

Distribution and frequency of wheat stem rust races (*Puccinia graminis* f. sp. *tritici*) in Ethiopia

Netsanet Bacha Hei^{1*} • Tsegaab Tesfaye¹ • Getaneh Woldeab¹ • Endale Hailu¹ • Bekele Hundie² • Daniel Kassa² • Fikirte Yirga² • Fufa Anbessa³ • Wubishet Alemu⁴ • Teklay Abebe⁵ • Miruts Legesse⁵ • Alemar Seid⁶ • Tesfaye Gebrekirstos⁷

¹Ethiopian Institute of Agricultural Research, Ambo Plant Protection Research Center, Ambo, Ethiopia.

²Ethiopian Institute of Agricultural Research, Kulumsa Agricultural Research Center, Assela, Ethiopia.

³Oromia Agricultural Research Institute, Bore Agricultural Research Center, Bore, Ethiopia.

⁴Oromia Agricultural Research Institute, Sinana Agricultural Research Center, Bale Robe, Ethiopia.

⁵Tigray Agricultural Research Institute, Alamata Agricultural Research Center, Alamata, Ethiopia.

⁶Southern Agricultural Research Institute, Areka Agricultural Research Center, Ethiopia.

⁷Tigray Agricultural Research Institute, Mekele Agricultural Research Center, Mekele, Ethiopia.

*Corresponding author. E-mail: netsanetbacha@yahoo.com.

Accepted 6th July, 2018

Abstract. Stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt) is one of the most important diseases of wheat in Ethiopia. The stem rust pathogen is capable to rapidly develop new virulence to resistance genes owing to mutation and genetic recombination. Ethiopian highlands are known hot spots for the rapid evolution and spread of new rust races. The present study was conducted to investigate the virulence diversity and spatial distribution of races of Pgt in the major wheat growing areas of Ethiopia. The physiologic races of the rust fungus were determined on seedlings of the standard wheat stem rust differentials following the international system of nomenclature. Three hundred and forty-seven samples were analyzed in 2014 and 2015 cropping seasons from Oromia, Amhara, Tigray and Southern Nations and Nationalities Peoples' regions. From 179 samples analyzed in 2014 six races which included TKTTF, TTKSK, TTTTF, TRTTF, PKTTF and TTTSF were identified. Seven races TKTTF, TTTTF, TRTTF, TTKSK, SJPQC, JRCFS and JRCQC were identified from 168 stem rust samples analyzed in 2015. Race TKTTF (Digelu race) was predominant and widely distributed in all the regions with 63.1 and 89.3% frequencies in 2014 and 2015, respectively. It was virulent on all the *Sr* genes except *Sr11*, *Sr24* and *Sr31*. The widest virulence spectrum was noted on race TTTTF making 90% of the *Sr* genes non-effective. This race also had wide spatial distribution next to TKTTF with frequencies of 32.4% in 2014 and 6% in 2015 cropping seasons. Race TTKSK (Ug99) was detected only in Oromia with frequency of less than 5% in the seasons. Most genes possessed by the differentials were ineffective against one or more of the tested isolates. Only the differential host carrying *Sr24* was effective to all the races identified in the regions. Hence, *Sr24* can be used as sources of resistance in wheat breeding programs of the country, preferable in combination with other genes.

Keywords: Race, stem rust, *Sr* resistance genes, virulence diversity, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the world's leading cereal grains used by more than one-third of its population as a staple food (Kumar *et al.*, 2011). It is grown from below sea level to elevations exceeding 3000 meters above sea level and at latitudes ranging from 30°

and 60°N to 27° and 40°S (Nuttonson, 1955). With more than 220 million hectares planted annually, wheat is the most widely cultivated cereal in the world (FAOSTAT, 2016). It is used for food and many industrial purposes.

Ethiopia is the largest wheat producer in sub-Saharan

Africa (FAOSTAT, 2014). In Ethiopia, wheat is cultivated on over 1.7 million hectares and accounts for 13.49% of the cropland, with an annual production of 4.5 million metric tons. Wheat contributes about 15.63% of the grain production in the country (CSA, 2017). In terms of area, wheat ranks fourth after teff (*Eragrostis tef* (Zucc.) Trotter), maize (*Zea mays* L) and sorghum (*Sorghum bicolor* (L.) Moench). In terms of total grain production, it ranks third after maize and teff (CSA, 2017). The crop is widely grown by subsistent farmers under rain-fed conditions and over one-third of cereal farm households are dependent on wheat farming (Shiferaw *et al.*, 2013). The mean wheat yield in the country is estimated to be 2.67 t ha⁻¹ (CSA, 2017), which is well below the world mean of 3.0 t ha⁻¹ (Hawkesford *et al.*, 2013). This is due to losses caused by biotic, abiotic and socioeconomic constraints (Abebe *et al.*, 2012; Haile *et al.*, 2012).

The major biotic factors that limit wheat production in the country include diseases, insect pests and weeds (Abebe *et al.*, 2012). Among the abiotic factors, soil fertility and moisture stress are the principal wheat production limiting factors in Ethiopia (Bogale *et al.*, 2011a; Bogale *et al.*, 2011b; Haile *et al.*, 2012). Among the diseases, stem rust (*Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn), leaf rust (*P. triticina* Eriks) and stripe rust (*P. striiformis* Westend. f. sp. *tritici* Eriks) are the most important diseases reducing wheat production in Ethiopia. Stem rust, also known as black rust, caused by the fungus *Puccinia graminis* f. sp. *tritici* (designated as Pgt here after), has been the most devastating disease of all wheat rusts in Ethiopia (Admassu *et al.*, 2012; Denbel *et al.*, 2013) causing up to 100% yield losses over wide areas during epidemic years (Park, 2007; Hodson, 2014).

The stem rust pathogen is known to produce a large number of spores which can be wind disseminated over long distances and infect wheat under favorable environmental conditions. This fact, together with the ability to change genetically is an opportunity for the force for created new races with increased aggressiveness on resistant wheat cultivars. In recent years, variable races of stem rust pathogen have been identified in wheat production areas in different continents (Singh *et al.*, 2008). According to Singh *et al.* (2006) and Periyannan *et al.* (2013), Ethiopia is considered as a hot spot for the development and spread of new stem rust races. These new races have reduced number of major rust resistance genes that are available for use (Kolmer, 2005).

Wheat stem rust can be effectively controlled by growing resistant varieties (DRRW, 2010). Development of resistance varieties, however, requires knowledge of the prevalent stem rust races in the country, and to identify resistance genes that are effective against the disease. According to Huerta-Spino (1994) and Park *et al.* (2011), race surveys provide essential information to determine the gene combinations to be considered by

breeding programs using major gene resistance. Race surveys are also needed to be carried out to detect new and highly virulent Pgt races before they build inoculum and cause epidemic more tragic in the country. Thus, the aim of this study was to determine the virulence diversity of *Puccinia graminis* f. sp. *tritici* races in Ethiopia.

MATERIALS AND METHODS

Collection of rust samples, isolation and multiplication of single-pustule isolates

Stem rust samples were collected from wheat fields and trial plots across the major wheat growing regions of Ethiopia: Oromia, Amahara, Tigray and Southern Nations and Nationalities Peoples' (SNNP) regions in 2014 and 2015 main cropping seasons. The time of collection was adjusted to coincide with the wheat growing periods of each region. The samples were collected every 5 to 10 km following main and feeder roads on preselected routes in areas where wheat is important and stem rust is known to be present.

Stems and/or leaf sheath of wheat plants infected with stem rust were collected, cut into small pieces of 5 to 10 cm in length using scissors and placed in paper bags after the leaf sheath was separated from the stem in order to keep stem and/or leaf sheath dry. This technique helps the samples reduce moisture so as the spores will not germinate before processing in the greenhouse. The samples collected in the paper bags were labeled with the name of the zone, district, and variety, GPS (altitude, latitude and longitude) data and date of collection and transported to Ambo Plant Protection Research Center's (APPRC) Laboratory for analysis.

Urediniospores collected from each field were suspended in lightweight mineral oil, Soltrol 170 (Chevron Phillips Chemical Company, The woodlands, Texas, United States) and inoculated using atomized inoculator on 7-day-old seedlings of variety McNair, which does not carry known stem rust resistance genes (Roelfs *et al.*, 1992). Inoculated seedlings were moistened with fine droplets of distilled water produced with an atomizer and placed in a dew chamber in darkness for 18 h at 18 to 22°C and 98 to 100% relative humidity. Upon removal from chamber, plants were exposed to 4 h of fluorescent light to provide condition for infection and allowed to dry their dew for about 2 h. Inoculated plants were then transferred to greenhouse benches where conditions were regulated at 12 h photoperiod, at temperature of 18 to 25°C and relative humidity (RH) of 60 to 70% (Stubbs *et al.*, 1986).

After seven to ten days of inoculation (when the flecks/chlorosis was clearly visible) leaves containing a single fleck that produce single pustule (single uredinial isolate) were selected from the base of the leaves and the remaining seedlings within the pots were removed

Table 1. Nomenclature of Pgt based on 20 differential wheat hosts.

Pgt- code	Set	Infection types produced on near-isogenic Sr lines			
		5	21	9e	7b
	Set 1	5	21	9e	7b
	Set 2	11	6	8a	9g
	Set 3	36	9b	30	17
	Set 4	9a	9d	10	Tmp
	Set 5	24	31	38	McN
B		Low ^a	Low	Low	Low
C		Low	Low	Low	High ^b
D		Low	Low	High	Low
F		Low	Low	High	High
G		Low	High	Low	Low
H		Low	High	Low	High
J		Low	High	High	Low
K		Low	High	High	High
L		High	Low	Low	Low
M		High	Low	Low	High
N		High	Low	High	Low
P		High	Low	High	High
Q		High	High	Low	Low
R		High	High	Low	High
S		High	High	High	Low
T		High	High	High	High

Source: Roelfs and Martens (1988); Jin *et al.* (2008).

^aLow = Infection types 0, ;, 1, and 2 and combinations of these values

^bHigh = Infection types 3 and 4 and a combination of these values

using scissors. Only leaves containing single pustules from each location were separately covered with cellophane bags and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunsmore, 2004).

Two weeks later (when the pustule was well developed), spores from each pustule were collected using power operated vacuum aspirator and stored separately in gelatine capsules. A suspension, prepared by mixing urediospores with Soltrol 170, was inoculated on seven-day-old seedlings of the susceptible variety McNair for multiplication purpose for each of the single pustules on separate pots following the procedures mentioned earlier. The urediniospores descending from one pustule made up a single pustule isolate. One isolate was developed from each wheat field and used for the final race analysis.

Inoculation of differential lines and race determination

Five seeds of each of the twenty wheat stem rust differentials with known stem rust resistance genes (*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr17*, *Sr21*, *Sr24*, *Sr30*, *Sr31*, *Sr36*, *Sr38*, *SrTmp*

and *SrMcN*) and a susceptible variety McNair were grown in 10 cm diameter pots. The susceptible variety 'McNair' (without Sr gene) was used to ascertain the viability of spores inoculated to the differential hosts. Each rust isolate derived from single pustule was suspended in Soltrol 170. The suspension was adjusted to 1×10^5 spores ml⁻¹ and inoculated onto seedlings of the differentials following the procedure described above.

Stem rust infection types (ITs) on the differential lines were scored 14 days after inoculation using a 0 to 4 scale (Stakman *et al.*, 1962). Infection types were grouped into two, where 0 to 2+ stands for low infection (resistance) and 3- to 4+ for high infection (susceptible). Five letters race code nomenclature system was used according to Roelfs and Marten (1988) and Jin *et al.* (2008). In this system the differential lines are grouped into five sub-sets in the following order: (i) *Sr5*, *Sr21*, *Sr9e*, *Sr7b* (ii) *Sr11*, *Sr6*, *Sr8a*, *Sr9g*, (lii) *Sr36*, *Sr9b*, *Sr30*, *Sr17*. (iv) *Sr9a*, *Sr9d*, *Sr10*, *SrTmp*, (v) *Sr24*, *Sr31*, *Sr38*, *SrMcN* (Table 1). An isolate that produces a low infection type on the four lines in a set is assigned with the letter 'B', while a high infection type on the four lines is assigned with a letter 'T'. Hence, if an isolate produces a low infection type (resistant reaction) on the 20 differential lines, the race will be designated with a five letter race code 'BBBBB'. Similarly, an isolate producing a high infection

Table 2. Distribution and frequency of Pgt races in Oromia, Amhara, Tigray and SNNP regions of Ethiopia in 2014 and 2015 main cropping seasons.

Races	Season	Oromia					Amhara			Tigray	SNNP
		Shewa	Arsi	Bale	Wellega	Borena	N. Shewa	Wello	Gondor	E. and S. Tigray	
TKTTF	2014	41(85.4) [†]	42 (85.7)	3 (14.3)	1 (33.3)	8 (29.6)	1(16.7)	1(100)	2 (100)	9 (75)	5 (50)
TTTTF	2014	4 (8.3)	3 (6.1)	17 (81)	2 (66.7)	19 (70.4)	5 (83.3)	-	-	3 (25)	5 (50)
TRTTF	2014	-	-	1(4.8)	-	-	-	-	-	-	-
TTKSK	2014	1 (2.1)	4 (8.2)	-	-	-	-	-	-	-	-
PKTTF	2014	1(2.1)	-	-	-	-	-	-	-	-	-
TTTSF	2014	1(2.1)	-	-	-	-	-	-	-	-	-
Total		48	49	21	3	27	6	1	2	12	10
TKTTF	2015	66 (88)	13 (92.9)	27 (81.8)	10 (100)					26 (100)	8 (80)
TTTTF	2015	4 (5.3)	1 (7.1)	4 (12.1)	-					-	1 (10)
TRTTF	2015	1 (1.3)	-	2 (6.1)	-					-	-
TTKSK	2015	2 (2.7)	-	-	-					-	-
SJPQC	2015	1 (1.3)	-	-	-					-	-
JRCFS	2015	1 (1.3)	-	-	-					-	-
JRCQC	2015	-	-	-	-					-	1 (10)
Total		75	14	33	10					26	10

[†]Figures in parentheses indicated the frequency (%) of a specific race in a respective zone; (-) indicated the race was not present in the respective zone; SNNP indicated Southern Nations and Nationalities Peoples' region

type (susceptible reaction) on the 20 lines will have a race code 'TTTTT' and race analysis was analyzed by using the descriptive statistics.

RESULTS

Virulence structure of stem rust pathogen

A total of 419 stem rust isolates were collected during 2014 and 2015 main cropping seasons. Of these, 179 isolates were viable and analyzed on to the 20 stem rust differential lines in 2014 while the race analysis was carried out on 168 isolates in 2015. From 347 isolates studied in the seasons, nine races namely TKTTF, TTTTT, TRTTF, TTKSK, PKTTF, TTTSF, SJPQC, JRCSF and

JRCQC were identified. Of these four races TKTTF, TTKSK, TTTTT and TRTTF were detected in both seasons. Races PKTTF and TTTSF were identified only in 2014 while SJPQC, JRCSF and JRCQC were detected only in 2015 main cropping season (Table 2).

Race TKTTF which is also known as 'Digelu' race was the most frequent and the predominant race with frequencies of 63.1% and 89.3% in 2014 and 2015, respectively (Table 2). It was most often found in samples collected from bread wheat varieties planted in farmers' fields. The second most frequent race was TTTTT. It had frequencies of 32.4% and 6% in 2014 and 2015 cropping seasons, respectively. This race was identified from samples collected from durum wheat, bread wheat and barley varieties. Races TKTTF and

TTTTF accounted for 95% of the stem rust population of Ethiopia in the study period. The remaining 7 races, TTKSK, TRTTF, PKTTF, TTTSF, SJPQC, JRCSF and JRCQC were the least abundant with frequency of less than 5%. Of these, races PKTTF, TTTSF, SJPQC, JRCSF and JRCQC were detected only at single locations in the seasons. Races PKTTF, SJPQC and JRCSF were identified from variety Kakaba while JRCQC was detected from Digelu. Similarly, race TTKSK was detected only from 7 samples in both seasons, which were collected from Hidase, Danda'a, Digelu and Kubsu varieties.

There was variation between the virulence spectra of the races within the regions. In 2014, the 48 isolates analyzed from Shewa zone were assigned to 5 races namely TKTTF, TTKSK,

Table 3. Wheat varieties affected by the Pgt races identified in 2014 and 2015 main cropping seasons and their frequency from the total Pgt population in Ethiopia in the seasons.

Race	Varieties/lines affected	Freq. of the race in 2014	Freq. of the race in 2015
TKTTF	Digelu, Danda'a, Hidasse, Kakaba, Ownless, Ogolcha, Kingbird and others ^a	113 (63.1) [‡]	150 (89.3)
TTTTF	Durum wheat, Digelu, Danda'a, Menze, Hidasse, Medawollabu, Kakaba, PBW 343, Barley (Holker, Grace)	58 (32.4)	10(6.0)
TTKSK	Hidasse, Danda'a, Digelu, Kubsu	5 (2.8)	2 (1.2)
TRTTF	Digelu, Kakaba	1 (0.6)	3(1.8)
PKTTF	Kakaba	1(0.6)	-
TTTSF	Unknown	1(0.6)	-
SJPQC	Kakaba	-	1(0.6)
JRCSE	Kakaba	-	1(0.6)
JRCQC	Digelu	-	1(0.6)
Total		179	168

^aOther included varieties, Kubsu, Alidoro, Shorima, Huluka, Tuse, Madawolabu, Tay and unknown bread wheat, durum wheat and barley cultivars

[‡]Figures in parentheses indicated the frequency (%) of a specific race in the season.

Table 4. Avirulence/Virulence spectra of the Pgt races identified in Ethiopia in 2014 and 2015 main cropping seasons.

Races	Virulence	Avirulence
TKTTF	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	11, 24, 31
TTTTF	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	24, 31
TRTTF	5, 21, 9e, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	8a, 24, 31
TTKSK	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38, McN	36, Tmp, 24
SJPQC	5, 21, 9e, 6, 8a, 36, 30, 17, 9a, 9d, McN	7b, 11, 9g, 9b, 10, Tmp, 24, 31, 38
JRCSE	21, 9e, 11, 6, 9g, 17, 9a, 9d, 10, 38, McN	5, 7b, 8a, 36, 9b, 30, Tmp, 24, 31
JRCQC	21, 9e, 11, 6, 9g, 17, 9a, 9d, McN	5, 7b, 8a, 36, 9b, 30, 10, Tmp, 24, 31, 38
PKTTF	5, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	21, 11, 24, 31
TTTSF	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, 38, McN	Tmp, 24, 31

TTTTF, PKTTF and TTTSF. Similarly, the 49 isolates studied in Arsi belonged to races TKTTF, TTKSK and TTTTTF while the 21 isolates analyzed from Bale belonged to races TKTTF, TTTTTF and TRTTF. The 27, 3, 6, 12 and 10 isolates from Borena, Wellega, North Shewa, Tigray and SNNP were assigned to two races TKTTF and TTTTTF in the season. On the other hand, the 75 isolates analyzed in 2015 from Shewa zone were assigned to 6 races namely TKTTF, TTTTTF, TRTTF, SJPQC, JRCSE and TTKSK. The 10 and 26 isolates studied in Wellega and Tigray belonged to race TKTTF while the 33 isolates analyzed from Bale belonged to races TKTTF, TRTTF and TTTTTF in the season. The 14 and 10 isolates from Arsi and SNNP were assigned to 2 (TKTTF and TTTTTF) and 3 races (TKTTF, TTTTTF and JRCQC) in 2015, respectively.

Of the races identified in Shewa zone, race TKTTF was predominant in both seasons with frequency of more than 85%, followed by race TTTTTF with frequencies of 8.3% in 2014 and 5.3% in 2015. In this zone, the remaining races were the least abundant, each with less than 5% frequency in both seasons. In 2015, the race frequency in

Arsi, Bale and Tigray showed a similar trend as TKTTF had the highest frequency followed by TTTTTF. On the other hand, the race pattern in 2014 in Bale, Wellega, Borena and North Shewa was different from that of the other zones. In these parts of the country TTTTTF was the dominant race in the season, accounting for 80.96, 70.4, 66.7 and 83.3% of the total race population of respective zones. The next most abundant race in the zones was TKTTF. Although most of the races were confined to specific locations, some had wider spatial distribution. Race TKTTF was present in most of the surveyed zones followed by race TTTTTF in both seasons. The races TRTTF, TTKSK, PKTTF, TTTSF, SJPQC, JRCSE and JRCSE were found only in Oromia region in the study period. Similarly, race JRCQC was detected only in SNNP region.

Virulence to Sr resistance genes

The races identified had wide virulence spectrum (Table 4). Of the genes possessed by standard differentials the

Table 5. Virulence frequency of Pgt races collected in Ethiopia in 2014 and 2015 to single gene wheat differentials.

Sr gene	Virulence frequency (%)	Sr gene	Virulence frequency (%)
5	77.8	30	77.8
21	88.9	17	100
9e	100	9a	100
7b	66.7	9d	100
11	66.7	10	77.8
6	100	<i>Tmp</i>	44.4
8a	66.7	24	0
9g	88.9	31	11.1
36	66.7	38	77.8
9b	66.7	<i>McN</i>	100

dominant race TKTF was avirulent only to *Sr11*, *Sr24* and *Sr31*, but virulent on the other genes. The widest virulence spectrum was noted on race TTTTF making 90% *Sr* genes non-effective. It was virulent to all except *Sr24* and *Sr31* genes. Similarly, races TKTF, TRTF, TTKSK and TTTSF defeated 85% of the *Sr* genes in the wheat differential lines. Of these, TKTF and TRTF varied from one another by single gene change, which were virulent to all differential lines except *Sr11*, *Sr24*, *Sr31* and *Sr8a*, *Sr24*, *Sr31*, respectively. Similarly, TTKSK and TTTSF varied from one another by single gene change, which were avirulent to *Sr36*, *SrTmp*, *Sr24* and *SrTmp*, *Sr24*, *Sr31*, respectively. Race PKTF defeated 80% of the *Sr* genes possessed by the differential lines. The narrowest virulence spectrum was recorded on race JRCQC with 45% of the *Sr* genes ineffective.

It was evident that the majority of the resistance genes were ineffective against most of the races identified in this study. For instance, 6 differential hosts carrying the resistance genes *Sr9e*, *Sr6*, *Sr17*, *Sr9a*, *Sr9d* and *SrMcN* were ineffective to all the races identified in both seasons (Table 5). *Sr21* and *Sr9g* were ineffective against 88.9% of the races in Ethiopia while *Sr5*, *Sr30*, *Sr10* and *Sr38* were ineffective against 77.8% of the races found across the country. *Sr7b*, *Sr11*, *Sr8a*, *Sr36* and *Sr9b* were ineffective against 66.7% of the races in Ethiopia. Differential host carrying *SrTmp* was resistant to less than 50% of the races. Only *Sr24* was found to be effective against all the races detected in this study. Differential host carrying *Sr31* gene was resistant to all races but not TTKSK.

DISCUSSION

Based on the 347 samples analyzed in both seasons nine races namely TKTF, TTTTF, TRTF, TTKSK, PKTF, TTTSF, SJPQC, JRCFS and JRCQC were identified. This indicated the presence of broad races with wider virulence spectrum within the Pgt population in

Ethiopia. This is in line with previous studies conducted in the country (Belayneh and Emebet, 2005; Admassu *et al.*, 2009; Hailu *et al.*, 2015). The continual wheat production and the favorable microclimates in the wheat production areas of Ethiopia could be the main reasons for the rapid evolution and high virulence diversity of the pathogen in the country. Sexual recombination may have also contributed to the virulence diversity of Pgt because *Berberry holstii*, alternate host of *P. graminis* f. sp. *tritici*, is present in proximity to wheat production areas of Ethiopia and the pathogen is able to complete its life cycle in the country (Getaneh *et al.*, 2016).

Two hundred and sixty three stem rust samples characterized for race type in this study revealed race TKTF, indicating that this race dominates the stem rust population in Ethiopia. It was widely distributed in the major wheat growing parts of the country in the seasons. TKTF was detected for the first time at a trace level in 2012 main cropping season from samples collected in Arsi and Bale zones of Oromia region and was found to be primary cause of the epidemics in the southeastern parts of the country in 2013 and 2014 cropping seasons (Hodson, 2015; Olivera *et al.*, 2015). The detection of this race in 2012 was the first report of virulence to *SrTmp* in the country. This non-effective gene is present in the most popular and widely grown bread wheat variety Digelu (Hodson, 2015).

According to Mert *et al.* (2012) races similar to TKTF occurred in Turkey in the 1990s and still predominate. TKTF has also been detected in Iran (2010), Lebanon (2012), Egypt (2013), Azerbaijan, Eriteria and Yemen (Olivera *et al.*, 2015). It is also detected in Kenya from samples collected in 2014 and 2015. The presence of stem rust race with identical virulence profiles throughout this vast region implies that there are inoculum exchanges and the race is a serious threat to the wheat production to wider scale (Hodson, 2016) and needs close monitoring, especially in countries which have cultivars carrying the *SrTmp* resistance gene. Studies indicated that TKTF does not belong to the Ug99 lineage based on avirulences to *Sr11* and *Sr31* and

molecular fingerprints (Olivera *et al.*, 2015).

The race TTTTF which had a wide virulence spectrum was also identified. It was the second most common race identified from samples collected in the seasons. TTTTF has a virulence formula which is almost similar to TKTF but is clearly different from stem rust race TTKSK as it has avirulence to *Sr31*. This race was detected from samples collected in 2009 in eastern Shewa zone of central Ethiopia at trace level (Lemma *et al.*, 2014). It was also detected in Iran from samples collected during 2010 to 2014 (Patpour *et al.*, 2014; Afshari *et al.*, 2015). TTTTF hit several thousands of hectares of durum wheat on the Italian Islands of Sicily in 2016, causing the largest stem rust outbreak that Europe has seen in decades. Without proper control, researchers caution, it could soon spread over long distances along the Mediterranean basin and the Adriatic coast (FAO, 2017).

The race TTKSK (Ug99) discovered in Uganda in 1999 represented a real threat to wheat production globally. Recognizing its potential threat to wheat production, Borlaug Global Rust Initiative (BGRI) was launched in 2005 which led to a concerted international collaborative effort to combat the emerging threat of wheat rusts. A key component of BGRI is surveillance and monitoring, which has successfully tracked various races with a broad geographic footprint (Hodson *et al.*, 2012). TTKSK was first time confirmed in Ethiopia in 2005. It had spread to most of the wheat growing areas of Ethiopia since its occurrence (Admassu *et al.*, 2009; Hailu *et al.*, 2015). However, this study recovered TTKSK only from 5 samples in 2014 and 2 samples in 2015. Most of the samples analyzed in the seasons have proven to be TKTF followed by TTTTF. This indicated that although the dominant wheat stem rust race in Ethiopia for a long time had been TTKSK, it is now over taken by the races TKTF and TTTTF. Stem rust samples collected from Arsi, Bale, South Tigray and West Shewa zones in 2015 and analyzed at United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Cereal Disease Laboratory (CDL), Minnesota also confirmed that TKTF dominated the race population in Ethiopia in the season (Y. Jin, Cereal Disease Laboratory, Minnesota, USA, Personal communication). Races TRTF, PKTF, TTTF and JRCQC were identified in Oromia region but at low frequencies. According to Olivera *et al.* (2012), race TRTF is also occurring in Yemen.

The pathogen population for virulence combinations varied from region to region. The differences in race compositions in the regions could possibly be due to variation over location and time, as the prevalence of races in a specific season and region depends on the type of wheat cultivars grown and to some extent on the predominant environmental conditions, especially temperature (Roelfs *et al.*, 1992). The long distance dispersal of rust races can also led to the shifts in the genetic structure of the rust population (Burdon and Silk, 1997; Kolmer, 2005; Hanzalova and Bartos, 2014).

Most of the races in the present study were virulent to

many of the resistance genes. *Sr9e*, *Sr6*, *Sr17*, *Sr9a*, *Sr9d* and *SrMcN* were ineffective to all the races identified in the present study. *Sr17*, *Sr9a* and *Sr9d* genes were reported to be ineffective for more than 90% of the races collected during 2006-2007 cropping season in the country (Admassu *et al.*, 2009). *Sr6*, *Sr17*, *Sr9a* and *Sr9d* were also reported to be susceptible to all races identified from 2013 stem rust isolates (Hailu *et al.*, 2015). On the other hand, lines carrying *Sr31* were resistant to most of the races identified in the present study. However, it is risky to use *Sr31* as a source of resistance as it is ineffective against Ug99. *Sr24* was found to be effective against all the races detected. It is important to note that a variant of Ug99 that added virulence on stem rust gene *Sr24* was not found in this study. Although a variant of Ug99 which has a combined virulence to *Sr24* and *Sr31* (PTKST) was reported from samples collected near Meraro district of Oromia region during 2007 and analyzed by the Cereal Research Centre, Winnipeg, AAFC, Canada (FAO, 2010), no virulent race was detected against *Sr24* gene in recent years by the Ethiopian scientists (Admassu *et al.*, 2009; Abebe *et al.*, 2012; Lemma *et al.*, 2014, Hailu *et al.*, 2015).

CONCLUSION

The Pgt populations in Ethiopia are highly variable. Therefore, it is necessary for the national wheat improvement program to monitor the virulence composition and dynamics in the stem rust population and utilize currently effective stem rust resistance genes in the improvement of wheat cultivars. The highly virulent race TTTTF was detected in this study with a higher frequency. Thus, this race should be a target of breeding for stem rust resistance in the country. For this reason, race TTTTF should be used as part of a race complex to evaluate advanced breeding materials in the breeding programs. Stem rust resistance gene *Sr24* was found to be effective to all races detected in the present study and hence can be considered as source of resistance, preferable in combination with other genes. On the other hand, to avoid fast breakdown of stem rust resistance genes in the wheat varieties, the breeding efforts of the country should focus on selecting for minor genes, based on adult plant resistance. This kind of resistance is especially important for countries like Ethiopia which are considered to be under high risk and where survival of the pathogen for several years is expected due to favorable environmental conditions.

ACKNOWLEDGEMENTS

This research received financial support from the Durable Rust Resistance in Wheat (DRRW) project. We thank the technical staff of wheat research section of Ambo Plant

Protection Research Center for their technical assistance.

REFERENCES

- Abebe T, Woldeab G, Dawit W (2012).** Analysis of pathogen virulence of wheat stem rust and cultivar reaction to virulent races in Tigray, Ethiopia. *Afr. J. Plan. Sci.* 6:244-50.
- Admassu B, Lind V, Friedt W, Ordon F (2009).** Virulence analysis of *Puccinia graminis* f. sp. *tritici* populations in Ethiopia with special consideration of Ug99. *Plant. Pathol.* 58:362-69.
- Admassu B, Friedt W, Ordon F (2012).** Stem rust seedling resistance genes in Ethiopian wheat cultivars and breeding lines. *Afr. Crop Sci. J.* 20:149-161.
- Afshari F, Aghaee M, Jalal KMR, Roohparvar R, Malhipour A, Khodarahmei M, Ebrahimbejad SH, Aghnum R, Chaichi M, Dadrezaei T, Dalvand M, Dehghan MA, Zakeri AK, Shahbazi K, Safavi SA, Tabatabaei N, Atahoseini M, Nabati E, Hooshyar R, Yasaei M, Nasrollahi M, Mehrabi R, Ghaffary T, Hashami M, Patpour M, Bayat Z (2015).** Surveillance and Pgt race analysis in Iran, 2014. Poster presented at Borlaug Global Rust Initiative Technical Workshop, 17-20 September 2015, Sydney, Australia.
- Belayneh A, Emebet F (2005).** Physiological races and virulence diversity of *Puccinia graminis* f. sp. *tritici* on wheat in Ethiopia. *Phytopathol. Mediterr.* 44:313-18.
- Bogale A, Kindie T, Geleto T (2011a).** Morphological and physiological attributes associated to drought tolerance of Ethiopian durum wheat genotypes under water deficit condition. *J. Bio. Environ. Sci.* 1:22-36.
- Bogale A, Kedir N, Seboka H (2011b).** Selection of some morphological traits of bread wheat that enhance the competitiveness against wild oat (*Avena fatua* L.). *World J. Agric. Sci.* 7: 128-135.
- CSA (2017).** Report on area and production of major crops (For private peasant holding, Meher Season Volume 1). Addis Ababa, Ethiopia: Central Statistical Agency.
- Denbel W, Badebo A, Alemu T (2013).** Evaluation of Ethiopian commercial wheat cultivars for resistance to stem rust of wheat race 'UG99'. *Int. J. Agron. Plant. Prod.* 4:15-24.
- DRRW (2010).** Phase II. Proposal for Bill and Melinda Gates Foundation. Office of International Programs, College of Agriculture and Life Sciences, Cornell University. USA.
- FAO (2010).** Wheat stem rust. Rust spore: A global wheat monitoring system. <http://www.fao.org/agriculture/crops/rust/stem/rust-report/stem-report/en/>. (Accessed on 20 April 2017).
- FAO (2017).** News article: Spread of damaging wheat rust continues: new races found in Europe, Africa, and Central Asia. <http://www.fao.org/news/story/en/item/469467/icode/>. (Accessed on 20 April 2017).
- FAOSTAT (2014).** FAO Statistical Databases. <http://faostat.fao.org/>. (Accessed on 5 July 2016).
- FAOSTAT (2016).** FAO Statistical Database. <http://www.fao.org/faostat/en/#data/QC>. (Accessed on 28 June 2018).
- Fetch TG, Dunsmore KM (2004).** Physiological specialization of *Puccinia graminis* on wheat, barley, and oat in Canada in 2001. *Can. J. Plant Pathol.* 26:148-55.
- Getaneh G, Endale H, Teklu N (2016).** Detection of Barberry plants (*Berberis holstii*) as an alternate host of stem rust (*Puccinia graminis*) of wheat in Ethiopia. *Pest Manage. J. Ethiopia.* 19:17-26.
- Haile JK, Hammer K, Badebo A, Singh RP, Roder MS (2012).** Haplotype analysis of molecular markers linked to stem rust resistance genes in Ethiopian improved durum wheat varieties and tetraploid wheat landraces. *Genet. Resour. Crop Evol.* 60:853-864.
- Hailu E, Woldeab G, Denbel W, Alemu W, Abebe A, Mekonnen A (2015).** Distribution of stem rust (*Puccinia graminis* f. sp. *tritici*) races in Ethiopia. *Plant* 3:15-19.
- Hanzalova A, Bartos P (2014).** Virulence surveys of wheat leaf rust in the Czech Republic and resistance genes in registered cultivars. *Czech J. Genet. Plant Breed.* 50:241-246.
- Hawkesford MJ, Arous J, Park R, Derini DC, Miralles D, Shen T, Zhang J, Parry MAJ (2013).** Prospects of doubling global wheat yields. *Food and En. Sec.* 2:34-48.
- Hodson DP, Gronbech-Hasen J, Lassen P, Alemayehu Y, Arista J, Sonder K, Kosina P, Moncada P, Nazari K, Park RF, Pretorius ZA, Szabo LJ, Fetch T, Jin Y (2012).** Tracking the wheat rust pathogens. In: RA McIntosh, ed. *Proceeding BGRI Technical Workshop*. Beijing. 11-12. <http://www.globalrust.org/sites/default/files/BGRI-2012-plenary.pdf>. (Accessed on 8 February 2017).
- Hodson D (2014).** Keeping track of rust. (Accessed on 4 February 2017). http://rusttracker.cimmyt.org/?page_id=40.
- Hodson D (2015).** Summary of Ethiopia 2014/15 rust situation. Recurrent, localized stem rust epidemics caused by race TKTTF ("Digalu" race) in Ethiopia. Extreme caution and vigilance needed in East Africa. *Rust tracker.org*, Global wheat rust monitoring system. <http://rusttracker.cimmyt.org/?p=6465>. (Accessed on 7 July 2016).
- Hodson D (2016).** Molecular diagnostics indicate presence of race TKTTF ("Digalu" race) in Kenya. *Rust tracker.org*, Global wheat rust monitoring system. <http://rusttracker.cimmyt.org/?p=6909>. (Accessed on 7 July 2016).
- Huerta-Spino J (1994).** Evolution of virulence combinations in wheat leaf and stem rust in Mexico. In: Saari EE, Hettel GP, eds. *Guide to the CIMMYT Wheat Crop Protection Sub-program*. *Wheat Special Report No. 24*. Mexico City, Mexico: CIMMYT. pp. 34-36.
- Jin Y, Szabo LJ, Pretorius ZA, Singh RP, Ward R, Fetch T Jr (2008).** Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Dis* 92:923-926.
- Kolmer JA (2005).** Tracking wheat rust on a continental scale. *Curr. Opin. Plant Biol.* 8:441-449.
- Kumar A, Mishra VK, Vyas RP, Singh V (2011).** Heterosis and combining ability analysis in bread wheat (*Triticum aestivum* L.). *J. Plant Breed. Crop Sci.* 3:209-217.
- Lemma A, Woldeab G, Semahegn Y, Dilnesaw Z (2014).** Survey and virulence distribution of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) in the major wheat growing areas of central Ethiopia. *Sci-Afric. J. Sci. Issues, Res. Essays* 2:474-478.
- Mert Z, Karakaya A, Dusunceli F, Akan K, Cetin L (2012).** Determination of *Puccinia graminis* f. sp. *tritici* races of wheat in Turkey. *Turk J. Agric. For.* 36:107-120.
- Nuttonson MY (1955).** Wheat-Climatic Relationships and the Use of Phenology in Ascertaining the Thermal and Photothermal Requirements of Wheat. American Institute of Crop Ecology, Washington, DC.
- Olivera, PD, Jin Y, Rouse M, Badebo A, Fetch TJ, Singh, RP, Yahyaoui A (2012).** Races of *Puccinia graminis* f. sp. *tritici* with combined virulence to *Sr13* and *Sr9e* in a field stem rust screening nursery in Ethiopia. *Plant Dis.* 96:623-628.
- Olivera PD, Newcomb M, Szabo LJ, Rouse MN, Johnson J, Gale S, Luster DG, Hodson D, Cox JA, Burgin L, Hort M, Gilligan CA, Patpour M, Justesen AF, Hovmoller MS, Woldeab G, Hailu E, Hundie B, Tadesse K, Pumphrey M, Singh RP, Jin Y (2015).** 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. *Phytopathol.* 105:917-928.
- Park R, Fetch T, Hodson D, Jin Y, Nazari K, Prashar M, Pretorius Z (2011).** International surveillance of wheat rust pathogens: progress and challenges. *Euphytica* 179:109-17.
- Park RF (2007).** Stem rust of wheat in Australia. *Aust J. Agric. Res.* 58:558-566.
- Patpour M, Afshari F, Hasan Bayat Z, Nazari K (2014).** Pathotype identification of *Puccinia graminis* f. sp. *tritici*, the Causal Agent of Wheat Stem Rust under Greenhouse Condition. Seed and Plant Improvement Institute-SPII, Karaj, Iran.
- Periyannan S, Moore J, Ayliffe M, Bansal U, Wang X, Huang L, Deal K, Luo M, Kong X, Bariana H, Mago R, McIntosh R, Dodds P, Dvorak J, Lagudah E (2013).** The gene *Sr33*, an ortholog of barley *Mla* genes, encodes resistance to wheat stem rust race Ug99. *Sci.* 341:786-788.
- Roelfs AP, Martens JW (1988).** An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. *Phytopathol.* 78:526-533.
- Roelfs AP, Singh RP, Saari EE (1992).** *Rust Diseases of Wheat: Concepts and Methods of Disease Management*. Mexico City, Mexico: CIMMYT.

- Shiferaw B, Kassie M, Jaleta M, Yirga C (2013).** Adoption of improved wheat varieties and impacts on household food security in Ethiopia. Food Policy. <http://dx.doi.org/10.1016/j.foodpol.2013.09.012>. (Accessed on 1 March 2014).
- Singh RP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Bhavani S, Singh D, Singh PK (2008).** Adult plant resistance in wheat to ug99 race of stem rust and its utilization. In: Singh GP, Prabhu KV, Singh AM, eds. Proceeding of International Conference on Wheat Stem Rust: Ug99-A Threat to Food Security. Indian Agricultural Research Institute, New Delhi, India. pp. 28-33.
- Singh RP, Rajaram S (2006).** Breeding for disease resistance in wheat. <http://www.fao.org/DOCREP/006/Y4011E/y4011e0b.htm>. (Accessed on 12 June 2016).
- Stakman EC, Stewart DM, Loeggering WQ (1962).** Identification of physiological races of *Puccinia graminis var. tritici*. Washington, USA: US Department of Agriculture, Agricultural Research Services E617 (revised).
- Stubbs RW, Prescott JM, Sarrri EE, Dubin HJ (1986).** Cereal Disease Methodology Manual. CIMMYT, El Batan, Mexico.

<http://www.sciencewebpublishing.net/jacr>