RESEARCH

Resistance to Race TTKSK of *Puccinia graminis* f. sp. *tritici* in Emmer Wheat

Pablo D. Olivera, Ayele Badebo, Steven S. Xu, Daryl L. Klindworth, and Yue Jin*

ABSTRACT

Race TTKSK (Ug99) of *Puccinia graminis* f. sp. *tritici* is a serious threat to wheat production worldwide because of its wide virulence on many cultivars and rapid spread. Emmer wheat [*Triticum turgidum* L. subsp. *dicoccon* (Schrank) Thell.] is known to be a source of resistance to stem rust but has not been evaluated against race TTKSK. In attempts to identify and characterize stem rust resistance genes effective against race TTKSK at the seedling stage, we evaluated 359 accessions of emmer wheat with race TTKSK and other races with broad virulence. A high frequency (31.8%) of accessions were resistant to TTKSK at the seedling stage with low infection types ranging from $2^{=}$ to 2^{+}. Thirty-seven accessions exhibited a resistant to moderately resistant response in Debre Zeit, Ethiopia, and St. Paul, MN, nurseries in 2010 and 2011. Studies were conducted to determine the inheritance of TTKSK resistance in five accessions at the seedling stage. Results from evaluating F_2 and $F_{2:3}$ generations revealed that resistance was conferred by single genes. One additional gene effective against race TTTTF was identified in the resistant parents. Results from this study indicated that emmer wheat is a source of resistance to race TTKSK and may contribute novel resistance genes. Since emmer wheat shares the same genome as durum [*Triticum turgidum* subsp. *durum* (Desf.) Husn.] wheat and is in cultivated form, resistance genes should be easily transferred to durum wheat by conventional breeding approaches.

P.D. Olivera, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108; A. Badebo, Ethiopian Institute of Agricultural Research (EIAR), Debre Zeit, Ethiopia; S.S. Xu and D.L. Klindworth, USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58108; Y. Jin, Department of Plant Pathology, University of Minnesota, St. Paul, MN, and USDA-ARS, Cereal Disease Laboratory, St. Paul, MN 51108. Received 7 Dec. 2011. *Corresponding author: (Yue.Jin@ars.usda.gov).

Abbreviations: APR, adult plant resistance; IT, infection type; NSGC, National Small Grain Collection.

Stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. and E. Henn., is one of the most destructive diseases of durum and common or bread wheat (*Triticum aestivum* L.) worldwide. In particular, a group of TTKS (or Ug99) related races possess broad virulence to wheat cultivars worldwide, and only a few genes in adapted cultivars are effective against these races (Jin and Singh, 2006; Jin et al., 2007; Pretorius et al., 2011). Since first reported in 1999 (Pretorius et al., 2000), TTKSK and its variants have been found throughout eastern and southern Africa (Jin et al., 2008; Singh et al., 2011; Visser et al., 2010; Wanyera et al., 2006; Wolday et al., 2011) and Iran (Nazari et al., 2009). The lack of resistance in adapted germplasm coupled with its rapid evolution and spread urgently requires the identification and introgression of effective resistance genes into adapted wheat. Wild and cultivated relatives of wheat are known to be a good source of stem rust resistance genes. A number of resistance genes derived from wild relatives of wheat appeared to be more effective against the TTKS races of *P. graminis* f. sp. *tritici* than genes of wheat origin (Jin et al., 2007; Singh et al., 2006). However, the use of

Published in Crop Sci. 52:2234–2242 (2012).
doi: 10.2135/cropsci2011.12.0645

resistance from cultivated wheat relatives may be preferred to hasten the introgression process.

Emmer wheat is a tetraploid ($2n = 4x = 28$, genome AABB) ancient hulled wheat. It is the oldest cultivated wheat (Kuckuck, 1970), and it is still grown in Ethiopia (Beteselassie et al., 2007), the Middle East, and Europe (Hammer and Perrino, 1984; Stallknecht et al., 1996). Emmer wheat is known to be a good source of resistance to wheat diseases (Gras, 1980; Oliver et al., 2008), pests (Liu et al., 2005), and environmental stresses (Sayed, 1985). Emmer wheat has contributed important genes for stem rust resistance, including *Sr2* from Yaroslav emmer (McFadden, 1930) and *Sr13* and *Sr14* from Khapli (CItr 4013) (Heermann and Stoa, 1956). Reaction of emmer wheat to the recently emerged race TTKSK and its variants has not been characterized. The high variability observed in emmer wheat for agronomic and quality traits (Damania et al., 1992; Pflüger et al., 2001; Sayed, 1985) opens the possibility for the identification of new and diverse stem rust resistance genes if germplasm collections are extensively screened. In addition, since emmer and durum wheat have the same genome constitution and share complete genomic compatibility (Yanchenko, 1985), resistance to stem rust from emmer could be easily introgressed into durum wheat. To identify novel stem rust resistance genes effective against TTKSK and its variants, we evaluated emmer wheat for resistance to stem rust races with broad virulence at the seedling and adult stage and conducted preliminary studies on the genetic basis of stem rust resistance.

MATERIALS AND METHODS

Germplasm

A total of 359 accessions of emmer wheat deposited at the USDA-ARS National Small Grain Collection (NSGC) (Aberdeen, ID) were evaluated in this study. Six emmer wheat accessions were selected for an inheritance and allelism study based on their reaction to races TTKSK, TRTTF, and TTTTF. Five accessions (PI 101971, PI 193883, PI 217640, PI 298582, and PI 319869) were used as the resistant parents, and one accession (CItr 7966) was used as the susceptible parent. In total, 11 crosses were developed to investigate the number of genes conferring resistance or to determine the relationship between resistant parents. The F_1 plants were grown and selfed to produce F_2 populations. Individual F_2 plants were then selfed to produce $F_{2:3}$ families.

Inoculation, Incubation, and Disease Assessment

Seedling Evaluations

The entire emmer wheat collection of the NSGC was evaluated for reaction to three *P. graminis* f. sp. *tritici* races with broad virulence and different geographic origin: TTKSK (Kenya), TRTTF (Yemen), and TTTTF (United States). Accessions exhibiting resistance to race TTKSK were further characterized for their reaction to six additional U.S. races (TPMKC, RKQQC, RCRSC, QTHJC, QCCLC, and MCCFC). The race designation is based on the letter code nomenclature system (Roelfs and Martens, 1988; Roelfs et al., 1993), modified to further delineate races in the TTKS group (Jin et al., 2008). Information about the stem rust isolates used in the disease phenotyping tests is summarized in Table 1. Five seedlings per accession were inoculated on fully expanded primary leaves 8 to 9 d after planting. Experimental procedures in inoculation and disease assessment were described by Jin et al. (2007). Wheat cultivar McNair 701 (Cltr 15288) was used as the susceptible control. All the assessments were done with one replicate and were repeated once.

Adult Evaluation

All the emmer entries were evaluated for resistance in field tests in stem rust nurseries planted in St. Paul, MN, (April to July 2010) and in Debre Zeit, Ethiopia (June to October 2010). Accessions rated as resistant with a maximum 30% stem rust severity and moderately susceptible infection response or lower in the 2010 Debre Zeit field nursery were further evaluated in the Debre Zeit and St. Paul nurseries in May 2011 and July 2011, respectively. In St. Paul, the nursery was inoculated with a composite of six U.S. races (TPMKC, RKQQC, RCRSC, QTHJC, QFCSC, and MCCFC). Accessions were planted in single 1-m-row plots. In 2010, the Debre Zeit nursery was artificially inoculated with race TTKSK and a bulk of Ethiopian isolates (with unknown race identities) collected from durum lines at the Debre Zeit Research Center at a ratio of 50:50 whereas in 2011 the inoculum was composed only of a bulk of Ethiopian isolates. Accessions were planted in double 1-m-row plots. In both nurseries, continuous rows of stem rust spreader (mixture of susceptible cultivars) were planted perpendicular to all entries to facilitate inoculum buildup and uniform dissemination. Spreader rows were artificially inoculated by needle injection two to three times at a weekly interval starting at stem elongation (stage Zadoks 31) (Zadoks et al., 1974). Urediniospores were suspended in distilled water plus one drop of Tween 20 per 0.5 L of suspension and delivered with a hypodermic syringe into the base of the stems. Disease assessment was done at the soft-dough stage of plant growth. Due to differences in maturity among emmer entries, three data points were recorded at 1 and 2 wk interval starting when the first entries reach the soft-dough stage. Plants were evaluated for their infection response (pustule type and size) (Roelfs et al., 1992) and stem rust severity following the modified Cobb scale (Peterson et al., 1948). Infection responses resistant and resistant to moderately resistant were considered as indicative of resistance, and infection responses moderately resistant, moderately resistant to moderately susceptible, and moderately susceptible with a maximum 30% stem rust severity of moderately or intermediate resistant.

Inheritance and Allelism Studies

To determine the genetic control of resistance to wheat stem rust at the seedling stage, crosses between resistant and susceptible emmer wheat accessions were evaluated. The F_1 plants were evaluated for response to race TTKSK to determine gene action. The F_2 populations were evaluated against races TTKSK, TRTTF, and TTTTF to determine the inheritance of resistance. The $F_{2:3}$ families were evaluated only against race TTKSK. The $F_{2:3}$ families from CItr 7966 × PI 217640 were also evaluated against race TRTTF. Twenty plants from each $F_{2:3}$ family were tested. According to Hanson (1958), this $F_{2:3}$

Table 1. Isolate designation, origin, and virulence phenotype of *Puccinia graminis* f. sp. *tritici* races used to evaluate resistance in emmer wheat (*Triticum turgidum* subsp. *dicoccon*).

Race	Isolate	Origin	Avirulence and virulence formula
TTKSK	04KEN156/04	Kenya	*Sr24 36 Tmp/Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 31 38 McN*
TRTTF	06YEM34-1	Yemen	*Sr8a 24 31/Sr5 6 7b 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp*
TTTTF	02MN84A-1-2	United States	*Sr24 31/Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp*
TPMKC	74MN1409	United States	*Sr6 9a 9b 24 30 31 38/Sr5 7b 8a 9d 9e 9g 10 11 17 21 36 McN Tmp*
RKQQC	99KS76A-1	United States	*Sr9e 10 11 17 24 30 31 38 Tmp/Sr5 6 7b 8a 9a 9b 9d 9g 21 36 McN*
RCRSC	77ND82A	United States	*Sr6 8a 9e 11 24 30 31 38 Tmp/Sr5 7b 9a 9b 9d 9g 10 17 21 36 McN*
QTHJC	75ND717C	United States	*Sr7b 9a 9e 24 30 31 38 36 Tmp/Sr5 6 8a 9b 9d 9g 10 11 17 McN*
QFCSC	06ND76C	United States	*Sr6 7b 9b 9e 11 24 20 31 36 38 Tmp/Sr 5 8a 9a 9d 9g 10 17 21 McN*
QCCLC	07WA140-17-1	United States	*Sr6 7b 8a 9b 9d 9e 10 11 24 30 31 38 Tmp/Sr5 9a 9g 17 21 McN*
MCCFC	59KS19	United States	*Sr6 8a 9a 9b 9d 9e 11 21 24 30 31 36 38/Sr5 7b 9g 10 17 McN Tmp*

family size has a 99% probability of distinguishing between segregating and nonsegregating families for monogenic inheritance. The allelism tests involved testing F_2 populations derived from crosses between two resistant accessions. The χ^2 test was applied to determine the goodness-of-fit to expected genetic ratios in the F_2 and $F_{2:3}$ generations. Additionally, χ^2 value also was calculated from a contingency table to assess the relationship of the reactions of $F_{2:3}$ families from the CItr 7966 × PI 217640 cross to races TTKSK and TRTTF.

Table 2. Number and percentage of emmer wheat (*Triticum turgidum* subsp. *dicoccon*) accessions exhibiting resistant, susceptible, and heterogeneous† reaction to *Puccinia graminis* f. sp. *tritici* races TTKSK, TRTTF, and TTTTF.

	TTKSK		TRTTF		TTTTF	
	No.	%	No.	%	No.	%
Resistant	107	31.8	123	36.6	148	44.8
Susceptible	215	64.0	199	59.2	176	53.3
Heterogeneous	14	4.2	13	4.2	6	1.8

†Accessions that contained both resistant and susceptible plants.

RESULTS AND DISCUSSION

Seedling Evaluation

A high frequency of resistance to the three races evaluated at the seedling stage was observed in this emmer wheat collection, as 107 (31.8%), 123 (36.6%), and 148 (44.8%) accessions exhibited a resistant reaction to race TTKSK, TRTTF, and TTTTF, respectively (Table 2). Ninety (25.1%) accessions were resistant to all three races. These results demonstrate that emmer wheat is a rich source of stem rust resistance. Resistance to wheat stem rust at the seedling stage was reported by Beteselassie et al. (2007) in Ethiopian emmer wheat, as 18 of 41 accessions were resistant to a bulk of six local isolates. The frequency of resistance to race TTKSK observed in this emmer collection was similar to the one reported in wild emmer [*T. turgidum* subsp. *dicoccoides* (Körn. ex Asch. & Graebn.) Thell.] (Olivera et al., 2009) and other cultivated tetraploids (Olivera et al., 2011). The characteristic infection types of the emmer wheat resistant accessions to the three races ranged from 2^- to 2^+, and lower infection types (ITs) (IT = 0; to 1) were observed in nine (8.2%), 15 (13.6%), and 33 (30.0%) accessions to races TTKSK, TRTTF, and TTTTF, respectively (data not shown). The predominance of infection types ranging from 2^- to 2^+ appears to be a common feature in cultivated tetraploid wheat as similar results were observed in wild emmer, Polish, Oriental, and Pollard wheat (P. Olivera and Y. Jin, unpublished data, 2011).

Ten different infection type patterns were obtained when the 107 TTKSK-resistant accessions were characterized with six additional U.S. races (Table 3). Eighty-two (76.7%) accessions were resistant to all evaluated races whereas 13 accessions were susceptible to only one race. Six patterns were not duplicated. These results suggest that resistance to TTKSK in emmer wheat is not highly diverse but may be useful against a broad spectrum of races. A higher level of diversity for stem rust resistance was observed in free threshing tetraploid wheat (McVey, 1991). In our study, we observed a lower level of diversity of stem rust resistance than that reported by McVey (1991), but this result may be explained by the fact that we characterized the accessions that were resistant to race TTKSK only. When we evaluated the accessions susceptible to race TTKSK against four additional U.S. races, we identified over 30 infection type patterns (data not shown). Emmer wheat has contributed race specific stem rust resistance genes. In particular, *Sr13* and *Sr14* were derived from Khapli emmer (Heermann and Stoa, 1956). These genes exhibit a moderate level of resistance (IT = 2^+) and susceptibility (IT = 4) to race TTKSK, respectively (Jin et al., 2007). In addition, *Sr13* exhibits a high reaction (IT = 3^+) to race TRTTF (Olivera et al., 2012). The infection types to races TTKSK, TRTTF, and TTTTF observed in the TTKSK resistant accessions indicate that resistance genes present in these accessions were likely not *Sr13* or *Sr14*.

Adult Evaluation

Minor differences in maturity were observed among the emmer entries. At both locations, about 80% of the accessions reached the soft-dough stage of plant growth on the same week (90–97 d after planting). Five percent of the entries reached the soft-dough stage 8 to 10 d before

Table 3. Number and infection type† patterns of emmer wheat (*Triticum turgidum* subsp. *dicoccon*) accessions resistant to race TTKSK of *Puccinia graminis* f. sp. *tritici* at the seedling stage.

Number of lines	Races								
	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	RCRSC	QTHJC	QCCLC	MCCFC
82	L	L	L	L	L	L	L	L	L
6	L	H	L	L	L	L	L	L	L
4	L	L	H	L	L	L	L	L	L
1	L	L	L	H	L	L	L	L	L
1	L	L	L	L	L	H	L	L	L
1	L	L	L	L	L	L	L	L	H
1	L	H	H	L	L	L	L	L	L
1	L	H	L	H	L	L	L	L	L
1	L	H	H	H	H	H	L	L	L
1	L	L	L	H	H	H	H	H	H

†Infection types observed on seedlings at 14 d postinoculation using a 0 to 4 scale according to Stakman et al. (1962), in which L stands for low infection types 0, ;, 1, 2, or combinations and H stands for high infection types 3 or 4.

Table 4. Number and percentage of emmer wheat (*Triticum turgidum* subsp. *dicoccon*) accessions† in field evaluations in Debre Zeit and St. Paul in 2010 and 2011 according to infection response and disease severity.

Disease evaluation		2010				2011			
		Debre Zeit		St. Paul		Debre Zeit		St. Paul	
Infection response‡	Severity§	No.	%	No.	%	No.	%	No.	%
R	T–30	13	4.0	51	16.0	0	0.0	25	15.9
R	31–60	0	0.0	0	0.0	0	0.0	0	0.0
RMR–MRR	T–30	11	3.4	25	7.9	5	3.2	22	14.0
RMR–MRR	31–60	0	0.0	5	1.6	0	0.0	0	0.0
MR	T–30	27	8.3	21	6.6	19	12.1	18	11.5
MR	31–60	0	0.0	8	2.5	0	0.0	0	0.0
MRMS–MSMR	T–30	74	22.7	17	5.3	20	12.7	11	7.0
MRMS–MSMR	31–60	7	2.1	28	8.8	1	0.6	3	1.9
MS	T–30	32	9.8	6	1.9	6	3.8	6	3.8
MS	31–60	10	3.1	25	7.9	1	0.6	14	8.9
MS	61–100	0	0.0	3	0.9	0	0.0	0	0.0
MSS–SMS	T–30	40	12.3	1	0.3	11	7.0	6	3.8
MSS–SMS	31–60	37	11.3	36	11.3	33	21.0	31	19.7
MSS–SMS	61–100	0	0.0	4	1.3	0	0.0	0	0.0
S	T–30	21	6.4	0	0.0	9	5.7	0	0.0
S	31–60	54	16.6	37	11.6	40	25.5	18	11.5
S	61–100	0	0.0	51	16.0	9	5.7	1	0.6
Total		326	100	318	100	157	100	157	100

†Accessions characterized as resistant to moderately resistant with a maximum 30% stem rust severity and maximum moderately susceptible infection response at the 2010 Debre Zeit field nursery were evaluated in 2011 in St. Paul and Debre Zeit.

‡Pustule type and size (Roelfs et al., 1992). R, resistant; RMR, resistant to moderately resistant; MRR, moderately resistant to resistant; MR, moderately resistant; MRMS, moderately resistant to moderately susceptible; MSMR, moderately susceptible to moderately resistant; MS, moderately susceptible; MSS, moderately susceptible to susceptible; SMS, susceptible to moderately susceptible; S, susceptible.

§Stem rust severity following the modified Cobb scale (Peterson et al., 1948). T, traces.

whereas 15 to 17% of the entries reached the soft-dough stage 2 wk after the main group. A higher frequency of susceptible accessions was observed in the late maturity group. Resistance to wheat stem rust at the adult stage was observed in emmer, as 164 (50.3%) and 161 (50.6%) accessions exhibited resistant to moderately resistant response in the 2010 Debre Zeit and St. Paul field nurseries, respectively (Table 4). Accessions characterized as resistant with a maximum 30% stem rust severity and maximum moderately susceptible infection response in the Debre Zeit field nursery in 2010 were further evaluated in both nurseries in 2011. Fifty-one (32.5%) and 85 (54.1%) accessions exhibited resistant to moderately resistant response in the 2011 Debre Zeit and St. Paul field nurseries, respectively (Table 4). The higher disease pressure and the inoculum composition in the off-season nursery (May 2011) in Debre Zeit may explain why only 32% of the accessions resistant in 2010 remained resistant in the 2011 evaluations. The lower frequencies of resistance in Debre Zeit compared to St. Paul in 2010 and 2011 may indicate the presence of races of *P. graminis* f. sp. *tritici* in the Debre Zeit nursery that overcome resistance genes in emmer wheat that are effective against U.S. races.

Table 5. Stem rust reaction of emmer wheat (*Triticum turgidum* subsp. *dicoccon*) accessions in field evaluations in Debre Zeit and St. Paul in 2010 and 2011, and seedling evaluations to races TTKSK, TRTTF, TTTTF, TPMKC, RKQQC, QTHJC, and MCCFC of *Puccinia graminis* f. sp. *tritici*.

	Field evaluation†				Seedling evaluation‡						
	Debre Zeit		St. Paul		TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
Accession	2010	2011	2010	2011	04KEN156/04	06YEM34-1	01MN84A-1-2	74MN1409	99KSD76A-1	75ND717C	59KS19
CItr 4013	40MSMR	30MRMS	30RMR	10RMR	33+	4	3+	3–3	2-;	2-;	;1-
CItr 12213	10MRR	20MR	30MR	20MR	2N	22-	;2-N	2+	2-	2-N	2-N
CItr 12214	20MRMS	30MR	30MR	30MR	2N	22-;	2-N	22+N	2-	2-N	2–2N
PI 41024	10R	20MR	40MRR	20MRR	2-N	2-	2-N	2-N	2-	2-	2-;N
PI 94624	20MR	20 MS	10R	10R	22+	4	;N2-	31;	;N2	2-	X
PI 94625	20MR	10 MR	5R	10RMR	33-	4	;13	31;	X-	;C	X-
PI 94626	10MR	15 MRMS	20R	15MRR	22+	3+	1;	3+	;C1-	2+	2-;C
PI 94635	10MR	10 MR, 20S		15RMR	;C2-	2=1;	2-;	;C2-	;C2-	2-;	;C
PI 94656	10R	20MRMS	20RMR	20MRR	2-N	2-	;2-	2-N	2-	2-	;N2-
PI 94665	20MRMS	30MSMR	20RMR	30MS	33+	33+	3+	2-/3–3	2=;	3-	3–3
PI 94674	30MRMS	30MS	30MR	5R	;2-N	;1	;N1-	2-;	;CN	2-;	;CN1-
PI 94747	5R	10MRR	30RMR	20RMR	2-N	2-;	2-;	2-N	2-	2-N	;N2-
PI 101971	20R	20RMR	30MR	25MR	2-N	2-;	;2-	2-N	2-	2-;N	2-N
PI 133134	5R	20MR	40MRR	20MR	22-	2-;	;2-	2-N	2-;	2-;N	2-;N
PI 193879	20MR	20 MR, 40SMS	50MR	40MRMS	2-N	2	;N	2-N	2-;N	2-;	;1
PI 193880	30MSMR	20MR, 50SMS	40MRMS	30MRMS	;12-	2–2=	;2-	;12-	;12-	;2–1	;1
PI 193881	30MSMR	30MSS	40MR	30MR	3+	3+3	33+	3–3	3	3–2+	3-
PI 193882	40MRMS	30MR, 30S	50MRMS	30MS	;2-	2-	;2-	2-	22-	2-;	;1
PI 193883	20MR	30MR, 40S	30MR	30MS	;2-	2–2	2-	2–2	2-;	;2-	;2-
PI 197481	20MSMR	20MSMR	30MRR	20MR	;	;1-	;-	;C	;C	;2–1	;CN
PI 217637	10R	20MRMS	30RMR	20RMR	2	22-	2-	2-N	2-	2-;	2-;N
PI 217639	5R	20RMR	30R	30RMR	2-N	2	2-;N	2-;	2-;N	;2-	2-N;
PI 217640	10R	20RMR	20R	30R	2-N	2-;	2-;N	2-;N	2-;	2-;	;N2-
PI 225332	10MR	20MSMR	10R	5R	3–2;	32;	;32	32;	;23	;32	;23-
PI 248991	20RMR	25MRMS	50MR	30MR	2-N	2-	3-	;N2-	2-	2-	2-;N
PI 254163	20MRMS	30MS	10R	10R	2-;N	4	3	3-;	2;N	3-;N	3-;
PI 254165	10MSMR	30MSMR		5R	32;	3+2;	3–2;	3–2;	3–2;	3–2;	32;
PI 254167	30MSMR	30MSMR	10R	10R	3	3+	;N	2+3	3-;	32;	13;
PI 254175	10MRMS	30MSMR	5R	5R	3–2;	4	32;	32;	23-;	32;	;3-
PI 275996	20MRR	30MR	30R	20MR	2+3-	2-	;3-	2+3-	2-	;3-	2–2
PI 298582	40MSMR	40MS	50MRMS	30MS	22+	22+	2	22+	22+	22+	2-;
PI 310471	20RMR	40MRMS	40MR	30MRR	2	22-	2-	2	2-	2-	2N
PI 319869	30MS	30MSS	50MR	30MR	22-	2	2-	2–2	2-	2-	2-;
PI 322232	20MR	30MRMS	40MR	30MR	22+	22+	2-;	2N	2-	2-N	2-;
PI 324076	20RMR	30MR	40MRMS	20MR	2+	22+	;2-	2–2N	2-;	2-N	2-N
PI 352548	20MR	20MR	20MR	20MSMR	22-	2	2	2-N	2-	2–2N	2-N
PI 355477	20RMR	30MRMS	30MR	20R	2	2	;C	2+2	2	2N	2-N
PI 470739	20MRMS	10MR, 5MS	5R	5R	32	32	32;	2–2	2-	2-	X-
PI 532304	10MRMS	20MRMS	40RMR	20MR	3+	3+	3+3	3-	3	2+	33-

†Plants evaluated for infection response (Roelfs et al., 1992) and severity (0–100) following the modified Cobb scale (Peterson et al., 1948). R, resistant; RMR, resistant to moderately resistant; MRR, moderately resistant to resistant; MR, moderately resistant; MRMS, moderately resistant to moderately susceptible; MSMR, moderately susceptible to moderately resistant; MS, moderately susceptible; MSS, moderately susceptible to susceptible; SMS, susceptible to moderately susceptible; S, susceptible.

‡Infection types observed on seedlings at 14 d postinoculation using a 0 to 4 scale according to Stakman et al. (1962), where infection types (ITs) of ;, 1, 2, or X are considered as a low IT and ITs of 3 or higher are considered as a high IT. N denotes excessive necrosis. "/" indicated accessions were heterogeneous with dominant type given first.

Forty-one (26.1%) accessions were resistant to moderately resistant in the two nurseries in 2011, and 39 accessions exhibited a resistant to moderately resistant response in the Debre Zeit and St. Paul nurseries in 2010 and 2011. Twenty-eight of these resistant accessions in field evaluations exhibited a resistant reaction to races TTKSK, TRTTF, TTTTF, TPMKC, RKQQC, QTHJC, and MCCFC in seedling evaluations (Table 5). Selection of resistance based on seedling tests can be effective, as resistance detected at the seedling stage remains effective at the adult stage. Four accessions (CItr 4013, PI 94665, PI 193881, and PI 532304) that were susceptible to races TTKSK, TRTTF, and TTTTF in seedling evaluations remained resistant to moderately resistant across the two

Table 6. Disease reaction of F_1 plants and segregation of F_2 populations of various crosses of emmer wheat (*Triticum turgidum* subsp. *dicoccon*) to race TTKSK of *Puccinia graminis* f. sp. *tritici*.

	F_1 plants		F_2 plants				
Cross†	No. plants tested	Infection type	Resistant (R)	Susceptible (S)	Ratio tested (R:S)	χ^2	*p*-value
CItr 7966 (S) × PI 101971 (R)	2	2^+3^-	81	24	3:1	0.257	0.612
CItr 7966 (S) × PI 193883 (R)	2	$3/3^+$	31	73	1:3	1.282	0.258
CItr 7966 (S) × PI 217640 (R)	2	2^+	92	29	3:1	0.069	0.793
CItr 7966 (S) × PI 298582 (R)	2	4	20	92	1:3	3.048	0.081
CItr 7966 (S) × PI 319869 (R)	2	$3/33^+$	25	98	1:3	1.434	0.231
PI 101971 (R) × PI 217640 (R)	2	2^-N	112	0	–	–	–
PI 193883 (R) × PI 298582 (R)	2	2	105	0	–	–	–
PI 193883 (R) × PI 319869 (R)	2	2^-	145	0	–	–	–
PI 319869 (R) × PI 298592 (R)	2	2^+	132	0	–	–	–
PI 217640 (R) × PI 193883 (R)	2	2^+	101	6	13:3	0.075	0.784
PI 298582 (R) × PI 101971 (R)	2	22^+	146	6	13:3	1.772	0.183

†Female parent × male parent; (R) and (S) indicate the resistant and susceptible parent, respectively.

evaluations performed at the adult stage (Table 5). These results may indicate the presence of genes for adult plant resistance (APR) in these accessions. *Sr2*, an important gene for stem rust resistance (McIntosh, 1988) was transferred to hexaploid wheat from Yaroslav emmer (McFadden, 1930). *Sr2* is reported in association with pseudo-black chaff, a black pigmentation that develops in the glumes and around stem internodes (Hare and McIntosh, 1979). Pseudo-black chaff and black internode were observed in a number of emmer accessions (PI 94624, PI 94625, PI 94626, PI 94674, PI 225332, PI 248991, PI 254163, PI 254165, PI 254167, PI 254175, PI 352548, and PI 532304). These accessions are being investigated further for the presence of *Sr2* based on stem rust evaluations in seedling and adult plant stages as well as available markers (Mago et al., 2011). Accessions CItr 4013, PI 94665, and PI 193881 did not exhibit the *Sr2* phenotype. Additional data are needed to confirm the presence of APR genes in these accessions and determine the genetic relationship between these genes and *Sr2*.

Inheritance and Allelism Studies

The infection types displayed by the F_1 plants (IT = 2^+ to 2^+3^-) from the crosses between the resistant accessions PI 101971 and PI 217640 and the segregation ratios observed in the resulting F_2 progeny (fit to a 3:1 ratio for resistant:susceptible) indicate that in both accessions, seedling resistance to race TTKSK was controlled by a single gene with partial dominance effect (Table 6). On the other hand, F_1 plants from the crosses between resistant accessions PI 193883, PI 298582, and PI 319869 and susceptible accessions exhibited ITs ($3/33^+$ to 4) that were similar to the susceptible parent (Table 6). The number of resistant:susceptible plants in F_2 fit a 1:3 ratio (Table 6), indicating that resistance to race TTKSK in accessions PI 193883, PI 298582, and PI 319869 was controlled by a single recessive gene. In the $F_{2:3}$ generation, the populations segregated in a 1:2:1 ratio for homozygous resistant:segregating:homozygous susceptible families, confirming that a single gene conferred resistance to race TTKSK (Table 7). The simple inheritance of TTKSK resistance in the five resistant emmer parents should simplify the transfer of resistance to durum and bread wheat. Accessions carrying the partially dominant gene (PI 101971 and PI 217640) exhibited a higher level of resistance at the adult stage comparing to the accessions that carry the recessive gene (PI 193883, PI 298582, and PI 319869) (Table 5). Five emmer accessions (CItr 12213, PI 41024, PI 94747, PI 133134, and PI 217639) exhibited a similar infection type pattern at the seedling stage and a level of resistance in field evaluations that is comparable to the one observed in accessions PI 101971 and PI 217640 (Table 5). These accessions may carry the same resistance gene effective against TTKSK. All these accessions are good candidates for use in wheat breeding programs.

In the allelism test, the F_2 population derived from the cross between the two resistant accessions carrying partially dominant genes (PI 101971 and PI 217640) produced only resistant progeny to race TTKSK (Table 6). This indicated that the accessions carried resistance alleles that were allelic or different genes that are linked to each other. A similar result was observed in the F_2 populations derived from crosses between the three resistant accessions carrying recessive genes (PI 193883, PI 298582, and PI 319869). On the other hand, segregation in F_2 populations derived from crosses between the resistant accessions carrying dominant and recessive genes (PI 217640 × PI 193883, and PI 298582 × PI 101971) fit a 13:3 ratio for resistant and susceptible plants (Table 6). This indicated that the dominant and recessive resistance genes segregated independently. Beteselassie et al. (2007) also reported the presence of diverse stem rust resistance genes in a group of 18 Ethiopian emmer wheat accessions. Genetic diversity for stem rust resistance has also been observed in wild emmer, in which six stem rust resistant accessions were postulated to each carry a different gene (Bai and Knott, 1994).

To identify additional stem rust resistance genes in the selected resistant parents, we evaluated the F_2 progeny

Table 7. Segregation of $F_{2:3}$ families of various crosses of emmer wheat (*Triticum turgidum* subsp. *dicoccon*) to race TTKSK of *Puccinia graminis* f. sp. *tritici*.

Cross†	Race	$F_{2:3}$ families‡				
		HR	Seg.	HS	Ratio tested (HR:Seg:HS)	*p*-value
CItr 7966 (S) × PI 101971 (R)	TTKSK	31	48	28	1:2:1	0.522
CItr 7966 (S) × PI 217640 (R)	TTKSK	37	80	33	1:2:1	0.644
CItr 7966 (S) × PI 298582 (R)	TTKSK	22	45	19	1:2:1	0.821
CItr 7966 (S) × PI 319869 (R)	TTKSK	22	38	19	1:2:1	0.843

†Female parent × male parent; (R) and (S) indicate the resistant and susceptible parent, respectively.

‡HR, homozygous resistant; Seg., segregating; HS, homozygous susceptible.

Table 8. Disease reaction of F_2 populations of various crosses of emmer wheat (*Triticum turgidum* subsp. *dicoccon*) to races TRTTF and TTTTF of *Puccinia graminis* f. sp. *tritici*.

Cross†	TRTTF				TTTTF			
	Resistant (R)	Susceptible (S)	Ratio tested (R:S)	*p*-value	Resistant	Susceptible	Ratio tested (R:S)	*p*-value
CItr 7966 (S) × PI 101971 (R)	78	32	3:1	0.322	129	12	15:1	0.267
CItr 7966 (S) × PI 193883 (R)	28	45	1:3	0.008	117	15	13:3	0.030
CItr 7966 (S) × PI 217640 (R)	49	17	3:1	0.145	209	34	13:3	0.057
CItr 7966 (S) × PI 298582 (R)	11	40	1:3	0.571	104	23	13:3	0.853
CItr 7966 (S) × PI 319869 (R)	38	11	3:1	0.507	125	15	13:3	0.015

†Female parent × male parent; (R) and (S) indicate the resistant and susceptible parent, respectively.

for their reaction to races TRTTF and TTTTF. No additional resistance gene was identified when crosses were evaluated against race TRTTF (Table 8). The χ^2 value obtained from the contingency table indicated a significant association in the reaction to races TTKSK and TRTTF in the $F_{2:3}$ families from the cross CItr 7966 × PI 217640 ($p = 1.40^{-39}$). In accession PI 319869, resistance to TRTTF was conferred by a single dominant gene (Table 8). This gene may be different from the one effective against race TTKSK (recessive gene) or may indicate a change in the dominance relationship in the resistance gene. Kolmer and Dyck (1994) reported a change in the expression of resistance in Thatcher lines from completely dominant to recessive when *Puccinia triticina* isolates were homozygous or heterozygous for avirulence. Two resistance genes effective against race TTTTF were identified in each of the five resistant parents. Progenies segregated in a 15:1 or 13:3 ratio for resistant:susceptible F_2 plants, indicating the presence of two dominant genes or one dominant and one recessive gene, to race TTTTF (Table 8). The existence of a number of stem rust resistance genes appears to be a common feature of tetraploid wheat as accessions with multiple stem rust resistance genes have been described in emmer wheat Khapli (*Sr13* and *Sr14*) (Heermann and Stoa, 1956), wild emmer (Knott et al., 2005; Olivera et al., 2011), and Persian [*Triticum turgidum* subsp. *carthlicum* (Nevski) Á. Löve & D. Löve] and Pollard (*T. turgidum* subsp. *turgidum*) wheat (Olivera et al., 2011). The use of races with different virulence spectrum and origin proved to be an efficient tool to identify multiple stem rust resistance genes in individual accessions.

CONCLUSIONS

The results of this study demonstrate that emmer wheat could serve as a source of resistance to race TTKSK. A number of accessions exhibited a resistant response in field evaluations and resistant reaction to race TTKSK and all the races evaluated at the seedling stage (Table 5). These accessions can provide with resistance genes that are effective against TTKSK and other races with broad virulence. Two genes effective against race TTKSK have been identified in this study. In particular, the partially dominant gene present in accessions PI 101971 and PI 217640 confers a high level of resistance in field evaluations. This gene is a good candidate for use in wheat breeding programs. Further genetic studies are required to confirm the presence of additional effective genes in the resistant accessions. Efforts should be made to incorporate these effective genes from emmer wheat into adapted backgrounds. Since emmer wheat shares the same genome as durum wheat and is in cultivated form, resistance genes should be easily transferred to durum wheat by conventional breeding approaches.

Acknowledgments

This research is funded by USDA-ARS and the Durable Rust Resistance of Wheat (DRRW), Cornell University. The authors acknowledge Lucy Wanschura, Sam Gale, and GebreHiwot Abraha for their technical assistance.

References

Bai, D., and D.R. Knott. 1994. Genetic studies of leaf and stem rust resistance in six accessions of *Triticum turgidum* var. *dicoccoides*. Genome 37:405–409. doi:10.1139/g94-057

Beteselassie, N., C. Fininsa, and A. Badebo. 2007. Sources of resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) in

Ethiopian tetraploid wheat accessions. Genet. Resour. Crop Evol. 54:337–343. doi:10.1007/s10722-005-5561-6

Damania, A.B., S. Hakim, and M.Y. Moualla. 1992. Evaluation of variation in *Triticum dicoccum* for wheat improvement in stress environments. Hereditas 116:163–166.

Gras, M.A. 1980. Disease resistance in wheat: I. *T. dicoccum* as a source of genetic factors against rust and mildew. Genet. Agrar. 34:123–132.

Hammer, K., and P. Perrino. 1984. Further information on farro (*Triticum monococcum* L. and *Triticum dicoccum* Schrank) in South Italy. Kulturpflanze 32:143–151. doi:10.1007/BF02002075

Hanson, W.D. 1958. Minimum family sizes for the planning of genetic experiments. Agron. J. 51:711–715. doi:10.2134/agronj1959.00021962005100120005x

Hare, R.A., and R.A. McIntosh. 1979. Genetic and cytogenetic studies of durable adult-plant resistances in 'Hope' and related cultivars to wheat rusts. Z. Pflanzenzuchtg. 83:350–367.

Heermann, R.M., and T.E. Stoa. 1956. New durum wheats resistant to 15B. N. Dakota Agric. Exp. Stn. Farm Res. 18:75–81.

Jin, Y., and R.P. Singh. 2006. Resistance in U.S. wheat to recent eastern African isolates of *Puccinia graminis* f. sp. *tritici* with virulence to resistance gene *Sr31*. Plant Dis. 90:476–480. doi:10.1094/PD-90-0476

Jin, Y., R.P. Singh, R.W. Ward, R. Wanyera, M. Kinyua, P. Njau, T. Fetch, Z.A. Pretorius, and A. Yahyaoui. 2007. Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 91:1096–1099. doi:10.1094/PDIS-91-9-1096

Jin, Y., L.J. Szabo, Z.A. Pretorius, R.P. Singh, R. Ward, and T. Fetch, Jr. 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis. 92:923–926. doi:10.1094/PDIS-92-6-0923

Knott, D.R., D. Bai, and J. Zale. 2005. The transfer of leaf and stem rust resistance from wild emmer wheats to durum and common wheat. Can. J. Plant Sci. 85:49–57. doi:10.4141/P03-212

Kolmer, J.A., and P.L. Dyck. 1994. Gene expression in the *Triticum aestivum-Puccinia recondita* f. sp. *tritici* gene-for-gene system. Phytopathology 84:437–440. doi:10.1094/Phyto-84-437

Kuckuck, H. 1970. Primitive wheats. In: OH Frankel and E. Bennett, editors, Genetic resources in plants – Their exploration and conservation. F. A. Davis Company. Philadelphia, PA. p. 249–266.

Liu, X.M., G.L. Brown-Guedira, J.H. Hatchett, J.O. Owuoche, and M.S. Chen. 2005. Genetic characterization and molecular mapping of a Hessian fly-resistance gene transferred from *T. turgidum* ssp. *dicoccum* to common wheat. Theor. Appl. Genet. 111:1308–1315. doi:10.1007/s00122-005-0059-3

Mago, R., G. Brown-Guedira, S. Dreisigacker, J. Breen, Y. Jin, R. Singh, R. Appels, E.S. Lagudah, J. Ellis, and W. Spielmeyer. 2011. An accurate DNA marker assay for stem rust resistance gene *Sr2* in wheat. Theor. Appl. Genet. 122:735–744. doi:10.1007/s00122-010-1482-7

McFadden, E.S. 1930. A successful transfer of emmer characters to *vulgare* wheat. J. Am. Soc. Agron. 22:1020–1034. doi:10.2134/agronj1930.00021962002200120005x

McIntosh, R.A. 1988. The role of specific genes in breeding for durable stem rust resistance in wheat and triticale. In: N.W. Simmonds and S. Rajaram, editors, Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico D.F., Mexico. p. 1–9.

McVey, D.V. 1991. Reaction of a group of related wheat species (AABB genome and AABBDD) to stem rust. Crop Sci. 31:1145–1149. doi:10.2135/cropsci1991.0011183X003100050012x

Nazari, K., M. Mafi, A. Yahyaoui, and R.P. Park. 2009. Detection of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) race TTKSK (Ug99) in Iran. Plant Dis. 93:317. doi:10.1094/PDIS-93-3-0317B

Oliver, R.E., X. Cai, R.W. Stack, T. Friesen, S. Halley, and S.S. Xu. 2008. Fusarium head blight resistance in tetraploid wheat (*Triticum turgidum* L.). Crop Sci. 48:213–222. doi:10.2135/cropsci2007.03.0129

Olivera, P.D., Y. Jin, A. Badebo, S. Xu, and D. Klindworth. 2011. Resistance to race TTKSK of *Puccinia graminis* f. sp. *tritici* in tetraploid wheat. Phytopathology 101:S132.

Olivera, P.D., Y. Jin, M. Rouse, A. Badebo, T. Fetch, R.P. Singh, and A. Yahyaoui. 2012. Races of *Puccinia graminis* f. sp. *tritici* with combined virulence to *Sr13* and *Sr9e* in a field stem rust screening nursery in Ethiopia. Plant Dis. 96:623–628.

Olivera, P.D., M. Rouse, and Y. Jin. 2009. Resistance to wheat stem rust in spelt wheat, wild emmer and triticale. Phytopathology 99:S97.

Peterson, R.F., A.B. Campbell, and A.E. Hannah. 1948. A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. Can. J. Res. Sect. C 26:496–500. doi:10.1139/cjr48c-033

Pflüger, L.A., L.M. Martín, and J.B. Alvarez. 2001. Variation in the HMW and LMW lutenin subunits from Spanish accessions of emmer wheat (*Triticum turgidum* ssp. *dicoccum* Schrank). Theor. Appl. Genet. 102:767–772. doi:10.1007/s001220051708

Pretorious, Z.A., Y. Jin, C.M. Bender, L. Herselman, and R. Prins. 2011. Seedling resistance to stem rust race Ug99 and marker analysis for *Sr2, Sr24* and *Sr31* in South African wheat cultivars and lines. Euphytica doi:10.1007/s10681-011-0476-0

Pretorius, Z.A., R.P. Singh, W.W. Wagoire, and T.S. Payne. 2000. Detection of virulence to wheat stem rust resistance genes *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Dis. 84:2003.

Roelfs, A.P., D.L. Long, and J.J. Roberts. 1993. Races of *Puccinia graminis* in the United States during 1992. Plant Dis. 77:1122–1125. doi:10.1094/PD-77-1122

Roelfs, A.P., and J.W. Martens. 1988. An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. Phytopathology 78:526–533. doi:10.1094/Phyto-78-526

Roelfs, A.P., R.P. Singh, and E.E. Saari. 1992. Rust diseases of wheat, concepts and methods of disease management. CIMMYT, Mexico D.F., Mexico.

Sayed, H.I. 1985. Diversity of salt tolerance in germplasm collection of wheat (*Triticum* ssp.). Theor. Appl. Genet. 69:651–657. doi:10.1007/BF00251118

Singh, R.P., D.P. Hodson, J. Huerta-Espino, Y. Jin, S. Bhavani, P. Njau, S.A. Herrera-Foessel, P. Singh, S. Singh, and V. Govindan. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu. Rev. Phytopathol. 49:465–481. doi:10.1146/annurev-phyto-072910-095423

Singh, R.P., D.P. Hodson, Y. Jin, J. Huerta-Espino, M.G. Kinyua, R. Wanyera, P. Njau, and R.W. Ward. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Res. 54:1–13.

Stakman, E.C., D.M. Steward, and W.Q. Loegering. 1962. Identification and physiologic races of *Puccinia graminis* var. *tritici*. USDA-ARS E-617. U.S. Gov. Print. Off., Washington, DC.

Stallknecht, G.F., K.M. Gilbertson, and J.E. Ranney. 1996. Alternative wheat cereals as food grains: Einkorn, emmer, spelt, kamut, and triticale. In: J. Janick, editor, Progress in new crops. ASHS Press, Alexandria, VA. p. 156–170.

Visser, B., L. Herselman, R.F. Park, H. Karaoglu, C.M. Bender, and Z.A. Pretorius. 2010. Characterization of two new *Puccinia graminis* f. sp. *tritici* races within the Ug99 lineage in South Africa. Euphytica 179:119–127. doi:10.1007/s10681-010-0269-x

Wanyera, R., M.G. Kinyua, Y. Jin, and R.P. Singh. 2006. The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in Eastern Africa. Plant Dis. 90:113. doi:10.1094/PD-90-0113A

Wolday, A., T. Fetch, Jr., D. Hodson, W. Cao, and S. Briere. 2011. First report of *Puccinia graminis* f. sp. *tritici* races with virulence to wheat stem rust resistance genes *Sr31* and *Sr24* in Eritrea. Plant Dis. 95:1591. doi:10.1094/PDIS-07-11-0582

Yanchenko, I. 1985. A study of interspecific hybrids of *Triticum durum* Desf. × *Triticum dicoccum* (Schrank) Schuebl. and ways of using them in durum wheat breeding. Naucho Tek. Eyul. Sibirskogo Old. Vashknil. 45:3–7.

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for the growth stage of cereals. Weed Res. 14:415–421. doi:10.1111/j.1365-3180.1974.tb01084.x