Kenyan Isolates of *Puccinia graminis* f. sp. *tritici* from 2008 to 2014: Virulence to *SrTmp* in the Ug99 Race Group and Implications for Breeding Programs

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ABSTRACT

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Frequent emergence of new variants in the *Puccinia graminis* f. sp. *tritici* Ug99 race group in Kenya has made pathogen survey a priority. We analyzed 140 isolates from 78 *P. graminis* f. sp. *tritici* samples collected in Kenya between 2008 and 2014 and identified six races, including three not detected prior to 2013. Genotypic analysis of 20 isolates from 2013 and 2014 collections showed that the new races TTHST, TTKTK, and TTKTT belong to the Ug99 race group. International advanced breeding lines were evaluated against an isolate of TTKTT (*Sr31*, *Sr24*, and *SrTmp* virulence) at the seedling stage. From 169 advanced lines from Kenya, 23% of lines with resistance to races TTKSK and TTKST were susceptible to TTKTT and, from two North American regional nurseries, 44 and 91% of resistant lines were susceptible. Three lines with combined resistance genes were developed to facilitate pathogen monitoring and race identification. These results indicate the increasing virulence and variability in the Kenyan *P. graminis* f. sp. *tritici* population and reveal vulnerabilities of elite germplasm to new races.

Wheat stem rust, caused by *Puccina graminis* f. sp. *tritici*, is an important fungal disease of common wheat (*Triticum aestivum*), durum wheat (*T. turgidum* subsp. *durum*), barley (*Hordeum vulgare*), and triticale (× *Triticosecale*). Wheat stem rust infection at high severities can result in a complete loss of a farmer's crop. Such dramatic reductions in yield have increased in frequency in Kenya since race TTKSK (Ug99, with virulence to *Sr31*) was found to be widespread throughout wheat production regions (Wanyera et al. 2006). Wheat crops in Kenya and elsewhere have historically been effectively protected from stem rust damage by the use of resistant cultivars (Ellis et al. 2014). However, the emergence of new combinations of virulence in *P. graminis* f. sp. *tritici* races has proven a challenge for wheat breeders. It has long been recognized that the deployment of race-specific resistance genes in wheat influences the prevalence of corresponding virulence in rust pathogen races (Johnson 1961), and this interplay between stem rust resistance deployed in the field and the predominant pathogen races is evident in Kenya. Following the spread of race TTKSK in Kenya, breeders and researchers developed new resistant cultivars. In 2001, three cultivars with resistance to TTKSK were approved for release by the national committee of the Ministry of Agriculture, including 'KS Mwamba', which became a popular cultivar. The estimated planted acreage of KS Mwamba, postulated to carry *Sr24*, increased to 50,000 ha in 2006 to 2007. In 2006, race TTKST (*Sr24* virulence) in the Ug99 race group was first detected in Kenya (Jin et al. 2008). Race TTKST rapidly spread and was associated with high disease severities on fields planted to KS Mwamba in 2007 (Singh et al. 2011).

It was not until 2011 that new cultivars ('Eagle 10' and 'Robin', with resistance to all known races in the Ug99 race group at that time) were again approved for release in Kenya. Six additional cultivars with resistance ('Kenya Hawk', 'Kenya Kingbird', 'Kenya Korongo', 'Kenya Wren', 'Kenya Sunbird', and 'Kenya Tae') were released in 2012. Farmers who planted Robin prior to 2013 reported high yields and large harvests and it became widely planted, comprising approximately 40% of the area planted to wheat by 2014 (S. Bhavani, unpublished data). Additionally, 67% of the total wheat seed sold by the Kenya Agricultural and Livestock Research Organization (KALRO) between 2011 and 2014 was that of Kenya Robin (G. Macharia, unpublished data). Robin has the pedigree of Babax/Lr42//Babax*2/3/Tukuru and is postulated to carry *Sr2* and *SrTmp* (Wheat Atlas by International Maize and Wheat Improvement Center [CIMMYT], http://wheatatlas.org/country/varieties/KEN/0). A monogenic source of *SrTmp* (CnsSrTmp, North American differential line) showed moderate resistance to TTKSK in two seasons in Kenya in a stem rust screening field nursery (infection responses of 40 moderately susceptible [MS] and 30 moderately resistant [MR]-MS) (Jin et al. 2007). Moreover, CnsSrTmp showed moderately low seedling infection types (IT) to TTKSK and TTKST

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(Jin et al. 2007, 2008), while 'Triumph 64' (source of *SrTmp*) showed resistance to TTTSK (Jin et al. 2009). Thus, *SrTmp* was resistant to the known races in the Ug99 race group in Kenya at the time when Robin was released. During 2013 and 2014, severe stem rust infection on Robin was reported. Patpour et al. (2015) reported the detection of race TTKTK, with virulence to *SrTmp*, in the Ug99 race group in samples collected in Kenya, Uganda, Rwanda, and Egypt in 2014.

Given the frequent emergence of new and virulent variants in the Ug99 race group in Kenya, stem rust surveillance has remained a high priority during the past decade. Knowledge of the races present across wheat-growing areas is critical for guiding the development and deployment of new cultivars. Moreover, when isolates are detected with new combinations of virulence, as in 2014 during the stem rust outbreak on Robin, it is equally critical to utilize these isolates to evaluate breeding germplasm to select candidate lines that are protected against all known races. Here, we report the results of 7 years of surveillance to monitor the *P. graminis* f. sp. *tritici* population in Kenya, with virulence and genotypic analyses of stem rust isolates, to inform wheat breeding programs of the spectrum of stem rust pathogen virulence in Kenya and the vulnerabilities of elite germplasm to newly identified races.

MATERIALS AND METHODS

Development of host differential lines with combined resistance genes. To monitor field populations in the Ug99 race group more effectively, we developed lines with gene combinations involving *Sr24*, *Sr31*, and *Sr36* that could differentiate races TTKSK, TTKST, and TTTSK in the field. Intercrosses were made between lines Agent/9*LMPG, Benno/6*LMPG, and CI12632/8*LMPG (Knott 1990) that carried resistance genes *Sr24*, *Sr31*, and *Sr36*, respectively. Selections were made by testing with multiple stem rust races at various generations, starting at F_3. Three fixed lines carrying gene combinations *Sr24+Sr31*, *Sr31+Sr36*, and *Sr24+Sr36* were advanced to F_8 and designated as CDLSr24Sr31 (PI 675464), CDLSr31Sr36 (PI 675465), and CDLSr24Sr36 (PI 675466). These lines were deposited at the United States Department of Agriculture–Agricultural Research Service (USDA-ARS) National Small Grains Collection (NSGC; Aberdeen, ID) and are available upon request.

The fixed lines were tested in advanced generations, with monogenic lines carrying *Sr31*, *Sr24*, and *Sr36* and susceptible control lines LMPG-6 and Chinese Spring, during 2011 to 2014 at a stem rust screening nursery at the Food Crops Research Centre, KALRO, Njoro. From 2011 to 2013, lines were assessed during the main wheat-growing season of June through October. In 2014, lines were assessed during both the main season and the off-season of January through May. The nursery location and environment, planting design, and stem rust inoculation method were previously described by Njau et al. (2010) and Rouse et al. (2011). The composition of the spreader row plants was modified to include cultivars carrying *Sr24* in addition to *Sr31* to select for *Sr24* and *Sr31* virulence in the *P. graminis* f. sp. *tritici* population in the nursery as described by Newcomb et al. (2013). Field disease ratings were assessed according to previously described methods (Newcomb et al. 2013; Njau et al. 2010; Rouse et al. 2011). Stem rust severities were estimated on a scale of 0 to 100% stem area affected by the pathogen, following a modified Cobb scale (Peterson et al. 1948). Infection responses were estimated in categories of resistant (R), MR, MS, susceptible (S), or combinations of each when multiple infection responses were observed within a plant stem (Roelfs et al. 1992).

Sample collection and storage. During the years 2008 to 2014, samples were collected from the stem rust screening nursery at the Food Crops Research Centre, KALRO, Njoro, and wheat and barley production fields ranging from the South, Central and North Rift areas and the Mount Kenya foothill region. Samples were shipped to the USDA-ARS Cereal Disease Laboratory (CDL), St. Paul, MN and the Foreign Disease-Weed Science (FD-WS) Research Unit, Ft. Detrick, MD under USDA Animal and Plant Health Inspection Service permits. Samples received at the CDL were stored at −80°C and were processed after 1 December of each year. Samples received at the FD-WS Research Unit were briefly stored at 4°C until being increased on susceptible wheat cultivar McNair 701 (CItr 15288) and selected differential lines. Resulting urediniospores were collected into gelatin capsules, dried, and stored at −80°C until they were shipped to the CDL in early December.

Race identification and characterization. Experimental procedures for inoculation, incubation, and disease assessment were conducted as described by Jin et al. (2007) and Olivera et al. (2015). Each sample was initially inoculated as a bulk sample onto 7- to 9-day-old seedlings of 20 single-gene North American differential lines (Jin et al. 2008). One to three single-pustule isolates were derived from each sample. Each isolate was evaluated two to four times on differential lines before a race designation was given using the international letter-code nomenclature described by Roelfs and Martens (1988) and modified by Jin et al. (2008). Isolates derived from samples from collection years 2013 and 2014 were also characterized with an additional set of 20 lines carrying important stem rust resistance genes (Olivera et al. 2015), including durum wheat 'Iumillo' (*Sr9g,12,*+), 'Leeds' (*Sr9e,13*,+), and Triumph 64 (*SrTmp*). Representative isolates derived from samples collected in 2013 and 2014 also were inoculated on 15 cultivars and breeding lines carrying genes important to Kenya breeding programs, including *SrTmp* (Lopez-Vera et al. 2014), *Sr9h* (Rouse et al. 2014), *SrCad* (Hiebert et al. 2011), *Sr28* (Rouse et al. 2012), and *SrND643* (Basnet et al. 2015).

Genotypic characterization. Infected wheat leaves containing uredinia of pure isolates were collected and dried. DNA was extracted as described previously (Olivera et al. 2015). Samples were genotyped using an Infinium custom single-nucleotide polymorphism (SNP) chip (PgtSNP 3.0k chip). This chip is an expansion of the original PgtSNP 1.5k chip (J. Johnson and L. Szabo, unpublished) and increases the coverage from 50 to 98% of the *P. graminis* f. sp. *tritici* assembled genome (Duplessis et al. 2011). The SNP chip assay was performed as described by the manufacturer (Illumina, San Diego, CA) using 500 ng of DNA per sample, and each sample was run in duplicate as described. Of the 3,072 candidate markers, 2,718 were successfully generated by Illumina. Of these, 2,665 markers produced call data above background level. This data set was further refined to 1,634 markers using the following criteria: GenTrain score > 0.6, 10%GC > 0.6, and ≥90% call rate. Inconsistency between replicates was treated as missing data (no calls). Phylogenetic analysis of the data were performed using R (version 3.2.1, R Core Development Team 2015), with the package 'Poppr', version 2.0.2 (Kamvar et al. 2015), as previously described (Olivera et al. 2015). A minimal spanning network was generated with a subset of 23 isolates (clade I) using 31 polymorphic markers. This subset contained only SNP loci that were polymorphic between isolates in clade I, when polymorphisms were due to nucleotide base change rather than missing data, and when the amount of missing data per locus was <5%. In order to remove background noise based on missing data, the nine data points scored as missing were converted to the most prevalent genotype for that marker, which resulted in converting 2.9% of the data points. A set of 15 *P. graminis* f. sp. *tritici* reference samples was included in the analysis, representing four common race groups in north and east Africa: clade 1/Ug99 race group, 04KEN156-04 (TTKSK), 06KEN19V-3 (TTKST), and 07KEN24-4 (TTTSK); clade II/JRCQC race group, 14YEM115-1, 14YEM123-1, and 14YEM149-5; clade III/TRTTF race group, 06YEM34-1 (TRTTF), 14ETH123-1 (RRTTF), and 14ETH136-3 (RRTTF); clade IV-A/TKTTF, 13ETH02-2, 13ETH18-1, and ETH128-1; and clade IV-B/TKTTF race group, 13ETH20-1, 14ETH126-1, and 14ETH132-2.

Evaluations of seedling resistance. Isolate 14KEN58-1 (combined virulence to *Sr31*, *SrTmp* and *Sr24*) was used to evaluate advanced breeding lines from Kenya, North America, and CIMMYT. Inoculations and seedling maintenance were as described above for evaluations of differential sets. Seedling IT were assessed 13 to 14 days after inoculation, using the 0-to-4 scale described by Stakman et al. (1962). IT < 3 were categorized as R, while those ≥3 were categorized as S. Each entry was evaluated for resistance to isolate 14KEN58-1 as well as races TTKSK (04KEN156/04) and TTKST (06KEN19-V-3). Resistance evaluations on these sets of breeding lines were conducted once per entry per race.

RESULTS

Race identification and characterization. From the 2008 to 2014 collections, 140 isolates derived from 78 viable samples were race typed at the CDL. Isolate identification numbers, collection information, IT for the five differential lines that distinguish the race types (*Sr24*, *Sr31*, *Sr36*, *Sr30*, and *SrTmp*), and race designations are given in Supplementary Table S1. For 74 of the 78 samples, all derived isolates from each sample were of the same race. A mixture of two races was found in four samples. All isolates collected during 2008 to 2012 were identified as races TTKSK, TTKST, or TTTSK and displayed IT expected of these races based on previous descriptions (Table 1). During this period (2008 to 2012), the predominant race changed from TTKSK to TTKST. In 2008, race TTKSK was isolated from five of eight samples, while TTKST was detected in only one sample. In 2009, race TTKST was detected in six of eight samples, while TTKSK was found in four of eight samples (two were mixed). In 2010 and 2012, race TTKST was isolated from all samples, and from seven of nine samples in 2011. Race TTTSK (virulence to *Sr36* and *Sr31*) was detected only in 2008 from two samples taken at the stem rust screening nursery in Njoro.

From the 2013 collections, four viable samples were characterized as race TTKST. In addition, one of the samples (13KEN21) showed two distinct IT on the *Sr30* differential line (BtSr30Wst), from which races TTHST and TTKST were isolated. Race TTHST is similar to race TTKST in virulence spectrum but produces a low IT (IT 2) on *Sr30* (Tables 1 and 2).

Four races were identified from 2014 collections: eight isolates of race TTKSK, eight of race TTKST, four of race TTKTK, and five of race TTKTT. Races TTKTK and TTKTT differ from previously described variants in the Ug99 race group only by high IT (S reactions) on differential CnsSrTmp and Triumph 64, the latter being the original source of *SrTmp* (Tables 1 and 2). In all, 12 isolates were derived from samples collected from Robin in 2014 and, of these, 5 were race TTKTT (with combined virulence to *SrTmp* and *Sr24*), 3 were race TTKTK (*SrTmp* virulence, *Sr24* avirulence), and 4 were race TTKSK (avirulence to both *SrTmp* and *Sr24*). High IT on multiple lines carrying *SrTmp* were consistently observed for isolates identified as races TTKTK and TTKTT (Table 3). The difference between the low IT (2 to 2+3) observed for races TTKSK, TTKST, and TTTSK and the high IT observed for races TTKTK and TTKTT (3 or 3+) on CnsSrTmp were repeatable and clearly distinguished between the expression of moderate resistance and full susceptibility (Fig. 1).

To further characterize the new variants in the Ug99 race group with virulence to *SrTmp*, we assembled a set of 15 lines that were either known or postulated to carry genes important to Kenya breeding programs for protection against the Ug99 races. The targeted genes included *SrTmp*, *SrND643*, *Sr9h*, and *SrCad*. Cultivars and lines Robin, 'Ripper', 'Overland', 'Ember', 'Guard-1', 'Shield', and 'Digalu' with *SrTmp* were all susceptible to races TTKTK and TTKTT (Table 3). The following lines were resistant to races TTKTK and TTKTT: Gabo56, SD4279, CDL001, Peace, AC Cadillac, Glupro 98601/9 (University of California [UC] Davis), and ND643 (UC Davis). A line postulated to carry both *SrTmp* and *Sr24* (PI 410954) displayed resistance to races TTKSK, TTKST, and TTKTK but an intermediate to high reaction to race TTKTT. The reduced IT observed on CDL001 (postulated genes *Sr9h* and *Sr2*8), relative to Gabo56 and SD4279 (postulated gene *Sr9h*), in response to races TTKTK and TTKTT indicated that these races were avirulent on both *Sr28* and *Sr9h*, which appear to show an additive effect in CDL001.

Stem rust severities and infection responses observed from 2011 to 2014 at the screening nursery in Kenya for advanced generations of the lines with gene combinations *Sr24+Sr31*, *Sr31+Sr36*, and *Sr24+Sr36* were in two distinct categories (Table 4). Low severities and resistant responses were observed each year for CDLSr31Sr36 (PI 675465) and CDLSr24Sr36 (PI 675466) and were consistent with the reactions observed for the two monogenic lines carrying *Sr36* (W2691SrTt-1 and CI 12632/8*LMPG) in all seasons, except for W2691SrTt-1 in 2011, which showed a reaction of 10 MS, possibly explained by a slight mixture in the seed source for this line. The high severities and susceptible infection responses observed for

TABLE 1. Seedling infection types observed on stem rust differentials for six races of *Puccinia graminis* f. sp. *tritici* in the Ug99 race group identified from samples collected in Kenya during 2008 to 2014[a]

Line	Genes	TTKSK	TTKST	TTTSK	TTKTK	TTKTT	TTHST
ISr5-Ra	*Sr5*	3+	3+	4	3+	3+	3+
CnS_T_mono_deriv	*Sr21*	33+	3+	3–	3+	33+	3
Vernstine	*Sr9e*	3+	3+	3+	3+	3+	3+
ISr7b-Ra	*Sr7b*	3+	3+	3+	3+	3+	3+
ISr11-Ra	*Sr11*	3+	3+	4	3+	3+	3+
ISr6-Ra	*Sr6*	3+	3+	4	3+	3+	3+
ISr8a-Ra	*Sr8a*	3+	3+	3+	3+	3+	3+
CnSr9g	*Sr9g*	4	3+	4	4	4	4
W2691SrTt-1	*Sr36*	0;	0	4	0	0;	0;
W2691Sr9b	*Sr9b*	33+	3+	4	3+	33+	33+
BtSr30Wst	*Sr30*	3+	3+	4	3+	33+	2–
Combination VII	*Sr17+Sr13*	2	22+	22+	2	22+	2
ISr9a-Ra	*Sr9a*	3+	3+	4	3+	3+	3+
ISr9d-Ra	*Sr9d*	3+	3+	4	3+	3+	3+
W2691Sr10	*Sr10*	3+	3+	4	3+	3+	3+
CnsSrTmp	*SrTmp*	2+3–	2+	2+3	33+	33+	2+3–
LcSr24Ag	*Sr24*	22–	3	2	22–	33+	3
Sr31/6*LMPG	*Sr31*	3+	3+	4	3+	3+	3+
VPM-1	*Sr38*	3+	3+	4	3+3	33+	3+
McNair 701	*McN*	4	4	4	4	3+	3+

[a] Infection types (IT) observed on seedlings at 14 days postinoculation using a 0-to-4 scale according to Stakman et al. (1962), where IT of 0, ;, 1, 2, or combinations thereof are considered to be a low IT and IT of 3 or higher are considered to be a high IT.

CDLSr24Sr31 (PI 675464) each year were similar to the monogenic line carrying *Sr31* (Sr31[Benno]/6*LMPG) and the two monogenic lines carrying *Sr24* (LcSr24Ag and Agent/9*LMPG). Moreover, these stem rust disease ratings for the gene-combination lines in the stem rust screening nursery corroborated with the race analysis results for the isolates derived from collections from Njoro in 2011 and 2012, all of which were characterized as race TTKST (virulent to *Sr31* and *Sr24*, avirulent to *Sr36*).

Genotyping. A set of 20 *P. graminis* f. sp. *tritici* isolates derived from 2013 and 2014 Kenyan collections were genotyped using a custom PgtSNP 3.0k chip, including 19 isolates characterized at the CDL and one isolate (CDL sample ID 14KEN230) from a 2014 Kenya sample that was race-typed at the Global Rust Reference Center (GRRC; Denmark; GRRC sample ID KE133b/14) (Patpour et al. 2015), then subsequently sent to the CDL for race confirmation and genotyping. A set of 15 reference isolates was also genotyped. After filtering the data, 1,634 SNP loci were used for analysis. All 20 Kenyan isolates from 2013 to 2014 collections clustered with the reference Ug99 race group isolates in clade I, which was well supported and distinct from the other three clades (Fig. 2). Minor substructure was observed within clade I but this substructure did not correlate with race phenotypes of the isolates.

To better understand the genetic relationship between the isolates in clade I, a subset of the 31 SNP loci was selected and analyzed using a minimum spanning network (Fig. 3). The 23 isolates formed a network of 12 multilocus genotypes (MLG). Seven of the MLG were represented by a single isolate and the remaining five MLG were represented by two, four, or six isolates. Each of the MLG composed of multiple isolates also consisted of two race phenotypes. The opposite was also true: each race that was represented by more than one isolate also consisted of more than one MLG. Race TTHST was represented by a single isolate (13KEN21-3, MLG.01), race TTKTK was represented by four isolates and two MLG (MLG.10, 14KEN47-1, 14KEN54-2, and 14KEN230; and MLG.11, 14KEN52-1), and race TTKTT was represented by two isolates and two MLG (MLG.04, 14KEN55-3; and MLG.07, 14KEN58-1). The most prevalent race (TTKST) in this set was represented by eight isolates and six MLG (MLG.02, reference isolate 06KEN19V-3; MLG.04, 14KEN45-3, 14KEN46-1, and 14KEN50-2; MLG.05, 14KEN53-1; MLG.06, 14KEN43-1-3; MLG.08, 14KEN56-1; and MLG.09, 14KEN92-1), followed by race TTKSK, which was

TABLE 2. Seedling infection types observed on lines carrying stem rust resistance genes for five races of *Puccinia graminis* f. sp. *tritici* from the Ug99 race group identified from samples collected in Kenya during 2013 and 2014[a]

Line	Genes	Type	TTKSK	TTKST	TTKTK	TTKTT	TTHST
SwSr22T.B.	*Sr22*	Bread wheat	;2–	2–	2–;	2–	2–
Agatha/9*LMPG	*Sr25*	Bread wheat	;2–	;2–	2–	2	2
Eagle (Australia)	*Sr26*	Bread wheat	;2–	;2–	2–;	2–	;2–
73,214,3-1/9*LMPG	*Sr27*	Bread wheat	;	0;	;	;	;
Federation*4/Kavkaz	*Sr31*	Bread wheat	3+	3+	3+	3+	3+
ER 5155	*Sr32*	Bread wheat	;2–	2–	2–	;2–	;2–
Mq(2)5XG2919	*Sr35*	Bread wheat	0;	0;	0;	;	0;
W3563	*Sr37*	Bread wheat	;13	31;	1+13;	31;	;13
RL6082	*Sr39*	Bread wheat	;	;2–	;2–	;1–	;1–
RL6088	*Sr40*	Bread wheat	;2–	;2–	2–	;2–	2–;
TAF 2	*Sr44*	Bread wheat	2–;	2–;	;2–	2–;	1
CSID 5406	*Sr45*	Bread wheat	;1–	;	;	;	;1–
Fed*3/Gabo*51BL.1RS-1-1	*Sr50*	Bread wheat	2–	2–	2–	2–	2–
DAS15	*Sr47 + SrAes7t*	Durum wheat	;	;	;1–	;	;
Satu	*SrSatu*	Triticale	0;	0;	;	0;	;
TAM 107-1	*Sr1RS^{Amigo}*	Bread wheat	2–	2–	2–	2–	2–
Iumillo	*Sr9g,12,+*	Durum wheat	0;	0;	;	0;	0;
Leeds	*Sr9e,13,+*	Durum wheat	0;	0;	;	0;	;
ST464	*Sr13*	Durum wheat	2+	2+	22+	2+	2+2
Triumph 64	*SrTmp,+*	Bread wheat	2+	2	33+	33+	2

[a] Infection types (IT) observed on seedlings at 14 days postinoculation using a 0-to-4 scale according to Stakman et al. (1962), where IT of 0, ;, 1, 2, or combinations thereof are considered to be a low IT and IT of 3 or higher are considered to be a high IT.

TABLE 3. Seedling infection types observed on select varieties, elite lines, and additional lines postulated to carry *Sr* genes utilized in Kenya breeding programs for five races of *Puccinia graminis* f. sp. *tritici* collected in Kenya[a]

Line	Postulated genes	TTKSK (04KEN156/04)	TTKST (06KEN19v3)	TTKTK (14KEN52-1)	TTKTT (14KEN58-1)	TTHST (13KEN21-3)
Robin	*SrTmp + Sr2*	2+3	2+2	3+	4	2
Ripper	*SrTmp*	2+	22+	33+	3+	2
Overland	*SrTmp*	2+	22+	3+	3+	2+3
Ember	*SrTmp*	2+3–	2+	3+	3+	22+
Guard-1	*SrTmp*	2+	22+	3+3	32+	2+
Shield	*SrTmp*	2+3–	2+	33+	3+	2
Digalu	*SrTmp*	2+	22+	33+	3+	2
Gabo56	*Sr9h*	2	2	2	2	22+
SD4279	*Sr9h*	2	2–	2–	2	Not tested
CDL001	*Sr9h + Sr28*	;1–	;1–	0	0;	Not tested
PI 410954	*Sr24 + SrTmp*	2–	2	2–	32+	Not tested
Peace	*SrCad*	0	1–	;	0	Not tested
AC Cadillac	*SrCad*	0	1–;	11+	0	Not tested
Glupro 98601/9 (UC Davis)	*SrND643*	2–	2+	2	2+	Not tested
ND643 (UC Davis)	*SrND643*	2–;	2+	No data	2+	Not tested

[a] Infection types (IT) observed on seedlings at 14 days postinoculation using a 0-to-4 scale according to Stakman et al. (1962), where IT of 0, ;, 1, 2, or combinations thereof are considered to be a low IT and IT of 3 or higher are considered to be a high IT.

represented by seven isolates and five MLG (MLG.01, reference isolate 04KEN156-04; MLG.06, 14KEN51-1; MLG.10, 14KEN59-1, 14KEN95-1, and 14KEN98-1; MLG.11, 14KEN57-1; and MLG.12, 14KEN48-2). Race TTTSK (reference isolate 07KEN24-4) was the only representative of MLG.03.

Isolates of the three new races (TTHST, TTKTK, and TTKTT) were located in different regions of the minimum spanning network, indicating that each of these were derived from different members of the Ug99 race group population (Fig. 3). Based on the 31 SNP loci used in the analysis, both 13KEN21-3 (TTHST) and reference isolate 04KEN156-04 (TTKSK) had an identical genotype (MLG.01). The four isolates of TTKTK were separated into two closely related genotypes (MLG.10, 14KEN47-1, 14KEN54-2, and 14KEN230; and MLG.11, 14KEN52-1), which were also shared with four TTKSK isolates (MLG.10, 14KEN59-1, 14KEN95-1, and 14KEN98-1; and MLG.11, 14KEN57-1). In contrast, the SNP genotypes of the two TTKTT isolates (14KEN55-3, MLG.04 and 14KEN58-1, MLG.07) were identical, or more closely related to MLG of isolates of race TTKST (MLG.04, 14KEN45-3, 14KEN46-1, and 14KEN55-3; and MLG.08, 14KEN56-1).

Resistance to races TTKSK, TTKST, and TTKTT in elite breeding germplasm. A selection of 1,055 lines from four international nurseries, including a nursery of Kenyan advanced lines and cultivars, was evaluated for resistance to races TTKSK (*Sr31* virulence), TTKST (*Sr31* + *Sr24* virulence), and TTKTT (*Sr31* + *Sr24* + *SrTmp* virulence). There were 80 entries in the 169 Kenyan advanced materials with resistance to both TTKSK and TTKST and, of these, 18 (23%) were susceptible to the new variant TTKTT (Table 5). There were 366 and 34 lines in the U.S. winter wheat and U.S. spring wheat selections, respectively, with resistance to both TTKSK and TTKST and, of these, 160 (44%) and 31 (91%) were susceptible to TTKTT. There were 119 CIMMYT entries that were resistant to both TTKSK and TTKST, and only 2 of these were susceptible to TTKTT, suggesting that *SrTmp* may not be an important source of stem rust resistance in this particular CIMMYT nursery. In contrast, the Kenyan results showed that *SrTmp* could be the source of resistance protecting approximately 23% of the material resistant to TTKSK and TTKST, indicating that race TTKTT poses a serious threat to wheat production in Kenya. The high degree of susceptibility in U.S. elite materials is a cause of concern should the incursion of race TTKTT into North America occur in the future.

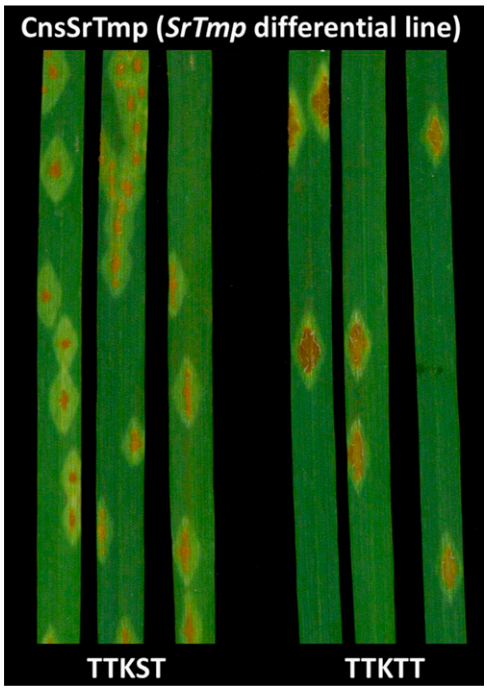

Fig. 1. Seedling infection types produced by *Puccinia graminis* f. sp. *tritici* races TTKSK and TTKTT, isolated from samples collected in Kenya in 2014, on differential line CnsSrTmp, distinguishing avirulence (TTKSK) from virulence (TTKTT) on *SrTmp*.

DISCUSSION

Annual collections and race evaluations of 78 stem rust samples from Kenya over the 7 years from 2008 to 2014 detected six races in the Ug99 group and provided information on how the virulence patterns and race structure changed over time. To date, 11 variants in the Ug99 race group have been reported in 13 countries in northern and eastern Africa and the Middle East (Patpour et al. 2015; Singh et al. 2015). In addition to the six variants described in this research, PTKSK and PTKST are two races in the Ug99 race group with avirulence to *Sr21* that have been reported in Kenya (Singh et al. 2015). The presence of these two races in Kenya was confirmed at the GRRC from collections made between 2011 and 2014 in side-by-side evaluations comparing reactions produced by races with and without *Sr21* virulence (M. Patpour and M. Hovmoller, unpublished data). Resistance conferred by *Sr21* has been shown to be influenced by temperature, environmental conditions, and genetic background, and Ug99 races could potentially be characterized as either virulent or avirulent on *Sr21* depending on the test environment (Chen et al. 2015). In total, 8 of the 11 variants in the Ug99 race group have been observed in Kenya, making it the most diverse country in the number of *P. graminis* f. sp. *tritici* races in the Ug99 race group. The eight variants of Ug99 that have been found in Kenya have been detected over a 10-year time span (2004 to 2014).

All samples characterized for race type in this study revealed races only in the Ug99 race group, indicating that this race group dominates the stem rust population in Kenya. Following 2008, race TTTSK maintained a low frequency below the level of detection in the Kenya national stem rust surveys, and its presence in the *P. graminis* f. sp. *tritici* population in the country remains uncertain. However, this race was detected in Tanzania, Ethiopia, Uganda, and Rwanda between 2009 and 2014 (Singh et al. 2015). Race TTKST,

TABLE 4. Stem rust severity and infection response for wheat differential monogenic lines and gene combination lines in the stem rust screening nursery at the National Plant Breeding Research Centre, Kenya Agricultural and Livestock Research Organization in Njoro, Kenya from 2011 to 2014[a]

Line	Genes	2011	2012	2013	2014 off-season	2014 main season
LMPG-6	…	35 S	60 S	70 S	60 S	80 S
Chinese Spring	…	10 S	15 S	40 MS-S	No data	50 S
LcSr24Ag	*Sr24*	45 S	40 S	40 MS-S	45 MS-S	50 S
Agent/9*LMPG	*Sr24*	40 S	60 S	60 S	60 S	No data
Sr31(Benno)/6*LMPG	*Sr31*	45 S	60 S	60 S	60 S	90 S
W2691SrTt-1	*Sr36*	10 MS	0	10 MR	5 MR-R	0
CI12632/8*LMPG	*Sr36*	Tr R	0	5 R-MR	10 MR-R	No data
CDLSr24Sr31	*Sr24, Sr31*	35 S	55 S	60 S	No data	90 S
CDLSr31Sr36	*Sr31, Sr36*	Tr R	0	5 R-MR	No data	0
CDLSr24Sr36	*Sr24, Sr36*	Tr R	0	5 R-MR	No data	0

[a] S = susceptible, MS = moderately susceptible, MR = moderately resistant, and R = resistant.

in contrast, increased in frequency to become a dominant race in the Kenyan *P. graminis* f. sp. *tritici* population.

The detection of race TTHST in 2013 is a first report of avirulence to *Sr30* in the Ug99 group, and is interesting with respect to the epidemiology of Ug99 and *P. graminis* f. sp. *tritici* in East Africa. Race TTHST was identified from a mixed sample that also yielded TTKST; thus, both races were coexisting, at least in the same field. Additional research on this isolate of race TTHST will be valuable for gaining better insights into the evolutionary trends within the Ug99 race group.

During 2013 and especially 2014, reports of high stem rust severity on the popular cultivar Robin led to great concern and a hypothesis that a new race had emerged and spread quickly, causing the breakdown in resistance in this cultivar. Both Robin and 'Digalu', a popular cultivar in Ethiopia, are reported to carry gene *SrTmp*. A newly detected race in Ethiopia (TKTTF, with virulence to *SrTmp*) was determined to be the cause of a stem rust epidemic in southern Ethiopia in 2013 to 2014 on Digalu (Olivera et al. 2015). This race could theoretically disperse to neighboring Kenya on prevailing winds. Race TKTTF was not detected in our samples from Kenya. Further sampling and race analysis is needed to determine whether TKTTF is present in Kenya, to better understand the distributions and frequencies of Ug99 variants present in Kenya, and to remain vigilant for early detection of new pathogen virulence. The need for coordinated plant pathogen surveillance efforts to help increase food security is becoming increasingly recognized, as are the high costs of such efforts for developing countries (Chakraborty and Newton 2011; Hodson 2011).

Kenya breeders have made progress, along with the international wheat community, incorporating sources of resistance to the known races of the Ug99 group into high-yielding lines. We tested a set of 15 lines that were either known or postulated to carry *SrTmp*, *SrND643*, *Sr9h*, *Sr28*, and *SrCad* for resistance to the Ug99 variants (Table 3). All lines carrying *SrTmp* consistently showed susceptible seedling reactions to new variants TTKTK and TTKTT, while lines carrying *Sr9h*, *Sr28*, *SrND643*, and *SrCad* showed seedling resistance to these races. Before the discovery of these two races, *SrTmp* has been valued as a source of resistance to Ug99 races. Because *SrTmp* originated from *T. aestivum* (McVey and Hamilton 1985), it is a source of resistance that can readily be incorporated into advanced lines without negative linkage drag. It has also been an important source of stem rust resistance in North American spring and winter wheats. However, the occurrence of these new races TTKTK and TTKTT in Kenya and TTKTK additionally in Uganda, Rwanda, and Egypt (Patpour et al. 2015) makes it important that growers and breeders shift to new cultivars and other gene deployment strategies as part of a broader integrated disease management approach.

Twenty isolates derived from *P. graminis* f. sp. *tritici* collections made in 2013 and 2014 in Kenya were genotyped using a custom SNP array (PgtSNP 3.0 chip). Phylogenetic analysis showed that isolates of TTHST, TTKTK, and TTKTT are part of the Ug99 race group (clade I) and distinctly different from isolates of TKTTF (clade IV). Minimum spanning network results indicated that isolates of these three new races have evolved from different genetic subgroups of clade I. The genotypic data may support the hypothesis that isolates of TTHST and TTKTK are more closely related to the TTKSK population and isolates of TTKTT are more closely related to the TTKST population. In the case of TTKTK isolates, the genotypic data indicate that the change in race

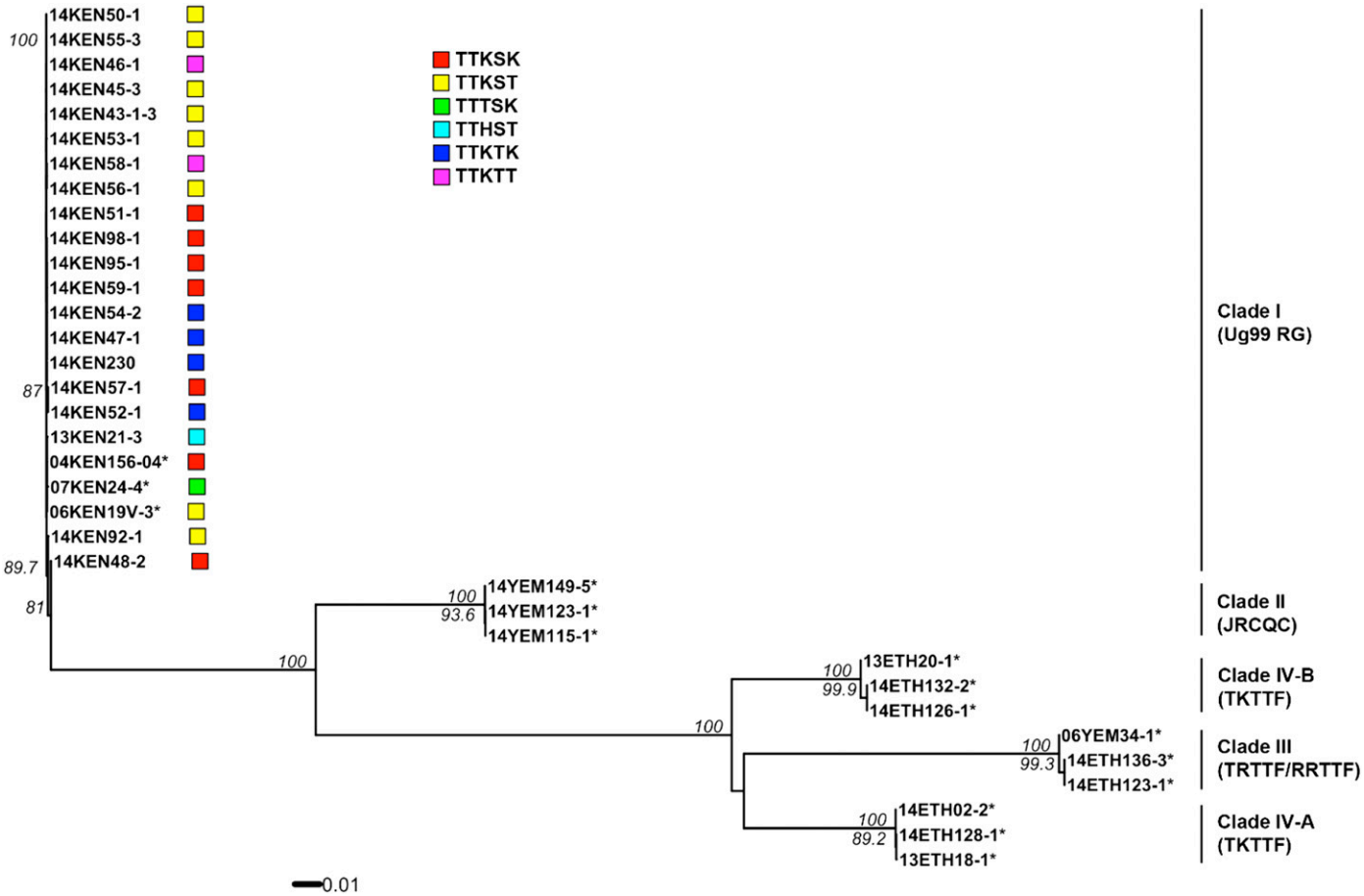

Fig. 2. Neighbor-joining phylogenetic tree of 20 Kenyan *Puccinia graminis* f. sp. *tritici* isolates from 2013 to 2014 based on 1,634 single-nucleotide polymorphism loci. Fifteen reference isolates were included in the analysis (*). Clades or subclades and corresponding race or race group are indicated. Race phenotypes for isolates in clade I are indicated by colors. Bootstrap values for 5,000 replicates are shown (>80%). Branch lengths are measured in number of substitutions per site.

phenotype occurred at least twice. It is interesting to note that the genotype of TTHST isolate (MLG.01) matched that of a reference isolate (04KEN156-4, TTKSK) collected in 2004, in contrast to isolates of TTKTK and TTKST, which corresponded to genotypes observed in the 2014 collections. This suggests that the isolate of race TTHST is part of an older lineage and raises the possibility that TTHST race type is not new but may have existed at low levels and gone undetected.

Given the genetic and phenotypic diversity of the Ug99 population in Kenya, it is clear that more extensive studies are necessary to understand the evolution of this race group. These studies should include genotyping of existing collections, as well as intensive sampling of the current population in Kenya and surrounding countries. A current bottleneck to intensive population studies is the lack of capacity to phenotype hundreds of samples per country per year to a race type. Increased capacity for race analysis within Kenya would enhance global capabilities and throughput of laboratories in Minnesota (USDA-ARS CDL), Denmark (GRRC), and Canada (Agriculture and Agri-Food Canada) that are currently race typing *P. graminis* f. sp. *tritici* samples from other countries around the world. Moreover, application of high-throughput SNP genotyping methods using single-pustule field collections will allow population studies with larger sample sizes.

An important outcome of detecting new races of *P. graminis* f. sp. *tritici* is that, if stored and maintained, they become new and useful tools for evaluating wheat germplasm for effective stem rust resistance. Information obtained from the resistance screening is critical for making decisions related to seed increases, cultivar releases, and breeding program planning. In this study, we first identified isolate 14KEN58-1 as TTKTT and, within 1 month, increased the isolate to sufficient quantity for resistance screening of breeding material. Results from seedling evaluations of 1,055 elite lines using race TTKTT indicate that this race, because of its virulence combination, is a major threat to both Kenya and North America. The proportion of cultivars and elite breeding lines from Kenya, and from the North American winter wheat and spring wheat regional nurseries, with seedling resistance to both TTKSK and TTKST but susceptibility to TTKTT indicates that *SrTmp* has been an important source of resistance to TTKSK and TTKST. It is promising that we detected a number of lines and sources of resistance that were effective against TTKTT. Alternative and additional sources of resistance other than *SrTmp* need to be considered, and the presence of TTKTT at multiple

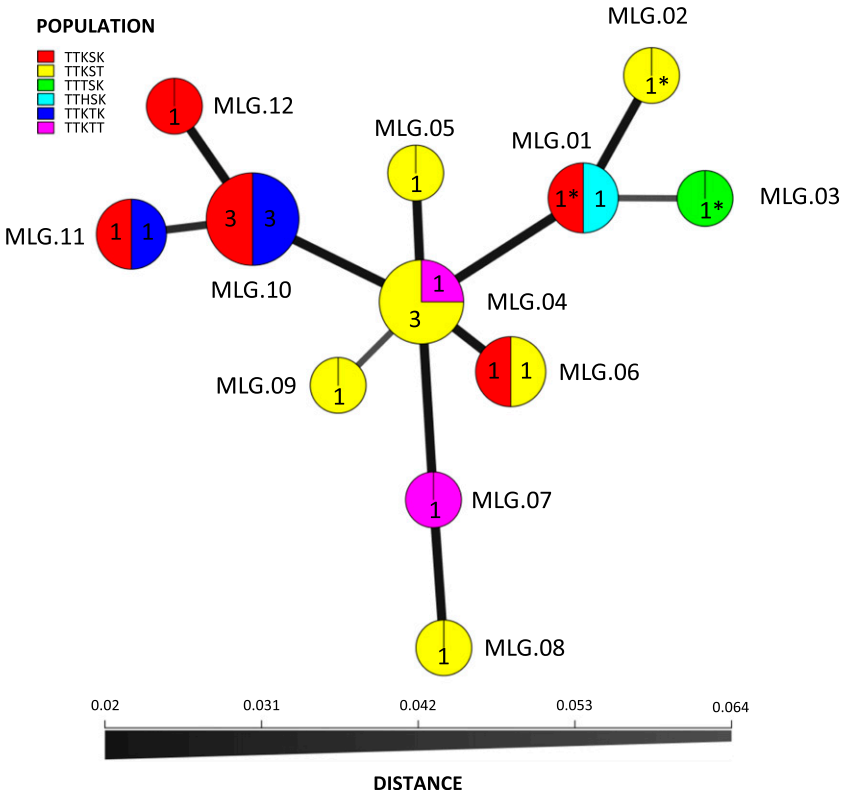

Fig. 3. Minimal spanning network of 20 Kenyan *Puccinia graminis* f. sp. *tritici* clade I isolates based on 31 single-nucleotide polymorphisms. In addition, three reference isolates were included in the analysis (*). Race phenotype of the isolates is indicated by color. Multiple locus genotypes (MLG) and number of isolates per race phenotype for each MLG are noted.

TABLE 5. Numbers of wheat (*Triticum aestivum*) lines screened at the seedling stage for resistance to races TTKSK (04KEN156/04), TTKST (07KEN24-4), and TTKTT (14KEN58-1) of *Puccinia graminis* f. sp. *tritici*, summarized by nursery groups

Nursery	Total lines screened with all three races	Resistant to TTKSK and TTKST	Resistant to TTKSK and TTKST and susceptible to TTKTT
Kenya elite material and breeding lines	169	80	18
U.S. winter wheat nursery selections	499	366	160
U.S. spring wheat nursery selections	107	34	31
Selections from CIMMYT 10th SRRSN	280	119	2

locations in Kenya needs to be considered during cultivar adoption and deployment planning.

In addition to characterizing races detected in Kenya between 2008 and 2014, we developed three lines with combinations of resistance genes *Sr31*, *Sr24*, and *Sr36* to monitor the *P. graminis* f. sp. *tritici* population in the field and facilitate race identification within the Ug99 race group. These lines are now deposited at the USDA-ARS NSGC and are available by request. Supplemental analyses were conducted to phenotypically and genotypically describe isolates from collections during 2013 and 2014. An isolate with combined virulence to *Sr31*, *Sr24*, and *SrTmp* was used to evaluate Kenyan and international advanced breeding lines for seedling resistance. These combined data inform wheat breeding programs of the spectrum of stem rust virulence in Kenya and increase our understanding of the epidemiology of the pathogen.

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