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Identification of physiological races of wheat stem rust disease in Guji zone, southern Ethiopia

Girma Teshome*

Bore Agricultural Research Center, Bore, Ethiopia.

ABSTRACT

Wheat (Triticum spp.) is one of the most important staple cereal crops cultivated for food globally including Ethiopia. However, production and productivity of this crop is hampered by wheat stem rust caused by Puccinia graminis f.sp. tritici considered as the main diseases of wheat hindering the production. Therefore, this study was aimed to identification of physiological race of wheat stem rust. Fifty stem rust isolates were analyzed on the twenty stem rust differentials lines and five races namely; TTKTF, TTKTT, TTTTF, TKTTF, and TKKTF. TTKTT, and TTKTF races were detected and their high virulence spectrum that makes 36(95%) and 38(85%). TTKTF was the dominant race being detected from 19 samples followed by TTKTT which was identified from 18 samples. The majority of resistance genes in differential host lines 95% were defeated with the race TTKTT. Thus, preferable to use of effective minor gene and major gene in combination through gene pyramid to track further virulence evolution

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Corresponding Author

Girma Teshome

E-mail: *girmateshome347@gmail.com*

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INTRODUCTION

Wheat (Triticum spp.) is one of the most important and major cereal crops in the world in terms of production and nutritional value. Wheat is the leading source of cereal proteins and primary staple food (Figueroa *et al*., 2017). It is the 2nd most important crop in the world next to rice (Ambika and Meenakshi, 2018). Globally, wheat is cultivated on over 787 million hectares of land with a production of about 774.9 million metric tons (FAO, 2021), with an average productivity of around 3.56t ha^{-1} with high variability among countries and regions (FAO, 2021).As compared to global wheat production potential (productivity 3.56t ha-1), wheat production in Sub-Saharan Africa is very minimal which is around 3.09 t ha-1(FAO, 2020). Ethiopia is the largest wheat producer in sub-Saharan Africa (Tadesse *et al*., 2018).

In Ethiopia, wheat is one of the most important cereal crops cultivated and considered as the most important strategic food security crops, which is largely grown in the mid and highlands of the country (Beyene et al., 2016). It ranks 3rd after tef, maize, and sorghum in area coverage and 2nd in total production after maize and teff (CSA, 2021). The national average productivity in Ethiopia is estimated to be 3.09t ha-1 (CSA, 2021) with low productivity as compared to the global average yield which was 3.56 t ha-1(FAO, 2021). The low productivity is principally because of biotic and abiotic stresses that are increasing in intensity and frequency associated with climate change (Tadesse *et al.*, 2018).In Ethiopia, more than 30 fungal diseases of wheat have been identified (Fekadu *et al*., 2004). Rust diseases; stem rust (P. graminis f sp. tritici), leaf rust (P. triticina), and yellow rust (P. striiformis f. sp. tritici) were reported as the major biotic wheat production constraints (Netsanet *et al*., 2017).

Moreover, wheat stem rust globally causing up to 100% yield losses over wide areas during epidemics. (Hodson, 2014). According to Netsanet *et al*. (2017) reported that in Ethiopia wheat stem rust causes up to 70.70% yield losses. Wheat stem rust populations in Ethiopia were reported to be highly variable. Hailu *et al*. (2015) identified 9 races in 2013 from 80 samples collected from Oromiya, Amhara & Tigray regions of the country. Abiyot *et al.* (2014) identified 16 races of wheat stem rust from samples collected from major durum and bread wheat growing areas of East Showa Zone in 2009 main cropping season. The intensity of wheat stem rust changes from year to year and from place to place depending on the type of variety grown and climatic conditions. Hence, generating information on the stem rust races and virulence's profile, incase new races developed or mutated is very important to utilize effective resistance genes in their breeding programs (Abebe *et al*., 2012).

The mobility of stem rust coupled with their inherent ability to change through mutation, genetic recombination and new introductions from other countries makes continual monitoring (Park, 2007). Moreover, some studies that were carried out in Ethiopia indicated that most previously identified races were virulent on most of the varieties grown in the country and they are among the most virulent in the world (Admassu *et al*., 2009). In Guji zone wheat stem rust was reported as a serious disease of wheat production and may cause high economic losses (Tolesa *et al*., 2014). Although Guji zone is one of the wheat producing areas in the country, but there was limited information regarding to the distribution, incidence, and severity of the wheat stem rust and the physiological races of Pgt. Nevertheless, recent information is scarce about intensity and virulence distribution of wheat stem rust races around Guji zone and was investigated in the current study.

Objective of the study: was to characterize physiological races of the *Puccinia graminis* f.sp. *tritici;*

MATERIAL AND METHOD

3.1. Description of the Study Areas

Wheat stem rust disease survey and crop sample collection were conducted in the Guji zone, Oromiya regional state during the 2020 main cropping season across major wheat producing districts (Anna Sora, Bore and Dama) (Table 2). Those districts were considered as the "hot spot" for wheat stem rust in the previous report (Tolesa *et al*., 2014).

Table 1: Description of the survey areas

Districts	Altitude(m.a.s.l)	Rainfall(mm)	Soil type	Temp (°C)	Crop type	Global position		Selected kebeles
						Latitude(N)	Longitude(E)	
Anna Sora	2100-2600	1200-1600	Nitosols	10-21	Wheat Potato Barley Maize	6° 10'	38° 43'	Raya boda, Sololo kobo, Bube korsa
Bore	2300-2950	1300-1800	Nitosols	9.3-18	Wheat Barley Potato Faba bean	6° 24'	38° 37'	Songo baricha, Gutu reji, Abayi kuture
Dama	2200-2900	1250-1750	Nitosols	9.5-20	Barley Wheat Faba bean Potato	6°17'33"	38°30'46"	Hada gurati, Dugo gujicha , Balo kadida

Source: National Meteorological Agency, Hawasa Branch Directorate and Agricultural and Natural Resource Management District Office (2008-2019)

3.3. Identification of Physiological Race of Wheat Stem Rust (*P. graminis* f.sp.*trittici*)

3.3.1. Sample collection

During the field survey stems and/or leaf sheath of wheat plants infected with stem rust were collected and cut into small pieces of 5 to 10 cm in length using sterilized scissors and placed in paper bags after the leaf sheath separated from core tissue (stem).This will help the samples to dry not to deteriorate before the analysis and avoids secondary infections. From 66 sample collected: Ana Sora 23, Bore 22 and Dama 21 as well as from three districts Ana Sora was more infected and at early dough growth stage was more samples were collected. After sample collection, the paper bags were labeled with the name of the zone, district and kebeles. Variety grown and weed infestations level per field were also recorded during the survey time. GPS information (altitude, latitude and longitude) and date of collection were recorded and samples were taken to Ambo Agricultural Research Center (AARC) laboratory for analysis.

3.3.2. Isolation and multiplication of single-pustules

Five seeds of universally susceptible wheat variety (McNair) were planted in 10 cm diameter pots filled with a mixture of sterilized soil, sand and manure in 2:1:1 by volume, respectively and preserved in the greenhouse at Ambo Agricultural Research Center. Urediniospores from each field were suspended in lightweight mineral oil, Soltrol 170 (Chevron Phillips Chemical Company, the woodlands, Texsas, United States) and sprayed onto 7-day-old seedlings of variety McNair according to Roelfs *et al*. (1992). Seven days after inoculation (when the flecks/symptoms become clearly visible) leaves containing a single fleck that produces a single pustule were selected from the base and removed using scissors. Only leaves containing single pustules from each location were 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, spores from each pustule were collected to prepare the suspension by mixing urediniospores with Soltrol 170 and inoculated on seven-day-old seedlings of the susceptible variety McNair for multiplication purpose for each of the single pustules in separate pots. Inoculated seedlings were then placed in incubation chamber in dark condition at 18-22 ºC for 18 hours and were exposed to light for four hours and transferred to the greenhouse. After 14 days, the spores of every single pustule were collected separately in gelatin capsules and inoculated on the standard differential lines.

3.3.3. Inoculation of isolates onto host differential lines

Seedlings of 19 wheat differential host lines (Table 3) with known stem rust resistance genes and a susceptible variety Mc Nair were raised in 10 cm diameter pots. Differential lines were originally brought to Ambo Agricultural Research Center from Cereal Disease Laboratory (CDL), Minesota, USA. Each rust isolate was suspended in Soltrol 170 and the suspension was sprayed onto seven-day old seedlings of the differentials using spore inoculators. Inoculated seedlings were preserved in a dew chamber for 18 hours at 18-25 ℃ and 98-100 % relative humidity. Then, plants were exposed to fluorescent light for four hours to enhance the infection process and were allowed to dry for about 2 hours. Finally, inoculated plants were transferred to greenhouse benches where the temperature and relative humidity is adjusted at 18 – 25 ℃ and 98-100 %, respectively (Stubbs *et al*., 1986).

Table 2: Wheat stem rust differential lines with their corresponding stem rust resistant gene used in the present study

Differential host lines	Stem rust genes	Pedigree
LcSr24Ag	24	Little Club/Agent (Cl 13523)
W2691SrTt-1	36	Cl12632 *T. timopheevii*
ISr7b-Ra	7b	Hope/Chinese Spring
ISr8a-Ra	8a	Rieti/Wilhelmina//Akagomughi
CnSSrTmp	Tmp	Triumph64(Cl13679)/ChineseSpring
Sr31(Benno)/6*LMPG	31	Kavkaz
CnS-T-.mono-deriv	21	Einkorn Cl 2433
Trident	38	Spear*4/VPM (Pl519303)
ISr9a-Ra	9a	Red Egyptian/Chinese Spring
ISr9d-Ra	9d	Hope/Chinese Spring
Combination VII	17	Esp 518/9
ISr5-Ra	5	Thatcher/Chinese Spring
ISr6-Ra	6	Red Egyptian/Chinese Spring
W2691Sr9b	9b	Kenya 117A
Vernsteine	9e	Little Club//3*Gabo/2*
W2691Sr10	10	Marquis*4/EgyptNA95/2/2*W2691
BtSr30Wst	30	Festival/Uruguay C10837
CnsSr9g	9g	SelectionfromKubanka(Cl1516)
ISr11-Ra	11	Kenya C6402/Pusa4/Dundee
McNair 701	McN	Cl 15288

Source: Ambo Agricultural Research Center.

3.3.4. Determination of stem rust races

Seedling infection types (ITs) were scored 14 days after inoculation using 0 to 4 scoring scale described by Stakman *et al*. (1962). The IT readings of 3 (medium-size uredia with/without chlorosis) and 4 (large uredia without chlorosis or necrosis) were regarded as susceptible. Other readings, i.e. 0 (immune or fleck), 1 (small uredia with necrosis) and 2 (small to medium uredia with chlorosis or necrosis) were resistant (Figure 5). The variations were refined by modifying characters as follows: **-**, uredinia somewhat smaller than normal for the infection type; +, uredinia somewhat larger than normal for the infection type. Hence, ITs were then grouped into low (“0”, “;”, “;1”, “1”, “1+”, “2-”, “2”, “2+”) and high (“3-”, “3”, “3+”, and “4”) infection types (Stackman *et al*., 1962).

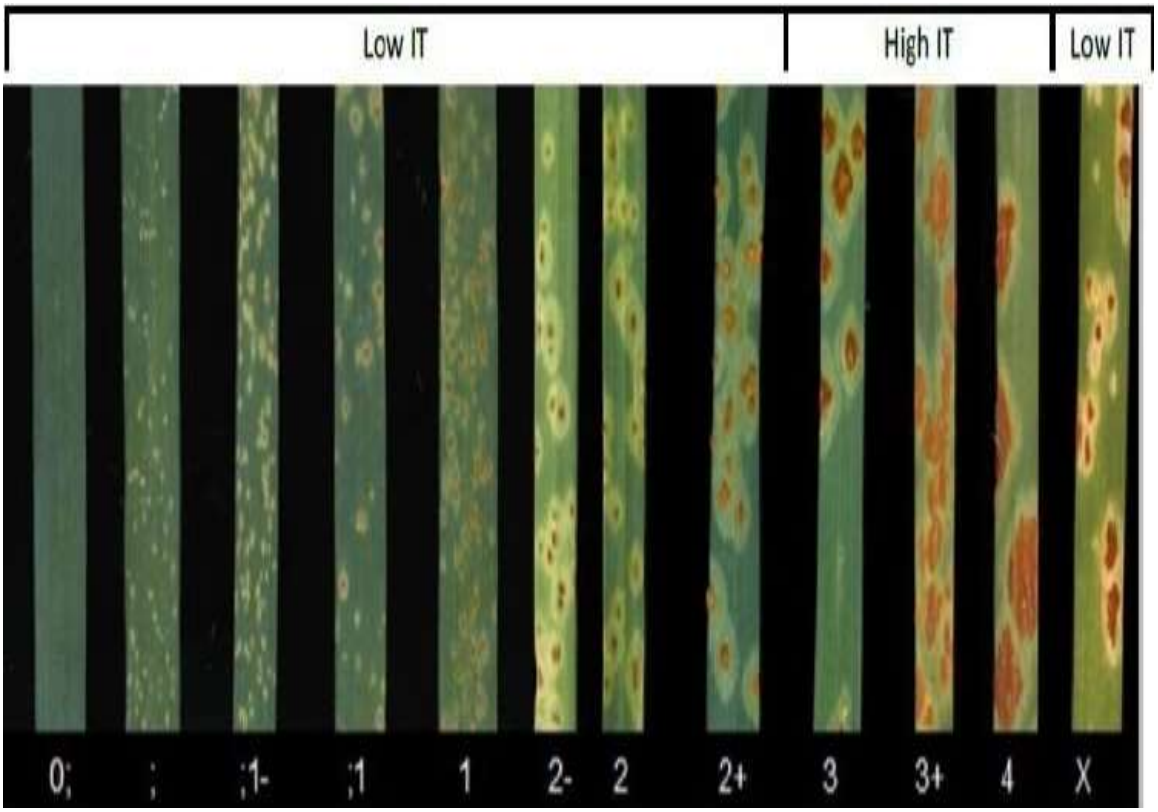

Figure 1: Pictorial infection types of *P. graminis* f.sp. *tritici* of stem rust and host response (Stackman *et al.* (1962)

Race identification was done using the North American nomenclature system for *P. graminis* f.sp. *tritici* (Roelfs and Martens 1988). Races were identified based on their reaction on differential hosts. Race designation was done by grouping the differential lines into five subsets as indicated in Table 4. Each isolate was assigned using a combination of a three letter code of Roelfs and Martens (1988) and an additional two letter race code by Jin *et al*. (2008) which finally give a five letter of designation based on its reaction on the differential lines (Fetch and Dunsmore, 2004). For instance, low infection type (IT) on all four hosts in a set was assigned the letter B, while high IT on the four hosts assigned T. Hence, an isolate produces low IT (resistant reaction) on each of the 20 differential lines, the race was designated with a five letter race code BBBBB. Similarly, an isolate that produces a high IT (susceptible reaction) on the 20 differential lines had a race code TTTTT. If an isolate produces a low IT on Sr*31* and Sr*24*, but a high infection type on the remaining 18 differential lines, the race was designated as TTTTF. The frequency of each race identified was also recorded.

Figure 2: Schematic overview of the protocols for inoculation of spores on McNair to lines evaluation in the greenhouse at AARC; A. Inoculation of field collected spores on McNair for multiplication, B. Development of wheat stem rust on McNair after 14 days, C. Development of a single pustule on McNair and D. Inoculated a single pustule on differentials

Table 3: Nomenclature of *P. graminis* f. sp*. tritici* based on 20 differential wheat host lines and one susceptible variety McNair

		Infection types produced on near-isogenic *Sr* lines			
Pgt- code	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

*Low/Resistant infection type (0 to 2+), High/ Susceptible infection type (3**-** to 4).

Data Analysis

Physiological race analysis was computed on the Microsoft excel by using the descriptive statistical analysis.

RESULT AND DISCUSSION

Physiological races and Virulence spectrum of *P. gramminis* f.sp *tritici*

In this study, a total of 66 infected wheat stem rust samples were collected from those selected districts. Of which, 16 did not yield viable isolates at the time of inoculation in the laboratory. The remaining 50 samples could produce single isolate and were used for the race spectrum analysis. Of these isolates, 5 races namely TTKTT, TTKTF, TTTTF, TKTTF, and TKKTF were identified (Table 14). Three races (TTKTT, TTKTF, and TTTTF) were commonly identified from three districts namely Ana Sora, Bore and Dama while TKKTF race was detected in Ana Sora and Dama districts and TKTTF was detected only in Ana Sora district. Five races were identified from Ana Sora district followed by Bore district that had 4 races. Variation in race composition over location and time depend on the type of wheat varieties grown and to some extent on the predominant environmental conditions, especially temperature (Roelfs *et al*., 1992).

TTKTF was the first dominant race being identified from 19 (38%) samples collected from altitude ranged from 2336-2771 m.a.s.l, which show that it is adapted to wider wheat growing agro-ecological districts followed by TTKTT that was identified from 18 (36%) samples(Table 13). This race (TTKTF) was detected from an altitude range of 2208-2796 m.a.s.l. Likewise TTTTF was detected from an altitude range of 2214-2822 m.a.s.l, which showed that, it is adapted to wider wheat growing agro-ecological districts. However, TKKTF and TKTTF race were the least dominant races among others, they were identified from 2(4%) and 1 (2%) sample with their altitude range of 2448-2702 and 2238, respectively (Table 12). TTKTT race was first reported in Ethiopia in 2018 from commercial wheat cultivars Shorima, Huluka, Ogolcho, Hidase, and Danda'a (Netsanet *et al*., 2020). It has the most virulence combination of all Ug99 (TTKSK) race groups. This race was first reported in Kenya in 2014 and its spread to different wheat growing areas of the world was highly significant (Patpour *et al*., 2016). TKKTF was reported in Germany and was among the races that caused an unusual wheat stem rust outbreak in 2013/14 cropping season (Olivera *et al*., 2017). TKTTF was first detected in 2012 in South eastern parts of Ethiopia and caused wheat stem rust epidemics in 2013/14 cropping season by attacking the popular variety Digelun which was resistant to TTKSK (Ug99 race) (Olivera *et al*., 2015). It was not good news for Ethiopian wheat cultivators for the reason that stem rust resistance gene *SrTmp* that is available in popular bread wheat variety (Digelu) was overcome by this virulent stem rust strain (Hodson, 2015).

The frequency of each race was calculated as a percentage of the total number of isolates analyzed. The races identified from major wheat grown areas in the districts of the zone had wide virulence spectra (Table 13). TTKTF race was the most frequently appeared in three districts being identified from 6 (12%), 510%) and 8(16%) in Ana Sora, Bore and Dama respectively (Table 12). The second most dominant race detected from Huluka variety was TTKTT which was identified from Ana Sora, Bore and Dama with 10 (20%), 5(10%) and 3(6%)in each district. On the other hand, TTTTF and TKKTF races appeared less frequently on Huluka and Senate varieties in each district. The least frequent races on Ogolcho variety were TTKTF identified from 1(2%) sample.

Table 4: Frequency of *Pg*t races identified from samples collected from the study area

Race	Identified from number of samples	Frequencies (%)
TTKTF	19	38
TTKTT	18	36
TTTTF	10	20
TKKTF	2	4
TKTTF	1	2

The most important dominant race TTKTF was isolated from 19 wheat fields planted with Kubsa, Digelu, Shorima, Ogolcho, Huluka and Senate of which 2 (40%), 3 (60%), 4 (80%), 1 (20%), 1 (20%), 2 (40%) were wheat varieties infected with this race in the same orders. For instance, 4 viable sampled wheat fields with Shorima bread wheat variety, 4(80%) were infected with TTKTF in all surveyed districts. Out of samples taken from Shorim variety, 3 (60%) of wheat fields were infected with TTKTF. This race was detected from an altitude range of 2693-2850 m.a.s.l, which show that it is adapted to wider wheat growing agro-ecological districts. This race was also detected in Ethiopia (Kitesa *et al*.,2020; Netsanet *et al*., 2020) The second important virulent race TTKTT was isolated from 18 wheat fields planted with Kubsa, Digelu, Huluka, Shorima and Senate of which 1 (2%), 3 (6%), 4 (8%), 7 (14), and 3(6%), wheat fields respectively. TTKTT race was first reported in Ethiopia in 2018 from commercial wheat cultivars Shorima, Huluka, Ogolcho, Hidase, and Danda'a (Netsanet *et al*., 2020). It has the most virulence combination of all Ug99 (TTKSK) race groups. This race was first reported in Kenya in 2014 and its spread to different wheat growing areas of the world (Patpour *et al*., 2016). The spatial distribution of the *Pgt* races was different among the three districts. Three races including TTKTT, TTTTF and TTKTF were present in three districts and the two races TKKTF and TKTTF were distributed in two districts.

Out of the 50 viable stem rust samples analyzed, TTTTF race was identified from 10 isolates and the third abundant races in the study areas in current the season 2020. TTTTF with virulence to Sr9e and *Sr13* attacked thousands of hectares of wheat, resulting in the largest burst of wheat stem rust in the world and a large number of spores produced by it may continue the epidemic (Bhattacharya, 2017). This race has a virulence formula which is almost similar to TKTTF but is clearly different from stem rust race TTKSK (Ug99) as it has avirulence to *Sr24* and *Sr31*. This race (TTTTF) was first identified from samples collected in 2009 in Eastern Shoa zone of central Ethiopia at trace level (Lemma *et al*., 2014). Likewise, it was identified in Iran from samples collected during 2010-2014 (Patpour *et al.,* 2014; Afshari *et al*., 2015). TTTTF knockout 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 (FAO, 2017).

Wheat varieties grown in Ana Sora, Bore and Dama districts were infected with one or more of *Pgt* races. Kubsa variety was infected with three Pgt races namely TTKTF, TTTTF, and TTKTT and Digelu was infected with TKTTF, TTKTT and TTTTF. Besides, Huluka variety was infected with multiple races (TTKTT, TTTTF and TTKTF). Similarly, Senate was infected with TTKTT and TTKTF and Shorima was infected with TTKTT, TKKTF, TTTTF and TTKTF. However, Ogolcho variety was infected with a single race (TTKTF).

Table 5: P. graminis f.sp. tritici races identified from samples collected and wheat varieties grown in varying altitude ranges of Guji zone main cropping season

Districts	Kebele	Detected Races	Number of isolate	Variety	Altitude(m)
Ana Sora	Raya Boda	TTKTF	2	Kubsa and Digalu	2436, 2450
		TTKTT	5	Huluka, Senate and Shorima	2438-2452
		TKKTF	1	Shorima	2448
	Sololo kobo	TTKTT	5	Senate, Huluka and Shorima	2208-2258
		TKTTF	1	Digelu	2238
		TTTTF	1	Huluka	2214
	Bube Korsa	TTTTF	1	Shorima	2357
		TTKTF	4	Digalu Ogolcho and Shorima	2347-2460
Bore	Songo	TTKTF	2	Shorima	2693, 2744
	Baricha	TTKTT	1	Kubsa and	2732
		TTTTF	2	Huluka and Digelu	2720, 2734
	Gutu Reji	TTKTF	3	Huluka and Shorima	2710, 2771
		TTKTT	1	Shorima	2661
		TKKTF	1	Shorima	2702
	Abayi Kuture	TTKTT	3	Huluka, Senate and Shorima	2705-2710
		TTTTF	2	Senate and Digelu	2709, 2788
Dama	Hada Gurati	TTTTF	3	Kubsa Shorima and Digelu	2787-2835
		TTKTF	2	Senate and Digelu	2797, 2819
	Dugo Guticha	TTKTF	2	Senate and Shorima	2750, 2803
		TTTTF	1	Shorima	2822
		TTKTT	1	Shorima and Digelu	2801
	Balo Kadida	TTKTF	4	Huluka, Digelu, Shorima and Kubsa	2750-2850
		TTKTT	2	Huluka and Shorima	2796, 2804
Total			50		

Wheat stem rust is considered as a re-emerging disease, having outbreaks and epidemics in East Africa, Europe, and Central Asia. Severe epidemics occurred in Ethiopia (2013-14), Kazakhstan and South Siberia (2015-16), outbreaks in Germany (2013), Italy (2016) and Sweden (2017) (Olivera *et al*., 2015). After the occurrence and spread of Ug99, new races with critical virulence have been occurring that have been posing a threat to both bread and durum wheat in many countries including Ethiopia.

4.2.1. Virulence spectrum of *P. graminis* f.sp *tritici* isolates

Wheat stem rust races identified in this study have a different virulence spectrum on stem rust resistance genes. The majority of resistance genes in differential host lines (40%-95%) were defeated with stem rust races identified in the study area. TTKTT race was virulent on 95% Sr genes except *Sr36*. Similarly, TTTTF, TKTTF and TKKTF races were virulent on 90%, 85% and 80% of Sr genes respectively (Table 14). TTTTF was virulent on all Sr genes except *Sr24* and *Sr31*. Unlike to present study, Lemma *et al*., (2014) reported that differential host line carrying *Sr24* was effective to all races identified in central Ethiopia. TKTTF and TKKTF races have almost similar virulence pattern since both are avirulent on Sr genes *Sr11*, *Sr24* and *Sr31*. The study confirmed the presence of high virulence spectrum and high variable populations among the five identified stem rust races.

Table 6: Virulence/Avirulence spectrum of *P. graminis* f.sp. *tritici* races collected from Guji zone

Races	Virulence/ineffective Sr genes	Avirulence spectrum (effective Sr genes)	No of isolates	Virulence of races on Sr gene (%)
TTKTF	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, TmP, 38, McN	36,24,31	19	85
TTKTT	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 24, 31, TmP, 38, McN	36	18	95
TKKTF	5, 21, 9e, 7b, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, TmP, 38, McN	11, 36, 24, 31	2	80
TTTTF	5, 21, 9e, 7b, 11, 6,8a, 9g, 9b, 30, 17, 9a, 9d, 10, 36, TmP, 38, McN	24, 31	10	90
TKTTF	5, 21, 9e, 7b, 6, 8a, 9g, 9b, 30, 17, 9a, 9d,10, 36, TmP, 38, McN	11, 24, 31	1	85
Total			50	

4.2.2. Virulence Frequency of *P. graminis* f.sp. *tritici* Isolates on Stem Rust Resistant Genes

The study shown that most of the races identified in the study area were virulent to many of the resistance genes and majority of resistance genes in differential host lines were ineffective against races identified in this study. Sixteen differential lines carrying stem rust resistance gene Sr*5,* Sr*21*, Sr*7b,* Sr*6, Sr8a* Sr*9g,* Sr9e, Sr*30,* Sr*17,* Sr*9a,* Sr*9d, Sr9b* Sr*10,* Sr*Tmp,* Sr*38* and Sr*McNair* were found to be 100% ineffective to all races. *Sr11* and *Sr36* were ineffective to 60% and 40% of races, respectively. While *Sr24* and *Sr31* were ineffective to 20% of races identified from Guji zone of Oromiya region in 2020 main cropping season. Admasu *et al*., (2009) reported that *Sr7a, Sr7b, Sr8b, Sr9a, Sr9b, Sr9d, Sr9g, Sr10* and *Sr17* were susceptible to the majority of stem rust races identified from Shewa, Arsi and Bale zones, Ethiopia. Abebe *et al*., (2012) also reported that most of the resistance genes possessed by differential lines were ineffective against one or more of stem rust isolates collected from Tigray region of Ethiopia. Moreover, other studies showed that virulence to *Sr6, Sr8b, Sr9a, Sr9d* and *Sr11* is common worldwide (Roelfs *et al*., 1992).

Differential host lines that caries *Sr24* and *Sr31* were effective to the majority of stem rust races detected in this study. Both of the lines were effective against 4 (80%) races (TTKTF, TTTTF, TKKTF and TKTTF) except TTKTT. Wheat stem rust resistance gene *Sr24* is effective against most races of *P. graminis* f. sp *tritici* and is used widely in commercial wheat cultivars worldwide (Jin *et al*., 2008). *Sr24* became ineffective for the first time in 2006 with TTKST race. Susceptible infection type response was observed on wheat lines and cultivars carrying *Sr24* in a field stem rust screening nursery at Njoro, Kenya (Jin *et al*., 2008). Besides, virulence to this effective stem rust resistance gene had been detected in different countries including South Africa (Pretorius *et al*., 2010), Eritrea (Wolday *et al*., 2011) and Ethiopia (Netsanat *et al*., 2020). Stem rust resistance gene *Sr31*-durable since 1980 was overcome due to a highly virulent race arisen in eastern Africa (Uganda) in 1999. The race was known as TTKSK (Ug99) and was virulent to the majority of the world's wheat cultivars (Pretorius *et al*., 2000). It has spread from Uganda throughout eastern Africa, Yemen, and Iran (Nazari *et al*., 2009; Singh *et al*., 2008; and Rouse *et al*., 2011).

Likewise, the differential line that caries Sr36 was effective to 3(60%) of the races identified in this study. It was resistant against, TTKTF, TTKTT, and TKKTF. The lowest resistance spectra were recorded on the differential line that caries *Sr11*. It was resistant to only 2(40%) races including TKKTF and TKTTF. Majority of stem rust resistance genes in differential host lines became susceptible to stem rust races identified in this study. Therefore, repeated wheat production and the favorable weather condition in the wheat production areas of Ethiopia could be the main reasons for the rapid growth and highly diversified 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). Therefore, searching for novel sources of resistance is pertinent to develop durable rust resistant wheat cultivars.

Table 7: Virulence frequency of Pgt isolates on 20 stem rust resistance genes

Stem rust resistance gene (Sr gene)	Virulence Frequency (%)	Stem rust resistance gene (Sr gene)	Virulence Frequency (%)
5	100	30	100
21	100	17	100
9e	100	9a	100
7b	100	9d	100
11	40	10	100
6	100	TmP	100
8a	100	24	80
9g	100	31	80
36	60	38	100
9b	100	McN	100

The detection of five races in the study area is an indicator of the great variability of pgt populations. The result of this finding is in agreement with previous studies conducted in different parts of wheat producing areas of the Ethiopia (Belayeneh *et al*., 2009; Abebe *et al*., 2012; Hailu *et al*., 2015 and Netsanet *et al*., 2017). In addition, some pathotypes identified in this study have more virulence combinations than preexisting races in the country. For instance, TTKTT and TTTTF races have 95% and 90% virulence spectra to stem rust resistance genes within differential lines. Resistance genes (Sr24) that is available in most of the commercial varieties worldwide became ineffective with these races. Roelfs *et al*., (1992) also stated that wheat stem rust is continued to be the main challenge of wheat production worldwide because of the great variability in the pathogen population. This could be created by different mechanisms like mutation and sexual recombination that enable the pathogen to overcome resistance genes within wheat genotypes.

SUMMARY AND CONCLUSION

Fifty stem rust isolates were analyzed on 20 stem rust differentials lines. Five races namely TTKTT, TTKTF, TTTTF, TKKTF, and TKTTF were identified. Among five races; TTKTF was a dominant race detected from 19 samples followed TTKTT race which was identified from 18 samples. However, TTTTF, TKKTF and TKTTF races were the least dominant races. The detection of five races in the study area is an indicator of the great variability of *Pgt* populations. For instance, TTKTT and TTKTF races has 95% and 85% virulence spectra to stem rust resistance genes within differential lines The study showed that The majority of resistance genes in differential host lines 95% were defeated with the race of TTKTT. Thus, preferable to use of effective minor gene and major gene in combination through gene pyramid to track further virulence evolution. The results revealed that the present distribution of the races remarkably on increasing trend, possibly associated with the evolution of new pathogen races, extensive cultivation of the susceptible varieties and the current climate change (warmer temperature, rainfall and humid conditions).

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