/CB175	2/3 LIF	60MSS
11050	ZEMAMMRA-1/5/HAHN/2*WEAVER/4/BOW/CROW//BUC/PVN/3/2* VEE # 10	2	60M
11079	SOVA/4/MILAN/3/JUP/BJY//URES	2/3	60S
11092	KANCHAN/AUQAB-2000//AS-2002	2	60M
NRL 0902	PASTOR/WBLL1	2	40M
NR 407	UP2338*2/VIVITSI/3/FRET2/TUKURU//FRET2/4/OASIS/SKAUZ//4*BCN/3/2*PASTOR	0/3 LIF	60MSS
IEYT(2011-12) 10	ABUZIG-11/CHAN-8/Fret-2/FRET2*2/BRAMBLING	2-	60M
IEYT(2011-12) 40	AUQAB-2000/WBLL1*2/BRAMBLING	;13/4	60MSS
B-1 (N)- 6	PBW343*2/KUKUNA*2//YANAC	0;/3	60MSS
B-1 (RF)- 12	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92/4/BERKUT...	0/3	60MSS
B-2 (RF)- 19	PASTOR/WBLL1	0	60MSS
NN-Gandam-2	WEEBIL/4/SABUF*2//PAVON 76/3/MH97	0/3+	70S
SD-998	TJ-83 x 4085/3	;	60M
V-106 (V-11156)	PSN/BOW//MILAN/3/2*BERKUT	;2-	60S
V-126 (V-10110)	KAUZ/CMH77A-308//BAU/3/INQ-91	0;	40MSS
V-130	KANZU	;1-	60MSS
V-11172	KANCHAN//INQALAB91*2/KUKUNA	0/3+	60M
V-12001	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	0/3	40M
CCRI-12	LIS "S"/KVZ/TRM//PTM/ANA X UP262	0;	50M
CCRI-13	F-3-71/TRM X F. SARHAD	2	30M
CCRI-16	CANDIAN/CUNNINGHAM//KENNEDY	23	60MSS

IT= Infection types where 0, ; , 1, 2, or combinations were considered low infection types (resistant), and 3 to 4 were considered high infection types (Stakman et al., 1962).

Table 5. Narrative of baseline resistance study (BRS) sets screened for *Puccinia graminis* f. sp. *tritici* at the seedling and adult plant stages.

Year	BRS designation	Total number of lines	No. of contributing institutes in Pakistan
2010-11	1st BRS	195	9
2011-12	2nd BRS	271	8
2012-13	3rd BRS	241	19

not shown). Adult plant resistance was exhibited by 101 lines with diverse field reactions (MS to RMR) and disease severities ranging from 5 to 40%, based on the modified Cobb scale (Table 3). All of these lines displayed seedling susceptibility (IT 33+ to 4), implying the presence of APR. Four entries (Sandal, DH-31, NIA-MN-8 and AZRC-12) displayed MR, with disease severities reaching 40%. Moderate responses (M or MRMS) were observed for most lines that displayed a severity of up to 30%. Resistant-to-moderately resistant responses and disease severities of up to 20% were observed for lines NR-405, SD-5, V-117, AZRC-16 and AZRC-17 (Table 3). These lines are the most desirable ones for use in breeding for durable resistance to stem rust. Previous research emphasized that lines showing seedling susceptibility but adult plant resistance to Ug99 in the field can be useful sources of Ug99 resistance (Nazari et al., 2008; Rouse et al., 2011; Singh et al., 2008). There were lines that showed resistance at seedling stage but were moderate susceptible in the field at the adult stage (Table 4). This type of response could be attributed either to major gene(s) operating only at the seedling stage, weather factors or disease/inoculum pressure in the field (Hickey et al., 2012). Understanding the resistance in these lines could facilitate their better use in breeding for stem rust resistance, possibly leading gene combinations for resistance against multiple *Pgt* races.

During the three years of study reported, the outcome-driven science collaboration under the Wheat Production Enhancement Program for Pakistan (WPEP) increased the frequency of stem rust resistance in Pakistan's national breeding programs. In 2010, only 15% of Pakistani wheat germplasm was resistant to Ug99 (TTKSK), but the number increased to 49% in 2012-13. The resistant germplasm provided national partners a range of sources of stem rust resistance and the associated knowledge to increase genetic gains for stem rust resistance in their breeding programs, with the release of two Ug99 resistant varieties NARC-2011 and Pak-2013 and a number of candidate in the pipeline.

Materials and methods

Experimental materials

Baseline resistance study (BRS) sets were prepared by collecting advanced spring wheat breeding lines and varieties from national wheat breeding programs across Pakistan. Three sets designated as 1BRS, 2BRS and 3BRS and having 195, 271 and 241 entries were assembled during 2010-11, 2011-12 and 2012-13, respectively, and used for this study (Table 5). These sets comprised old and newly-released wheat varieties and advanced lines, as well as germplasm introduced to Pakistan from CIMMYT.

Pgt races used in seedling and field evaluation

All the lines were tested for seedling resistance at the cereal disease laboratory, University of Minnesota-St. Paul, using the eastern African *Pgt* race (TTKSK = Ug99). Lines that were initially resistant to race TTKSK were further screened against the variant TTKST. The seedlings were fertilized at emergence stage with 20-20-20 liquid fertilizer and shifted to a clean (rust free) glass house until inoculation. Briefly, seedlings were inoculated when plants fully expanded primary leaves (7-9 days of sowing) with urediniospores of stem rust isolates suspended in mineral oil (~5.0 mg of rust in 0.8 ml oil). After inoculation, plants were dried for ~10 minutes and shifted to dew chamber for 14-16 hours in darkness at 18-21^{o}C temperature and 100% relative humidity. Next day plants were slowly dried and exposed to fluorescent lights for 4 hours. After dew period, plants were moved to a dew chamber at 18-22^{o}C and 16 hours of light for 6 days and were scored on the 14th day after inoculation. For field evaluation spreader rows were planted perpendicularly and around the experimental plots and also inoculated with a bulk collection of Ug99 TTKSK and TTKST with added virulence to *Sr24* to create an artificial disease epidemic. Urediniospores were collected from inoculum increase plots planted with wheat varieties possessing both *Sr31* and *Sr24*, which are expected to select for *Pgt* race TTKST, the Ug99 variant with virulence to both *Sr31* and *Sr24* (Njau et al., 2012).

Seedling evaluation

For seedling evaluation five to six untreated seeds of each variety/line were planted in pots at a depth of 1 cm, along with seeds of susceptible control McNair701 during 2010-11 (1BRS), 2011-12 (2BRS) and 2012-13 (3BRS). Procedures for inoculation and disease assessment followed Jin et al. (2007). Plants were scored on the 14th day after inoculation using a 0-4 Stakman et al. (1962) scale. Infection types 0-2++ were considered low, indicating host resistance, while ITs 3-4 were considered high, indicating host susceptibility. When low and high ITs were present on the same leaf, the plant was considered resistant. Lines were classified as heterogeneous when both resistant and susceptible plants were present.

Field evaluation

Wheat lines were sown in double rows on plots 1 m in length for field screening at the wheat research institute facility of Kenya Agricultural and Livestock Research Organization (KALRO) in Njoro, Kenya, during 2010-11, 2011-12 and 2012-13. Lines were scored at the soft dough stage for stem rust severity on a scale of 0-100% stem area affected using the modified Cobb scale (Peterson et al., 1948). Infection response was scored using categorical scores based on the size of the stem rust pustules and the amount of associated chlorosis and necrosis. Infection response categories included the following, either singly or in combination: resistant (R), moderately resistant (MR), intermediate (M), moderately susceptible (MS), and susceptible (S) (Nzuve et al., 2012). Combinations of categories were recorded when two distinctly different infection responses occurred on a single stem (i.e., MR-MS ratings indicated MR pustules on the same stem as MS pustules). The predominant category was listed first, such that MR-MS differed from MS-MR. When lines with different infection responses or severities on the same plant were observed, a comma (,) was used to separate the readings.

DNA marker analysis

The three BRS sets were screened with a 'Gb' molecular marker linked to *Sr25/Lr19* (Prins et al., 2001) at the USDA-ARS Eastern Regional Genotyping Laboratory, Raleigh, NC. Methods for genotyping followed Olson et al. (2010).

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