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Research Article

Analysis of Physiological Races of Wheat Stem Rust (*Puccinia graminis f. sp. tritici*) in Gurage Zone, Ethiopia

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Abstract

Wheat is one of the important cereal crops of Ethiopia. It is threatened by abiotic and biotic constraints. Stem rust caused by *Puccinia graminis f. sp. tritici* is amongst the biotic factors which can cause up to 100% yield loss if susceptible cultivar grown and epidemic occurs. The highland of Ethiopia is considered as a hot spot for the development of stem rust diversity. Guraghe highlands are one of the considerable areas for wheat production in the country. However, the production of wheat in this region is threatened by wheat stem rust disease. So, this study was conducted with the following objectives: to determine pathogenic variability of *Puccinia graminis f.sp. tritici* populations in Guraghe highlands. Field survey was carried out in four districts of Guraghe zone. Twenty five (25) peasant associations were assessed in each districts and diseased plant parts were collected and kept in labelled paper bags. All samples from the fields were inoculated on universally susceptible wheat cultivar for aggressiveness of isolates. Then virulence isolates were used for race analysis. It is inoculated on differential lines of wheat stem rust. Races TKTTF and TKTTC from the area were most virulent with frequency of 46.67% and 13.33%. Sr11 and Sr24 resistance genes were found to be effective 100% to all races detected. A variant of Ug99 virulent against the stem rust resistance gene Sr24 was not detected in this study. The survey revealed high occurrence and distribution of wheat stem rust in the study area. Race TKTTF was the most prevalent race following with race TKTTC in the study area. Differential hosts carrying Sr24 and Sr11 were an effective gene which confers resistance to all pathogen races. Therefore, it is imperative for the national agricultural research center to replace the susceptible cultivars with currently effective resistance genes. The high frequency of occurrence for specific pathogen race indicated that there is low level of variability in the Pgt population of the surveyed areas. Moreover, it is better to monitor pathogen populations over time to track further virulence evolution and to ensure that currently effective resistance genes are applied within a system of resistance-gene management.

Keywords: Stem rust; Race; *Puccinia graminis f.sp. tritici*; Differential lines; Resistance genes; Virulence/ avirulence genes; Isolates

Abbreviations

CSA: Central Statistical Authority; EWRTN: Ethiopian Wheat Rust Trap Nursery; SR: Stem Rust Resistance Gene; SNNP: Southern Nations Nationalities People

Introduction

Wheat is an important cereal crop in Ethiopia that is widely cultivated in a wide range of altitude [1]. It is the main staple food for about 36% of the Ethiopian population [2,3]. The area under wheat production is estimated to be about 1.4 million hectares, which makes the country the largest wheat producer in Sub-Saharan Africa [1,2]. Wheat ranks third in area coverage and total production after tef and maize. Bale region is one of the major wheat producing areas in Ethiopia and it is recognized as one of the wheat belts in eastern Africa. About 226,292 hectares of land are devoted to wheat production by subsistence farmers and profit oriented state farms in Bale [2]. In Ethiopia, wheat is produced in many regions of the country. Oromia, Amhara, SNNP and Tigray are the major wheat producing regions in the country with area coverage of 875641.45, 529609.63, 137294.72 and 108865.39 ha, respectively. Furthermore, 47,259 farmers were involved with unestimated area coverage in Gurage Zone in 2015 main production season [4]. The average productivity is lower than the average yield of other wheat producing areas in the country.

The national average yield of wheat in Ethiopia is about 1.4 t/ha[2]. This is by far below the world's average, which is about 2.5t/ha [5]. Multifaceted biotic and abiotic factors are responsible for this low yield. Cultivation of unimproved low yielding varieties, insufficient and erratic rainfall, poor agronomic practices, diseases and insect pests are among the most important constraints to wheat production in Ethiopia [1,6].

Cereal rusts are the most destructive diseases of wheat worldwide [7,8]. Stem rust caused by the fungus *Puccinia graminis f.sp. tritici* Eriks. and E. Henn, has been the most devastating of all wheat diseases under favorable conditions. This disease is also known as black rust of wheat due to the abundant production of shiny black teliospores that form at the end of the season or with unfavorable conditions [9].

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Table 1: List of differential lines used in variability study.

Differentials (set, line)		Sr - gene
I	ISr5-Ra	5
	Cns-T -mono-deriv	21
	Vernstein	9e
	ISr7b – Ra	7b
II	ISr-11-Ra	11
	ISr6- Ra	6
	ISr8-Ra	8a
	CnSr9g	9g
III	W2691SrTt-1	36
	W2691Sr9b	9b
	BtSr30Wst	30
	Combination V	17
IV	ISr9a-Ra	9a
	ISr9d-Ra	9d
	W2691Sr10	10
	CnsSrTmp	Tmp
V	LeSr24Ag	24
	Sr31/6*LMPG	31
	VPM1	38
	McNair701	McN

Under favorable environmental conditions, stem rust can cause yield losses of up to 100% in susceptible wheat varieties [10]. The yield loss due to this disease is usually greatest when the disease becomes severe before the grain is completely formed, but yield losses are generally influenced by the resistance level of the cultivar grown, the weather conditions and the onset of the disease [11,12]. In Ethiopia, yield losses due to stem rust have been reported to be in the range of 61-100% depending on the susceptibility of the variety and environmental conditions [13,14].

Epidemics of stem rust of wheat often occur in different parts of the world. Resistant cultivars are continuously being developed to prevent such epidemics, although these become susceptible to new pathogenic races sooner or later. A number of physiologic races are known to occur in *P. graminis f.sp. tritici* [15]. An epidemic of stem rust of wheat occurred in 1972, in Ethiopia, due to the loss of resistance in cultivar Lakech, which was grown on large area. Similarly, the cultivar Enkoy went out of production in the country after the epidemics in 1992. Currently cultivar Kubsa has become highly susceptible to stem rust. Thus monitoring of the races and their virulence is important part of rust management strategy to avoid crop losses [16-18]. There are limited reports on the distribution of stem rust races in some parts of Ethiopia [19,20]. Even though Gurage zone is one of the wheat production areas in the country; there is no any information on the virulence and race composition of wheat stem rust disease. Therefore, this study was conducted with the following objectives:

- To analyze the pathogenic variability of *Puccinia graminis f.sp. tritici* populations.
- To determine the occurrence and distribution of pathogen races in the study area.

Table 2: Races of *Puccinia graminis f.sp. tritic* iidentified from Guraghe zone in 2017 and their virulence spectra.

Races	Virulence Spectrum (ineffective Sr resistance genes)	No.	%
TKTTF	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	7	46.7
TKTTC	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, McN	3	20
TKSTF	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp, 38, McN	2	13.3
TKSTC	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp, McN	2	13.3
TKSTH	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp, 31, McN	1	6.67
	Total	15	100

Materials and Methods

Site description

The study was conducted during 2017, in major wheat producing areas of Gurage zone and all the laboratory activities were conducted at Ambo plant protection research center. Geographically, Guraghe Zone is located between 7.8°-8.5° North latitude and 37.5°C-38.7° East longitude of the equator. Wolkite, the capital of the zone, is 155km away from Addis Ababa to south west direction. Gurage zone has a total area of 5932km^2. It has 13 woredas' with a total population estimated about 1343246[4]. The zone comprises altitudes ranging from 1,001 to 3,500 meters above sea level (m.a.s.l). It is classified into three agro-climatic zones: Dega (high altitude) covers 28.3% of the area and ranges between 2,500-3662 m.a.s.l, Woindega (mid-altitude) at 1,500-2,500 m.a.s.l, encompasses about 64.9% of the area, and Kolla (lowland) at 1,000-1,500 m.a.s.l covers 6.8% of the area. The mean annual temperature of the zone ranges between 13-30°c and the mean annual rain fall ranges 600-1600mm. The rainfall pattern in the Gurage Zone is bimodal in which 80% of rain falls in the Kremt period of June to August whereas 20% in the Belg period of February to May. According to land utilization data of the region, 298,369 ha cultivated land, 67,678ha forest, bushes and shrub covered land, 70,249.31ha grazing land, and 14,234ha of land, were covered by others [21].

The pathogen sample collection

Samples were collected from farmer fields and the EWRTN. Forty one (41) samples were collected during the main cropping season of 2017. Sterile scissor with alcohol was used in each field to cut samples. Three to four infected wheat stem tissues with length of 3 to 4 cm were collected and kept in labeled paper bags and transported to Ambo plant protection research center and stored in the refrigerator at 4-5°C until used for variability study. The collected samples were bulked together based on their area of collection and type of cultivars.

Growing test plant in the greenhouse

Five to six seeds of susceptible wheat cultivar (Morocco) were planted in to 7cm diameter plastic pots containing soil, sand and compost mixtures in a 2:1:1 (v/v/v) ratio. The seedlings were allowed to grow until the first leaves were fully emerged. During inoculation, leaves were firstly rubbed gently between moistened fingers to remove the waxy layer from the surface which hinders the penetration of the germ tube of the pathogen spores. Bulk spores from each sample were suspended in distilled water with a drop of Tween^{20} and then

sprayed until runoff using an atomizer. Hands and all materials used during inoculation were disinfested with 70% ethyl alcohol after each inoculation. The seedlings were incubated at relative humidity of about 100% and temperature of about 22°C in a plastic chamber for 24 hours. Then, it was transferred to a greenhouse bench having temperature of 18-27°C. Seedlings were kept for 11-14 days for development of symptom. Samples with viable spores were selected to multiply inoculums for generation of monopustule isolates on the susceptible cultivar, Morocco.

Generation of monopustule isolates

According to the procedures mentioned above, five to six seeds of the universally susceptible wheat variety (Morocco) was planted in plastic pots and then allowed growing for 6-7 days until they produced the first leaves. Seedlings were inoculated very lightly with a population of urediospores of each sample to obtain monopustule isolates.

Urediospores were scrapped from sample leaves/stems in to sterile water in watch glasses using sterile scalpel and were inoculated on seedlings by spatula method of inoculation. The inoculated seedlings were immediately transferred to incubation chamber having saturated atmosphere (95-100%RH) and was kept for 24 hours there at 10-15°C. Thereafter, the seedlings were transferred to greenhouse benches having temperature 18-27°C which is maintained with air conditioners. Seedlings were kept there until urediospores developed. In case where the pustules were aggregated and no isolated pustule occurred, inoculation was repeated on susceptible variety until separate pustules developed and monopustule isolates were generated. A clear plastic cage with a mesh cloth on the top was used to isolate and maintain each isolate.

Multiplication of monopustule isolates

The mono-pustule isolates (Urediospores) were multiplied on the variety "Morocco". Seedlings were raised following the procedures mentioned in above to multiply mono-pustule isolates. Inoculation of seedlings were done in isolation hood. For the multiplication of spores, each isolate was inoculated until sufficient urediospores were collected for differential host test.

Determination of variability

Seedlings of 20 North American stem rust standard differentials were raised with universal susceptible variety "Morocco". Greenhouse inoculations were done using the methods and procedures developed by Stakman et al. (Table 1) [22].

Seven-days-old seedlings of all entries were inoculated with spore suspension of monopustule isolates when their first leaf is fully expanded. Each entry was inoculated separately with each mono-pustule isolate of the pathogen. Seedling inoculation was carried out by spraying spore suspension with an atomizer. The inoculated seedlings were incubated in plastic cage with small layers of water at the bottom for overnight at 18-24°C. After incubation; seedlings were transferred to the greenhouse benches.

Disease assessment

Stem rust Infection Types (IT) were scored 14 days after inoculation using the 0-4 scale of Roelf et al., [20] as follows:- Meaning that; (**0**) no uredia or other macroscopic sign of infection, (**;**) no uredia, but hypersensitive necrotic or chlorotic flecks present, (**1**) Small uredia surrounded by necrosis, (**2**) Small to medium uredia often surrounded by chlorosis; green islands may be surrounded by chlorotic or necrotic border, (x) Random distribution of variable-sized uredia on single leaf, (**3**) Medium sized uredia that may be associated with chlorosis, (**4**) Large uredia without chlorosis. Infection types with 0, ";", 1 and 2 indicate low infection types while 3 and 4 indicates high infection type.

Table 3: Virulence frequency of *P. graminis f.sp. tritici* isolates on the 20 Sr genes.

Differential line	Sr gene	Frequency (%)
ISr5-Ra	5	100
Cns-T-mono	21	100
Vernstein	9e	100
ISr7b-Ra	7b	100
ISr-11-Ra	11	0
ISr6- Ra	6	100
ISr8-Ra	8a	100
CnSr9g	9g	100
W2691SrTt-1	36	100
W2691Sr9b	9b	100
BtSr30Wst	30	100
Combination V	17	66.67
ISr9a-Ra	9a	100
ISr9d-Ra	9d	100
W2691Sr10	10	100
CnsSrTmp	Tmp	100
LeSr24Ag	24	0
Sr31/6*LMPG	31	6.67
VPM1	38	60
McNair701	McN	100

Race analysis

Race designation was done by grouping 20 differential hosts into five subsets and race nomenclature of *P. graminis f.sp. tritici* was analyzed based on five letters systems of nomenclature. Stem rust infection types were scored after 14 days of inoculations based on Stakman et al., [22]. Zero to four scales was used in which 0-2 stands for low infection whereas 3-4 for high infection. To name races, five letters of code nomenclatures were used based on Roelfs and Martens [23] and Jin et al., [24].

Results and Discussion

Race of *Puccinia graminis f.sp. tritici* identified

Of 41 stem rust samples collected from the Guraghe zone, 26 did not yield viable isolates at the time of inoculation in the laboratory. Hence, the virulent 15 isolates were used for the final race analysis. Out of 15 wheat stem rust isolates, 5 types of races were identified with different frequency of occurrence and virulence spectrum. The most abundant and widely distributed race across the study areas was race TKTTF (Digelu race) with frequency of 46.67%. The second most abundant and virulent race was race TKTTC with frequency of

20%. These two races accounted for almost 66.67% of the stem rust population. The remaining races composed were TKSTF, TKSTC and TKSTH which were detected only at one or more location each (Table 2). The identification of 5 races from 15 samples is a clear indication of high virulence diversity within the *Puccinia graminis f. sp. tritici* population in Guraghe zone. Admassu and Fekadu [25] reported that there is high *Puccinia graminis f.sp. tritici* population variability in Ethiopi.

Most of the races identified were virulent to many of the resistance genes (Table 3). For instance, the differential host carrying the resistance gene 5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 9a, 9d, 10, Tmp and McN were ineffective to all of the races. Sr-38 was ineffective to races such as TKTTF and TKSTF and confirms resistance to the rest three races. Sr-31 was indicates ineffective reaction only to race TKSTH, but it was resistant to all races. The 40% of the races were virulent over Sr-17 whereas the rest of races were ineffective against Sr-17. On contrary, Sr11 and Sr24 resistance gene were found to be effective to all races detected in this study and hence can be considered as source of resistance. Resistance gene Sr24 was found to be effective to the fifteen isolates analyzed in this study; and hence, this confirms Roelfs et al. [20] and Borlaug [26], they mentioned as this gene, is amongst the effective genes which have an adequate and some immediate values to almost all races in the world. Stem rust resistance gene Sr24 was effective against all of the isolates tested in Ethiopia. Admassu et. al., [27] also indicated that no virulent race was detected against Sr24 gene in Ethiopia. Use of this gene for breeding in Ethiopia is pertinent [28].

In general, the virulence spectrum of pathogen confirmed the presence of wider range of virulence in the study area and is in line with previous studies conducted in Ethiopia [29,25]. A comparison of the races identified in the present study with these earlier reports revealed differences. This could be due to variation over location and time, as the prevalence of races in a specific season and region depends on the type of wheat cultivars grown and to some extent on the predominant environmental conditions, especially temperature [20].

Summery and Conclusion

Stem rust survey and sample collection were carried out along in four Districts of Guraghe zone during the 2017 main growing season. These were Soddo, Cheha, Ezha and Gumer districts. The prevalence and intensity of the disease was variable with location, crop type, variety, altitude range and the growth stage of the crop.

The variability of the pathogen resulted in fifteen (15) virulent isolates which were assigned to five (5) races. Of these races, TKTTF was the most prevalent race which accounts for 46.67% of the races identified. Race TKTTC with a frequency of 20% and the highly virulent race TKSTF with a proportion of 13.33% were also amongst the important races which were identified in the area. Differential hosts carrying Sr24 and Sr11 were an effective gene which confers resistance to 100% of the races identified in the area followed by Sr31 which was lightly affected by the races identified. In contrast, all differential hosts were ineffective gene to all races identified except Sr38which was effective for 40% of the races. Therefore, it will be helpful to another investigator to include resistance gene found in differential host lines Sr24, and Sr11 into breeding program meant to manage the stem rust disease; especially by focusing on the race Ug99 and newly emerging variants of Ug99 which becoming virulent on different types of resistance genes.

Generally, the area under wheat production in Guraghe has dramatically increased over the years. The level of the disease has also increased. Due to the impact induced by stem rust and other diseases the productivity of the crop has remained low and this needs greater attention if national goal for food self-sufficiency is to be attained. Therefore, it is imperative for the national agricultural research center to monitor pathogen populations over time, to track further virulence evolution and to ensure that currently effective resistance genes are applied within a system of resistance-gene management.

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