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Survey of Rust and *Septoria* Leaf Blotch Diseases of Wheat in Central Ethiopia and Virulence Diversity of Stem Rust *Puccinia graminis* f. sp. *tritici*

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Abstract

Wheat is one of the most important cereal crops in Ethiopia and produced across large area of the country. Production of the crop constrained by several infection diseases including rust and Septoria leaf blotch diseases which are the major bottle neck of wheat production in Ethiopia. The objective of this study was to ass distribution, incidence and severity of wheat rusts and Septoria leaf blotch in west and South West Shewa zones and identification of Puccinia graminis f.sp. *tritici* virulences in Ethiopia. The survey was made in 2013 main cropping season (from September to October) following the main roads and accessible routes in each survey district, and stops were made at every 5 km intervals based on vehicles odometers as per wheat fields available. Five stops were made in each wheat field by moving "W" fashion at each stop interval using quadrants and data were collected from each. Race analysis was carried out by inoculating single uredinial isolates on to the 20 differential hosts. The result indicated that, stem, leaf and yellow rust mean incidence value 54.7%, 19.4% and 7.7% were recorded in the surveyed areas, respectively and mean severity value of 7.0%, 9.7% and 5.5% in the same order. Septoria leaf blotch was the most prevalent disease with100%. Mean incidence of 83% and 0.44 disease index of Septoria leaf blotch were recorded. The most widely grown Varieties Digelu and Kakaba showed susceptible reaction to stem and leaf rust whereas Meda wolabu were free both rusts. Variety Kubisa were susceptible to the three rusts (stem, leaf and yellow). Out of 20 isolates, two races namely TTKSK and TKTTF were identified. Race TTKSK was the most frequent with 95%. Stem rust resistance genes *Sr36*, *SrTmp* and *Sr24* were effective against TTKSK and while, *Sr8a*, *Sr24* and *Sr31* were effective against TKTTF (Digelu race). Most of the genes possessed by the differentials were ineffective against one or more of the tested isolates except *Sr24*. *Sr24* which confer resistance against most of the races detected and prevalent in Ethiopia can be used in breeding for resistance to stem rust in the country.

Keywords: Race; *Puccinia graminis* f. sp. *Tritici*; *Sr* genes

Introduction

Bread wheat (*Triticum aestivum* L. em. Thell) is the world's leading cereal grain where more than one-third of the population of the world uses as a staple food. It is one of the most important cereal crops of Ethiopia [1,2]. It ranked fourth in land coverage and total production after tef, maize and sorghum [3]. Wheat is produced across a wide range of agro ecological and crop management regime. The most suitable area for wheat production falls between 1900-2700 m.a.s.l [1]. Despite the large area under wheat in Ethiopia the national average yield is 2.11 t/ha [3], which is far below the average of African and world yield productivity. The low productivity is attributed to a number of factors including biotic (diseases, insect pest and weeds) and a biotic (moisture, soil fertility, etc) and adoption of new agricultural technologies [4]. Among these factors, diseases play a significant role in yield reduction.

Wheat is susceptible to many diseases including the highly destructive ones like rusts (*Puccinia spp.*), *Septoria* leaf blotches (*Septoria tritici*), *Fusarium* head blight (*Fusarium graminearum*), tan spot (*Pyrenophora tritici repentis*), smut (*Ustilago tritici*) and powedery mildew (*Erysiphe graminis* f.sp.*tritici*) [5]. Over 30 diseases have been reported on wheat in Ethiopia [6]. Of these, fungal diseases like rusts (stem, stripe and leaf rust), *Fusarium* head blight (FHB), *Septoria* blotch, *Helminthosporium spp*., and tan spot are the dominant ones that were reported over time [6-9]. Rusts are the most important disease of wheat worldwide, in spite of great progress made in their control in many countries [10] and considered the major diseases of wheat since no other wheat disease could result in greater loss over large area in a given year [11]. Rusts can cause up to 60 percent of yield loss for leaf or stripe (yellow) rust and 100 percent loss for stem rust. The persistence of rust as a significant disease in wheat can be attributed to specific characteristics of the rust fungi. These characteristics include a capacity to produce a large number of spores which can be wind-disseminated over long distances and infect wheat under favorable environmental conditions and the ability to change genetically, thereby producing new races with increased aggressiveness on resistant wheat cultivars.

In West and South West Shewa zones, wheat is highly exposed to wheat rust and *Septoria* blotch damages. Most probable farmer of the area use the most susceptible wheat cultivars like Kubsa and Dashen, the involvement of new wheat rust races and most of the farmer use double cropping system can be used as green bridge for wheat rusts.

Therefore, disease monitoring and surveillance are of paramount significant for sustainable wheat production and tackle food insecurity. Hence, the present research aims to survey of rusts and *Septoria* leaf blotch of wheat in West and South West Shewa zones in addition to

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identify *Puccinia graminis* f.sp. *tritici* virulences in the surveyed areas of Ethiopia.

Materials and Methods

Survey of wheat diseases

Wheat diseases survey was conducted in 2013 cropping season in two major wheat producing districts of South West and West Shewa zones. A total of 65 fields were assessed in 10 districts in two zones. The surveys were made following the main roads and accessible routes in each survey district, and stops were made at every 5-10 km intervals based on vehicles odometers. Three to five stops were made in each wheat field along a diagonal move at each stop interval. Disease prevalence, incidence and severity were recorded for three rusts (stripe, leaf and stem) and *Septoria* leaf blotch. The disease prevalence was calculated using the number of fields affected divided by the total number of field assessed and expressed in percentage. Incidence was calculated by using the number of plants infected and expressed as percentage of the total number of plants assessed. Severity was scored visually using the modified Cobb's Scale [12] for the three rusts, and the double digit scale (00-99) for *Septoria* leaf blotch [10,13]. The first scale (0-9) in the double digit scale represents the blotch development up the plant height (5 if the disease reached at the middle height, 8 for flag leaf and 9 for spike), and the second digit stands for severity (1=10%, 2=20% and 9=90%). For each score, the disease severity percentage was calculated based on the following formula [14].

$$\% \text{ Disease severity (DS)} = (D1/9) \times (D2/9) \times 100.$$

The mean incidence and severity of each field was computed from three to five stops. The results of the survey were summarized by districts and varieties. The geographic coordinates (latitude and longitude), and altitude were recorded using Geographic Positioning System (GPS) unit. The latitude and longitude coordinates were used to map the distributing of the three rusts and *Septoria* leaf blotches of wheat in the survey areas using the Environmental Systems Research Institute (ESRI) Arc View 3.0.

Stem rust race analysis

Race analysis was conducted only for stem rust. Samples were collected from South West and West Shewa zone of Oromia region. A total of 20 samples were collected and analyzed. Rusted stems were cut into small pieces of 5 to 10 cm in length using scissors and placed in paper bags after the leaf sheath was separated from the stem in order to keep the leaf sheath dry.

Urediniopores were collected from samples using atomizer collector in capsule and suspensions were prepared by mixing spores with lightweight mineral oil (Soltrol). For each isolate, the prepared spore suspension was inoculated using atomized inoculator on seven-days-old seedlings of the universally rust susceptible variety "Morocco" which does not carry known stem rust resistance to get enough amounts of spores to inoculate on stem rust differentials. Greenhouse inoculations were done using the methods and procedures developed by Stakman et al. [11]. The mono-pustule (single uredinial isolate) was further multiplied to get enough spores for the differentials. Seedlings were moistened with fine droplets of distilled water produced with an atomizer and placed in dew chamber for 18 h dark at 18 to 22°C followed by exposure to light for 3 to 4 h to provide condition for infection and seedlings were allowed to dry their dew for about 2 h. Then, the seedlings were transferred from the dew chamber to glass compartments in the greenhouse where conditions was regulated at 12 h photoperiod, at temperature of 18 to 25°C and relative humidity (RH) of 60 to 70%.

After two weeks of inoculation, urediniospores of each single pustule were collected in separate capsule and inoculated on the twenty standard differential sets. Five seeds of the twenty wheat stem rust differentials with known resistance genes (*Sr5*, *Sr6*, *Sr7b*, *Sr8a*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9g*, *Sr10*, *Sr11*, *Sr31*, *Sr17*, *Sr21*, *Sr30*, *Sr36*, *Sr38*, *Sr24*, *SrTmp*, and *SrMcN*) and one susceptible variety Morocco were grown in 3 cm diameter pots separately in greenhouse. The single pustule derived spores was suspended in soltrol inoculated onto seven-day-old seedlings using atomizers and/or an air pump. After inoculation, the formal procedure was repeated in dew chamber room. Upon removal from the dew chamber, plants were placed in separate glass compartments in greenhouse.

Infection types were scored after 14 days from inoculations based on 0-4 scale as described by Stakman et al. [11] where 0-2 stands for low infection and 3-4 for high infection. Five latters race code nomenclature system was used according to Roelfs and Martens and Jin et al. [15,16].

Results and Discussion

Survey of wheat stem, leaf and yellow rust

The survey result showed that the overall stem rust, leaf rust and yellow rust prevalence in the surveyed areas (West and South West Shewa) were 76.7%, 33.7% and 13%, respectively (Table 1).

Stem, leaf and yellow rust mean incidence of 6.4%, 26.9% and 5.3% were recorded in South West Shewa zone whereas 5.4, 19.6 and 7.7 was recorded in West Shewa zone in this order. The maximum incidence with 20%, 67% and 30% of stem rust, leaf rust and yellow rust, were recorded in Dawo, Seden Sodo and Chelia districts, respectively. In contrary, no leaf rust and yellow rust were observed in Ameya and Bacho districts (Table 1).

Over all mean stem, leaf and yellow rust severity with 6.5%, 9.7% and 5.5%, respectively were recorded in the surveyed areas. The maximum mean stem and leaf rust severity of 15% and 25%, respectively were recorded in Dawo district while, 20% severity of yellow rust was recorded in Chelia district (Table 1).

In general, the distribution of stem, leaf and yellow rust in both zones were less. This may be due to the unfavorable weather condition during 2013 cropping season. However, districts like Dawo, become hot spot for stem and leaf rust and attention should be given for such areas in the feature to manage loss due to the rust diseases. Chalia district was also affected by yellow rust which may became main treat for the feature unless resistant wheat varieties used.

Reaction of wheat varieties to rust diseases

Wheat varieties Digelu, Bonde, Dashen, Danda'a, Emer wheat, Gisso, Kekeba, Kubsa, Kumute and Medawelabu were grown in the surveyed areas. Among the wheat varieties, Digelu was the most popular and widely grown variety in two zones followed by Kubisa. Stem rust severity up to 20MS and 15S were recorded on variety Digelu and Kubisa, respectively. Zero stem rust severity was recorded on Danda'a, Gisso and Medawelabu Varieties. Varieties Digelu, Kekeba and Kumute showed moderately susceptible reactions while, Bonde, Emer wheat and Kubsa showed susceptible reactions to stem rust. On contrary, variety Dashen and Medawelabu showed moderately resistant reactions to stem rust (Table 2).

All varieties grown in the areas except Danda'a were affected by leaf

Zone	Districts	Stem rust			Leaf Rust			Yellow rust		
		Prevalence (%)	Incidence (%)	Severity (%)	Prevalence (%)	Incidence (%)	Severity (%)	Prevalence (%)	Incidence (%)	Severity (%)
South west Shewa	Ameya	67	5	5	0	0	0	0	0	0
South west Shewa	Bacho	100	5	10	0	0	0	0	0	0
South West Shewa	Dawo	100	20	15	75	40	25	50	25	10
South west Shewa	Seden Sodo	100	5	5	67	67	20	0	0	0
South west Shewa	Wolisso	100	5	10	40	54	17	0	0	0
south west Shewa	Wenchi	60	5	5	40	22	10	10	2	5
Mean		**87.8**	**7.5**	**8.3**	**37.4**	**30.5**	**12**	**10**	**4.5**	**2.5**
West Shewa	Ambo	100	5	5	40	5	10	20	10	10
West Shewa	Chelia	20	1	5	65	3	5	30	30	20
West Shewa	Dendi Toke	100	2	5	10	1	5	10	5	5
West Shewa	kutaye	20	1	5	40	2	5	20	5	5
Mean		**60.0**	**2.3**	**5**	**38.8**	**2.8**	**6.3**	**20**	**12.5**	**10**
G mean		**76.7**	**54.7**	**7.0**	**37.7**	**19.4**	**9.7**	**14**	**7.7**	**5.5**

Table 1: Distribution of Stem, Leaf and Yellow rust of wheat in South West and West Shewa zones.

Variety	Altitude (masl)	Stem rust				Leaf Rust				Yellow rust			
		Incidence		Severity		Incidence		Severity		Incidence		Severity	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Digelu	1896-2843	0-20	5	0-20MS	5.5MS	0-5	1	05MR-Ms	1	0-5	1	0-5R	0.5R
Bonde	2306	2	2	5 S	5S	5	5	10MS	10	5	5	5MR	5MR
Dashen	1967-2346	0-5	4	0-10R	5R	0-100	98	0-60mss	54	0	0	0	0
Danda'a	2679	0	0	0	0	0	0	0	0	0	0	0	0
Emer wheat	2148	1	1	5S	5S	100	100	80S	80	0	0	0	0
Gisso	2378	0	0	0	0	5R	5	5MS	5	5R	5	5MS	5MS
Kekeba	2132-2361	5-10	7.5	5MS	5MSS	0-100	50	0-40MS	20	0	0	0	0
Kubsa	2016-2817	0-60	10	0-15S	10S	0-100	25	0-40mss	12	0-100	33	0-70MSS	20mss
Kumute	2612	5	5	5MS	5MS	5R	5	5R	5	0	0	0	0
Medawelabu	2758-2870	0	0	0	0	0-5	3	0-5R	2.5	0	0	0	0

Table 2: Response of improved wheat varieties to stem, leaf and yellow rust.

rust. The maximum leaf rust severity 80S were recorded on Emer wheat. Leaf rust severity with 60S, 40S, 40S were recorded on variety Dashen, Kekeba and Kubisa. Varieties Digelu showed moderately resistant to moderately susceptible reaction to leaf rust. Bonde, Dashen, Gisso, Kekeba and Kubisa wheat varieties showed moderately susceptible to susceptible reaction while, varieties Kumute and Medawelabu showed resistant reactions (Table 2).

Varieties Digelu, Bonde, Gisso and Kubsa were affected by yellow rust while, the rest varieties were free yellow rust. The highest yellow rust severity 70MS were recorded on Kubsa.Kekeba and Kubissa varieties, showing moderately susceptible reaction. On other hand Bonde and Digelu wheat varieties showed moderately resistant and resistant reaction, respectively (Table 2).

Varieties Danda'a and Meda Walabu were resistant against the three rusts (stem, leaf and yellow) while, the widely grown and popular variety Digelu showed susceptible reaction to stem rust. So wheat growers should be advised to grow resistance varieties Danda'a and Meda walabu replacing to the susceptible varieties like Digelu.

Survey of *Septoria* leaf blotch of wheat

Septoria leaf blotch was found to be among the most destructive disease observed during the growing season across surveyed areas (Table 3). The overall distribution/prevalence of the disease in the ten districts reached 100%. The disease was prevalent in all surveyed areas of the zones. The highest mean incidence (100%) was recorded in Wolisso and Toke kutaye districts followed by the mean (90%) in Chelia. The overall mean incidence 83% was recorded in the surveyed areas in which mean incidence 81% and 85% were recorded in South West and West Shewa zone, respectively. The disease index ranges from 0.19-0.6 in the surveyed areas. The maximum severity index value 0.6 was obtained in Wanchi districts of South west Shewa zone. The overall severity index was 0.44 (Table 3). This indicated that *Septoria* leaf blotch became the most important disease in the surveyed areas.

Stem rust race analysis

Out of 20 stem rust samples collected and analyzed, 2 races were identified. Ten *P. graminis f. sp. tritici* isolates collected from West Shewa zone were assigned to one race and the rest 10 from South West Shewa zone were assigned to 2 races (Table 3). The highly virulent race called Ug99 (TTKSK) was the most abundant and widely distributed race across both zones with a frequency of 95%. The race TKTTF was detected in South west Shewa zone with frequency value 5%. The identification of two races from 20 samples is a clear indication of high virulence diversity within the *P. graminis* f. sp. *tritici* populations in Ethiopia. Admassu and Fekadu reported that there is high variability of *P. graminis* f. sp. *tritici* populations in Ethiopia [17].

The two races were virulent to one or more of the resistance genes (Table 5). For instance, the differential host carrying the resistance gene *5, 21,9e, 7b, 11, 6, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38* and *McNair* (*SrMcN*) were susceptible to both of the races. Race TTKSK was virulent on resistance gene *8a* and *31* whereas TKTTF was virulent on resistance

Zone	Woreda	Altitude (m.a.sl)	Septoria leaf blotch			
			Prevalence (%)	Incidence (%)	Severity (%)	Disease index
South west Shewa	Ameya	**1896-2006**	100	87	45	0.25
	Bacho	2161-2293	100	80	76	0.52
	Dawo	2148-2306	100	82	76	0.52
	Seden Sodo	2292-2413	100	83	54	0.25
	Wolisso	1967-2415	100	100	76	0.52
	Wenchi	2100-2817	100	78	77	0.60
	Mean	**1967-2817**	**100**	**85**	**66**	0.44
West Shewa	Chelia	2383-2870	100	90	65	0.37
	Dendi	2227-2497	100	64	84	0.40
	Toke kutaye	2247-2413	100	100	54	0.25
	Ambo	2073-2679	100	70	35	0.19
	Mean	**2073-2679**	**100**	**81**	**65**	0.37
Grand mean		**1967-2817**	**100**	**83**	**66**	**0.44**

Table 3: Distribution of *Septoria* leaf blotch of wheat in South West and West Shewa zones.

***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

*Low: Infection types 0, 1, and 2 and combinations of these values. **High: Infection types 3 and 4 and a combination of these values [15,16]

Table 4: Nomenclature of *Puccinia graminis* f. sp. *tritici* (*Pgt*) based on 20 differential wheat hosts.

Race	Virulence spectrum (ineffective Sr resistance genes)	No	%
Oromia TTKSK	*5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10, 31, 38,MCN*	19	95
TKTTF	*5,21,9e,7b,11,6,9g,36,9b,30,17,9a,9d,10,TMP,38,MCN*	1	5
	Total	20	100

Table 5: Races of *P. graminis* f.sp. *tritici* identified and their virulence spectrum in west and south west Shewa zones, Oromia region, Ethiopia in 2013.

gene *36* and *Tmp*. Only three of the differential lines carrying resistance gene *Sr36*, *SrTmp* and *Sr24*, were effective against the most dominate race TTKSK (Ug99) whereas only *Sr8a*, *Sr24* and *Sr31* were effective against the most virulent race TKTTF. *Sr24* gene was found to be effective to all races detected in this study and hence can be considered as source of resistance (Table 5).

In general out of two races identified the most dominant and virulent race were TTKSK. Most of the genes were ineffective except *Sr36*, *SrTmp* and *Sr24* against TTKSK race but *Sr36* and *SrTmp* susceptible to TKTTF. The discovery of the race Ug99 with Virulence to *Sr31* in Uganda in 1999 [18] represented a real threat to wheat production in the world, including Ethiopia where stem rust epidemics had not occurred since the resistant cultivar 'Enkoy, lost its resistance in 1993. In Ethiopia Ug99 was first detected in 2003 at six dispersed sites [16]. In this study also this race is widely distributed in the central part of the country. Previous study also indicated that Ug99 were predominantly distributed in the southern and central parts of the country than in northern west of Ethiopia [19].

Sr24 was effective against most of the isolates tested in Ethiopia. Admassu et al., also indicated that no virulent race detected against *Sr24* gene in Ethiopia [19]. Use of this gene for breeding in Ethiopia is permanent [20,21]. Countries like Ethiopia in which stem and yellow rust severely occur every year and the majority of wheat grown by subsistence farmers, for whom use of chemical fungicide against

stem rust is not economical, continuous supply of resistance varieties decidedly needed to avoid wheat rust epidemics.

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