Original Research Article||

Distribution and virulence diversity of wheat stem rust races (*Puccinia graminis* f.sp. *tritici*) in Amhara and Oromia regions, Ethiopia

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Abstract

Wheat stem rust (*Puccinia graminis* f.sp. *tritici*) is the major constraint to wheat production worldwide. Because of the sudden changes in stem rust pathogen race patterns, commercial wheat varieties become vulnerable to the pathogen. The present study aimed to identify the races of stem rust pathogen and examine virulence diversity in the pathogen population in the study areas. To achieve these, surveys were conducted in 464 wheat fields across the Amhara and Oromia regional states of Ethiopia during the 2017 cropping season. Four to six wheat stem samples infected with *Puccinia graminis* f.sp. *tritici* (Pgt) were collected from each farmer and experimental station, where wheat is a key crop and stem rust is known to occur following the international stem rust live sampling collection protocol. Sixty stem rust-infected wheat samples were collected from the study areas, of which only 48 were viable. Three races of the stem rust pathogen, namely, TKTTF, TTKSK, and TTRTF were identified from 48 isolates. Of these, TTRTF which was detected in both regions is a new race for Ethiopia. Most of the genes possessed by the differentials were ineffective against one or more of the tested races except Sr24. The most important resistance gene Sr24, present in the majority of Ethiopian wheat cultivars, was effective against all races identified in this study. Thus, the use of effective Sr genes, either individually or in combination, will be crucial for developing wheat cultivars with a broader base of stem rust resistance. Regular monitoring of further virulence evolution is recommended to identify the emergence of new races for future breeding programs. Due to the limited number of samples analyzed in this study, future study with a large sample sizes is recommended to draw more conclusive findings regarding the Pgt population in the study areas.

Key words: Phenotype, Race analysis, Virulence evolution, Wheat rust

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is among the leading cereal crops grown worldwide. Annually, it is produced on an area of over 220 million hectares globally (FAOSTAT, 2021). The crop is grown at altitudes up to 3000 meters above sea level and latitude ranges of 30o and 60oN to 27o and 40oS (Deng et al., 2005). Wheat is among the major crops cultivated in the Ethiopian highlands. Ethiopia is the leading wheat producer in Sub-Saharan Africa (FAO, 2021). Wheat is produced on over 1.8 million hectares of land under rainfed conditions and over 3 million ha under irrigation with about 3.1 metric tons/ha annual average production which is by far below the world's average yield (3.5 tons /ha) (CSA, 2022).

Biotic factors such as diseases, insect pests and weeds are among the factors that constraints wheat production in Ethiopia (Abebe et al., 2012). Among these, wheat stem rust (*Puccinia graminis* f. sp. *tritici*) (Pgt) is the most prevalent of all wheat rusts in the country (Admassu et al., 2012; Denbel et al.,

2013). The disease causes complete destruction of the crop over wide areas during epidemic years (Hodson, 2015; Mulu et al., 2022). In Ethiopia, the average yield loss due to stem rust during 2013-2014 was 51% but the disease caused 100% yield loss in some fields and forced the widely cultivated wheat variety Digelu to be out of production (Olivera et al., 2015).

The ability of the stem rust pathogen to quickly form new races makes it difficult to control the disease. According to Singh et al., (2015), genetic recombination and mutation are important mechanisms of new race formation for rust pathogens. Ethiopian highlands are hot spots for the development and dissemination of new stem rust races (Leppick, 1970; Singh and Rajaram, 2006; Periyannan et al., 2013). Most of the previously identified Pgt races were virulent on wheat varieties grown in the country (Admasu et al., 2009; Belayneh and Emebet, 2005). Resistant varieties can be used as an effective method for controlling stem rust (DRRW, 2010). Knowledge of the pathogen virulence diversity, distribution of races and the identification of resistance genes in the host are required for the development of rust-resistant varieties. In Ethiopia, wider virulence diversity of races, the frequent evolution of the pathogen, and the narrow genetic base in wheat lines make the released wheat varieties break up their resistance within short period.

Virulence surveys and pathotype analysis are important to studying the evolution of new races and forecasting the virulence shifts in a pathogen population. These can be used to assesses the origin, occurrence, and dissemination of new pathotypes and to understand how new pathotypes develop. Such studies also help to determine the degree and range of pathogenic variation in particular regions. This study was therefore carried out to identify the physiological race(s) of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) in the selected regions of Ethiopia and determine the virulence diversity of the races.

MATERIALS AND METHODS

Description of the Study Area

The wheat stem rust surveys were carried out in the main wheat growing areas of Amhara and Oromia regional states of Ethiopia during the 2017 cropping season (Fig. 1). The two regional states contribute 70% of domestic wheat production. A total of 464 wheat fields were surveyed, of which 224 fields were from Oromia and 240 from Amhara Regional States. The study areas in the Amhara region are located within 10.06172 to 11.96160 N latitude and 36.51448 to 39.64268 E longitudes and had altitude ranges of 1804 to 3450 m.a.s.l. In Oromia regional state, the disease surveys were carried out at altitudes between 1704 and 3334 m.a.s.l, within latitudes of 6.55038 to 8.93800 N and longitudes of 37.16835 to 39.28679 E.

Zones and districts from both regional states were selected based on their wheat production potential. The Bale, East Arsi, and West Arsi areas in Oromia are traditionally referred to as “The Ethiopian Basket of Bread” while East Gojjam, West Gojjam, and North Wello areas in the Amhara regional state are commonly known as “Wheat belts of Amhara”.

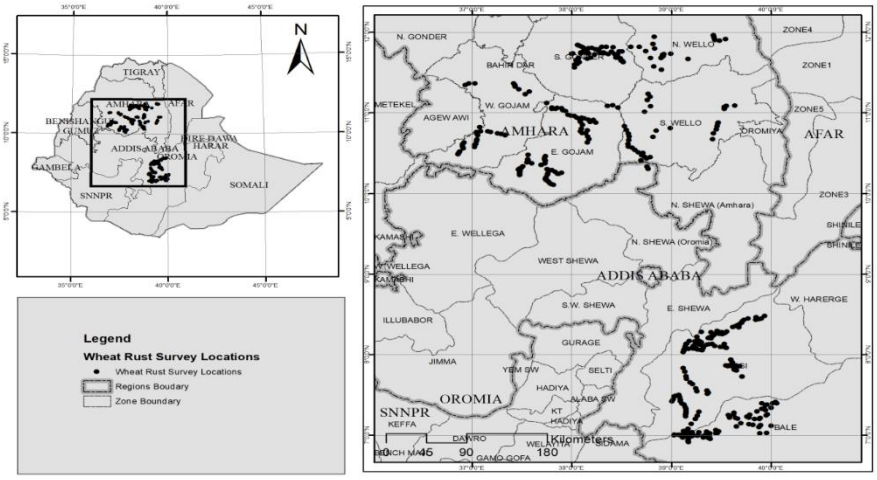

Figure 1. Map showing wheat stem rust survey areas

Rust Samples Collection

Four to six stem samples infected with *Pgt* were collected from farmers' fields and experimental stations, where wheat is important and stem rust is known to occur. The international stem rust live sampling collection protocol was followed during sampling (Park et al., 2013). A total of 464 wheat fields were surveyed of which 224 fields were from Oromia and 240 from Amhara regions. The number of sample was limited to only 464 fields due to budget constraint. During the survey, 60 stem rust-infected wheat samples were collected from the study areas, of which only 48 were viable. The 24 viable samples were collected from Oromia and the remaining 24 viable samples were from Amhara regions. Accordingly, the core tissue and sheaths of the stem samples were removed and cut into small pieces of 5-10 cm. The stem rust infected core sheaths were then put in a glycine paper bag. This method facilitated the easy drying of the samples and prevented prior spore germination before the actual race analysis in the greenhouse. After every sampling and labeling, cutting materials and hands were disinfected using 75% ethanol according to the international rust live sampling protocol (Park et al., 2013). The samples collected were labeled with relevant information, including the name of the zone and district, cultivar name, date of collection, and GPS data (latitude, longitude, and altitude) recorded during the survey. The samples were collected at every 5 km intervals along the main and feeder roads.

Isolation and Multiplication of Single-Pustule Isolates

The urediospores from the collected infected stem samples were harvested into a motorized collector known as "a vacuum and diaphragm pump atomizer" (Vacuubrand Technology, Berlin, Germany). The spores were then multiplied on the universally rust-susceptible variety "Mc Nair701" which does not carry stem rust resistance genes (Roelfs et al., 1992). This variety was obtained from the University of Minnesotra, Cereal Disease Laboratory and CIMMYT wheat rust disease research team. Before using the spore inoculators and collectors, they were decontaminated by 95 % alcohol suspension. They were then immersed in alcohol suspension and stayed for 24 hrs before being used for the green house experiment. Five seedlings grown from seeds of this wheat variety which was confirmed to be stem rust free were raised in 8 cm diameter pots containing sterilized potting media with 2:1:1 ration of soil, sand, and manure, respectively. Greenhouse inoculation was carried out using the methods and procedures developed by Stalkman et al. (1962). Three mg of *Pgt* spores in 1ml of solTrol-130 were inoculated onto seven-day old wheat seedlings using vacuum and diaphragm pump atomizer. Distilled water was used to moisten plants and the moistened plants were then placed in an incubation chamber for 14 hrs in the dark at a temperature of

18-24^{0}C. After incubation, seedlings were supplied with fluorescent light for 4 hrs to provide favorable conditions for infection, and seedlings were allowed to dry/remove their dew/ for about 1-2 hrs. The inoculated seedlings were then placed in glass compartments of the greenhouse, which maintained a 12 hrs photoperiod, a temperature range of 18-25 ^{0}C, and a relative humidity of 60-70%. The remaining rust spores were stored at -80^{0}C and used to replace samples that fail to produce infection on the universally susceptible line Mc Nair701.

After seven to ten days of inoculation (when the flecks/symptoms were clearly visible), leaves containing a single fleck that had produced single pustule were selected from the base of the plant and the remaining seedlings within the pot were removed using scissors. Only 2-3 leaves per pot which contain single pustule were left. Each pot with single pustule was covered with cellophane bags (145 x 235mm) and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunsmore, 2004). After two weeks (14-15 days) following inoculation (when the mono pustule was developed), each mono pustule was collected using spore collector under vacuum and stored in a separate gelatin capsule. Seven-day old seedlings of a universally susceptible variety "Mc Nair " was inoculated with one gelatin capsule of pgt spore suspension prepared by mixing 3-5 mg urediospores with 1ml of mineral oil (solTrol-130). After inoculation, the seedlings were placed in a humid chamber in dark condition at 18-22^{0}C for 18 hrs and then in the light for 3-4 hrs, after which they were transferred to a greenhouse with temperature of 18 - 25^{0}C and RH of 60 - 70% for 14-15 days. After inoculation, the multiplied spores of each mono pustule/isolate were collected in separate test tubes and stored at -80^{0}C till they were inoculated on the standard differential sets. This procedure was repeated till sufficient number of spores were produced in order to inoculate the set of stem rust differential hosts.

Inoculation of Wheat Stem Rust Differential Lines

Five seeds of each of the 19 stem rust differential hosts with known resistance genes (*Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr24, Sr30 Sr31, Sr36, Sr38,* and *SrTmp*) along with the susceptible line "Mc Nair" (Table 1) were sown separately in 3cm diameter pots and placed in a greenhouse at Ambo Agricultural research center WRRAL (Wheat Rust Race Analysis specialized Laboratory. Twenty pots were used for each of the differential lines and the experiment was arranged in a completely randomized design (CRD) with three replications. Seven-day old seedlings were inoculated with 3-5 mg *Pgt* spores derived from single pustule isolates in 1 ml of distilled water or solTrol-130 using a vacuum pump inoculator, following the procedure described under section 2.3. Once inoculated, plants were supplied with moisture using fine droplets of distilled water produced with an atomizer and placed in an incubation chamber for 14 hrs in the dark at 18-24^{O}C and then the plants in the incubation chamber were supplemented with 4 hrs of additional cool white fluorescent tubes. Once removed from the dew chamber, plants were placed in glass compartments in a greenhouse at temperature of 18-24^{o}C and the relative humidity of 70% until disease develops and evaluated. The experiment was repeated three times to confirm the result.

Data Collection

Scoring for wheat stem rust infection types was carried using the 0-4 scale (Stalkman et al., 1962) 14 days after inoculation. Infection types were grouped into two, where, Low infection type (resistance reaction; 0-2+) are considered as the pathogens are avirulent/low infection. and High infection type (susceptible reaction; 3 to 4 scores) considered as the pathogens are virulent (Figure 2).

Table 1. List of stem rust differential lines used in the study, their corresponding Sr genes and Origin/pedigree.

No.	Differential host	*Sr* genes	Origin/pedigree
1	Lc*Sr*24Ag	24	Little Club/Agent (Cl 13523)
2	W2691*Sr*Tt-1	36	Cl12632 T.timopheevii
3	I*Sr*7b-Ra	7b	Hope/Chinesen Spring
4	I*Sr*8a-Ra	8a	Rieti/Wilhelmina//Akagomughi
5	CnSSrTmp	Tmp	Triumph 64(C/13679)/Chinese Spring
6	*Sr*31 (Benno)/6*LMPG	31	Kavkaz
7	CnS-T-mono-deriv	21	Einkorn Cl 2433
8	Trident	38	Spear *4/VPM(p1519303)
9	I*Sr*9a-Ra	9a	Red Egyptian/Chinese spring
10	I*Sr*9d-Ra	9d	Hope/Chinese spring
11	Combination VII	17	Esp 518/9
12	I*Sr*5-Ra	5	Thatcher/Chinese Spring
13	I*Sr*6-Ra	6	Red Egyptian/Chinese spring
14	W2691*Sr*9b	9b	Kenya 117A
15	Vernsteine	9e	Little club//3*Gabo/2*
16	W2691*Sr*10	10	Marquis*4/Egypt NA95 /2/2*W2691
17	Bt*Sr*30Wst	30	Festival/Uruguay C10837
18	Cns*Sr*9g	9g	Selection from Kubanka (C11516)
19	I*Sr*11-Ra	11	Kenya C6402/pusa4/Dundee
20	McNair701	McN	C115288

Seed source: Ambo Plant Protection Research Center (2017)

Table 2. Naming of *Puccinia graminis* f. sp. *tritici* based on 20 differential wheat

		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*	Low	Low	Low
C		Low	Low	Low	High**
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); *Low: Infection types 0;, 1, and 2 and combinations of these values; **High: Infection types 3 and 4 and a combination of these values.

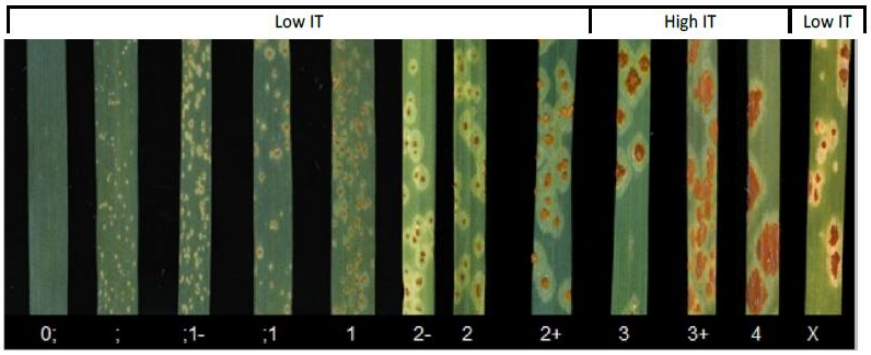

Figure 2. Pictorial description of infection types used in classifying the reactions of stem rust on leaves of wheat seedlings (Stalkman et al., 1962).

Designation of Races

The Northern American Nomenclature System for *Puccinia graminis* f. sp. *tritici* (Roelfs and Martens, 1988) was used to designate races by grouping differential lines, each containing a unique single resistance gene. This approach allows for distinguishing races based on their qualitative reactions to different pathogen isolates. The differentials were put in the order as shown in (Table 2). Set I: Sr5, Sr21, Sr9e, Sr7b, Set II: Sr11, Sr6, Sr8a, Sr9g, Set III: Sr36, Sr9b, Sr30, Sr17, Set IV: Sr9a, Sr9d, Sr10, SrTmp, Set V: Sr24 Sr31, Sr38, SrMcN. The five-letter code was given to each isolate of a race based on the reaction on the differential lines (Fetch and Dunsmore, 2004). For example, letter 'B' and 'T' were assigned for low and high infection types on the four lines, respectively. Hence, when an isolate had a resistant reaction (produce low infection type) on the 20 differential lines, the race was assigned with 'BBBBB' race code. When an isolate produced a high infection type (susceptible reaction) on the 20 wheat differential lines, it was given 'TTTTT' race code. An isolate which produced a low infection type on Sr11, Sr24, and Sr31, but a high infection type on the remaining 17 differential lines was designated as TKTTF. Infection types that were consistent across the differential lines in all three replications were solely used for data analysis. When an infection type of 0, indicating an immune reaction, was observed, the experiment was repeated to rule out the possibility of disease escape.

Data Analysis

SPSS IBM20 and Arc GIS version 10.5.1 computer software programs were used to compute the mean, statistical analysis and to generate maps.

RESULTS

Virulence Structure of Stem Rust Pathogen

Of the total of 60 stem rust isolates collected, 48 were viable and the remaining 12 samples did not yield viable spores after inoculation on the susceptible check McNair 701 in the greenhouse. Therefore, the race analysis was done using 48 viable isolates. The identified races in different wheat varieties and their detection frequencies across the study zones are presented in table 3 and 4, respectively.

Three races namely TKTTF (Digelu race), TTKSK (Ug-99) and TTRTF were identified from the samples collected in the two regions. Among these, TKTTF was the predominant race in the surveyed regions, with a frequency of 75%. The other two races, TTRTF and TTKSK, were detected at frequencies of 22.91% and 4.16%, respectively. Variation in the virulence spectra of these races was observed within the regions. In Oromia, TKTTF was detected in all three surveyed zones (Bale, East Arsi, and West Arsi). The race was most frequent in the East Arsi (45%) followed by Bale (33.33%) zone, while, it was the least abundant in West Arsi zone (8.3%). TKTTF was obtained from the diseased samples from several varieties, including Hidase, Ude, Tesfaye, Ogolcho, Senbete, Kubsa, Dashen, Danda'a, Crossing lines, Ravi-18 and some unknown local varieties in the region.

The race TTKSK (Ug-99) was detected in the East Arsi and West Arsi zones, each with a frequency of 4.2%, from the varieties Ogolcho and Kakaba. However, this race was not observed in Bale zone. In total, the race TIKSK accounted for 8.33% of the *Pgt* population in the surveyed zones. The race TTRTF was detected at a single location in East

Arsi zone with a frequency of 4.2%, specifically from the variety Hidase but it was not detected from samples collected in Bale and West Arsi zones.

In Amhara region, two races, namely TKTTF and TTRTF were detected from the 24-stem rust infected samples analyzed. TKTTF was the predominant race detected in all surveyed zones of the Amhara region. It was most frequently detected in East Gojjam, North Wello, and South Wello zones with frequencies of 20.8%, 16.7%, and 12.5%, respectively. However, it was least abundant (4.2%) in South Gondar.

Race TKTTF was detected from the varieties Digelu, Danda'a, PBW-343, Pavon-76 and some other unknown varieties. In contrast, the race TTKSK was not detected in any of the five zones in the Amhara region. The new race TTRTF was detected in all surveyed zones except West Gojjam, with the highest frequency of 20.8% in South Gondar. TTRTF was obtained from stem rust samples of varieties Digelu, Danda'a, Pavon-76, Israel, Triticale and some Unknown varieties in the region.

Table 3. Wheat varieties infected by the Pgt races identified in selected districts in Oromia and Amhara regions during 2017 main cropping season

Region	Pgt Races identified	Varieties
Oromia	TKTTF	Hidase Ude Tesfaye Ogolcho Senbete Kubsa Dashen Danda'a Crossing line Ravi-18 Unknown
	TTKSK	Ogolcho
	TTRTF	Hidase
Total		
Amhara	TKTTF	Danda'a Unknown Digelu PBW-343 Pavon-76
	TTKSK	-
	TTRTF	Unknown Digelu Danda'a Pavon-76 Israel Triticale
Total		

Table 4. Summary of number of samples collected, analyzed and races obtained from the study areas.

Region	Zone	No of samples	TKTTF	*Freq. (%)	TTKSK	Freq. (%)	TTRTF	Freq(%)
Oromia	Bale	8	8	33.33	-	-	-	-
	East Arsi	13	11	45.83	1	4.17	1	4.17
	West Arsi	3	2	8.33	1	4.17	-	
Sub total		24	21	87.50	2	8.33	1	4.17
Amhara	East Gojjam	6	5	20.83	-	-	1	4.17
	West Gojjam	2	2	8.33	-	-	-	
	North Wello	6	4	16.67	-	-	2	8.33
	South Wello	4	3	12.50	-	-	1	4.17
	South Gondar	6	1	4.17	-	-	5	20.83
Sub total		24	15	62.50	-	-	9	
Total		48	36	75.00	2	4.17	19	

*Freq: Frequency

Virulence Spectra to Sr Resistance Genes

The finding of the present study revealed that 85% of the stem rust resistance genes (Sr) were ineffective against all the races detected, while 30% were effective against one or more of the races identified (Table 5). Fourteen differential hosts with the resistance genes *Sr5, Sr21, Sr9e, Sr7b, Sr6 Sr8a, Sr9g, Sr9b, Sr17, Sr9a, Sr9d, Sr10, Sr38* and *McN* were ineffective to the three races detected, exhibiting a virulence frequency of 100% on these *Sr* genes (Table 6). In contrast, the differential host with the resistance gene *Sr11* was found to be effective against the race TKTTF but ineffective against TTKSK and TTRTF. Similarly, the differential host with *Sr36* was ineffective to race TKTTF and TTKSK, though it was effective against TTRTF. Additionally, the differential host having the *SrTmp* resistance gene was not effective against the races TKTTF and TTRTF but effective against TTKSK. The differential host carrying resistance gene *Sr31* was effective against both TKTTF and TTRTF races, but not against TTKSK. The most important resistant gene Sr24, which the majority of Ethiopian commercial varieties possess (Belayneh, 2010) was found to be effective against all races identified in the study areas. Overall, there was variation in the virulence spectrum among the races identified in the present study.

Table 5. Virulence spectra of the Pgt races identified in Oromia and Amhara regions in 2017

Race	Virulence	Avirulence
TKTTF	*5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*	*11, 24,31*
TTKSK	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38, McN*	*36, Tmp, 24,*
TTRTF	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, 38, McN*	*30, 24, 31*

Table 6. Virulence frequency of Pgt races collected from the study area to single-gene wheat differentials.

Stem rust resistance gene	Virulence frequency (%)	Stem rust resistance gene	Virulence frequency (%)
*Sr5	100	Sr 30	66.67
Sr 21	100	Sr 17	100
Sr 9e	100	Sr 9a	100
Sr 7b	100	Sr9d	100
Sr 11	66.67	Sr 10	100
Sr 6	100	SrTmp	66.67
Sr 8a	100	Sr 24	0
Sr 9g	100	Sr 31	33.33
Sr 36	66.67	Sr 38	100
Sr 9b	100	McN	100

*Sr: Stem rust

DISCUSSION

From the 48 samples analyzed in this study, three Pgt races (TKTTF, TTKSK, TTRTF) were identified. In contrast, previous studies have reported a broad range of Pgt races in Ethiopia (Belayneh and Emebet 2005; Admasu et al., 2009; Hailu et al., 2015; Abebe et al., 2012; Hei et al., 2018). The smaller number of Pgt races detected in the present study might be due to the limited number of samples analyzed.

The race TTRTF was reported in Georgia in 2014 (Olivera et al., 2019). The race TTRTF identified in this study was the first report of this race in wheat in Ethiopia by Tsegaab et al. (2019). The detection of TTRTF race in Ethiopia concurrent with the massive government ambition of expanding irrigated wheat calls for developing resistant wheat varieties against the race and improving surveillance systems, or implementing policy changes to support farmers.

The race TKTTF is widely distributed in the Middle East region and it has been confirmed to be present in nine countries (Turkey, Iran, Lebanon, Egypt, Ethiopia, Georgia, Azerbaijan, Eritrea, Yemen) (Olivera et al., 2015). This race was the dominant one in Pgt population in Ethiopia and was the major cause for the wheat stem rust epidemics that happened during 2014–15 in Ethiopia (Olivera et al., 2015). The race caused complete yield losses in some fields and led to the widely cultivated wheat variety Digelu being removed from production (Olivera et al., 2015).

The other race identified in this study was race TTKSK (Ug-99). This race pre-dominated the Pgt population of Ethiopia and threatened wheat production in the country (Endale et al., 2016). It had virulence on *Sr31* gene which was resistant for over 40 years. According to Endale et al. (2016) TTKSK was the most dominant and widely distributed Pgt race in Ethiopia after 2003 until the Digelu race (TKTTF) took over the dominance. Similarly, Olivera et al. (2015) reported the dominance of the race TTKSK in Arsi and Bale, before the Digelu (TKTTF) race. The authors also reported that the TTKSK race was responsible for epidemics in 2012. However, in the present study it was detected only from two stem rust samples. This suggests that TTKSK was not predominant in the study areas and this might be due to the release of multiple resistant wheat varieties targeting this race. However, it might be difficult to come up with a conclusive remark due to the limited number of samples collected.

Based on the findings of the present study, Sr 24 is the only stem rust resistance gene that remains resistant against the three races i.e. TKTTF, TTKSK and TTRTF. Virulence on *SrTmp* is considered as the main factor for the complete susceptibility of the variety “Digelu” to the race. Moreover, the detected races had a wider range of virulence spectrum. Broader virulence diversity of Pgt races has also been reported in Ethiopia (Belayneh and Emebet, 2005; Belayneh, 2010). Favorable environmental conditions and continuous wheat production might be the probable reasons for the occurance of virulence diversity in Pgt population in Ethiopia. Similar virulence diversities within Pgt population have been reported in other countries, including

Mexico, USA and Canada and South Africa (Jin, 2005).

The two races TKTTF and TTRTF identified in the present study varied by single gene changes. Both races exhibited avirulence to *Sr24* and *Sr31*. However, a single gene change in virulence occurred on *Sr30 and Sr11*. This entails *Sr30* was broken by the race TKTTF but remained resistant to the race TTRTF and *Sr11* gave up its resistance to the race TTRTF but remains resistant to the race TKTTF. Similarly, Belayneh (2010) and Teklay et al. (2012) noted that single-gene changes are major factors for the variations amongst most Pgt races in Ethiopia. Such single-step changes in virulence have been recognized as a primary mechanism of evolutionary change in *Puccinia graminis* f. sp. *tritici* populations. The findings of the present study are also in agreement with the report of Teklay et al. (2012), which stated that Sr24 is among the effective genes to all stem rust races collected from the northern Ethiopia. However, virulence to Sr24 gene was reported in Kenya in 2006 (Wanyera et al., 2010) and later identified in Ethiopia from samples collected in 2018 (Hei et al., 2020). A variant of Ug99 group (TTKST) that added virulence on stem rust resistance gene Sr24 (Ug99+Sr24 virulence) has further increased the vulnerability of wheat to stem rust worldwide (Jin et al., 2008). Previous reports also indicated the breakdown of the Sr31 resistance gene in Ethiopia (Belayneh, 2010; Teklay et al., 2012; Endale et al., 2016) and this serves as evidence for the existence of the race TTKSK (Ug99) in the country.

CONCLUSIONS

This study revealed that the nature and distribution of diversified soil types along the toposequence of the Qenberenaweti sub-watershed were influenced by the degree of variations in typical topographic positions and key slope features (steepness, aspect, and form). Because these pedogenesis factors directly affect the erosion-deposition and eluviation-illuviation of soil materials via controlling the action of water moving laterally across the surface and percolating vertically into the subsoils, respectively. Generally, the absence of inclusive evidence on the formation, development, and distribution of soils at a site-specific physiographic condition is often a constraint to the improvement of agriculture. Thus, the outputs of such detailed soil characterization, classification, and mapping work give a vital clue for proper planning, management, and utilization of the soil resources at local topographic variability level. However, further research should be done to ensure sustainable agricultural production in the study area.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

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