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Distribution of Wheat Stem Rust *(Puccinia Graminis F. Sp. Tritici)* in West and Southwest Shewa Zones and Identification of its Phsiological Races

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Abstract

Stem rust (black rust) caused by Puccinia graminis f.sp.tritici is one of the most important air borne diseases of wheat (*Triticum aestivum*) in the central high lands of Ethiopia, including west and southwest Shewa zones. The pathogen is capable to produce new physiological races that attack resistant varieties and develop epidemic under favorable environmental conditions which results in a serious yield loss. However, information on the status of stem rust distribution and races in west and southwest Shewa zones is lacking. Therefore, the present studies were based on stem rust survey to compute the prevalence and intensity of disease; race analysis via inoculation of stem rust isolates and multiplication of single-pustule of the pathogen and race designation by inoculating on wheat differential lines. Eighty six wheat fields were assessed in 12 districts of west and south west Shewa zones with altitude ranges between1925-2915 m.a.s.l. Seventy five (87.2%) wheat fields infected with stem rust had the overall mean of 33% incidence and 10.8% severity. The mean prevalence of stem rust was 96.3% in southwest and 83.1% in west Shewa zones, whereas, the mean incidence was 34.7% and 31.2% in west and southwest Shewa zones, respectively. Similarly, mean severity was 14.5% in west and 7.1% in southwest Shewa zones. Forty five stem rust samples collected during the survey were analyzed on the twenty standard stem rust differentials and resulted in identification of 5 races (TTTTH, TTKSK, TKTTF, HKPPF & HKNTF). Of these, 88.4% of the isolates were TKTTF (Digalu race) followed by 4.7% of the isolates by TTKSK (Ug99). Among the five races, the most virulent, which made 18 *Sr* genes non-effective was TTTTH. TKTTF and TTKSK races were virulent on 85% of *Sr* genes. Differential host carrying *Sr*24 was an effective gene which confers resistance to all of the races identified in the area. On the other hand, the wheat differential hosts carrying the resistance genes *Sr* McN, *Sr*10, *Sr*9a, *Sr*30, *Sr*9g, *Sr*8a, *Sr*6, *Sr*7b and *Sr*21 were ineffective to 100% of the isolates tested. Hence, the *Sr* resistance gene *Sr*24 can be used as sources of resistance in wheat breeding program.

Keywords: Wheat stem rust; Race; *Puccinia graminis f.sp.tritici*; *Sr* genes; Disease prevalence; Disease severity; Disease incidence

Introduction

Ethiopia is the largest wheat producer in sub-Saharan Africa [1].West and southwest Shewa zones are among the major wheat producing areas in Oromia region [2]. Wheat is the staple food for 4.5 billion people in the world [3]. Its popularity comes from the versatility of its use in the production of a wide range of food products, such as "Injera", breads, cakes, Pastas, cookies, etc .,[4].

Although the productivity of wheat has increased in the last few years in Ethiopia, it is still very low as compared to other wheat producing countries. The national average productivity is estimated to 2.4 tons/ha [2], which is by far below the world's average of 3.3 tons/ha [5]. The low productivity is attributed to a number of factors including: Biotic (Diseases, insect pests, and weeds), abiotic (moisture, soil fertility, etc.,) [6]. Among biotic factors, rusts are the most important diseases of wheat, cause up to 60% loss of wheat yield for leaf or stripe (yellow) rust and 100% loss for stem rust [7].Wheat and rusts have co-evolved for thousand years and resulted in the accumulation of wide spectrum of the pathogens in Ethiopia [8].

However, Stem rust or black rust (caused by *Puccinia graminis* f. sp. *tritici*) is a serious wheat disease causing a decrease of wheat production in many areas of the world [9]. Yield loss due to stem rust in Ethiopia was estimated to reach up to 100% on susceptible wheat varieties at times of disease epidemics [10].According to Leppik [11] and Singh et al. [12] the highland of Ethiopia is considered as a hot spot for the development of stem rust races diversity.

In a study conducted in Germany, Admassu et al. [13] reported 22 stem rust races from 152 collections made in Ethiopia in 2006. Similarly, due to lack of infrastructure, race analyses of stem rust samples collected in Ethiopia 56 was done in St. Paul and Winnipeg. Surveys made from 1996 to 2005 in Bale indicated that stem rust was the most damaging to the crop with severity levels of 40% in 'Genna' and 90% in 'Bona' [14]. Similarly, Wheat stem rust disease was recorded with 44.1% prevalence, 19.2% incidence and 11.3% of severity in west and southwest Shewa zones in 2008 cropping season [15]. Moreover, due to sudden changes in stem rust race patterns, commercial varieties tend to become vulnerable. Hence, detailed information on the wheat stem rust status and physiological race variability have been essential in the west and southwest Shewa zones of Oromia region.

Materials and Methods

Description of the study area

The wheat stem rust survey was carried out in West and Southwest

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Shewa zones of Oromia Regional State in Ethiopia. West Shewa zone is located at 8°57´N latitude and 38°07´ E longitude and within elevation ranges between 1380-3300 m.a.s.l. Annual mean maximum and minimum rain fall is 1900 mm and 600 mm, respectively. The mean minimum and maximum air temperature of the area is 11.7°C and 25.4°C, in that order. Southwest Shewa zone is located at 8°16-9° 56´ N latitude and 37° 05´-38° 46´ E longitude and altitude ranging from 1600-3576 m.a.s.l. It receives annual rainfall ranging from 900 -1900 mm. The mean minimum and maximum air temperature of the area is 10°C and 35°C, respectively. Stem rust race analysis was done in Ambo Plant Protection Research Center (APPRC). It is located at 08⁰ 96' 885" N latitude and 37⁰ 85' 923" E longitude and at an altitude of 2147 m.as.l. The annual average temperature and rain fall is 27.54⁰C and 1077.68 mm, respectively.

Wheat stem rust field survey

A total of twelve districts that included seven from West Shewa zone (Ambo, Dendi, Chelia, Tokaye Kutaye, Dire Inchine, Dawo, Ejere) and five from Southwest Shewa zone (Woliso, Suden Sodo, Bechio, Amaya and Wonchi) were surveyed. The districts were selected based on wheat area coverage and followed systematic sampling every 5-10 km intervals. The survey was conducted following main and feeder roads on pre-planned routes in areas where wheat is predominantly grown. Stem rust assessment was made once at the vital growth stage of the crop per field, along the two diagonals (in an "X" pattern) of the field at five points using 0.5 m × 0.5 m (0.25 m^2) quadrant. In each field, wheat plants within the quadrant were counted and recorded as diseased/infected and healthy/non-infected and intensity of stem rust was calculated. 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 and recorded the average incidence.

$$\textbf{Plant disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total Number of plants in quadrant}} \times 100$$

The disease severity under field condition was recorded as percentage of leaf/stem area covered by rust disease followed modified Cobb's scale as developed by Peterson et al.[16] According to this scale, at 100% disease severity, the actual leaf/stem area covered by rust pustules is 37%. Disease severity was assessed by selecting 10 plants from a single quadrant and five quadrants were used for the estimation of disease severity from a single wheat field.

$$\textbf{Disease severity (\%)} = \frac{\text{Area of plant tissue affected}}{\text{Total area}} \times 100$$

The prevalence of rust disease was measured by using the number of fields affected divided by total number of fields and expressed in percentage. It is calculated as:

$$\textbf{Disease prevalence} (\%) = \frac{\text{No. of infected fields}}{\text{Total number of fields assessed}} \times 100$$

In addition, data on geographical information (latitude, longitude and elevation) of each field was recorded using GPS (e Trex Legend GPS system, Garmin). Crop growth stage was assessed based on the decimalized key developed by Zadoks et al. [17].

Collection of stem rust samples

Stems and/or leaf parts of wheat plants infected with stem rust were cut in to small pieces of 5-10 cm using scissors and put in paper bags after the leaf sheath was separated from the stem in order to keep stem and/or leaf sheath dry. The samples collected in the paper bags were tagged with the name of the Zone, district, variety and date of collection. The samples within the paper bags were air dried and kept in refrigerator at 4°C for race analysis purpose in the greenhouse until the survey in all districts between zones completed. A total of 45 stem rust samples (27 and 18 from West and Southwest Shewa zones of Oromia regions, respectively) were collected.

Isolation and multiplication of single-pustules

The inoculum was multiplied and maintained on standard rust susceptible variety" McNair " which does not carry stem rust resistant genes [18]. Five seedlings of this variety for each samples were raised in suitable 8 cm diameter clay pots that was filled with a mixture of steam sterilized soil, sand and manure in the ratio of 2:1:1, respectively. Seven-day old seedlings or when the primary leaves were fully expanded and the second leaves beginning to grow, the leaves were rubbed gently with clean (disinfected with 97% alcohol) moistened (with distilled water) fingers.

Green house inoculations were carried out using the methods and procedures developed by Stakman et al. [19]. Uredio spores of the stem rust were collected from the diseased wheat parts by using motorized spore collector in a capsule container and diluted by using lightweight mineral oil (SolTrol 130) chemicals and then [20] to make rust uredial spore more uniform. These were sprayed on to the seedlings of Mc Nair from a distance with clean motorized stem rust inoculator. For incubation, inoculated plants were moistened with fine droplets of distilled water by using atomizer after twenty minutes of inoculation and placed in dew chamber for 18 hr dark period at 18-22⁰C followed by exposure to light at least for 4 hr to provide favorable condition for stem rust infection. Seedlings were allowed to dry/remove their dew/moisture for about 3-4 hr. Following this, the seedlings were transferred from dew chamber to glass compartments in the green house where conditions were regulated at 12 hr photoperiod, at temperature range of 18-25⁰C and RH of 60-70%.

After seven to ten days of inoculation (when the flecks/symptoms was clearly visible) leaves containing single fleck that produce single pustule was selected from the base of the leaves and the remaining seedlings within the pots were eliminated using hand scissors. Only 2-3 leaves which contain single pustule were left and each of them was covered with cellophane bag (145 × 235 mm) and tied up at the base with a rubber band to avoid cross contamination [21].

After two weeks of inoculation (when the monopustule was well developed) each monopustule was sucked using electric power operated machine (vacuum pump) and collected in capsule container separately. A suspension, prepared by mixing urediospores of the monopustule in lightweight mineral oil, was inoculated on seven-day-old seedlings of the susceptible variety 'McNair' for multiplication purpose on the separate pots. Soon after inoculation, the seedlings were placed in a humid chamber in dark condition and transferred to a green house following the earlier mentioned procedure.

After inoculation of 15 days, the spores of each monopustule/isolate were collected in separate test tubes and stored at 4°C until they were inoculated on the standard differential lines. This procedure was repeated till sufficient amount of spores are produced in order to inoculate the stem rust differential lines. By following this procedure a total of 43 monopustules/isolates were developed from 45 wheat stem rust samples. A schematic overview of the general protocol used for race analysis in the greenhouse has been given in appendix (Figure 1).

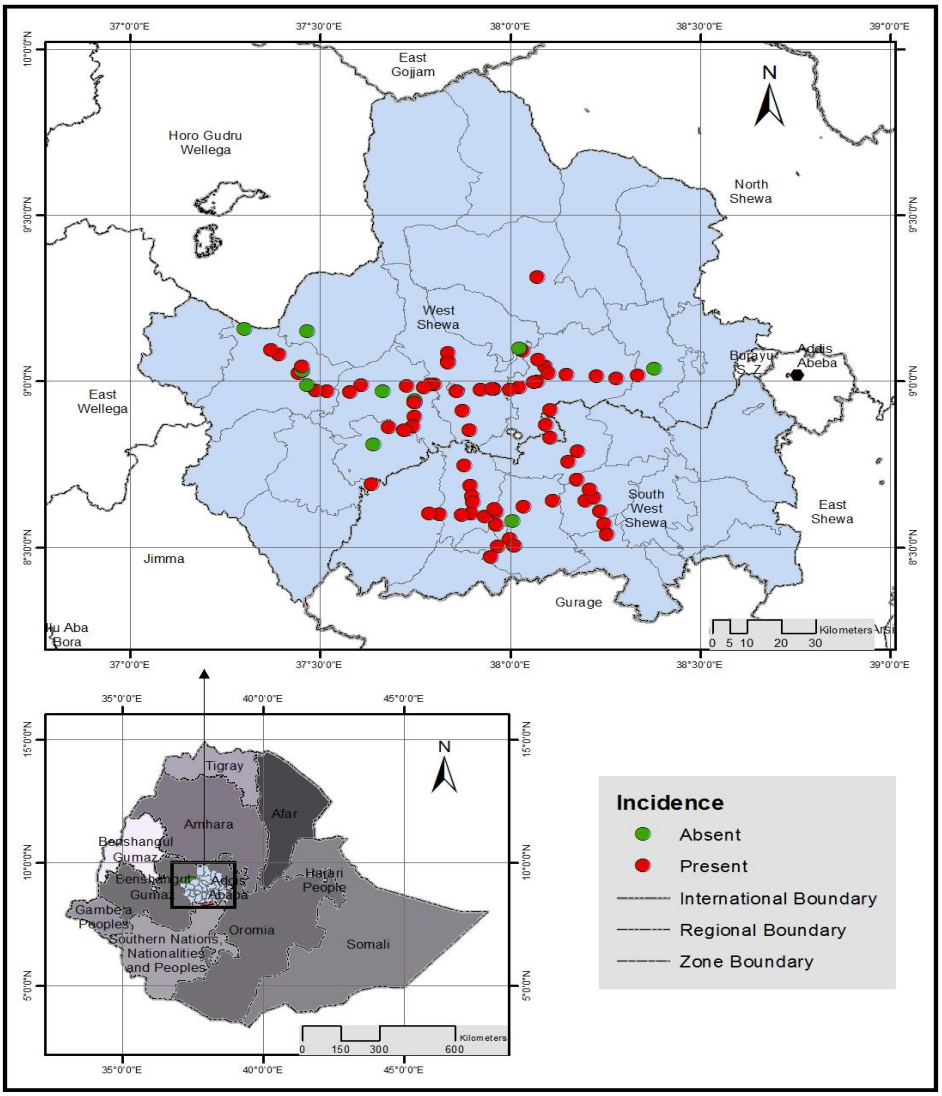

Figure 1: Map showing wheat stem rust survey areas in west and southwest Shewa zones of Oromia region in 2014.

Inoculation of wheat stem rust isolates on the differential lines

Five seeds each of the 20 stem rust differential lines including the susceptible variety (Table 9) were grown in 3 cm diameter pots separately in the growth chamber. The Susceptible variety was used to determine the viability of spores inoculated on the differential hosts and as a check. The single pustule spores/ isolate/ mixed with lightweight mineral oil (approximately 4 mg of spores per 1 ml) was sprayed/inoculated on to seven-day-old seedlings. Similar methods of inoculation, incubation and green house condition were applied as mentioned in section 2.4. Natural day light was supplemented with additional 4 hr/day that emitted by cool white fluorescent tubes arranged directly above plants in the green house.

Stem rust infection types were scored 14 days after inoculation using the 0-4 scale (Table 1) of Stakman et al. [19]. Infection types were grouped in to two, where, Low (resistance) = incompatibility (infection phenotype 0, 0; (fleck), 1, 2, and 2^+) and High (Susceptible) = compatibility (infection phenotype, 3^-, 3^+ & 4).

Designation of races

Race designation was done by grouping the 20 differential lines in to five subsets in the following order (Table 2).

Each isolates was assigned a five letter race code based on its reaction on the differential lines [21]. For example, low infection types on the four lines in a set is assigned with the letter 'B' while high infection types on the four lines is assigned with letter 'T'. Hence, if an isolate produces low infection type (resistant reaction) on the 20 differential lines, the race will be designated with a five letter race code 'BBBBB'. Similarly, an isolate which produces a high infection type (susceptible reaction) on the 20 wheat differential lines will have a race code 'TTTTT'. If an isolate produces a low infection type on *Sr11, Sr24*, and *Sr31*, but a high infection type on the remaining 17 differential lines, the race will be designated as TKTTF (Table 2). The experiment was repeated once, and only differential lines that produced similar infection types in the two experiments were considered for the data analysis. When there was infection type 0 (immune reaction), the test was done again to exclude the possibility of disease escape.

Class	IT	Description of symptoms
Immune	0	No sign of infection on the naked eye
Very Resistant	0	No uredia, but distinct flakes of varying size, usually a chlorotic yellow but occasionally necrotic
Resistant	1	Small uredia surrounded by yellow chlorotic and necrotic area.
Moderately Resistant	2	Small to medium sized uredia, typically in a dark green island surrounded by a chlorotic area
Mesothentic/ Heterogeneous	x	A range of infection type from resistant to susceptible scattered randomly on a single leaf caused by a single isolate not mixture
Moderately Susceptible	3	Medium sized Uredia. Usually surrounded by a light green chlorotic
Susceptible	4	Large uredia with a limited amount of chlorosis: may be diamond shaped
Modified characters		
Lower uredia	=	Uredia much smaller than typical and at the lower limit of the infection type
Small Uredinia	_	Uredia smaller than normal
Large Uredinia	+	Uredia larger than normal
Largest Uredinia	++	Uredia much larger than typical and at the upper limit for the infection type

IT=infection type.

Table 1: Description of infection types used in classifying the reactions of stem rust on leaves of wheat seedlings.

Infection phenotype of pathogen and wheat *Pgt* gene					
Pgt-code	Set1	5	21	9e	7b
	Set2	11	6	8a	9g
	Set3	36	9b	30	17
	Set4	9a	9d	10	Tmp
	Set5	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

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

Table 2: Code for the 20 differential lines for *P. graminis* f.sp. *tritici* in ordered sets of five.

Data analysis

Survey data (prevalence, incidence and severity) were analyzed by using the descriptive statistical analysis (means) over districts, varieties, altitude range and crop growth stages. Similarly, race analysis was analyzed using the descriptive statistics.

Result and Discussion

Survey of wheat stem rust in west and southwest shewa zones of oromia region

Survey of wheat stem rust was carried out in west and southwest Shewa zones in October, 2014. A total of 86 wheat fields were surveyed mainly for assessment of wheat stem rust intensity. During the surveys, the crop was at flowering to hard dough growth stages (Table 3). From 86 fields inspected, 21 (24.4%), 42 (48.8%), 7 (8.1%), 4 (4.7%) and 12 (14%) of wheat fields were at flowering, milk, soft dough, dough and hard dough stages, respectively. In the same order, stem rust was observed in 17 (81%), 38 (90.5%),7(100%), 4 (100%) and 9 (75%) of 21, 42, 7, 4 and 12 wheat fields inspected in the mentioned growth stages. Thirteen wheat varieties were grown by farmers such as Digelu, Kakaba, Danda'a, Kubsa, ET-13A2, Shorima, Kulutu, Kilinto, Roma awn less, Hidasie, Bedu Gela, Gisoo, and Chofero (Table 3). Out of 86 inspected wheat fields, 48 (55.8%), 14 (16.3%), 7 (8.1%) and 5 (5.8%) fields were sown by Digalu, Kakaba, Danda'a and Kubsa, respectively. ET-13A2, Shorima and Kulutu were sown in two fields (2.3%) each. Similarly, six varieties (Roma awn less, Hidasie, Bedu Gela, Gisoo, and Chofero) were planted with 1 (1.2%) of assessed fields for each. Thirteen wheat varieties have been grown in west Shewa zone whereas only 3 varieties (Digelu, Kakaba, Kubsa) were sown in southwest Shewa zone. Disease survey was carried out at altitude ranges of 1925-2915 m.a.s.l in west and 1935-2859 m.a.s.l in southwest Shewa zones.

Intensity of stem rust across locations: Of the 86 wheat fields assessed in the two zones, 87.2% were infected by the stem rust disease (Figure 1). The mean field prevalence of stem rust was 96.3% in southwest and 83.1% in west Shewa zones (Table 4). Whereas, the mean incidence was 34.7% and 31.2% in west and southwest Shewa zones, respectively. Similarly, mean severity was 14.5% in west and 7.1% in southwest Shewa zones. The assessed wheat fields showed susceptible (S), moderately susceptible (MS) and resistance (R) types of responses to stem rust infection. Hundred percent stem rust prevalence was recorded from 7 districts i.e., 4 districts from southwest and 3 from west Shewa zones. The least field prevalence was observed in west Shewa zone from Ejere (50%) and Chelia (54.5%) districts, respectively (Table 4).The mean incidence of stem rust in the areas varied between 1.4% in Chelia to 78% in Bechio districts (Table 4).

The overall mean incidence of wheat stem rust in both zones was 33%. The highest stem rust incidences (100%) were recorded in Ambo (Senkale locality), Bechio (Soyoma Guenji), Dawo (Uluma Busa, Girmi), Dendi (Cherto Kogn, jemjem lagabatu, Arera Kurae, Degawuchi), Ejere (Temoye, Kalana Imbortu) , and Tokaye Kutaye (Birbisana Duguma), while the lowest (zero) were recorded in Woliso (Obi,), Ejere (Chere, Tosegne gefere) , Tokaye Kutaye (Kele Boredu, Birbsana duguma), Chelia (Wegdi Kortu, Chobi tulu ,Tulu goseru,Mida kegn) and Dire Inchine (Woledo Hign) Districts.

The mean severity of stem rust ranged from 0.9% in Chelia to 35% in Dawo district. The overall mean severity of the disease was 10.8%. A maximum disease severity of 80% was recorded in Dendi (jemjem lagabatu locality) followed by Tokay Kutaye (Birbisana Duguma & Koleba) and Dawo (Girmi) districts with 60% of each. In general, most of the assessed wheat fields lied between 1 to 20% for stem rust severity (Figure 2).

Intensity of stem rust in different altitude ranges: The survey was conducted in the altitude ranges between 1925-2915 m.a.s.l. Based on CSA [22] altitude agro-ecology classification, out of 86 wheat fields

observed, 5 (5.8%), 69 (80.2%) and 12 (14%) fields were found at low-altitude (1500-2000), mid-altitude (2001-2500) and high-altitude (2501-3560 m.a.sl.), respectively. Of the 5 wheat fields inspected in the altitude ranges between 1500-2000 m.a.s.l, stem rust was observed in 5 (100%) wheat fields which had 30.8% mean incidence and 9.6% mean severity. Of the 69 wheat fields surveyed in the elevation that ranges between 2001-2500 m.a.s.l, black rust was recorded in 62 (89.9%) fields, with mean incidence and severity of 36.3% and 13.5%, following the same order mentioned. Similarly, 66.7% of stem rust disease prevalence was recorded at high-altitude which had 3.4% mean incidence and 1.5% mean severity. The survey result indicated that, mean incidence and severity increased from low-altitude to mid-altitude and decreased at high altitude (Table 5). Maximum stem rust disease severity (80%) was recorded at mid-altitude followed by 40% at low-altitude. Similarly, maximum stem rust incidence (100%) was recorded at mid and low-altitude. The highest level of stem rust infection has been cited in literatures in the altitude ranges of 1600 and 2500 m.a.s.l. Ayele et al [23]. Abebe et al. [24] showed that stem rust occurred in the altitude ranges of 1494-1800 m.a.s.l in southern Tigray. Dagnatchew [25] also mentioned as stem rust of wheat disease was very important at altitude below 2300 m.a.s.l. In Kenya, stem rust had been recorded and known to occur mainly in the low altitude areas of 1800 m.a.s.l [26] Even though stem rust has been seemed more important at mid and low-altitude, it also occurred at higher elevation as shown below in the data. This indicates, wheat stem rust has been extensive in the wide altitude ranges through times; and this might be due to climate change, widely cultivation of susceptible varieties and appearance of new races. Hence, wheat stem rust survey could be carried out in the wide altitude ranges in order to know disease distribution and race variability before going to out of control.

Intensity of stem rust in different wheat varieties grown in the surveyed areas: Of the 86 assessed wheat fields, only six (7%) fields were covered by five different local varieties and the remaining 80 (93%) were covered by eight different released varieties. The local varieties such as Bedu Gela and Chofero have shown resistance response to stem rust infection during survey in west Shewa zone. The absence of stem rust in local varieties may probably be due to their relative resistance and/or may be cultivated at a relatively higher altitude (≥ 2810 m.a.s.l) (Table 6), where stem rust disease is not a threat to

Zone	District	No. of fields observed	Varieties	Altitude range (m.a.s.l.)	Growth stage
SWS	Wonchi	7	Digelu, Kakaba, Kubsa	2079-2859	FS-MS
	Amaya	2	Digelu,	2009, 2038	MS
	Woliso	11	Kubsa, Digelu, Kakaba	1935-2353	FS-HDS
	Bechio	5	Digelu, Kakaba	2172-2223	MS-HDS
	Suden Sodo	2	Digelu	2268,2360	MS-HDS
WS	Ambo	12	Digelu, Danda'a, Kakaba, Kubsa, Kilito,ET-13A2	1925- 2904	FS-HDS
	Dawo	3	Digelu,	2173-2399	FS-MS
	Dendi	12	Roma, Digelu, Kakaba, Kubsa	2172-2773	FS-HDS
	Ejere	4	Kakaba, Digelu, Bedu Gela	2149-2915	FS-DS
	Tokaye Kutaye	13	Danda'a, Kakaba, ET-13A2, Digelu, Gisoo,	1949-2399	MS-HDS
	Chelia	11	Digelu, Kakaba, Hidasie, Shorima, Kulutu, Chofero,	2261-2891	FS-MS
	Dire Inchine	4	Digelu, Shorima, Kakaba,	2373-2462	FS-MS
Total		86		1925-2915	FS-HDS

FS: Flowering stage; MS: Milk stage; DS: Dough stage; HDS: Hard dough stage;
SWS: South west Shewa; WS: West Shewa

Table 3: Number of fields, varieties, altitude ranges and growth stages of wheat by zones and districts, 2014.

Zone	District	No. of fields inspected	Prevalence (%)	Incidence (%)		Severity (%)		Host response
				Range	Mean	Range	mean	
SWS	Wenchi	7	100	1-20	6.3	1-5	2.3	MS
	Amaya	2	100	2,10	6	2,5	3.5	MS
	Woliso	11	90.9	0-30	5.8	0-10	2.3	MS-R
	Bechio	5	100	50-100	**78**	10-30	20	MS
	Suden Sodo	2	100	60,60	60	5,10	7.5	MS
Subtotal/ mean		27	96.3	0-100	31.2	0-30	7.1	MS-R
WS	Ambo	12	100	1-100	33.3	1-40	12.7	MS-R-S
	Dendi	12	100	1-100	54.3	1-80	20.2	MS-S
	Ejere	4	50	0-100	50	0-50	20	MS-R-S
	Dawo	3	100	30-100	76.7	5-60	35	MS-S
	Tokaye kutaye	13	84.6	0-100	22.2	0-60	10.5	MS-R-S
	Chelia	11	54.5	0-10	**1.4**	0-5	0.9	MS-R
	Dire Inchine	4	75	0-15	5.3	0-5	2	MS-R
Subtotal/ mean		59	83.1%	0-100	34.7	0-80	14.5	MS-R-S
Grand total/Mean		86	87.2	0-100	33	0-80	10.8	MS-R-S

MS: Moderately Susceptible; R: Resistance; S: Susceptible; SWS: South west Shewa; WS: West Shewa.

Table 4: Intensity of wheat stem rust in 12 districts of west and southwest Shewa zones, Oromia region in 2014.

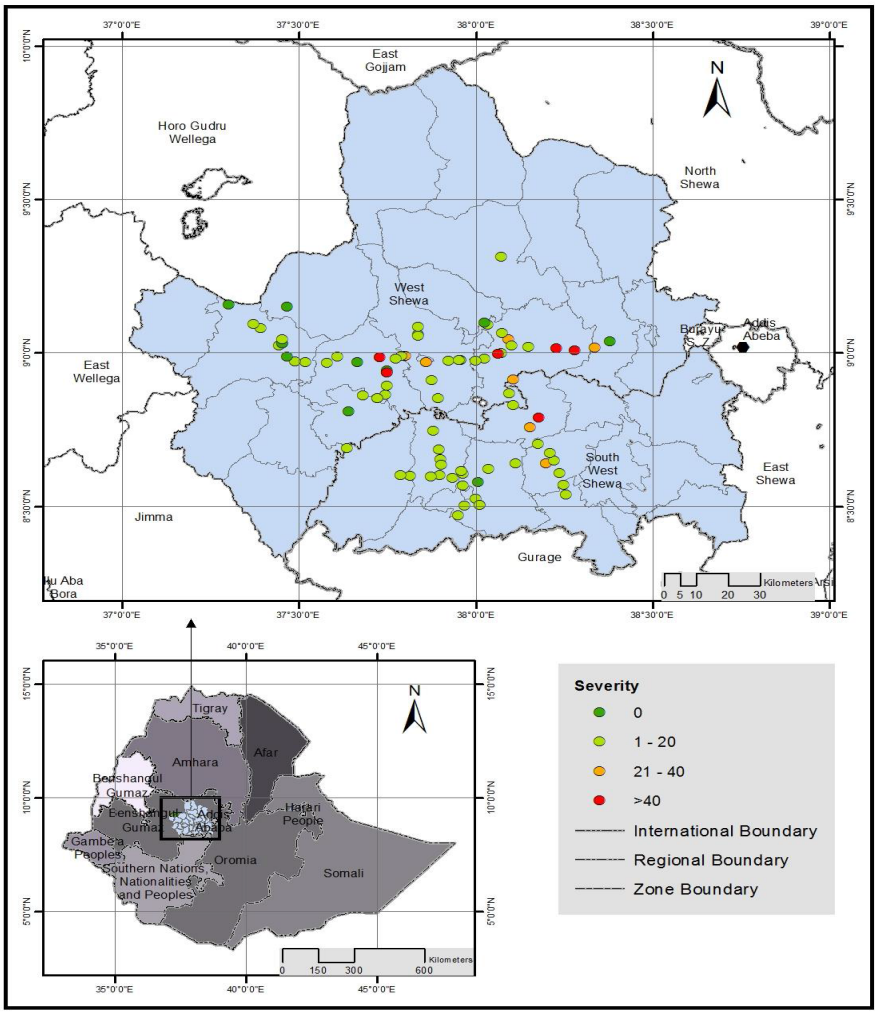

Figure 2: Map showing stem rust severity in west and southwest Shewa zones of Oromia region in 2014.

Altituderange (m.a.s.l)	Class Name (Traditional)	No. of fields inspected	Prevalence		Incidence (%)		Severity (%)	
			No	%	Range	Mean	Range	Mean
1500-2000	Low- altitude	5	5	100	1-100	30.8	1-40	9.6
2001-2500	Mid-altitude	69	62	89.9	0-100	36.3	0-80	13.5
2501-3560	High-altitude	12	8	66.7	0-20	3.4	0-5	1.5

Table 5: Intensity of stem rust based on different altitude ranges.

wheat crop [25] The most widely grown wheat variety was Digalu and it covered 55.8% of surveyed wheat fields in west and south west Shewa zones, Oromia region with 1 to 80% ranges of stem rust severity (Table 6). It showed susceptible to moderately susceptible reactions with 39.2% mean incidence and 14.5 mean severity. The second commonly grown variety Kakaba was also infected with stem rust at different intensity levels and its coverage was 16.3% surveyed wheat fields in both Zones. This variety showed moderately susceptible to resistance stem rust reaction with mean incidence and severity of 12.5% and 3.4%, respectively. Variety Danda'a was the third widely grown (8.1% wheat fields) in west Shewa zone only and it also showed similar field response as Kakaba for stem rust disease with 12.7% mean incidence and 2.1% mean severity. Hidasie variety was released in 2012 by KARC/EIAR and was not widely cultivated in the assessed areas except one field in Chelia district. This variety has shown resistance response for stem rust disease in west Shewa zone during surveying time. Most improved wheat varieties have shown moderately susceptible type of reaction to wheat stem rust disease in surveyed areas in 2014 main crop growing season.

Of the 48 inspected Digalu variety fields, 100% stem rust incidence was recorded in 12 (25%) fields. Similarly, the highest disease severity of 80% was recorded on Digalu variety followed by Kilinto with 40%. From eight improved wheat varieties in the assessed areas, stem rust disease was observed on 7 (87.5%); and of the five local varieties, stem rust appeared on 3(60%) varieties. Likewise, Out of 75 (87.2) infected wheat fields, 72 (83.7%) stem rust disease prevalence was recorded on the improved wheat varieties whereas 3 (3.5%) recorded on the local varieties.

In general, the survey result indicated that the intensity of stem rust varied across locations, elevation, varieties, growth stage. In addition, out of 75 (87.2%) stem rust disease infected wheat fields, only 2 (2.7%) wheat fields were sprayed with fungicide (Tilt 250EC). This low percentage use of fungicide by farmers was due to lack of awareness, unaffordable price of the fungicide and low technical support from agricultural experts according to farmers.

Physiological races and virulence diversity of stem rust on wheat in west and southwest zones

Race analysis is done based on the reaction of differential lines which contain 20 monogenic resistance genes. These genes are race specific and they show different response for various race groups. Race analysis provided essential information in determining the range of pathogenic variation in a specific region, screening for resistance in varieties, confirming that host responses are due to race changes, understanding the mechanism of variation as well as in determining the direction of research and breeding programs before the pathogen became a threat to wheat crop production in a specific region (District).

In this study, of the total 45 stem rust samples, 43 from farmer's fields and 2 from experimental plot of Ambo Plant Protection Research Center (APPRC) were collected. Of these, 2 samples from west Shewa zone did not yield viable spores at the time of inoculation on the susceptible check McNair701 in the green house. Forty-three viable isolates were identified and further multiplied on differential line for final race analysis.

Virulence and physiological race composition of wheat stem rust: Of the 43 isolates tested, 5 races were identified from west and southwest Shewa zones. The result showed that, most of the isolates collected from different wheat fields belonged to the same race group, except Ambo and Woliso districts .Three races namely TKTTF, TTKSK and TTTTH were identified from west Shewa zone. Similarly, 4 races (TKTTF, HKPPF,TTKSK and HKNTF) were identified from southwest Shewa zone. TKTTF is common race and detected from all districts of the two zones (Table 7). It was identified from 38 isolates while 4 (TTKSK, TTTTH, HKPPF, and HKNTF) races were identified only from 5 isolates from those particular districts (Ambo and Woliso). Four races were identified from Woliso followed by Ambo district (3 races). Among the identified races, 4 races such as TKTTF, TTTTH, HKPPF and HKNTF were identified for the first time in the sampling zones. The most important race TTKSK (Ug99) was isolated from two fields grown with ET-13A2 in Ambo Plant protection research center, on station experimental plots and Kakaba in Woliso district. Out of 43 viable stem rust collected wheat fields, 88.4% fields were infected

Zone	Variety	Elevation Range (m.a.s.l.)	No. Of fields inspected	Prevalence (%)	Incidence (%)		Severity (%)		Variety Responses
					Range	Mean	Range	Mean	
W & SW Shewa	Digelu	1925-2904	48	100	1-100	39.2	1-80	14.5	MS-S
W & SW Shewa	Kakaba	2059-2575	14	71.4	0-50	12.5	0-20	3.4	R-MS
W. Shewa	Danda'a	1949-2460	7	85.7	0-50	12.7	0-5	2.1	R-MS
W & SW Shewa	Kubsa	1935-2291	5	100	1-10	3	1-5	2	MS
W. Shewa	ET-13A2	2147, 2373	2	50	0,60	30	0, 30	15	R-S
W. Shewa	Shorima	2434, 2495	2	50	0, 1	0.5	0, 1	0.5	R-MS
W. Shewa	Kulutu	2821, 2891	2	50	0, 1	0.5	0, 1	0.5	R-MS
W. Shewa	Roma	2588	1	100	20	20	5	5	MS
W. Shewa	Bedu gela	2915	1	0	0	0	0	0	R
W. Shewa	Hidasie	2493	1	0	0	0	0	0	R
W. Shewa	Chofero	2810	1	0	0	0	0	0	R
W. Shewa	Gisoo	2202	1	100	1	1	1	1	MS
W. Shewa	Kilinto	2154	1	100	90	90	40	40	S

Table 6: Stem rust intensity in different wheat varieties grown in west and southwest Shewa zones of Oromia region in 2014.

Zone	District	Race	No.Of isolates	Altitude (masl)	Variety
WS	Ambo	TKTTF	7	1925-2460	Danda'a, Kakaba, Digalu, Kilinto
		TTKSK	1	2147	ET-13A2
		TTTTH	1	2154	Kilinto
	Dawo	TKTTF	1	2199	Digalu
	Dendi	TKTTF	7	2172-2588	Digalu, Kakaba, Roma awn less, Kubsa
	Ejere	TKTTF	1	2160	Digalu
	Tokaye kutaye	TKTTF	4	1949-2332	Digalu, Danda'a
	Chelia	TKTTF	1	2261	Digalu
	Dire Inchine	TKTTF	2	2417, 2434	Digalu, Shorima
SWS	Wonchi	TKTTF	5	2079-2575	Digalu, Kakaba, Kubsa
	Amaya	TKTTF	2	2009, 2038	Digalu
	Woliso	TKTTF	4	2005-2326	Digalu
		HKPPF	1	2054	Digalu
		TTKSK	1	2059	Kakaba
		HKNTF	1	2073	Digalu
	Bechio	TKTTF	3	2172-2223	Digalu, Kakaba
	Suden Sodo	TKTTF	1	2360	Digalu

WS: West Shewa; **SWS:** South west Shewa

Table 7: Races of wheat stem rust across district, altitude and wheat variety in west and southwest Shewa zones.

by TKTTF race and the remaining 11.6% fields infected by other races such as TTKSK, TTTTH, HKPPF, and HKNTF. Twenty three and fifteen sampled wheat fields were infected with TKTTF in west and southwest Shewa zones, in the mentioned order. Out of 27 samples taken from Digalu variety, 25 (92.6%) fields were infected with TKTTF. Similarly, 5 (83.3%), 3 (75%), 2 (100), 1 (100%), 1 (100) and 1 (100%) of Kakaba, Danda'a, Kubsa, Kilinto, Roma and Shorima sampled wheat fields were infected with TKTTF, respectively. On the other hand, other three new races such as TTTTH, HKPPF, and HKNTF were detected only at single location of each (Table 7). HKNTF and HKPPF races were identified from Digalu; and TTTTH race identified from Kilinto (Durum wheat type).

In general, the new, TKTTF race was distributed in the altitude range of 1925-2588 m.a.s.l in 12 districts of west and south west Shewa zones, Oromia region. This showed that, TKTTF is the most virulent race on wheat varieties and it is rapidly spreading to a wide altitude ranges. This might be due to favorable environmental conditions as well as cultivation of susceptible wheat varieties in those districts. In contrast, other 3 new races were found in the altitude range of 2054-2154 m.a.s.l. only from two districts (Table 7). The race TTKSK (Ug99) was detected from elevations of 2059 and 2147 m.a.s.l.

Out of 5 races, the most frequently and predominantly occurred race was TKTTF with a frequency of 88.4% (Table 8). The second frequently race was TTKSK with a frequency of 4.7%. This might be widely growth of resistant variety like Digalu for this race in those districts and/or it might be dominated by virulent race like TKTTF. However, it was reported by Admassu et al.[13] reported that TTKSK race was dominant throughout the country including west and south west Shewa zones at a frequency of 26.6%. The least frequently occurring races were TTTTH, HKPPF and HKNTF with a frequency of 2.3% each.

The observed/recorded virulence spectrum varied between 13-18 *Sr* genes (Table 8). The most wide virulence spectrum was recorded on the race of TTTTH that exhibited virulence on 18 *Sr* genes. The second broad virulence spectrum was recorded on the TKTTF and TTKSK races that showed virulence on 17 *Sr* genes. The most devastating stem rust race TTKSK (commonly known as Ug99) virulence on gene *Sr*31 was first detected in Uganda in 1999 [27] and had been spread to most of the wheat growing areas of Kenya in 2002 and Ethiopia in 2003 [28]. In 2005, Ethiopian reports confirmed its presence in six dispersed locations [29] and was spread to most of wheat growing areas in the country and becoming the main threat for wheat production [30]. Similarly, TTKSK has been reported by Teklay in southern Tigray zone with a virulent spectrum on the 17 resistance gene of differential lines [24] The least virulence spectrum was recorded on the HKPPF and HKNTF races that they caused 13 stem rust resistance genes ineffective each (Table 8).

TTKSK (Ug99) was avirulent to *Sr36*, *Sr24*, and *SrTmp* (Table 8). In the same way, the new race TKTTF (Digalu race) was avirulent to *Sr11*, *Sr31*, and *Sr24*. Virulence on the resistance gene **SrTmp** is considered the main factor behind the complete susceptibility of the variety "Digalu" to this new race. This race, before the present study, had not been detected in the 2 zones. The assumption therefore is that this is either a foreign incursion (most likely by wind) or a mutation in-country. At present, very little is known about the regional and global distribution of *Pgt* race TKTTF and members of this genetic lineage. The race was reported in Turkey previously [31] TTTTH, HKNTF and HKPPF races were avirulent to *Sr24*, *Sr38*; *Sr5*, *Sr9e, Sr11, Sr9b, Sr17, Sr24, Sr31*; and *Sr5, Sr9e, Sr11, Sr9b, Sr9d, Sr24, Sr31* genes, respectively (Table 8).

Generally, the identified races had wider range of virulence in the study areas (Table 8). High virulence diversity of stem rust races were reported earlier in Ethiopian [8,30,32] .Co-evolution of *Pgt* along with wheat being the reason for high virulence diversity in Ethiopian *Pgt* populations [33]. This might be due to variation over location and time, as the races found in a specific season and region depend on the type of wheat varieties grown [29] and to some extent on the predominant environmental conditions, especially temperature [18]Virulence diversities within *Pgt* were also reported from countries such as South Africa, Mexico, USA and Canada [34].

The race spectrum in Ethiopia was clearly different from other parts of the world. For example surveys in Canada [21,34-36] USA, Russia and South Africa detected fewer races such as 15, 5, 6 and 7, respectively. Whereas, more races were identified from Ethiopia, i.e. 60, 41, 17, 44, 22 and 20 [24, 30, 31, 37-39] at different times and locations in the country.However, the present study is dissimilar to the previous works that have been done in Ethiopia. It is evident that only 5 races have been identified from two zones and TKTTF was the most dominant across the locations and it covered 88.4% of the race frequency occurrence in those 12 districts.

Most of Ethiopian races varied from one another by single gene/step changes Belayneh et al.[30] Abebe et al.[24] also reported that, 40% of the races that were identified from Southern Tigray in 2010 cropping season varied by single gene changes. Such single step changes in virulence were reported to be the main process of evolutionary change in *P. graminis* f. sp. *tritici* populations [40].However, the present study showed that all identified races were not varied by single step changes (Table 8). There might be other factors for race variation in the studied area like, parasexualism, migration, selection pressure and gene combination.

Virulence frequency of *P. graminis* f. sp. *tritici* isolates to *Sr* resistance genes: The results showed that the majority of the stem rust resistance genes were found ineffective against most of the isolates tested in this study. 85% of the *Sr* genes were ineffective to 88.4% of the isolates. The wheat differential line that carry the resistance genes *Sr*McN, *Sr*10, *Sr*9a, *Sr*30, *Sr*9g, *Sr*8a, *Sr*6, *Sr*7b and *Sr*21 were ineffective to 100% of the isolates tested (Table 9). In the same way, three differential lines that carry resistance genes *Sr*17, *Sr*9d and *Sr*38 were ineffective to 97.7% of the tested isolates each. However, two differential lines carrying resistance genes *Sr*31 and *Sr*11 were ineffective with the least

Race	Virulence (ineffective Sr genes)/Avirulence (effective Sr genes) spectrum	No.Of isolates	Frequency (%)
TKTTF	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN/11, 24, 31	38	88.4
TTKSK	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38, McN/36,Tmp, 24	2	4.7
TTTTH	5,21,9e, 7b,11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 31, McN/24, 38	1	2.3
HKPPF	21, 7b, 6, 8a, 9g, 36, 30, 17, 9a, 10, Tmp, 38, McN/5, 9e, 11, 9b, 9d, 24 , 31	1	2.3
HKNTF	21, 7b,6, 8a, 9g, 36, 30, 9a, 9d, 10, Tmp,38, McN/5, 9e, 11, 9b, 17, 24, 31	1	2.3
Total		**43**	**100**

Table 8: Virulence/Avirulence spectrum and frequency of races of *P. graminis* f. sp. *tritici* collected from west and south west Shewa zones of Oromia region in 2014.

virulence frequency of 7% each to the tested isolated. Belayneh et al. [30] reported similar finding that McNair 701 (*Sr*McN) was susceptible to all of the races identified. According to these authors five stem rust resistance gene in the differential lines; *Sr*9a, *Sr*9g, *Sr*10, *Sr*7b and *Sr*9d were infective for more than 96% of isolates that were collected from Shewa, Arsi, Bale, and northwest regions of Ethiopia, during 2006-2007 cropping season. Similarly, Abebe et al. (2010) also reported that, McNair 701 (*Sr*McN) was susceptible to 95% of the races identified and about 55% of the *Sr* genes were ineffective to more than 60% of the isolates. This report indicated that, Six differential lines carrying resistance genes *Sr*9d, *Sr*21, *Sr*6, *Sr*10, *Sr*9g and *Sr*9b were ineffective with virulence frequency of 65.6, 78.1, 75, 81.2, 87.5 and 93.8% to the isolates tested, respectively. Roelfs et al. [9] also reported that Virulence for *Sr*6, *Sr*9a, and *Sr*9d are common worldwide. However, the present study showed that, the virulence frequency of stem rust identified races are a little bit higher on the most tested differential line genes than earlier studies. This could be due to emerge of new virulent stem rust races and extensive cultivation of susceptible varieties in west and southwest Shewa zones of Oromia region as well as in the country.

In contrast, the stem rust resistance gene *Sr*24 was found effective to all 43 stem rust isolates collected from west and south west Shewa zones of Oromia region (Table 9). This was previously confirmed by the reports of Roelfs et al.[9] Abebe et al.[24] and CIMMYT [41] as *Sr*24 gene is amongst the effective genes in different countries. Even though, in Kenya 2006, a lineage of Ug99 called TTKST added virulence on stem rust gene *Sr*24 has further increased the vulnerability of wheat to the rust worldwide [42]. Based on the present study, *Sr11* and *Sr31* resistance genes were found to be effective against most of stem rust races detected in both zones. Differential lines that carry *Sr31* and *Sr11* were resistant to 93% of the isolates tested. *Sr31* and *Sr11* genes were resistant to 3 common (TKTTF, HKPPF, and HKNTF) races (Table 8). Whereas, differential lines that carry *Sr36*, *Sr9e, Sr9b, SrTmp* and *Sr5* showed resistance to 4.7% of the isolates tested. It was found to be effective to 59.4% of the isolates collected from Southern zone of Tigray [24] even though there was a historical damage of *Sr36* by the race emerged in Ethiopia in the variety Enkoy in 1993/94, CIMMYT [39] and Belayneh et al.[30] also reported in their finding that *Sr36* and *SrTmp* were effective for 81.6 and 76.3% of the isolates tested, respectively, for samples collected during 2006-2007 cropping season in Arsi, Shewa, Bale and northeast regions of Ethiopia. But, these authors reports were not similar to the present study due to more effectively resistance of *Sr36* and *SrTmp* genes to their isolates. Therefore, the effective genes such as *Sr11, Sr31* and *Sr24* can be used as a source of resistance genes, in wheat breeding programs in west and southwest Shewa zones of Oromo region as well as in Ethiopia. Besides, genes that confer seedling and/or adult plant resistance to Ug99 include *Sr2, Sr13, Sr14, Sr22, Sr28, Sr29, Sr32, Sr33, Sr35, Sr37, Sr39, Sr40* and *Sr44* [43] are used as a source of genetic material in breeding program.

Conclusion

The study confirmed the presence of high virulence spectrum among the five identified wheat stem rust races. This indicated that, West and southwest Shewa zones are hot spot areas for appearance of virulent genetic diversity of stem rust races. Therefore, regular assessment and physiological stem rust race identification will be mandatory for virulence and/or avirulence information in west and southwest Shewa zones. *Sr24* gene was the only effective gene that showed resistant for all identified races. Hence, the *Sr* resistance gene *Sr24* can be used as sources of resistance in wheat breeding program.

Differential line	*Sr* gene	Frequency (%)
I*Se*5-Ra	5	95.3
CnS-T-mono-deriv	21	100
Vernsteine	9e	95.3
I*Sr*7b-Ra	7b	100
I*Sr*11-Ra	11	7
I*Sr*6-Ra	6	100
I*Sr*8a-Ra	8a	100
Cns*Sr*9g	9g	100
W2691*Sr*Tt-1	36	95.3
W2691*Sr*9b	9b	95.3
Bt*Sr*30Wst	30	100
Combination V	17	97.7
I*Sr*9a-Ra	9a	100
I*Sr*9d-Ra	9d	97.7
W2691*Sr*10	10	100
CnS SrTmp	Tmp	95.3
Le*Sr*24Ag	24	0
*Sr*31 (Benno)/6*LMPG	31	7
VPM1	38	97.7
McNair701	McN	100

Table 9: Virulence frequency of *P. graminis* f. sp. *tritici* isolates on the 20 *Sr* genes.

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