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Research Article

Distribution and Physiological Races of Wheat Stem Rust (*Puccinia graminis* f. sp *tritici*) in North and East Shoa Zones of Ethiopia

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ABSTRACT

Wheat (*Triticum aestivum* L.) is the most important staple crop in temperate zones and is in increasing demand in countries undergoing urbanization and industrialization. However, its production is affected by many biotic and abiotic factors. Among biotic production constraints; wheat stem rust (*Puccinia graminis* f.sp *tritici*) is the most important one. This study was (i) to assess the importance of wheat stem rust in North and East Shoa zones of central Ethiopia and (ii) to identify physiological races. Purposive multistage sampling was used to select major wheat growing districts and farmers associations from each zone. Wheat stem rust race identification was carried out via inoculation of isolates on susceptible line (McNair); single pustule isolation; inoculation on standard differential sets and infection type evaluation of each line fourteen days after inoculation. One hundred fifty wheat fields (75 from each zone) were assessed. Wheat stem rust was observed in 71 (94.7%) and 52 (73.3%) of wheat fields in East and North Shoa zones, respectively. Disease incidence and severity were significantly different ($p < 0.0001$) between the two zones. Six physiological races of *Puccinia graminis* f.sp *tritici* (*pgt*) namely; TKTTF, TTTTF, TKKTF, TTKTT, TTKTF and TTTTT were identified. TKKTF was the dominant race which was detected from 40 (48.2%) samples followed by TKTTF (Digelu race) which was identified from 28 (33.7%) samples. But, TTTTT, TTKTT and TTTTF were less frequent races. They were identified from 1 (1.2%), 2 (2.4%) and 4 (4.8%) samples, respectively. The majority of resistance genes in differential host lines (80-100%) were defeated with the races. Resistance genes Sr24 and Sr31 were effective to majority of races identified. Hence, they can be used as source of resistance in breeding program.

Keywords: Incidence; Prevalence; Race; Severity; Stem rust

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important staple crop in temperate zones and is in increasing demand in countries undergoing urbanization and industrialization. It is a major source of starch, energy and provides substantial amounts of several components that are essential or beneficial for health like protein, vitamins B, dietary fiber, and phytochemicals that have health benefits for humans [1]. Wheat represents approximately 19% of global major cereal crop production. East African countries, North Africa, and the Middle East consume over 150% of their wheat production and are heavily dependent on imports to meet their food security [2]. Ethiopia is the largest wheat producer in sub-Saharan Africa [3]. In Ethiopia, wheat ranks fourth inland coverage and total production after tef, maize, and sorghum. It is grown on around 1.7 million ha of land [4] and is grown primarily as a rain-fed crop by subsistence farmers in mid to high land areas. The low productivity is principally because of abiotic and biotic stresses which are increasing in intensity and frequency associated with climate change [3]. The average yield of wheat in Ethiopia is 2.8 t/ha [4] is lower than global wheat average yield which is 3.43t/ha [5].

Stem rust caused by *Puccinia graminis* f.sp. *tritici* (*Pgt*) is one of the major production constraints in most wheat growing areas of the country, causing yield losses of up to 100% during epidemic years [6]. Wheat stem rust populations in Ethiopia were reported to be highly variable. Hailu, et al. [7] identified 9 races in 2013 from 80 samples collected from Oromia, Amhara, and Tigray regions of the country. Besides, Hei, et al. [8] reported six races out of 179 samples collected from Oromia, Amhara, Tigray and Southern

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Nations and Nationalities Peoples' regions in 2014 cropping season and seven races from 168 stem rust samples analyzed in 2015 main cropping seasons. Races prevalent in the central highlands of Ethiopia are among the most virulent in the world [9]. The study also revealed that the wheat stem rust pathogen consists quite complex pathotypes in central high lands of Ethiopia. Lemma, et al. [10] identified 16 races from samples collected from major durum and bread wheat growing areas of East Shoa zone in 2009 main cropping season. Nevertheless, recent information is limited about intensity and virulence distribution of wheat stem rust disease in North and East shoa zones of central Ethiopia. Because of quick alteration of genetic makeup of wheat stem rust pathogen, it has to be surveyed regularly to know the status of the disease and to monitor shift in virulence. In addition, the evolution of new stem rust races with broad virulence on many of the resistance genes deployed in wheat breeding is a driving force to search for new sources of resistance. Therefore, regular virulence analysis of the pathogen is mandatory to monitor shift in virulence. This in turn helps to get ready and combat in case any new strains of the pathogen have occurred. Thus, knowledge of resistance genes in cultivars is important for deploying cultivars with different genes to reduce damage. The study was therefore undertaken to assess the distribution and physiological races of stem rust (*Pgt*) in North and East Shoa zones of Ethiopia.

MATERIALS AND METHODS

Survey of wheat stem rust in major wheat producing districts of East and North Shoa zones

Surveys were conducted using purposive multistage sampling that was used to select major wheat growing districts and peasant associations from each zone. Ten districts (five from each zone) were selected depending on area coverage. Farmers associations where wheat is predominantly grown were selected from all districts and three wheat fields were assessed from each peasant associations at 5-10 km interval. The map of the study area where the surveys were carried out is indicated below (Figure 1).

Disease assessments

Disease assessment was made at five points along the two diagonals (in an "X" pattern) of each field using 0.5 m × 0.5 m (0.25 m^2) quadrate. In each field, wheat plants within the quadrant were counted and recorded as diseased/infected and healthy/non-infected and the intensity of stem rust was calculated. Accordingly, the Incidence of stem rust was calculated by using the number of infected plants and expressed as a percentage of the total number of plants assessed.

Disease prevalence

Disease prevalence was calculated as number of fields affected by stem rust over the total fields assessed and expressed in percentages. Disease severity was examined visually on the whole plants within the quadrates as the percentage of plant tissue affected and recorded according to modified Cobb's scale [11]. In order to take into account the different sizes of pustules and their distribution, this scale provides four series of diagrams (each series containing twelve actual diagrams) covering a wide range of combinations of pustule size and distribution. According to modified Cobb's scale, severity (percentage of the plant infected) and response (type of disease reaction) was recorded together. Intervals (Trace, 5, 10, 20, 40, 60, and 100) was used to estimate percentage of plant tissue infected. The host plant response/infection type to infection was scored using the description of Roelfs et al. [12]. The coefficient of infection (CI) was calculated by multiplying the level of disease severity and the constant value of infection type. The constant values for infection types were R = 0.2, MR = 0.4, MR-MS = 0.6, MS = 0.8, MS-S = 0.9, S = 1 [13]. Besides, data on variety, field

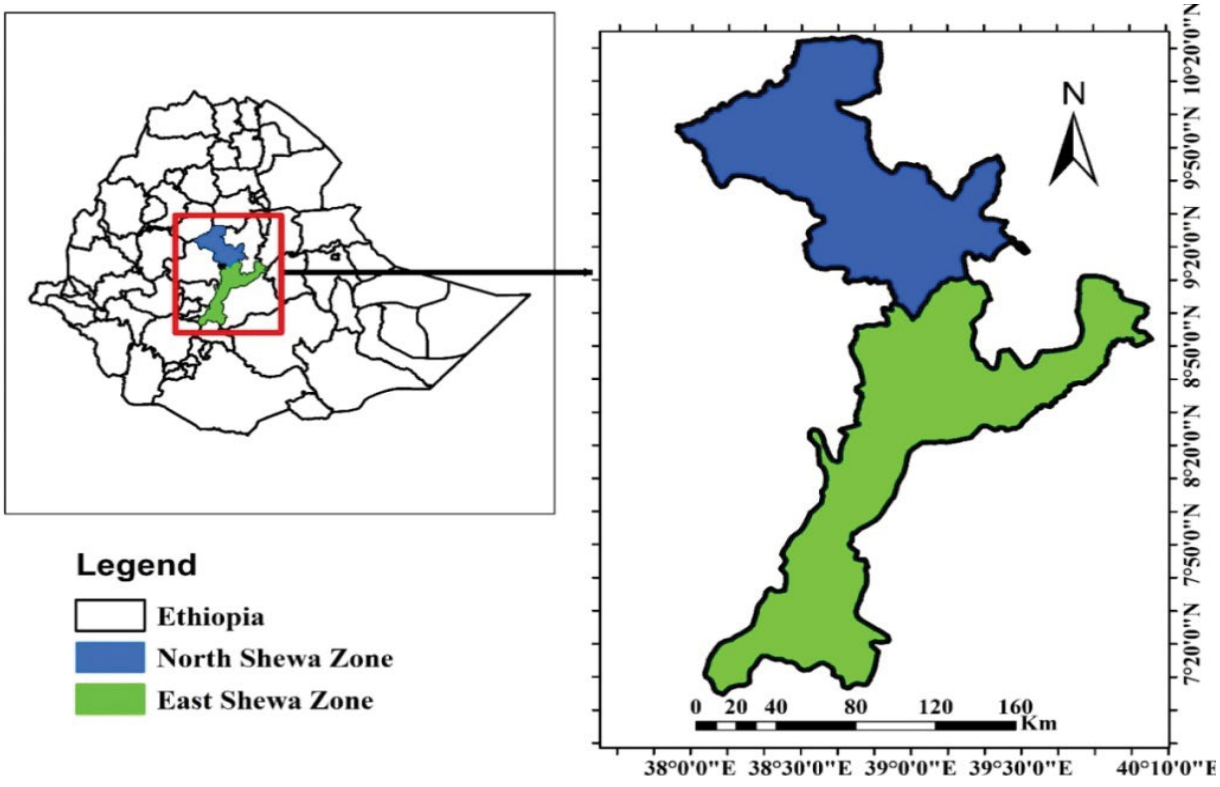

Figure 1: Map of the study area where the surveys were carried out.

history, crop growth stage using Zadok's scale [13] and geographical information (latitude, longitude, and elevation) using GPS were recorded for each field.

Physiological races and Virulence Spectrum of *Puccinia graminis* f.sp. *tritici*

Collection of infected tissue samples: Samples of infected stems (one sample per field) were collected at 5-10 km interval from wheat fields from North and East Shoa zones, Ethiopia. Wheat fields along the main and feeder (accessible) roadsides were assessed in the surveyed areas. Stem and/or leaf sheath of wheat plants infected with stem rust was cut into small pieces of 5 to 10 cm in length using scissors and placed in paper bags after the leaf sheath separated from the core tissue (stem) to dry the samples. This technique helps the samples not to deteriorate before analysis [14]. Thereafter, samples collected in the paper bags were labeled with the name of the zone, district, variety, GPS information (altitude, latitude and longitude) and date of collection and was taken to Ambo Agricultural Research Center (AARC) laboratory for analysis.

Isolation and multiplication of single-pustule isolates: Five seeds of universally susceptible wheat variety (McNair) were sown in 10 cm diameter pots filled with a mixture of sterilized soil, sand, and manure in 2:1:1 by volume, respectively. Seedlings were grown in the greenhouse with a temperature and relative humidity of 18-25°C and 98-100% relative humidity. Urediniospores from each field was 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 [12]. Seven days after inoculation (when the flecks/symptoms become clearly visible) leaves containing a single fleck that produces a single pustule was selected from the base of the leaves and the remaining seedlings within the pots were 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 [15]. Two weeks later, spores from each pustule was collected to prepare the suspension by mixing urediospores with Soltrol 170. Then after, it was inoculated on seven-day-old seedlings of the susceptible variety McNair for multiplication purpose for each of the single pustules in separate pots.

After inoculation, seedlings were placed in incubation chamber in dark condition at 18-22°C 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.

Inoculation of wheat stem rust differential lines

The seedlings of 20 wheat differential host lines with known stem rust resistance genes that and a susceptible variety McNair were grown in 10 cm diameter pots. Differential lines were originally brought to Ambo Agricultural Research Center from Cereal Disease Laboratory (CDL), Minnesota, USA. Each rust isolate was suspended in Soltrol 170. The suspension was sprayed onto seven-day old seedlings of the differentials using spore inoculators. Inoculated seedlings were put in a dew chamber for 18 hours at 18-25°C and 98-100% relative humidity. Then, plants were exposed to fluorescent light for four hours to provide a conducive condition for infection and were allowed to dry for about 1-2 hours. Finally, inoculated plants were transferred to greenhouse benches where the temperature and relative humidity is adjusted at 18 – 25°C and 98-100% [13], respectively.

Determination of races

Seedling infection type was scored 14 days after inoculation using a 0 to 4 scale [16]. 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 regarded as low infection type or resistance reaction. The variations were refined by modifying characters like -, uredinia slightly smaller than normal for the infection type; +, uredinia slightly larger than normal for the infection type [16].

Race designation was done by grouping the differential lines into five subsets as indicated in Table 1. Each isolate was assigned using a five-letter designation based on its reaction on the differential lines [17,18].

Data analysis

Survey data was arranged using three stage nested design with the model:

$$y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \gamma_{k(ij)} + \varepsilon_{l(ijk)}$$

Where: γijk is the wheat stem rust disease intensity whereas peasant association k is nested within district J nested within Zone i, μ is the overall mean, τi is the effect of the i^{th} zone, βj(i) is the effect of the j^{th} district within the i^{th} zone, and γk(ij) is the effect of the k^{th} peasant association within the j^{th} district and i^{th} zone, and εl(ijk) is the error term. Analysis of variance (ANOVA) was performed using SAS version 9.4 Software package (SAS, 2012). Means were separated using LSD test at the alpha level of 5%. The associations of disease incidence and severity with independent variables viz. altitude, variety and growth stage was computed by Pearson's correlation using SAS version 9.4 Software package. Each of the independent variables were tested with the incidence and severity of stem rust as the dependent variable. Linear regression analysis was done by plotting disease severity against altitude. Determination of regression intercept, slope and coefficient of determination were computed using SAS version 9.4 Software package. Race analysis data were analyzed by using Microsoft Spread Sheet software.

RESULTS AND DISCUSSION

Distribution and intensity of wheat stem rust on North and East Shoa Zones

A total 150 wheat fields were assessed in North (75 fields) and East Shoa (75 fields) zones during the 2019 cropping season. Wheat stem rust disease was prevalent all over surveyed districts of North and East Shoa zones. Out of 150 fields assessed, 110 (73.3%) of the fields were infected with wheat stem rust disease. The disease was observed in 71 (94.7%) and 52 (73.3%) of wheat fields in East and North Shoa zones, respectively. The highest (100%) disease prevalence was recorded in Akaki district followed by Adea, Gimbichu, Liben and Liben where the disease was equally (93.3%) prevalent (Table 2).

Disease incidence and severity were significantly different ($p < 0.0001$) between the two zones. The overall mean incidence and severity in the zones were 23.1 and 10.3, respectively. Mean disease incidence of 41.6 and 5.7 were recorded in East and North Shoa zones, respectively. Moreover, mean disease severity of 17.9 and 3.3 were recorded from East and North Shoa zones, respectively.

Wheat stem rust incidence was significantly different ($p < 0.0001$) across districts of North and East Shoa zones. The highest mean incidence was recorded in Liben and Lume districts 50.7 and 47.5, respectively with no significant difference between the two districts. Likewise, the highest mean severity (21.3 and 20.7) was recorded in Liben and Lume districts with a significant difference recoded between them. However, the lowest mean disease intensity (2.5, 2.5, 2.7, 3.3) and severity 1.5, 2, 2, 2.2) was recorded in Kimbibit, Abichuna Gna'a, Basona werana, Siyadebrina Wayu districts, respectively with no significant difference among the districts (Table 3). Furthermore, the highest range of wheat stem rust incidence was recorded in Lume, Adea, Liben and Akaki districts with incidence range of 0-100, 0-90, and 0-80, respectively. On the other side, the lowest incidence range of wheat stem rust disease was recorded in Siyadebrina Wayu and Abichuna Gna'a districts with a similar incidence range value of 0-20 followed by Kimbibit district with an incidence range of 0-30. Besides, the maximum wheat stem rust severity range (0-70S) was recorded in Kimbibit district followed by Liben district (0-60 MSS). This study revealed that there was high disease pressure in East Shoa than Northern Shoa. The higher disease intensity in East Shoa might be due to mid-altitude ranges (1593 m-2289 m) in East Shoa zone that is suitable for stem rust pathogen [12]. However, the higher altitudes range (2306 m-3034 m) and cold weather conditions in North shoa which was less favorable for the pathogen. Ismail, et al. [19] reported relatively lower wheat stem rust severity in high altitude areas of more than 2200 m.a.s.l. than lower altitude areas of less than 1900 m.a.s.l in North rift regions of Kenya. The variation in crop disease intensity between and within specific locations could be due to diversity in wheat variety grown, time of disease onset, the virulence of the pathogen and favorable environmental conditions [20].

Intensity of wheat stem rust across altitude ranges

The survey was carried out in an area with altitude range of (1593-3034 m.a.s.l) which fall under two altitude classes namely mid-altitude (1593 m-2289 m) and high altitudes (2306-3034 m.a.s.l) [21]. Wheat stem rust disease prevalence and intensity varied with altitude ranges. Accordingly, wheat stem rust was more prevalent in mid altitudes than higher altitudes. Out of 62 wheat fields assessed in the mid-altitude area, 58 (93.5%) fields where infected. But, Out of 88 wheat fields assessed from highland areas; 52 (59.1) fields where infected with wheat stem rust disease. In the same manner, wheat stem rust intensity was significantly different ($p < 0.0001$) between mid and high altitude ranges. The maximum mean disease incidence and severity of 44.4 and 18.7 were recorded in mid and high altitudes, respectively. Meanwhile, the lower mean disease incidence and severity of 8.9 and 4.9 were recorded in mid and high altitudes, respectively (Table 4).

This study showed that the higher the altitude the lower wheat stem rust prevalence and intensity, and vice versa. Wheat stem rust is quite important at low and mid altitudes (<2400 m). However, it could be important at higher altitudes on late sown and/or late-maturing wheat varieties, specially grown on vertisols [22].

Table 1: Nomenclature of *Puccinia graminis* f. sp. *tritici* based on 20 differential wheat host lines.

		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

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

[a]**Low** = Infection types 0, ;, 1, and 2 and combinations of these values.

[b]**High** = Infection types 3 and 4 and a combination of these values.

Table 2: Prevalence of wheat stem rust disease across districts of North and East Shoa Zones in 2019 main cropping season.

Zone	Districts	Number of fields assessed	Number of fields infected	Prevalence (%)
East Shoa	Adea	15	14	93.3
	Gimbichu	15	14	93.3
	Liben	15	14	93.3
	Lume	15	14	93.3
	Akaki	15	15	100
	Sub total	75	71	94.7
North Shoa	Siyadebrina Wayu	15	7	46.7
	Basoana Warana	15	5	33.3
	Kimbibit	15	8	53.3
	Abichuna Gna'a	15	8	53.3
	Aleltu	15	11	73.3
	Sub total	75	39	52
	Total/mean	150	110	73.3

Table 3: Mean incidence and severity of wheat stem rust across wheat producing districts of East and North shoa zones.

Zones	Districts	Disease Incidence		Disease Severity	
		Range	Mean	Range	Mean
East Shoa	Liben	0-80	50.7[a]	0-60MSS	21.3[a]
	Lume	0-100	47.5[a]	0-70S	20.7[a]
	Akaki	10-80	40[b]	5MS-70S	14.5[b]
	Adea	0-90	39.3[b]	0-40MS	16.9[b]
	Gimbichu	0-60	27.3[c]	0-70S	14.8[b]
North Shoa	Aleltu	0-60	14.6[d]	0-40MS	7.3[c]
	Siyadebrina Wayu	0-20	3.3[e]	0-20MS	2.2[d]
	Basona Werana	0-40	2.7[e]	0-40MS	2[d]
	Abichuna Gna'a	0-20	2.5[e]	0-20MSS	2[d]
	Kimbibit	0-30	2.5[e]	0-30MSS	1.5[d]
Overall mean			23.1		10.3
LSD (0.05)			6.3781		3.0433
CV %			26.02		28.01

Means with the same letter are not significantly different at p<0.05

Table 4: Prevalence and intensity of wheat stem rust across altitude ranges.

Altitude range	Class name	Number of fields inspected	Number of fields infected	Prevalence (%)	Mean Incidence	Mean Severity
1593 m-2289 m	Mid-altitude	62	58	93.5	44.4[a]	18.7[a]
2306 m-3034 m	High altitude	88	52	59.1	8.9[b]	4.9[b]
Total/overall mean		150	110	76.2	26.7	11.8
LSD (0.05)					2.718	1.2969
CV					27.8	29

Means with the same letter are not significantly different at p<0.05

The correlation analysis between altitude and incidence (r = -0.76) and severity (r= -0.7) of wheat stem rust disease were also highly significant (p < 0.001) and negative in the present study (Table 5). The same scenario was also reported by different authors. Abebe, et al. [23] and Hailu, et al. [7] reported higher wheat stem rust disease intensity at a lower altitude than at higher altitude ranges. Ismail, et al. [19] also reported a highly significant negative correlation between stem rust severity and altitude in Kenya. Various studies also showed that wheat stem rust disease is more important in low and mid-altitude areas than high land areas in Ethiopia. Hailu, et al. [7] reported that higher wheat stem rust disease intensity (incidence and severity) in low land (1500-200 m.a.s.l) and mid-altitude (2001-2500 m.a.s.l) than high land (2501-3560 m.a.s.l) areas of West and South West Shoa zones of Ethiopia. Likewise, Regasa et al. [24] reported higher wheat stem rust incidence and severity in the mid-attitude range of (1568 m-2300 m) than high altitude range (2301 m-3008 m) in the Southern Tigray region of Ethiopia.

A negative relationship was observed between stem rust disease severity and altitude ranges with regression analysis. As elevation increases (in meter), stem rust disease severity is reduced by 0.02 (Figure 2). The negative relationship between altitude and disease

severity implied that the disease was more important at lower and mid altitudes resulting in decreased intensity at higher altitudes [25].

Wheat stem rust prevalence and intensity across wheat varieties grown in the study area

A total of 9 wheat varieties (Utuba, Dashen, Kakaba, Mangudo, Kubsa, Kingbird, Hidase, Danda'a, Digelu and ET-13) and Unknown varieties were encountered during the survey. Wheat stem rust prevalence was varied among varieties. Accordingly wide range wheat stem rust sverity was observed on different varieties during the survey. The disease was 100% prevalent on varieties Utuba, Dashen, Mangudo, Kingbird and Digelu. Moreover, disease prevalences of 82.6%, 68.8%, 66.7%, 66.7% and 50% were recorded from Kakaba, Danda'a, Kubsa, Hidase and ET-13, respectively. The disease was 68% prevalent in unknown varieties grown in the study area (Table 6). Likewise, there was a significant difference in wheat stem rust disease incidence and severity among varieties grown in North and East Shoa zones. The highest mean disease incidence (70%) was recorded from Utuba variety. The second highest mean disease incidence (50%) was recorded on Dashen variety followed by Kakaba, Mangudo, Kubsa, and kingbird with a mean incidence value of 40%. However, the lowest mean disease incidence (7.5%) was recorded from a variety ET-13. The maximum mean disease severity (36%) was recorded from Utuba variety followed by Kubsa, Kakaba and Dashen with mean incidence values of 17.3, 16.6 and 16%, respectively. However, the lowest mean disease severity (2%) was recorded on ET-13 variety followed by Digelu, Danda'a, Hidase and Kingbird varieties with mean disesse severity value of 8, 9.4, 12.5 and 14%, respectively. The mean disease severity recorded from Unknown varieties was 11.1%. Wide ranges of recently released commercial wheat varieties released in Ethiopia are vulnerable to rust disease shortly after their release. This might be due to virulence variability in the pathogen population and deployment of major gene resistance in the majority of wheat cultivars [26].

Wheat stem rust prevalence and intensity by growth stage of wheat varieties grown in the study area.

Different growth stages (flowering to dough) were observed during the survey time. Out of 150 fields assessed, 73 fields were at dough stage, 40 at soft Dough stage, 32 at milk stage and 5 fields were at the flowering stage. Significantly different wheat stem rust intensity was recorded at different stages of the crop in wheat growing areas of North and East Shoa zones of Ethiopia in 2019 main cropping season. The highest mean disease incidence (35.7) was recorded at Dough growth stage followed by Soft Dough growth stage with a mean incidence value of (16.6). However, the lowest mean disease incidence (6) was scored at the flowering stage followed by milk stage with a mean disease incidence value of 10.6. In the same way, the maximum mean disease severity of 15.4 was recorded at Dough followed by and Soft Dough (8.3) growth stages. The lowest mean disease severities of 4.8 and 5.4 were scored at flowering and milk stage, respectively. However, there was no significant difference between the two growth stages (Table 7). On the other hand, the correlation analysis between growth stage and disease intensity indicated that there was a significant ($p < 0.001$) and positive correlation between the wheat growth stage and stem rust severity ($r = 0.43$) and incidence ($r = 0.49$). This showed that wheat stem rust intensity increased with increasing stage of crop from flowering to dough stage. Roelfs et al. [12] also stated that, the late

Table 5: Pearson's correlation coefficients between wheat stem rust intensity with altitude, growth stage and weed status.

Variables	DS	DI	ALT	GS
DS	1		-0.7***	0.43***
DI		1	-0.76***	0.49***
			1	-0.58***
				1

DS- Disease Severity; DI- Disease Incidence; ALT- Altitude; GS- Growth Stage;
*Significant level at p<0.05; **Significant level at p<0.01; ***Significant level at p<0.001

Table 6: Wheat stem rust prevalence and intensity across wheat varieties grown in the study area.

Variety	No. of fields inspected	No. of fields infected	Prevalence (%)	Mean incidence	Mean severity
Utuba	2	2	100	70^{a}	36^{a}
Dashen	1	1	100	50^{b}	16^{cb}
Kakaba	23	4	82.6	40.3^{b}	16.6^{b}
Mangudo	1	1	100	40^{b}	13.5^{bcd}
Kubsa	3	1	66.7	40^{b}	17.3^{b}
Kingbird	4	4	100	40^{b}	14^{bcd}
Hidase	6	4	66.7	21^{cd}	12.5^{bcd}
Unknown	25	17	68	24c	11.1^{bcd}
Danda'a	80	55	68.8	18.7^{cde}	9.4^{cd}
Digelu	3	3	100	11.7^{de}	8^{d}
ET-13	2	1	50	7.5^{e}	2^{e}
LSD (0.05)				11.59	5.59
CV				26.2	27.85

Means with the same letter are not significantly different at p<0.05

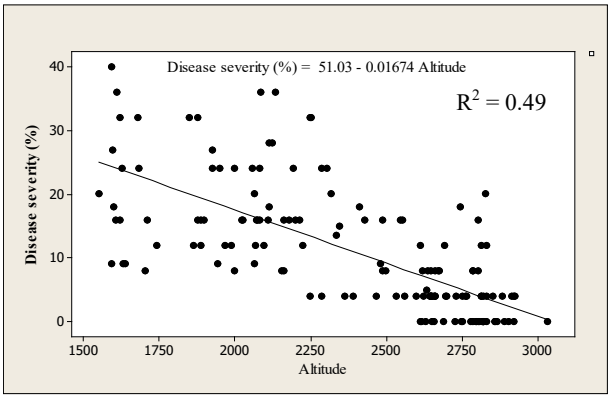

Figure 2: Regression relationship between wheat stem rust severity (%) and altitude.

growth stages of the crop are a crucial time for stem rust disease to reach its maximum severity level. Similarly, Regasa, et al. [24] also reported the positive correlation of wheat stem rust intensity and crop growth stage.

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

A total of 110 stem rust samples (39 from North and 71 from East Shoa zones) were collected in 2019 main cropping season. Of these, 83 live samples were analyzed following the procedure of Roelfs and Marthens to identify the physiological races of the stem rust pathogen. However, the remaining samples were not viable because no visible symptoms were produced after being inoculated

Table 7: Wheat stem rust intensity across wheat growth stages in North and East Shoa Zones, 2019.

Growth stage	Number of fields	Mean incidence	Mean severity
Flowering	5	6.7[c]	5.5[c]
Milk	32	10.6[c]	5.24[c]
Soft Dough	40	16.6[b]	8.33[b]
Dough	73	35.7[a]	15.4[a]
LSD (0.05)		4.64	2.13
CV		28.15	28.42

Means with the same letter are not significantly different at p<0.05

Table 8: Wheat stem rust races identified from different wheat varieties and their frequencies.

Zone	Varieties	Race	Number of samples	Frequency (%)
East Shoa	Danda'a	TKKTF	10	12.04
		TTTTF	3	3.6
		TKTTF	6	7.2
		TTKTF	2	2.4
	Kakaba	TKTTF	9	10.8
		TTTTF	2	2.4
		TKKTF	2	2.4
		TTKTT	1	1.2
	Mangudo	TKKTF	1	1.2
	Hidase	TKKTF	2	2.4
	Dashen	TTTTF	1	1.2
	Kubsa	TKKTF	3	3.6
		TTTTF	1	1.2
		TKTTF	1	1.2
	Utuba	TKKTF	2	2.4
	Unknown	TKKTF	7	8.4
		TTTTF	1	1.2
		TTKTF	2	2.4
		TKTTF	2	2.4
North Shoa	Danda'a	TKKTF	11	13.3
		TKTTF	6	7.2
		TTKTT	1	1.2
		TTTTT	1	1.2
	ET-13	TKTTF	1	1.2
	Digelu	TKKTF	1	1.2
	Hidase	TKTTF	1	1.2
	Unknown	TKTTF	1	1.2
		TKKTF	1	1.2
Total		**6**	**83**	

Table 9: Frequency of *pgt* races identified from samples collected from the study area.

Race	Identified from number of samples	Frequency (%)
TKTTF	28	33.7
TKKTF	40	48.2
TTTTF	8	9.6
TTKTT	2	2.4
TTKTF	4	4.8
TTTTT	1	1.2

Table 10: Virulence/Avirulence spectra of the *Pgt* races identified from North and East Shoa Zones.

Races	Virulence/ineffective Sr genes	Avirulence/effective Sr genes
TKTTF	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	11, 24, 31
TKKTF	5, 21, 9e, 7b, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	11, 36, 24, 31
TTTTF	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	24, 31
TTKTT	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Tmp, 24, 31, 38, McN	36
TTKTF	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	36, 24, 31
TTTTT	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 24, 31, 38, McN	-

Table 11: 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 (%)
Sr5	100	Sr30	100
Sr21	100	Sr17	100
Sr9e	100	Sr9a	100
Sr7b	100	Sr9d	100
Sr11	66.7	Sr10	100
Sr6	100	SrTmp	100
Sr8a	100	Sr24	33.3
Sr9g	100	Sr31	33.3
Sr36	50	Sr38	100
Sr9b	100	SrMcN	100

on susceptible wheat line (McNair). Six *pgt* races namely TKTTF, TTTTF, TKKTF, TTKTT, TTKTF and TTTTT were identified. TKKTF was the dominant race being identified from 40 (48.2%) samples followed by TKTTF (Digelu race) that was identified from 28 (33.7%) samples. However, TTTTT and TTKTT race were the least dominant races. They were identified from 1 (1.2%) and 2 (2.4%) samples, respectively (Table 8). TKKTF was reported in Germany and was among the races that caused an unusual wheat stem rust outbreak in 2013/14 cropping season [27]. TKTTF (Digelu race) was the dominant race in major wheat producing districts of East and North Shoa zones of Ethiopia.

TKTTF was first detected in 2012 in Southeastern parts of Ethiopia and caused wheat stem rust epidemics in 2013/14 cropping season by attacking the popular variety Digelu which was resistant to TTKSK (*Ug99* race) [6]. It was bad news for Ethiopian wheat growers because stem rust resistance gene *SrTmp* that is available in popular bread wheat variety (Digelu) was defeated by this virulent stem rust strain [28]. TTKTT is virulent on all resistance genes in differential lines except *Sr36*. The spatial distribution of the *pgt* races was different among the two zones. Five races including TKKTF, TTTTF, TTKTF, TKTTF and TTKTT were distributed in East Shoa zone. Lemma et al., [10] also reported a wider variability of *pgt* populations in central Ethiopia. They identified 16 races from samples collected from major wheat producing districts of East Shoa zone. Four races namely TKKTF, TKTTF, TTKTT and TTTTT races were distributed in North Shoa zone. Out of the six races identified in this study; TTTTT was detected only in North Shoa zone while TTKTF and TTTTF were identified only in East Shoa zone.

Wheat varieties are grown North and East Shoa zones were infected with one or more of *pgt* races. Danda'a variety was infected with four *pgt* races namely TKKTF, TTTTF, TKTTF and TTKTF in East Shoa zone. Similarly, TKKTF, TTTTF, TTKTT and TTTTT races were identified from this variety in North Shoa zone. Besides, Kubsa variety was infected with multiple races (TKKTF, TTTTF and TKTTF) in East Shoa zone. Similarly, Hidase was infected with TKKTF race in East Shoa zone and TKTTF in North Shoa zone. However, Mangudo variety was infected with a single race (TKKTF). TKKTF race was the most frequently appeared in North and East shoa zones being identified from 11 (13.3%) and 10 (12.04%) in East Shoa zones, respectively. The second most dominant race detected from Danda'a variety was TKTTF (Digelu race) which was identified from 6 (7.2%) in each zones. On the other hand, TTKTF and TTTTF races appeared less frequently on Danda'a variety in East Shoa zone being identified from 2 (2.4%) and 3 (3.6%) stem rust samples, respectively. The least frequent races on Danda'a variety were TTKTT and TTTTT each identified from 1 (1.2%) samples. Furthermore, Kakaba (bread wheat variety) was infected with four *pgt* races (TKTTF, TTTTF, TKKTF and TTKTT) in East Shoa zone. Among these races, TKTTF (Digelu race) had a

high frequency being identified from 9 (10.8%) samples. However, TTKTT, TKKTF and TTTTF were less frequent races on Kakaba variety. TTKTT was identified from 1 (1.2%) while TKKTF and TTTTF races were each identified from 2 (2.4%) samples (Table 8). TTKTT race was first reported in Ethiopia in 2018 from commercial wheat cultivars Shorima, Huluka, Ogolcho, Hidase, and Danda'a [29]. 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 [30]. TTTTT *pgt* race was identified from a sample collected from Danda'a (bread wheat variety) in North Shoa zone. TTTTF race was reported from samples collected in 2009 from the Eastern Shoa zone of central Ethiopia [10]. It was also detected in Iran in 2010 [31]. Moreover, TTTTF race caused a wheat stem rust outbreak in Italy hitting several thousands of hectares of durum wheat [32] (Table 9).

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 (2013-2014), Kazakhstan and South Siberia (2015-2016), outbreaks in Germany (2013), Italy (2016), and Sweden (2017) [33-35]. 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. The epidemic of stem rust has occurred in Ethiopia in 2013/14 on popular variety Digelu have caused up to 100% yield loss. It was caused by a new strain of the pathogen called TKTTF or Digelu race during the main cropping season [6].

Virulence spectrum of *Puccinia graminis* f.sp *tritici* isolates

Wheat stem rust races identified in the study area have a variable virulence spectrum on stem rust resistance genes. The majority of resistance genes in differential host lines (80%-100%) were defeated with stem rust races identified in the study area. Unusual virulence was noted by race TTTTT which defeated 100% Sr genes in differential lines. Likewise, 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. TTTTF was virulent on all Sr genes except *Sr24* and *Sr31*. Unlike to present study, Lemma et al., [10] reported that differential host line carrying *Sr24* was effective to all races identified in central Ethiopia. TKTTF and TKKTTF races have almost similar virulence pattern since both are virulent on Sr genes *Sr11*, *Sr24* and *Sr31*. TKKTF race have a similar virulence profile to race TKTTF. Nevertheless, low infection type was displayed on *Sr36* in a former race (Table 10).

The majority of resistance genes in differential host lines were ineffective against races identified in this study. Differential hosts carrying *Sr5, Sr21, Sr9e, Sr7b, Sr6, Sr8a, Sr9g, Sr9b, Sr30, Sr17, Sr9a, Sr9d, Sr10, SrTmp, Sr38* and *SrMcN* were ineffective to all races identified from stem rust samples collected in the season. *Sr11* and *Sr36* were ineffective to 66.7% and 50% of races, respectively. While *Sr24* and *Sr31* were ineffective to 33.3% of races identified from North and East Shoa zones of Ethiopia in 2019 main cropping season. The result of the present study is in agreement with previous findings. Admasu et al., [9] 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., [23] 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, various studies showed that virulence to *Sr6, Sr8b, Sr9a, Sr9d* and *Sr11* is common worldwide [12].

Stem rust resistance gene *Sr24* is effective against most races of *Puccinia graminis* f. sp*tritici* and is used widely in commercial wheat cultivars worldwide [18]. *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 [18]. Besides, virulence to this effective stem rust resistance gene had been detected in different countries including South Africa [36], Eritrea [37] and Ethiopia [29]. Durable Stem rust resistance gene *Sr31* since 1980 was overwhelmed due to a highly virulent race arisen in eastern Africa (Uganda) in 1999. The race was known as TTKSK or (Ug99) and is virulent to the majority of the world's wheat cultivars [38]. It has spread from Uganda throughout eastern Africa, Yemen, and Iran [39-41].

On the other hand, 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 (66.7%) races (TKTTF, TTTTF, TKKTF and TTKTF) except TTKTT and TTTTT. Likewise, the differential line that caries *Sr36* was effective to 3 (50%) of races identified in the study area. It was resistant against TKKTF, TTKTT and TTKTF. The lowest resistance spectra were recorded on the differential lines that caries *Sr11*. It was resistant to only 2 (33.3%) including TKTTF and TKKTF. Unlike to present study, Lemma et al. [10] reported that differential host line carrying *Sr24* was effective to all races identified in central Ethiopia. However, there was no effective *Sr* gene to all races identified in this study (Table 11). All/majority of stem rust resistance genes in differential host lines became susceptible to stem rust races identified in this study. Therefore, searching for novel sources of resistance is pertinent to develop durable rust-resistant wheat cultivars.

The detection of six 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 country [42]. Also, some pathotypes identified in this study have more virulence combinations than pre-existing races in the country. For instance, TTKTT and TTTTT races have 95% and 100% 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., [12] 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

Wheat is one of the major cereal crops with tremendous nutritional value and is one of the staple crops in many parts of the world. African countries are heavily dependent on imports to meet their food security. Ethiopia is a leading country in wheat production from sub-Saharan countries. Never the less, its production is con-

strained by many biotic and abiotic factors. Of these, wheat stem rust disease is the most important biotic constraint since a long time ago. Present study showed that wheat stem rust was prevalent all over surveyed districts and different level of disease intensity was notified. Disease incidence and severity were significantly different ($p < 0.0001$) between the two zones. Mean disease incidence of 41.6 and 5.7 were recorded in East and North Shoa zones, respectively. Moreover, mean disease severity of 17.9 and 3.3 were recorded from East and North Shoa zones, respectively. Moreover, wheat stem rust disease intensity was significantly varied among varieties grown, altitudes ranges and weed infestation levels. Eighty three stem rust isolates were analyzed using 20 standard differential lines. Six races namely TKTTF, TTTTF, TKKTF, TTKTT, TTKTF and TTTTT were identified. TKKTF was the dominant race being detected from 40 samples followed by TKTTF (Digelu race) which was identified from 28 samples. However, TTTTT and TTKTT races were the least dominant races. The detection of six races in the study area is an indicator of the great variability of *pgt* populations. In addition, some of pathotypes identified in this study have more virulence combinations than pre-existing races in the country. For instance, TTKTT and TTTTT races have 95% and 100% virulence spectra to stem rust resistance genes within differential lines. *Sr24* resistance gene that is available in most of the commercial varieties worldwide became ineffective to these races. Hence, it needs high emphasis to reduce the potential spread of this virulent *pgt* strains to another wheat producing regions of the country and beyond. On the other hand, stem rust resistance gene *Sr24* and *Sr31* were effective against majority of races identified in present study. Therefore, they can be used as source of resistance in breeding program. The rapid evolution of new races within the rust population is a bottleneck to rust management worldwide. Therefore, regular surveillance work and identification of physiological races is mandatory to know the importance of the disease and to monitor shift in virulence pattern.

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