Phenotypic and Genotypic Characterization of Race TKTTF of *Puccinia graminis* f. sp. *tritici* that Caused a Wheat Stem Rust Epidemic in Southern Ethiopia in 2013–14

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ABSTRACT

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A severe stem rust epidemic occurred in southern Ethiopia during November 2013 to January 2014, with yield losses close to 100% on the most widely grown wheat cultivar, 'Digalu'. Sixty-four stem rust samples collected from the regions were analyzed. A meteorological model for airborne spore dispersal was used to identify which regions were most likely to have been infected from postulated sites of initial infection. Based on the analyses of 106 single-pustule isolates derived from these samples, four races of *Puccinia graminis* f. sp. *tritici* were identified: TKTTF, TTKSK, RRTTF, and JRCQC. Race TKTTF was found to be the primary cause of the epidemic in the southeastern zones of Bale and Arsi. Isolates of race TKTTF were first identified in samples collected in early October 2013 from West Arsi. It was the sole or predominant race in 31 samples collected from Bale and Arsi zones after the stem rust epidemic was established. Race TTKSK was recovered from 15 samples from Bale and Arsi zones at low frequencies. Genotyping indicated that isolates of race TKTTF belongs to a genetic lineage that is different from the Ug99 race group and is composed of two distinct genetic types. Results from evaluation of selected germplasm indicated that some cultivars and breeding lines resistant to the Ug99 race group are susceptible to race TKTTF. Appearance of race TKTTF and the ensuing epidemic underlines the continuing threats and challenges posed by stem rust not only in East Africa but also to wider-scale wheat production.

Additional keywords: dispersal model, surveillance.

Ethiopia is the largest wheat producer in sub-Saharan Africa (FAOSTAT 2014). Wheat is a traditional staple food crop, cultivated by 5 million households on 1.6 million ha of land under rain-fed conditions. Wheat rusts, primarily stem rust caused by *Puccinia graminis* f. sp. *tritici* and stripe rust caused by *P. striiformis* f. sp. *tritici*, are major biotic constraints to wheat production in Ethiopia. Repeated rust epidemics have occurred in the last 20 years, notably stripe rust on 'Dashen' in 1988 (Zewde et al. 1990), and stem rust 'Enkoy' in 1993 and 1994 (Shank 1994). In 2010, a devastating stripe rust epidemic affected more than 600,000 ha and widely grown 'Kubsa' and 'Galema', and the *Yr27*-virulent strain of *P. striiformis* f. sp. *tritici* was attributed to be a major cause of this epidemic (www.wheatrust.org).

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Following the identification and spread of the *P. graminis* f. sp. *tritici* the Ug99 race group in East Africa, major national and international efforts have been made to develop and promote stem-rust-resistant cultivars in Ethiopia (Singh et al. 2011). Heavy losses to stripe rust in 2010 provided a strong impetus for Ethiopian wheat farmers to adopt new rust-resistant cultivars. 'Digalu' (released in 2005) possesses good stripe rust resistance, high yield, and resistance to known races in the Ug99 group of *P. graminis* f. sp. *tritici*. Fast-track seed multiplication made it available quickly, and Digalu became a popular variety with Ethiopian farmers after 2010. By the 2013–14 main wheat season, Digalu was the most widely grown bread wheat cultivar in Ethiopia, planted on an estimated 500,000 ha. Intensive surveys undertaken in July 2013 during the short rain (Belg) season in southeastern and southern Ethiopia (Arsi, Bale, and SNNPR) found no stem rust infections on Digalu. Observations in field plots at Assasa, West Arsi on 10 October provided the first indication of increased susceptibility on Digalu, with stem rust severity scores of up to 50 disease severity and moderately susceptible (MS) to susceptible (S) responses (for more

information on severity scoring, see Peterson et al. [1948]). No further observations of high susceptibility on Digalu were reported until November. High incidence but low severity of stem rust on Digalu was observed on 15 and 16 November in Robe district, Arsi zone. Stem rust at epidemic proportions on Digalu was subsequently reported from two adjacent districts (Agarfa and Gasera) in Bale zone on 23 November, although stem rust was likely present in these districts for several weeks prior to the formal reports. Agarfa and Gasera districts represented the core of the epidemic; however, subsequent spread was observed during the period of November 2013 to January 2014. In total, 27 districts in southeastern and southern Ethiopia were affected to some extent based on field surveys (Fig. 1). Over 100,000 ha were planted to wheat in these districts, with an estimated 20,000 to 40,000 ha likely planted to Digalu and affected by stem rust. Digalu was observed to be highly susceptible, with 80 to 100% disease severity and moderately susceptible to susceptible infection responses throughout the affected region. Wheat production in this region of Ethiopia is asynchronous with the majority of Ethiopian wheat-growing areas, being 2 to 3 months later due to the presence of a prior Belg season. This asynchrony resulted in no further spread beyond southeastern and southern Ethiopia during the 2013–14 crop season because the majority of wheat crops in other areas had already been harvested. A crop loss assessment in three affected districts (Agarfa, Gasera, and Sinana) in Bale zone recorded losses of up to 92% on Digalu compared with harvested yields in 2012. Measured harvested yields in the worst-affected district (Gasera) were as low as 0.3 t/ha. Average losses on Digalu across all three districts, compared with reported yields on Digalu from the same fields in 2012–13, were 51%.

The objectives of this study were to identify and characterize the races of *P. graminis* f. sp. *tritici* that caused the stem rust epidemic in southeast Ethiopia, genotype representative isolates, and determine the level of vulnerability of Ethiopian and international bread wheat breeding materials to this potentially new virulence combination.

MATERIALS AND METHODS

Sample collection and storage. Dried samples of *P. graminis* f. sp. *tritici*-infected wheat tissue were mailed to two rust diagnostic laboratories: United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Cereal Disease Laboratory (CDL), St. Paul, MN and Aarhus University, Global Rust Reference Center (GRRC), Flakkebjerg, Denmark. Duplicated samples were also received by the Ethiopian Institute of Agricultural Research Ambo Plant Protection Research Center. Fifty-nine samples (13ETH01 to -59) of infected stems collected from bread and durum wheat cultivars and lines from 2 October 2013 to 21 January 2014 were sent to CDL, and five samples (13ETH60 to -64) collected in October to November 2013 were mailed to GRRC. Collection sites were located in eight Ethiopian wheat-growing zones: Arsi, West Arsi, Bale, Selti, East Shewa, West Shewa, North Shewa, and West Gojam (Fig. 2). Passport data of the samples are given in Table 1. Each sample consisted of 10 to 15 pieces of stem tissue of approximately 10 cm in length bearing moderately susceptible to susceptible pustules. Stem and leaf sheath tissue were kept in glassine or paper bags and air dried at room temperature. Dried samples were mailed to the two laboratories using an international express courier service with a transit time of approximately 5 days.

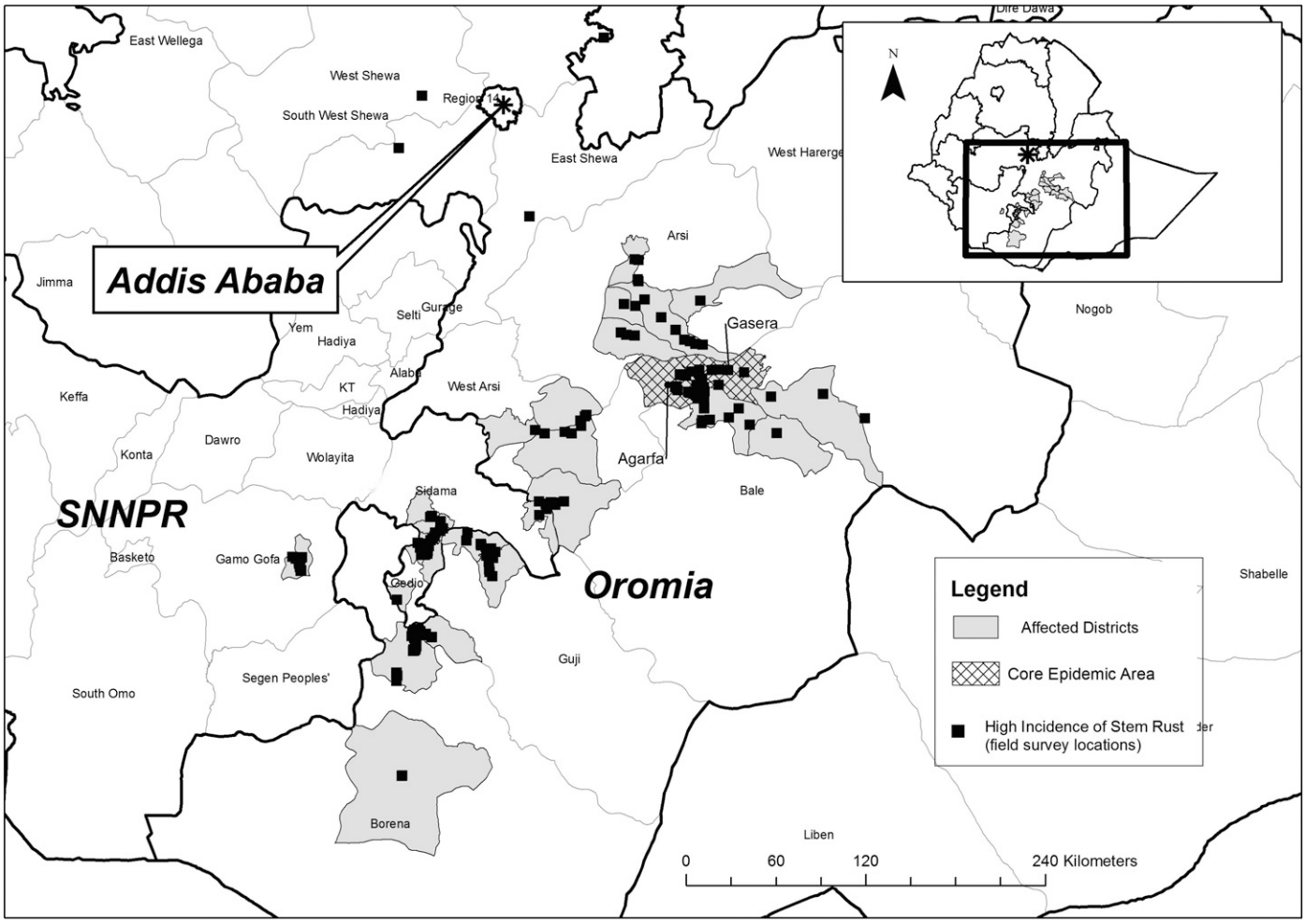

Fig. 1. Districts affected by a stem rust epidemic caused by race TKTTF of *Puccinia graminis* f. sp. *tritici* in Ethiopia from November 2013 to January 2014.

Shipping protocol was followed according to USDA Animal and Plant Health Inspection Service permit conditions for handling international cultures of *P. graminis* f. sp. *tritici*. Samples that arrived at the CDL before 1 December were stored in a –80°C freezer, whereas samples received after 1 December were processed upon arrival. Urediniospores from each sample were collected into three to four gelatin capsules (size 00) and stored at –80°C. Samples arriving at GRRC were processed upon arrival and recovered spore samples were stored in liquid nitrogen until further use.

Race identification. The North American stem rust differential set (Roelfs et al. 1993; Roelfs and Martens 1988) that was modified to further delineate *P. graminis* f. sp. *tritici* races in the TTKS race group (Jin et al. 2008) was used for race identification. In addition, all samples at CDL were further characterized on 20 monogenic lines carrying the following resistance genes: *Sr7a*, *Sr13*, *Sr22, Sr25, Sr26, Sr27, Sr31, Sr32, Sr33, Sr35, Sr37, Sr39, Sr40, Sr44, Sr45, Sr47, Sr50, SrSatu, SrTt-3,* and the 1A.1R translocation in winter wheat. Durum wheat 'Iumillo' (*Sr9g,12,*+) and 'Leeds' (*Sr9e,13*,+) were also included in the evaluation. One capsule per sample was removed from the –80°C freezer, heat shocked (45°C for 15 min), and then placed in a rehydration chamber (80% relative humidity maintained by a KOH solution) for a period of 4 h (Jin et al. 2008). Five seedlings of each differential and additional resistant lines were inoculated with a bulk collection of spores on fully expanded primary leaves at 8 to 9 days after planting. Experimental procedures for inoculation, incubation, and disease assessment were done as described by Jin et al. (2007). Single-pustule isolates were derived from individual plants after evaluation on the differential and additional lines. One to four pustules were isolated from each original collection. Incubation and collection of urediniospores from each single pustule was done as described by Jin et al. (2008). Urediniospores from the original samples and the pure cultures derived from single-pustule procedure were increased on the susceptible wheat Line E and McNair 701 (CItr 15288) in pots enclosed in cellophane bags (Zellglas Boden-Beutel, Germany) and stored at –80°C. Pure cultures were evaluated two to three times on differential lines before a race name was designated. Race designation was based on the letter code proposed by Roelfs and Martens (1988).

Race identification at GRRC and Ambo generally followed the procedures described above, except that at GRRC isolates were recovered on seedlings of 'Morocco' wheat. The seedlings were treated with 5 ml of 0.5% Antergon MH180 growth regulator (Nordisk Alkali, Randers, Denmark) to prevent further leaf formation and enhance spore production. After removal from the 24-h dew chamber, inoculated plants for spore increase and for differential set assays were incubated in spore-proof, climate-controlled cabinets at 19 to 21°C (day) and 16 to 18°C (night) with gradually changing temperature and a 16-h photoperiod from natural and supplemental light at 300 µE/m²/s PAR.

In order to increase the probability of recovering *P. graminis* f. sp. *tritici* isolates with specific virulence that might be present at a low frequency, 110 to 120 plants of lines ISr11-Ra (*Sr11*) and Sr31/6*LMPG (*Sr31*) were included in the evaluation for 15 samples collected from Bale and Arsi regions. At the time of disease assessment, the number of uredinia and infection sites

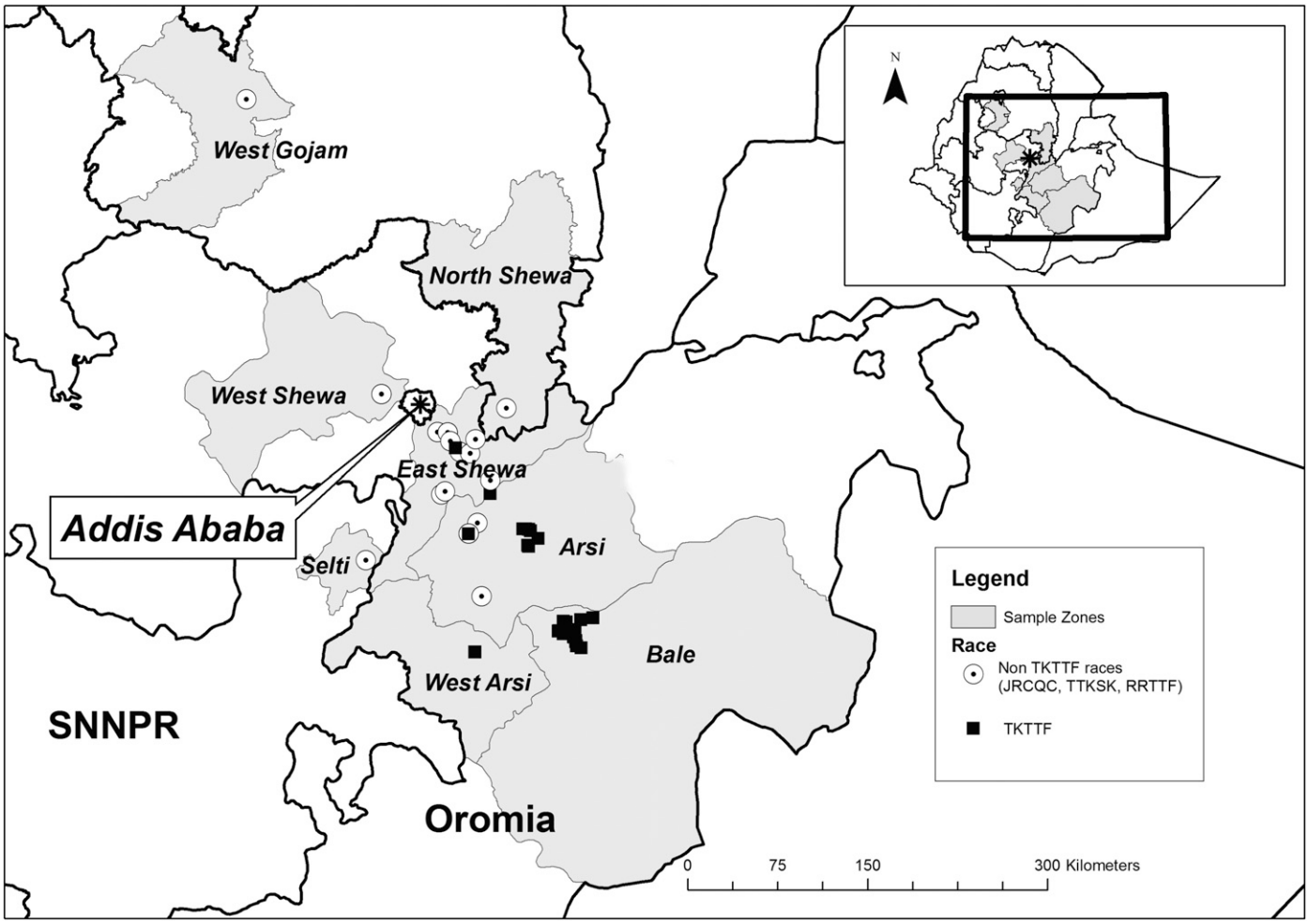

Fig. 2. Wheat stem rust sample collection sites (October 2013 to January 2014) and the distribution of race TKTTF and non-TKTTF races of *Puccinia graminis* f. sp. *tritici*.

on 20 randomly selected leaves was counted from both sets of plants, infection types (ITs) were recorded, and the frequency of each IT was calculated. Uredinia of *P. graminis* f. sp. *tritici* exhibiting high ITs were randomly selected, and urediniospores were collected and race-typed following the abovementioned procedures.

Genotyping of *P. graminis* f. sp. *tritici* isolates. DNA was extracted from either purified *P. graminis* f. sp. *tritici* urediniospores

TABLE 1. Races of *Puccinia graminis* f. sp. *tritici* identified from samples collected in Ethiopia from October 2013 until January 2014, according to collection date and site

Sample ID	Collection date	Collection site					Host	Variety	Races identified[b]
		Zone	Location[a]	Latitude	Longitude	Altitude (m)			
13ETH60	3 October 2013	Arsi	Kulumsa	8.01514	39.1579	2,214	Bread wheat	Hidasse	TTKSK
13ETH61	3 October 2013	East Shewa	Debre Zeit	8.77164	39.00023	1,897	Bread wheat	*Sr31* differential	TTKSK
13ETH62	10 October 2013	West Arsi	Assasa	7.125422	39.20741	2,381	Bread wheat	Digalu	TKTTF
13ETH49	10 October 2013	West Arsi	Assasa	7.125422	39.20741	2,381	Bread wheat	Digalu	TKTTF
13ETH50	10 October 2013	West Arsi	Assasa	7.125422	39.20741	2,381	Bread wheat	Digalu	TKTTF + TTKSK
13ETH15	19 October 2013	West Goham	Adet R.C.	11.278	37.4912	2,242	Bread wheat	Digalu	RRTTF
13ETH13	20 October 2013	Arsi	Kulumsa R.C.	8.0151	39.1579	2,214	Bread wheat	Hidase	TTKSK + TKTTF
13ETH24	21 October 2013	East Shewa	Denbi R.S.	8.7739	38.925	1,910	Bread wheat	Unknown	TTKSK
13ETH25	21 October 2013	East Shewa	Denbi R.S.	8.7739	38.925	1,910	Durum wheat	Unknown	JRCQC
13ETH26	22 October 2013	East Shewa	Debre Zeit R.C.	8.7716	39.0002	1,897	Bread wheat	Danda'a	TTKSK
13ETH27	22 October 2013	East Shewa	Debre Zeit R.C.	8.7716	39.0002	1,897	Bread wheat	Combination	JRCQC
13ETH28	22 October 2013	East Shewa	Debre Zeit R.C.	8.7716	39.0002	1,897	Bread wheat	Kota/6*LMPG	JRCQC
13ETH29	22 October 2013	East Shewa	Debre Zeit R.C.	8.7716	39.0002	1,897	Bread wheat	LcSr25Ars	JRCQC
13ETH30	22 October 2013	East Shewa	Debre Zeit R.C.	8.7716	39.0002	1,897	Bread wheat	RL5405	JRCQC
13ETH31	22 October 2013	East Shewa	Debre Zeit R.C.	8.7716	39.0002	1,897	Emmer wheat	Unknown	JRCQC
13ETH37	23 October 2013	East Shewa	Alem Tena	8.3098	38.9524	1,644	Durum wheat	Unknown	TTKSK
13ETH38	23 October 2013	East Shewa	Welenchiti	8.3323	38.9808	1,602	Bread wheat	Kubsa	TTKSK
13ETH32	24 October 2013	East Shewa	Debre Zeit	8.7055	39.0218	1,891	Bread wheat	Kubsa	TTKSK
13ETH33	24 October 2013	East Shewa	Mojo	8.6251	39.0927	1,806	Bread wheat	Kubsa	TTKSK
13ETH34	24 October 2013	East Shewa	Adama	8.616	39.1686	1,887	Bread wheat	Kubsa	TTKSK
13ETH35	24 October 2013	East Shewa	Deda	8.7194	39.2105	2,150	Bread wheat	Danda'a	TTKSK
13ETH36	24 October 2013	North Shewa	Minjar R.S.	8.955	39.4401	1,757	Durum wheat	Unknown	TTKSK + RRTTF
13ETH12	24 October 2013	Selti	Lanfro	7.815053	38.388203	1,872	Bread wheat	Jefferson	TTKSK
13ETH10	25 October 2013	East Shewa	Ude	8.6599	32.059	1,864	Bread wheat	Digalu	TKTTF
13ETH08	25 October 2013	Arsi	Shakee Sharara	8.09521	39.223837	2,209	Bread wheat	Hidase	TTKSK
13ETH09	25 October 2013	Arsi	Dhera R.S.	8.319	39.3209	1,686	Bread wheat	Kakaba	TKTTF + TTKSK
13ETH11	25 October 2013	Arsi	Melkassa R.C.	8.4141	39.3208	1,533	Bread wheat	Unknown	TTKSK
13ETH03	28 October 2013	Arsi	Kulumsa R.C.	8.0151	39.1579	2,214	Bread wheat	Shorima	TTKSK
13ETH05	28 October 2013	Arsi	Kulumsa R.C.	8.0151	39.1579	2,214	Bread wheat	Shorima	TTKSK
13ETH01	29 October 2013	Arsi	Kulumsa R.C.	8.0151	39.1579	2,214	Bread wheat	Kingbird	TTKSK
13ETH02	29 October 2013	Arsi	Kulumsa R.C.	8.0151	39.1579	2,214	Bread wheat	Danda'a	TTKSK
13ETH06	29 October 2013	Arsi	Kulumsa R.C.	8.0151	39.1579	2,214	Bread wheat	Danda'a	TTKSK
13ETH07	29 October 2013	Arsi	Kulumsa R.C.	8.0151	39.1579	2,214	Bread wheat	Digalu	TKTTF + TTKSK
13ETH04	30 October 2013	Arsi	Kulumsa R.C.	8.0151	39.1579	2,214	Bread wheat	Shorima	TTKSK
13ETH14	30 October 2013	Arsi	Bekoji	7.5443	39.2559	2,812	Bread wheat	Unknown	TTKSK
13ETH16	4 November 2013	West Shewa	Holetta R.C.	9.0607	38.5051	2,401	Bread wheat	Unknown	RRTTF
13ETH17	4 November 2013	West Shewa	Holetta R.C.	9.0607	38.5051	2,401	Durum Wheat	Unknown	JRCQC + RRTTF
13ETH63	8 November 2013	Arsi	Arsi Robe	7.884212	39.628016	2,443	Bread wheat	Unknown	TTKSK
13ETH64	8 November 2013	Arsi	Meraro	7.410474	39.257877	2,978	Bread wheat	Unknown	TTKSK
13ETH18	26 November 2013	Bale	Agarfa	7.237	39.9546	2,382	Bread wheat	Danda'a	TKTTF + TTKSK
13ETH19	26 November 2013	Bale	Agarfa	7.3595	39.8688	2,377	Bread wheat	Danda'a	TKTTF + TTKSK
13ETH20	26 November 2013	Bale	Agarfa	7.2136	39.964	2,365	Bread wheat	Digalu	TKTTF + TTKSK
13ETH21	26 November 2013	Bale	Agarfa	7.2391	39.9514	2,422	Bread wheat	Digalu	TKTTF + TTKSK
13ETH22	26 November 2013	Bale	Agarfa	7.2864	39.8375	2,410	Bread wheat	Digalu	TKTTF + TTKSK
13ETH23	26 November 2013	Bale	Agarfa	7.3517	39.8934	2,322	Bread wheat	Digalu	TKTTF + TTKSK
13ETH39	03 December 2013	Bale	Sinana	7.17241	39.96988	2,406	Bread wheat	Digalu	TKTTF
13ETH40	3 December 2013	Bale	Agarfa	7.23993	39.94786	2,420	Bread wheat	Danda'a	TKTTF + TTKSK
13ETH41	3 December 2013	Bale	Agarfa	7.26406	39.86922	2,510	Bread wheat	Danda'a	TKTTF
13ETH42	3 December 2013	Bale	Agarfa	7.284	39.83201	2,442	Bread wheat	Digalu	TKTTF
13ETH43	3 December 2013	Bale	Agarfa	7.35281	39.88284	2,376	Bread wheat	Hidasse	TKTTF
13ETH44	3 December 2013	Bale	Robe	7.15747	40.00434	2,444	Bread wheat	Digalu	TKTTF + TTKSK
13ETH45	3 December 2013	Bale	Gasera	7.36907	40.0018	2,340	Bread wheat	Digalu	TKTTF + TTKSK
13ETH46	3 December 2013	Bale	Gasera	7.38352	40.09188	2,337	Bread wheat	Digalu	TKTTF
13ETH47	3 December 2013	Bale	Robe	7.20527	39.96315	2,377	Bread wheat	Danda'a	TKTTF
13ETH48	3 December 2013	Bale	Sinana	7.294738	39.958571	2,373	Bread wheat	Digalu	TKTTF
13ETH51	21 January 2014	Arsi	Diksis	8.0531	39.5657	2,685	Bread wheat	Digalu	TKTTF
13ETH52	21 January 2014	Arsi	Diksis	8.0516	39.587	2,635	Bread wheat	Digalu	TKTTF
13ETH53	21 January 2014	Arsi	Sude	8.0381	39.6229	2,539	Bread wheat	Danda'a	TKTTF
13ETH54	21 January 2014	Arsi	Hule, Sude	7.9817	39.68	2,416	Bread wheat	Danda'a	TKTTF
13ETH55	21 January 2014	Arsi	Diksis	8.0481	39.6088	2,582	Bread wheat	Digalu	TKTTF
13ETH56	21 January 2014	Arsi	Diksis	8.0481	39.6088	2,582	Bread wheat	Digalu	TKTTF
13ETH57	21 January 2014	Arsi	Robe	7.9204	39.6086	2,462	Bread wheat	Digalu	TKTTF
13ETH58	21 January 2014	Arsi	Robe	7.9301	39.6048	2,462	Bread wheat	Digalu	TKTTF
13ETH59	21 January 2014	Arsi	Robe	7.9301	39.6048	2,462	Bread wheat	Digalu	TKTTF

[a] R.C. = Research Center and R.S. = Research Station.
[b] Race nomenclature was based on Jin et al. (2007), and predominant race in the sample was listed first.

(25 to 50 mg) or a 25-mm section of *P. graminis* f. sp. *tritici*-infected dry wheat leaf material. Starting material was pulverized with 1-mm glass beads (Lysing matrix C; MP Biomedicals, Santa Ana, CA) by shaking in a FastPrep-24 5G (MP Biomedicals) at a speed setting of 4 for 20 s, twice. DNA isolations were performed using the OmniPrep DNA extraction kit (G-Biosciences, St. Louis) as described by the manufacturer. Amount and purity of the DNA was determined using a Nanodrop Model ND-1000 (NanoDrop Products, Wilmington, DE).

A custom 1,536-single-nucleotide protein (SNP) GoldenGate Chip (PgtSNP Chip) (L. Szabo and J. Johnson, unpublished data) was used for genotyping. 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. Chips were read on an Illumina iScan instrument and genotyping calls were made using Illumina GenomeStudio software (v2011.1), with Genotyping module (v1.9.4). The SNP data set was refined from 1,536 to 918 by removing markers with GenTrain score < 0.6, 10%GC < 0.6, missing data (no calls), and inconsistency between replicates.

Phylogenetic analysis of the data was performed using R (version 3.1.2; R Core Team, 2014), with the package 'Poppr' version 1.1.2 (Kamvar et al. 2014) installed. The following packages and their libraries were also imported for analysis: Ape v3.1-4 (Paradis et al. 2004), Adegenet v1.4-2 (Jombart 2008), and Pegas v 0.6 (Paradis 2010). Trees were constructed with the 'aboot' function. Parameters were Nei's distance (Nei 1972, 1978), neighbor-joining (Saitou and Nei 1987), a sample of 5,000 bootstrap replicates, and cutoff value of 80%. Discriminant analysis of principal components (DAPC) (Jombart et al. 2010) was carried out using the 'dapc' function in Poppr. The first four principal components were retained for the analysis, which accounted for over 98% of the cumulative variance. Data were displayed using 'scatter' and 'compoplot' functions. A minimum spanning network was generated using 'msn' function with Nei's distance matrix of a subset of 41 *P. graminis* f. sp. *tritici* isolates from Ethiopia, which was organized by sample location.

Seedling evaluation of wheat germplasm. Isolate 13ETH18-1 derived from a sample collected in Bale zone was increased on the *Sr36* differential line W2691SrTt-1 for use in seedling screening assays conducted inside a University of Minnesota Biosafety Level 3 facility. Seedling screening entries included 66 Ethiopian cultivars and 66 Ethiopian advanced breeding lines that are candidates for being replacements of the current cultivars, as well as selections from the eighth and ninth Stem Rust Resistance Screening Nurseries (SRRSN) from the International Maize and Wheat Improvement Center (CIMMYT). The SRRSN selections were based on field stem rust resistance evaluated at the Kenya Agricultural Research Institute stem rust nursery at Njoro in the preceding years.

Methods for planting, seedling maintenance, and inoculations were the same as described above for race analysis on differential sets. All inoculations were conducted with fresh urediniospores collected from secondary increases on the susceptible wheat line McNair 701. Seedling ITs were determined at 12 to 14 days postinoculation following the 0-to-4 scale developed by Stakman et al. (1962). ITs greater than or equal to were categorized as susceptible reactions, while those less than 3- were categorized as resistant reactions. Seedling assays were conducted a single time per entry.

Modeling airborne spore dispersal. In order to assess the risk of pathogen spread via airborne dispersal of spores from the hypothesized locations of initial infection in Agarfa and Gasera within the Bale zone, we used the U.K. Met Office Numerical Atmospheric-dispersion Modeling Environment (NAME) model (Jones et al. 2007). NAME is a Lagrangian, stochastic particle dispersion model designed to predict the atmospheric transport and deposition of airborne substances to the ground surface. The model accounts for solar-induced loss of spore viability during transport, and for wet and dry spore deposition processes. The dispersal processes were driven by high-resolution meteorological data (interpolated from 25 km) from the U.K. Met Office Unified Model (Brown et al. 2012), with 3-hourly input data for a range of variables that include air temperature, wind speed and direction, and precipitation rate. Spore release was simulated daily between 09:00 and 15:00 local time. The maximum spore lifetime during transport was limited to 3 days, consistent with Singh et al. (2008), with the proportion of viable spores decreasing exponentially to a threshold of 1% by 3 days, giving a half-life of approximately 11 h. Sensitivity analysis, extending the survival to a maximum of 10 days, resulted in an extended dispersal range; however, wind directions were such that the qualitative radial patterns of regions receiving high and low spore deposition densities around the infection sources did not change. Parameters for wet and dry deposition were selected to be appropriate for spore sizes (Jones et al. 2007) and the spatial resolution of the spore deposition-sampling grid was set to 5 arcmin, approximately 9 km at the equator.

RESULTS

From 59 *P. graminis* f. sp. *tritici* samples collected in Ethiopia from October 2013 through January 2014 and sent to the CDL, 101 single pustules were derived. In addition, five isolates were derived from the samples submitted to GRRC in 2013. From these isolates, four races of *P. graminis* f. sp. *tritici* were identified: TKTTF, TTKSK, JRCQC, and RRTTF (Table 1).

Zones of East, North, and West Shewa and West Gojam. In the zones of Shewa and Gojam, the wheat-growing period extends from June to November, with stem rust samples collected from October through early November. Both bread wheat and durum are cultivated in these regions. In samples collected from the zones of West Gojam and West, North, and East Shewa, *P. graminis* f. sp. *tritici* races TTKSK, JRCQC, and RRTTF predominated; only one sample collected at Ude, East Shewa on 25 October was confirmed to be race TKTTF. The most frequent race observed was TTKSK, which was most often found in samples collected from bread wheat varieties planted in farmer's fields (Table 1). Isolates of race RRTTF were found in four geographic zones in late 2013 (Table 1). This race was identified from samples collected from both bread wheat and durum. The virulence profile of race RRTTF from samples collected in Ethiopia in 2013 (Table 2) was identical to that of isolates of race RRTTF first reported in Ethiopia and Yemen in 2007 (Fetch 2009) and Pakistan in 2009 (Iqbal et al. 2010). Isolates of race JRCQC were observed primarily at the Debre Zeit Research Center, where it is used as inoculum in the durum stem rust field nursery. It was also collected from durum wheat at Holetta Research Center (West Shewa) and Denbi Research Station (East Shewa), the later located less than 10 km from the Debre Zeit stem rust nursery. All the JRCQC isolates from different locations had an identical virulence profile compared with each other (Table 2) and with previously described collections made in 2009 at the Debre Zeit stem rust nursery (Olivera et al. 2012).

Zones of Arsi, West Arsi, Bale (Oromia region), and Selti (SNNPR). In the southeast zones of Arsi, West Arsi, Bale, and Selti, bread wheat is the predominant crop, and Digalu is currently one of the most important wheat varieties, occupying approximately 30 to 40% of wheat acreage. In Arsi, West Arsi, and Selti, the majority of wheat is planted at the same time as in Shewa, and stem rust samples were collected in October. Pockets of wheat in Arsi zone (e.g., Arsi Robe, Diksis, and Sude districts) were late planted in August to September and stem rust samples were collected from November through January. In Bale zone and parts of SNNPR, wheat is grown in two seasons: a short Belg season planted in March to April and harvested in July, and a main season planted in August and harvested in November to January. Stem rust samples are usually collected in either July (Belg season) or from November to January (main season).

Race TKTTF was first identified from stem rust samples collected on Digalu from Assasa, West Arsi in early October 2013 (10 October). In late October, race TKTTF was identified from three samples collected from Arsi (Kulumsa Research Center, 20 and 29 October; and Dhera Research Station, 25 October) (Table 1). From the remaining 11 Arsi samples collected in October, the only race identified was TTKSK. However, once the localized stem rust epidemic started in Bale and Arsi zones of Ethiopia, 27 of 29 samples collected in the period November 2013 to January 2014 yielded only race TKTTF (16 samples) or were collected where isolates of race TKTTF were dominant (11 samples) (Table 1). Race TTKSK was identified from two samples only, which were not collected from Digalu.

Isolate 13ETH18-1 (race TKTTF) produced high ITs on differential lines carrying *Sr5*, *Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g*, *Sr10, Sr17, Sr21, Sr30, Sr36, Sr38, SrTmp*, and *SrMcN* (Table 2). The differences between isolates of race TKTTF and TTKSK on these differential lines is shown in Figure 3. In a few replications, intermediate ITs on *Sr9b* inoculated with isolate 13ETH18-1 were observed. Thus, it is uncertain whether isolates of TKTTF are actually virulent on *Sr9b*. *P. graminis* f. sp. *tritici* race TKTTF is highly virulent on Digalu, producing disease severities up to 100-S in unsprayed fields (data not shown). Isolates of race TKTTF were avirulent to all the additional stem rust resistance genes tested in this study (Table 3). The uredinial morphology of *P. graminis* f. sp. *tritici* isolates of race TKTTF is similar to that observed in isolates of race TRTTF (Olivera et al. 2012) and RRTTF, in that the color of uredinia is darker than normal and the rupture of the epidermal tissue over the uredinia is delayed. These morphological characteristics are distinct from other common *P. graminis* f. sp. *tritici* isolates.

In some stem rust samples in which race TKTTF was predominant, the presence of pustules with high IT on differential lines ISr11-Ra (*Sr11*) and Sr31/6*LMPG (*Sr31*) was observed at low frequencies. These "off types" suggested that these samples contained *P. graminis* f. sp. *tritici* TTKSK-like races. In order to

TABLE 2. Infection types (ITs) observed on stem rust differentials produced by inoculating races TKTTF, TTKSK, JRCQC, and RRTTF of *Puccinia graminis* f. sp. *tritici* collected in Ethiopia in the period during October 2013 to January 2014

		IT[a]			
Line	Gene	TKTTF (13ETH18-1)	TTKSK (13ETH04-1)	JRCQC (13ETH29-2)	RRTTF (13ETH15-2)
ISr5-Ra	*Sr5*	3+	4	;	4
Cns_T_mono_deriv	*Sr21*	33+	33+	3+	3+
Vernstein	*Sr9e*	3+	3+	3+	2
ISr7b-Ra	*Sr7b*	3+	4	2	3+
ISr11-Ra	*Sr11*	2-	4	3+	3+
ISr6-Ra	*Sr6*	3+	3+	3+	4
ISr8a-Ra	*Sr8a*	3+	3+	2	2-
CnsSr9g	*Sr9g*	4	4	3+	4
W2691SrTt-1	*Sr36*	4	0	0;	4
W2691Sr9b	*Sr9b*	3+	3+	2+	3+
BtSr30Wst	*Sr30*	3+	3+	2-	4
Combination VII	*Sr17* (+*Sr13*)	2+	2	2+3	2+3
ISr9a-Ra	*Sr9a*	4	3+	3+	3+
ISr9d-Ra	*Sr9d*	3+	3+	3+	3+
W2691Sr10	*Sr10*	3+	4	1+3;	4
CnsSrTmp	*SrTmp*	3+	2+	2-	3+
LcSr24Ag	*Sr24*	2-	2-	2-	2
Sr31/6*LMPG	*Sr31*	2-	4	2-	;2-
VPM1	*Sr38*	3+	3+	11+;	3+
McNair 701	*McN*	4	4	4	4

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

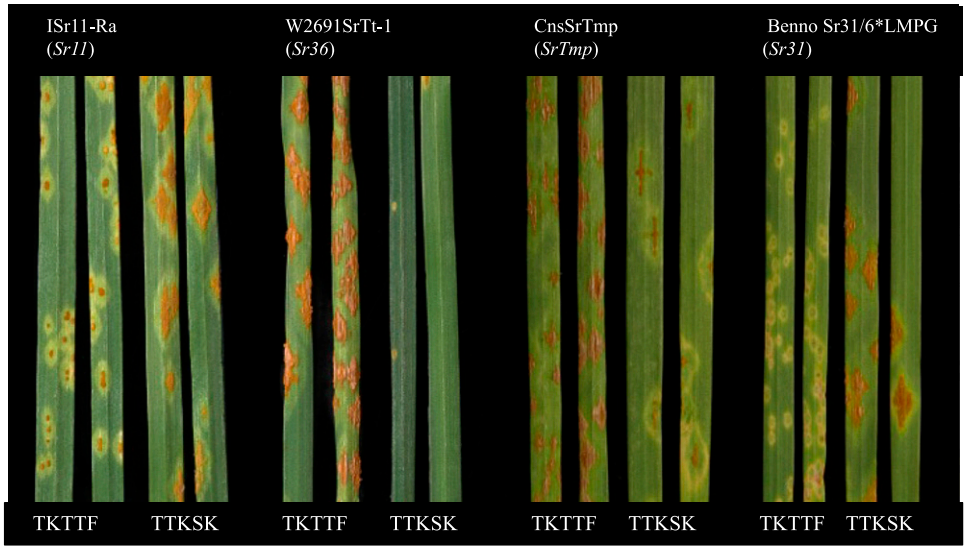

Fig. 3. Infection types on four differential lines that differentiate between races TKTTF and TTKSK of *Puccinia graminis* f. sp. *tritici*.

test this, bulk uredinial collections from 15 samples (Bale and Arsi zones) were inoculated onto approximately 120 seedling plants of ISr11-Ra (*Sr11*) and Sr31/6*LMPG (*Sr31*). The number of uredinia and infection sites on 20 randomly selected leaves per line was counted, and the frequencies of high IT uredinia on *Sr11* and *Sr31* were calculated. In all cases, the frequencies of the high IT uredinia were less than 5% (Table 4), indicating low frequencies of *P. graminis* f. sp. *tritici* races that were not TKTTF. Randomly selected uredinia from high-IT pustules only produced isolates of race TTKSK.

Genotyping. In all, 41 isolates of 2013 Ethiopian *P. graminis* f. sp. *tritici* (29 isolates of race TKTTF, 6 of race TTKSK, 3 of race RRTTF, and 3 of race JRCQC) and 6 reference isolates, which included representatives of the Ug99 race group (TTKSK, TTKST, TTTSK, and TTKSF), TRTTF, and JRCQC, were genotyped using a custom PgtSNP Chip (Table 5). After filtering, 918 SNP loci were used for analysis. Sixteen multilocus genotypes (MLGs) were identified in this set of 47 isolates. Phylogenetic analysis divided these isolates into four well-supported clades, with bootstrap values of 100% (Fig. 4). Three of these clades contained *P. graminis* f. sp. *tritici* isolates of the Ug99 race group (clade I), JRCQC (clade II), TRTTF or RRTTF (clade III), and the corresponding reference isolates for each of these races. The fourth clade (clade IV) contained the 29 TKTTF isolates and was composed of two well-supported branches: subclade IV-A contained 12 isolates and subclade IV-B contained 17 isolates. Although all isolates of subclade IV-A were composed of a single MLG (MLG.01), subclade IV-B contained five MLGs and was further subdivided into three well-supported branches (IV-B1, IV-B2, and IV-B3). Subclade IV-B1 contained two isolates (13ETH40-2 and 13ETH47-1), with each being a separate MLG (MLG.06 and MLG.07,

TABLE 3. Infection types (ITs) observed on lines carrying additional resistance genes to race TKTTF (isolate 13ETH18-1) of *Puccinia graminis* f. sp. *tritici* collected from Bale region of Ethiopia[a]

Line	Gene	Type	TKTTF (13ETH18-1)
NA101/MqSr7a	*Sr7a*	Bread wheat	31;
ST464	*Sr13*	Durum wheat	22+
SwSr22T.B.	*Sr22*	Bread wheat	2-;
Agatha/9*LMPG	*Sr25*	Bread wheat	22+
Eagle	*Sr26*	Bread wheat	2-;
73,214,3-1/9*LMPG	*Sr27*	Bread wheat	;1
Federation*4/Kavkaz	*Sr31*	Bread wheat	2-;
ER 5155	*Sr32*	Bread wheat	2
Tetra Canthatch/Ae. squarrosa	*Sr33*	Bread wheat	;2-
Mq(2)5XG2919	*Sr35*	Bread wheat	;
W3563	*Sr37*	Bread wheat	1;3-
RL6082	*Sr39*	Bread wheat	;2-
RL6088	*Sr40*	Bread wheat	2
TAF 2	*Sr44*	Bread wheat	;1
CSID 5406	*Sr45*	Bread wheat	;1-
DAS15	*Sr47*	Durum wheat	;N
Fed*3/Gabo*51BL.1RS-1-1	*Sr50*	Bread wheat	22-
Satu	*SrSatu*	Triticale	;
Fed/SrTt-3	*StTt-3*	Bread Wheat	1+1
TAM 107	*1A.1R*	Bread wheat	2-;
Leeds	*Sr9e,13,*+	Durum wheat	;
Iumillo	*Sr9g,12,*+	Durum wheat	;

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

TABLE 4. Frequency of pustules exhibiting high infection type (IT) on wheat lines ISr11-Ra (*Sr11*) and Sr31/6*LMPG (*Sr31*) inoculated with the original stem rust samples[a]

Sample	Collection date	Predominant race	High IT pustules (%) *Sr11*	High IT pustules (%) *Sr31*
13ETH18	26 November 2013	TKTTF	0.51	0.77
13ETH19	26 November 2013	TKTTF	4.42	3.81
13ETH20	26 November 2013	TKTTF	0.53	0.51
13ETH21	26 November 2013	TKTTF	1.37	1.75
13ETH22	26 November 2013	TKTTF	0.89	0.66
13ETH23	26 November 2013	TKTTF	4.05	3.37
13ETH51	21 January 2014	TKTTF	0.00	0.00
13ETH52	21 January 2014	TKTTF	0.00	0.00
13ETH53	21 January 2014	TKTTF	0.00	0.00
13ETH54	21 January 2014	TKTTF	2.33	2.80
13ETH55	21 January 2014	TKTTF	0.00	0.00
13ETH56	21 January 2014	TKTTF	0.00	0.00
13ETH57	21 January 2014	TKTTF	0.00	0.05
13ETH58	21 January 2014	TKTTF	0.00	0.00
13ETH59	21 January 2014	TKTTF	0.00	0.00

[a] ITs observed on seedlings at 14 days postinoculation using a 0-to-4 scale according to Stakman et al. (1962), where ITs of 3 or higher are considered as a high IT.

TABLE 5. Isolates of *Puccinia graminis* f. sp. *tritici* used for genotyping.

Sample ID	Isolate ID	Zone, country	Year	Race	Genetic type[a]
13ETH07	13ETH07-1	Arsi	2013	TKTTF	Type A
13ETH09	13ETH09-1	Arsi	2013	TKTTF	Type A
	13ETH09-2	…	…	…	Type A
13ETH10	13ETH10-1	East Shewa	2013	TKTTF	Type A
	13ETH10-2	…	…	TKTTF	Type B
	13ETH10-3	…	…	TKTTF	Type B
13ETH13	13ETH13-1	Arsi	2013	TKTTF	Type A
	13ETH13-2	…	…	TKTTF	Type A
	13ETH13-4	…	…	TKTTF	Type A
13ETH15	13ETH15-1	West Goham	2013	RRTTF	na
	13ETH15-2	…	…	RRTTF	na
13ETH16	13ETH16-1	West Shewa	2013	RRTTF	na
13ETH17	13ETH17-2	West Shewa	2013	JRCQC	na
13ETH18	13ETH18-1	Bale	2013	TKTTF	Type A
13ETH19	13ETH19-1	Bale	2013	TTKSK	na
	13ETH19-2	…	…	TKTTF	Type B
13ETH20	13ETH20-1	Bale	2013	TKTTF	Type B
13ETH21	13ETH21-1	Bale	2013	TKTTF	Type B
13ETH22	13ETH22-1	Bale	2013	TKTTF	Type A
	13ETH22-2	…	…	TKTTF	Type A
13ETH23	13ETH23-1	Bale	2013	TKTTF	Type B
13ETH25	13ETH25-1	East Shewa	2013	JRCQC	na
13ETH31	13ETH31-2	East Shewa	2013	JRCQC	na
13ETH35	13ETH35-2	East Shewa	2013	TTKSK	na
13ETH39	13ETH39-1	Bale	2013	TKTTF	Type B
13ETH40	13ETH40-2	Bale	2013	TKTTF	Type B
13ETH41	13ETH41-1	Bale	2013	TKTTF	Type B
13ETH42	13ETH42-1	Bale	2013	TKTTF	Type B
13ETH43	13ETH43-1	Bale	2013	TKTTF	Type A
13ETH44	13ETH44-2	Bale	2013	TKTTF	Type B
13ETH45	13ETH45-2	Bale	2013	TKTTF	Type B
13ETH46	13ETH46-2	Bale	2013	TKTTF	Type B
13ETH47	13ETH47-1	Bale	2013	TKTTF	Type B
13ETH48	13ETH48-1	Bale	2013	TKTTF	Type B
13ETH49	13ETH49-1	West Arsi	2013	TKTTF	Type B
13ETH50	13ETH50-2	West Arsi	2013	TKTTF	Type A
13ETH60	13ETH60-1	Arsi	2013	TTKSK	na
13ETH61	13ETH61-1	East Shewa	2013	TTKSK	na
13ETH62	13ETH62-1	West Arsi	2013	TKTTF	Type B
13ETH63	13ETH63-1	Arsi	2013	TTKSK	na
13ETH64	13ETH64-1	Arsi	2013	TTKSK	na
…	04KEN156-4[b]	Kenya	2004	TTKSK	na
…	06KEN19-V-3[b]	Kenya	2006	TTKST	na
…	06YEM34-1[b]	Yemen	2006	TRTTF	na
…	07KEN24-4[b]	Kenya	2007	TTTSK	na
…	09ZIM01-5[b]	Zimbabwe	2009	TTKSF	na
…	87KEN3018-4[b]	Kenya	1987	JRCQC	na
…	75-36-700[b]	United States	1975	SCCLC	na
…	78-21-BB463[b]	United States	1978	DFBJ	na

[a] Abbreviation: na = not applicable.
[b] Isolate included as reference.

respectively). Subclade IV-B2 contained a single isolate (13ETH41-1, MLG.08) and appears to represent an intermediate branch between subclades IV-B1 and IV-B3. Subclade IV-B3 was made up of 13 isolates and two MLGs (MLG.05, 12 isolates and MLG.04, 1 isolate).

In order to further evaluate the population structure of this set of *P. graminis* f. sp. *tritici* isolates, DAPC was used (Jombart et al. 2010). The 47 *P. graminis* f. sp. *tritici* isolates formed four discrete genetic clusters corresponding to the four phylogenetic clades I to IV described above (Fig. 5). All isolates had a membership probability of 1.0, reflecting the highly clonal nature of *P. graminis* f. sp. *tritici* and indicating a lack of recent genetic exchange between these clusters. Furthermore, the 41 Ethiopian isolates were examined using Minimum Spanning Network analysis. The four distinct clades identified in the phylogenetic analysis are clearly shown (Fig. 6). This analysis supports the relationships within clade IV, with subclades IV-B1 and IV-B2 being intermediate between IV-A and IV-B3. Furthermore, the Minimum Spanning Network analysis suggests that clade III (TRTTF and RRTTF) is more closely related to subclade IV-B than IV-A. Given the large distances between the clades, additional *P. graminis* f. sp. *tritici* isolates will need to be genotyped in order to better resolve this network and understand the evolutionary relationships.

Genetic variation between *P. graminis* f. sp. *tritici* isolates within the same race phenotype was also observed in the other three clades (Fig. 4; Table 5). Of the six Ethiopian isolates of race TTKSK (clade I), five of them had the same MLG (MLG.10) as the reference isolate (04KEN156-4), while one (13ETH61-1) was different (MLG.09). Each of the three remaining reference isolates for the Ug99 race group (isolate 06KEN19-V3 of race TTKST, isolate 07KEN24-4 of race TTTSK, and isolate 09ZIM1-5 of race TTSKF) represented distinct MLGs. No substructure was identified within this clade. Clade III (TRTTF and RRTTF) was similar, in that the three Ethiopian isolates (13ETH15-1, 13ETH15-2, and 13ETH16-1) of race RRTTF were represented by two MLGs (MLG.2 and MLG.3).

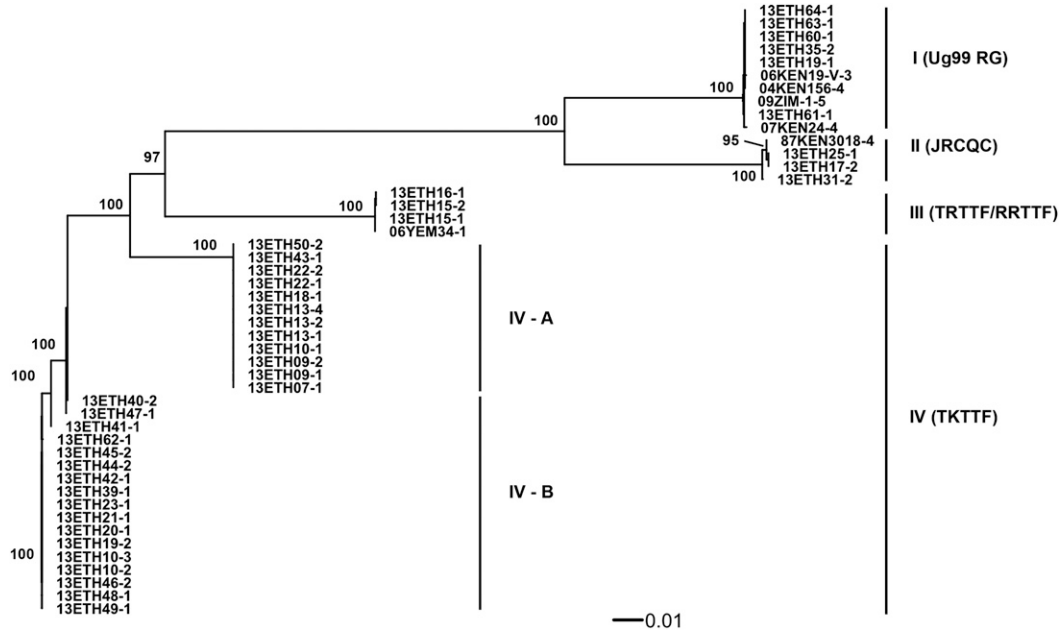

Fig. 4. Neighbor-joining phylogenetic tree of 47 isolates of *Puccinia graminis* f. sp. *tritici* based on data from 918 single-nucleotide protein loci. Clades or subclades and corresponding race or race groups are indicated. The tree is unrooted. Bootstrap values for 5,000 replicates are shown, if greater than 80%. Branch lengths are measured in the number of substitutions per site.

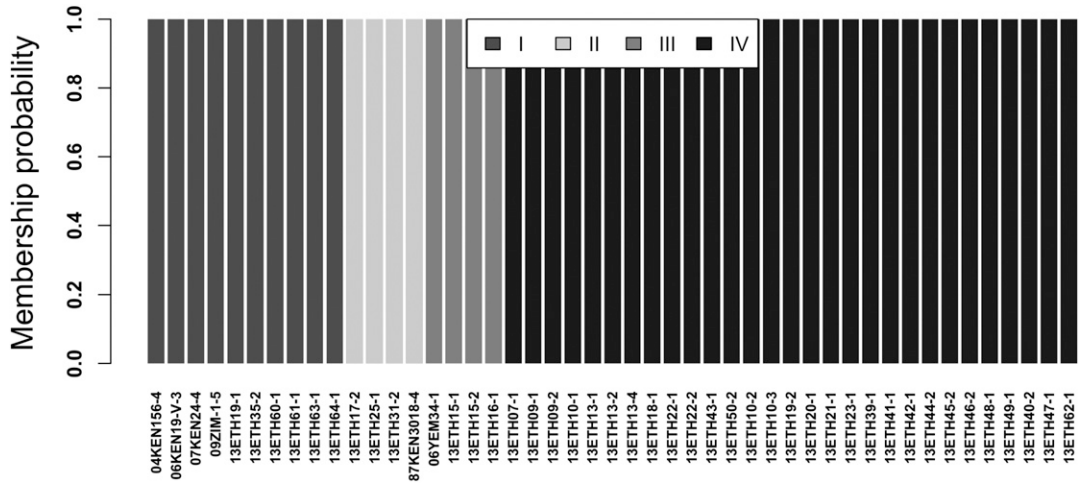

Fig. 5. Plot of membership probability of each isolate to each genetic cluster for 47 isolates of *Puccinia graminis* f. sp. *tritici*. Discriminant analysis of principal components program with the first four principal components analyses, which accounted for greater than 98% of the cumulative variance. Each shade represents a genetic cluster and each vertical bar represents an individual.

As in clade I, no substructure was observed. However, in clade II (JRCQC), well-supported substructure was observed between Ethiopian isolates with different MLGs. Isolate 13ETH31-2 (MLG.14) formed a subclade distinct from 13ETH17-2 and 13ETH25-1 (MLG.16) and the reference isolate 87KEN3018-4 (MLG.15).

The majority of the clade IV-B isolates (13 of 17 isolates) was collected from Bale zone (Table 5; Fig. 6). The remaining isolates were from West Arsi (2 isolates) and East Shewa (2 isolates) zones. Clade IV-A isolates were from Arsi (6 isolates), West Arsi (1 isolates), Bale (4 isolates), and East Shewa (1 isolates) zones. For six of the Ethiopian samples, multiple isolates per sample were genotyped. Only one of these samples (13ETH10) contained representatives of both IV subclades: IV-A, 13ETH10-1 and IV-B, 13ETH10-2 and 13ETH10-3. Two different MLGs were observed from isolates derived from the sample 13ETH15 (13ETH15-1, MLG.02 and 13ETH15-2, MLG.03). Isolates derived from the sample 13ETH19 were of different *P. graminis* f. sp. *tritici* races: 13ETH19-1 (TTKSK) and 13ETH19-2 (TKTTF). For the other three cases (13ETH09, 13ETH13, and 13ETH22), no differences in race or MLG were observed between different isolates derived from the same sample. The number of isolates of race TKTTF genotyped in this study is too small to determine whether there is significant geographical stratification between the MLGs within Ethiopia.

Reactions of breeding germplasm. Ethiopian cultivars (66 entries), elite breeding lines (66 entries) from several Ethiopian research centers, and the most recent SRRSN (the eighth and ninth SRRSN, with 274 and 457 entries, respectively) from CIMMYT were evaluated against *P. graminis* f. sp. *tritici* isolates of race TKTTF and TTKSK (Table 6). Of the 66 Ethiopian cultivars, 12 were resistant to isolates of race TTKSK, and 5 of these TTKSK-resistant lines were susceptible to isolates of race TKTTF. None of the 13 most widely grown cultivars in Ethiopia were resistant to isolates of both races TTKSK and TKTTF (Table 7). Of the 66 Ethiopian breeding lines, 50 showed seedling resistance to isolates of TTKSK, and 11 of these

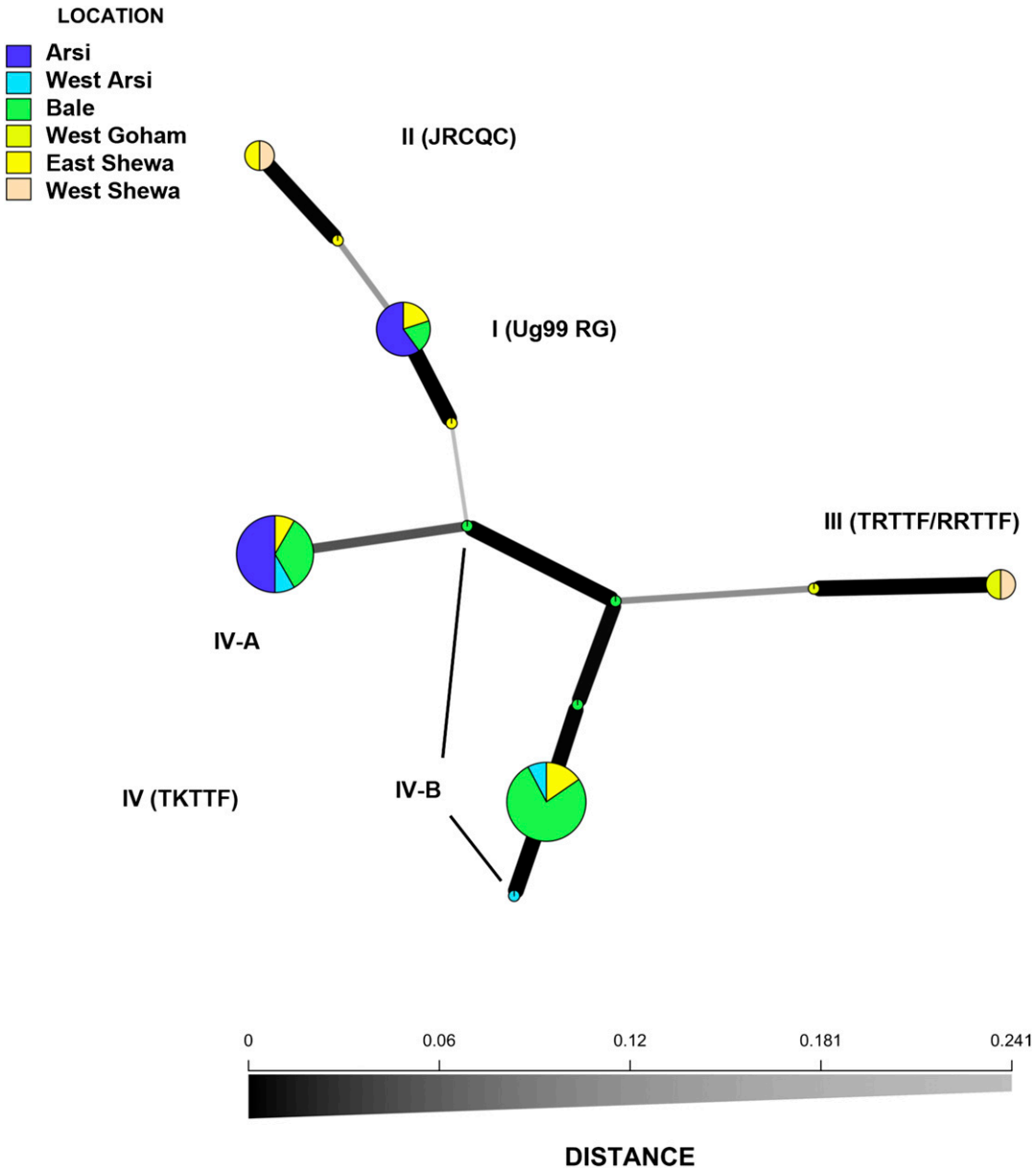

Fig. 6. Minimum spanning network of 41 Ethiopian isolates of *Puccinia graminis* f. sp. *tritici*. Origin of isolates is indicated by colors. Phylogenetic clades or subclades and corresponding race or race groups are shown. Distance is measured in the number substitutions per site.

TTKSK-resistant lines were susceptible to isolates of TKTTF. In total, 221 and 198 of the 274 and 457 lines from the eighth and ninth SRRSN, respectively, showed seedling resistance to isolates of TTKSK. Of the 221 and 198 TTKSK-resistant lines, 21 and 31%, respectively, were susceptible to isolates of TKTTF. If these TKTTF-susceptible lines lack adult plant resistance, they will be vulnerable to the *P. graminis* f. sp. *tritici* race TKTTF if released as cultivars.

Modeling airborne spore dispersal. The results from the simulation model indicate that airborne spore dispersal during October and November 2013 occurred in the largest quantities to the southwest of the source region in Bale zone (Fig. 7). The regions of greatest simulated spore deposition concentration are coincident with the locations of the positive disease survey sites to the southwest of the Bale zone and highest levels of disease incidence on Digalu. The regions of lower simulated spore deposition concentration are coincident with the locations of the negative disease survey sites to the northwest of the Bale zone. The results are consistent with the hypothesis that the locations of high stem rust incidence on Digalu to the southwest of the core of the epidemic are the result of airborne spore dispersal from the initial outbreaks in the Bale zone.

TABLE 6. Number of wheat (*Triticum aestivum*) lines with resistant (R) or susceptible (S) reactions at seedling stage to races TTKSK (04KEN156/04) and TKTTF (13ETH18-1) of *Puccinia graminis* f. sp. *tritici*

	Seedling reaction to TTKSK (number of lines)[a]							
	Ethiopian breeding lines		Ethiopian cultivars		CIMMYT eighth SRRSN		CIMMYT ninth SRRSN	
Seeding reaction to TKTTF	R	S	R	S	R	S	R	S
R	39	7	7	29	175	34	136	182
S	11	9	5	25	46	19	62	77
Total	50	16	12	54	221	53	198	259

[a] SRRSN = Stem rust resistance screening nursery from CIMMYT (International Maize and Wheat Improvement Center, Mexico D.F., Mexico. Infection types (ITs) observed on seedlings at 14 days postinoculation using a 0-to-4 scale according to Stakman et al. (1962), where ITs of 0, ;, 1, 2, or combinations are considered as a low IT (resistant reaction) and ITs of 3 or higher are considered as a high IT (susceptible reaction).

TABLE 7. Seedling infection types (ITs) observed on common Ethiopian cultivars to isolates of *Puccinia graminis* f. sp. *tritici* races TTKSK and TKTTF

		IT[a]	
Cultivar	2012 Acreage (ha)[b]	TTKSK (04KEN156/04)	TKTTF (13ETH18-1)
Digalu	522,274	2+	3+
Kubsa	247,500	3+	3+
Kakaba	213,596	3+	3+
Tusie	126,042	3+	0;
Madda Walabu	116,220	3+	0;
Danda'a	89,720	3+	33+
ET-13 A2	40,923	33+	;1+3/33+/2-
Galema	33,000	3+	3+
Pavon 76	32,738	3+	;2-
Dashen	16,369	3+	;2-
Sofumar	14,732	3+	0;
K6295-4A	6,548	3+	33+
Simba	4,911	3+	2-

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

[b] Acreage data obtained from the CIMMYT wheat atlas on 8 May 2014 (http://wheatatlas.org/).

DISCUSSION

The Ethiopian countrywide epidemic of stripe rust in 2010 caused on-farm losses close to 100% and estimated national losses in wheat production of at least 15%. This epidemic accelerated the rapid adoption of stripe-rust-resistant Digalu, with high yield potential and stem rust resistance to *P. graminis* f. sp. *tritici* Ug99 race group. Based on seed production estimates, Digalu occupied over 0.5 million ha (approximately 31% of the production acreage) in Ethiopia in 2013 and made a major contribution to the record wheat harvest of 3.92 million tons in the 2013–14 season. Resistance to stripe rust was a key factor that enabled farmers to benefit from the above-average rainfall in the 2013–14 season (Abeyo et al. 2014). Other rust-resistant cultivars, notably recent releases such as Kakaba and Danda'a and older varieties such as Mada Walbu, ET-13, and K-6295-4A also contributed to rust control (stripe rust and Ug99 race group) in Ethiopia.

The stem rust resistance in Digalu is postulated to be due to *SrTmp*. Although *SrTmp* is effective against reported *P. graminis* f. sp. *tritici* races in the Ug99 race group, the gene is ineffective against *P. graminis* f. sp. *tritici* race TKTTF. The narrow genetic basis of stem rust resistance in Digalu (*SrTmp*), although effective against *P. graminis* f. sp. *tritici* race TTKSK, created a situation of vulnerability if strains with different virulence spectra appear in the country. The high virulence of *P. graminis* f. sp. *tritici* race TKTTF on *SrTmp,* coupled with appearance in a stem-rust-conducive environment at a relatively early crop growth stage (anthesis), resulted in a rapid stem rust epidemic on Digalu in southeast Ethiopia. Major production losses resulted in the area affected by the epidemic. Initial production forecasts made by CSA in October 2013 (preepidemic) for Bale zone (core of the epidemic) were 546,213 tons, whereas actual production (postepidemic) for Bale zone reported by CSA in May 2014 was only 439,384 tons. No other mitigating factors were observed in Bale zone; hence. the negative difference of over 100,000 tons is believed to be the outcome of the stem rust epidemic caused by *P. graminis* f. sp. *tritici* race TKTTF.

A high-throughput SNP array (PgtSNP Chip) was used to genotype selected isolates derived from *P. graminis* f. sp. *tritici* collections made in Ethiopia during the 2013–14 season. Phylogenetic analysis showed that TKTTF isolates formed a unique clade (clade IV) that was clearly distinct from the other three clades representing isolates of common *P. graminis* f. sp. *tritici* race types (clade I, Ug99 race group; clade II, TRTTF and RRTTF; and clade III, JRCQC) found in Ethiopia. Clade IV (TKTTF) was further subdivided into two groups (IV-A and IV-B) and composed of six MLGs. Genetic variation between isolates of the same race phenotype was also observed in samples of TTKSK, JRCQC, and RRTTF. Preliminary studies indicate that clade III (TRTTF and RRTTF) and clade IV (TKTTF) may be part of a larger lineage, and SNP genotyping of an expanded set of *P. graminis* f. sp. *tritici* isolates is being performed. This study should provide a better understanding of the relationships and evolution of these clades. However, this phylogenetic study clearly demonstrates that isolates of *P. graminis* f. sp. *tritici* race TKTTF are not a result of recent mutations from common races in Ethiopia, and suggests that *P. graminis* f. sp. *tritici* TKTTF was either recently introduced into Ethiopia from an outside regions or has existed in Ethiopia but at a low frequency and, therefore, has gone undetected.

Very little is known about the *P. graminis* f. sp. *tritici* population structure and dynamics in Ethiopia. In the last decade, there has been a dramatic increase in stem rust surveillance in Ethiopia and the surrounding region due to epidemics caused by *P. graminis* f. sp. *tritici* races in the Ug99 race group. The majority of stem rust samples that have been collected and characterized have come from breeding nurseries. Admassu et al. (2009) was the first to study the *P. graminis* f. sp. *tritici* population in Ethiopia and analyzed

approximately 150 isolates collected from wheat fields in Arsi, Bale, and Shewa zones and northwest Ethiopia during 2006 and 2007. In this study, 22 *P. graminis* f. sp. *tritici* races were identified, with race TTKS (Ug99) being the most predominant. None of the isolates characterized by Admassu et al. (2009) had a virulence profile that matched TKTTF.

A limited set of stem rust samples (n = 33) was collected from Arsi and Bale zones in Ethiopia during 2012 (August to November). Recently, single-pustule isolates from this collection were race phenotyped at the GRRC (M. Hovmøller and M. Patpour, unpublished results). One of the isolates, derived from a sample from Afrisha (Bale zone), was identified to be race TKTTF. Genotyping of DNA from this sample indicated that it belongs to TKTTF subclade IV-A (L. Szabo and J. Johnson, unpublished). These results indicate that *P. graminis* f. sp. *tritici* race TKTTF was present in Ethiopia prior to the 2013 epidemic; however, this does not preclude the possibility that aerial incursion of urediniospores from outside of Ethiopia did occur that contributed to the epidemic.

At present, very little is known about the regional and global distribution of *P. graminis* f. sp. *tritici* race TKTTF and members of this genetic lineage. The race was reported in Turkey previously (Mert et al. 2012). Clearly, increased efforts are needed to collect and characterize *P. graminis* f. sp. *tritici* populations not only in Africa but also globally. Advances in SNP genotyping technologies now allow the analysis of *P. graminis* f. sp. *tritici*-infected wheat tissue from greenhouse-derived samples, and application of this method to single-pustule field samples should greatly enhance population genetic studies. In addition, development of SNP marker sets will provide diagnostic tools for rapid identification of critical strains of *P. graminis* f. sp. *tritici*.

To assess the vulnerability of Ethiopian and international bread wheat breeding materials to race TKTTF, 863 Ethiopian and CIMMYT cultivars and elite breeding lines were evaluated for seedling reaction to isolate 13ETH18-1 of TKTTF from Ethiopia. Results of these seedling evaluations indicated that 26% (16 of 62) of the Ethiopian lines with seedling resistance to TTKSK were susceptible to race TKTTF. Similarly, 21 and 31% of the eighth and ninth SRSSN lines, respectively, with seedling TTKSK resistance were susceptible to TKTTF. A stem rust field-screening nursery with race TKTTF as the inoculum will allow determination of the presence and level of adult plant resistance. Race analyses from this study also showed that other *P. graminis* f. sp. *tritici* races with significant virulence combinations such as TTKSK and RRTTF are present in Ethiopia. Wheat breeding programs in Ethiopia need to focus on developing and releasing cultivars with genes that confer resistance to a broad spectrum of virulence combinations. The use of seedling resistance genes with major effects can prevent inoculum buildup and limit the exposure of cultivars to high disease pressures in an epidemic. This study identified a group of Ethiopian advanced

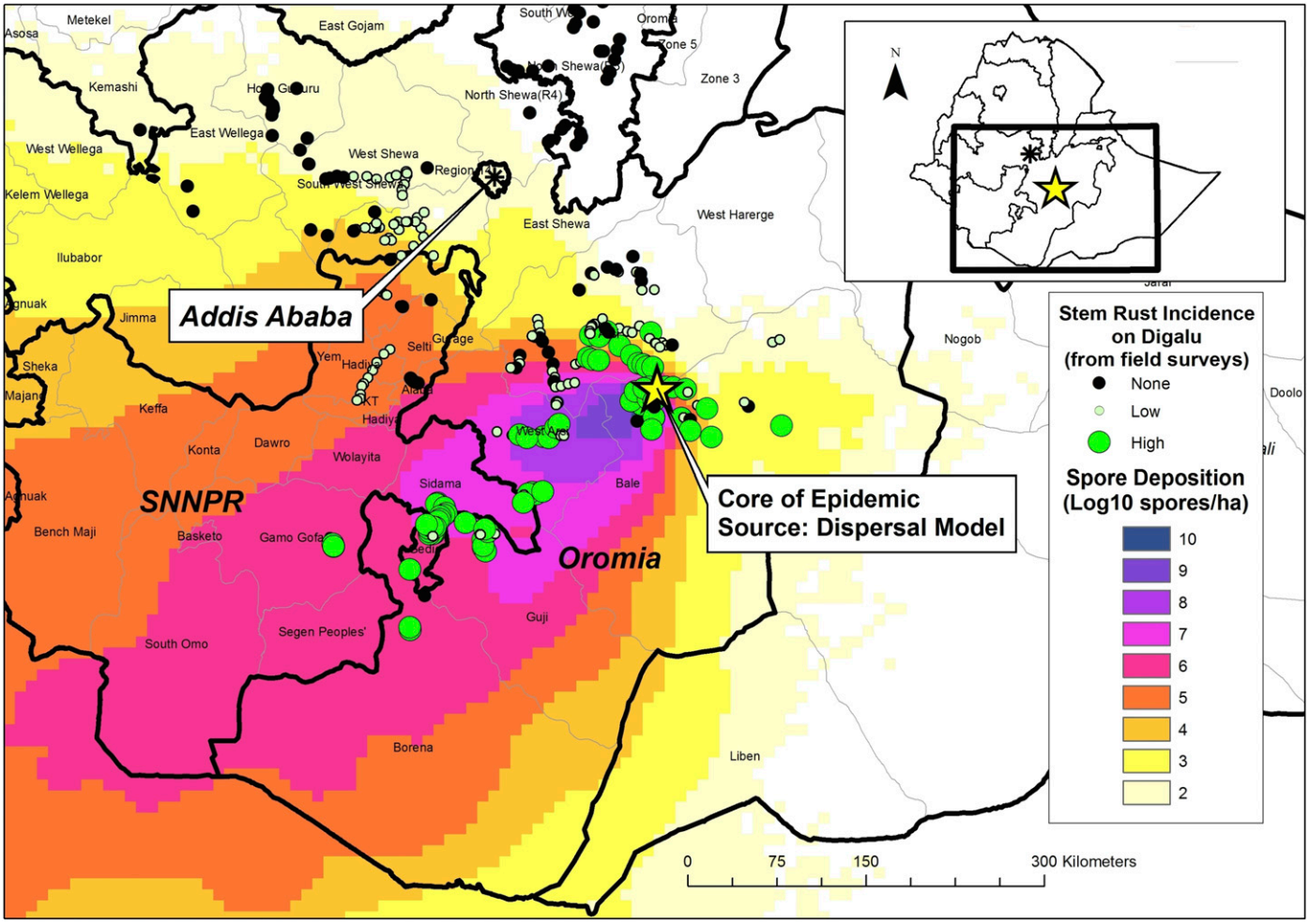

Fig. 7. Simulated airborne spore deposition for spores released from the core of epidemic (Gasera, Bale zone) in November 2013. with recorded infection sites for *Puccinia graminis* f. sp. *tritici* on Digalu. Spore deposition is displayed as the logarithm (base 10) of the spatial concentration (viable spores ha^{-1}) in each grid cell (5 arcmin, approximately 9 km^2 at the equator). Spore deposition concentration is not shown below a lower threshold of $\log_{10}$ 10^2 spores ha^{-1}. Incidence of *P. graminis* f. sp. *tritici* on Digalu is derived from georeferenced field surveys. High incidence (>40%) on Digalu is considered indicative of the presence of race TKTTF.

breeding lines and cultivars that possess resistance to both *P. graminis* f. sp. *tritici* races TKTTF and TTKSK at the seedling stage

The stem rust epidemic on Digalu in southeastern and southern Ethiopia in 2013–14 developed very rapidly and spread to cover a relatively large area. A large amount of inoculum was produced and there is considerable potential both for recurrent infections in Ethiopia and also dispersal to neighboring countries. In Ethiopia, it is a near certainty that large areas will be planted to Digalu in the forthcoming seasons. Risk of interseason inoculum carry over is high, and there is an urgent need to identify and deploy diverse sources of resistance in Ethiopia. The use of a meteorologically driven airborne spore dispersal model has indicated the potential to predict disease spread in real time. Further work, not reported here, has established that meteorological conditions for spore release and dispersal are consistent among years in Ethiopia and surrounding countries during critical periods for spread. Therefore, there is a potential to develop a regional forecasting scheme using a combination of field sampling and epidemiological modeling informed by meteorological models.

The severe stem rust epidemic in southeastern and southern Ethiopia in 2013–14 on the popular variety Digalu triggered a rapid national and international response to determine the contributing factors and underlying causes. Within months of the epidemic breaking out, the causal race had been identified, likely dispersal patterns had been elucidated, and a germplasm screening program had been initiated. This is a result of worldwide efforts in recent years in stem rust surveillance to detect and monitor Ug99 and other significant races that pose a threat to wheat production and in germplasm screening to identify effective stem rust resistance for wheat improvement.

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