Advances in Crop Science and Technology

Hailu et al., Adv Crop Sci Tech 2015, 3:3
http://dx.doi.org/10.4172/2329-8863.1000173

Research Article Open Access

Distribution of Stem Rust (*Puccinia graminis* f. sp. *tritici*) Races in Ethiopia

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Abstract

Wheat is one of the most important cereal crops of Ethiopia. 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. This study was carried out to determine virulence diversity and race distribution of *P. graminis f. sp. tritici* in Ethiopia. One hundred wheat stem rust samples were collected in 2013 cropping season in the Oromia, Amhara and Tigray region. Of sample collected, 66 were viable and analyzed on to the 20 stem rust differentials lines. A total of 9 races were identified, which includes TTKSK, TTKTF, TTKTK, JRCQC, TKTTF, TTKSC, TRTTF, SRKSC and RRKSF. Race TTKSK was predominant and widely distributed in the country with 52% frequency except in Tigray region. The most virulent and new race, TKTTF which causes localized stem rust epidemic in Bale and Arsi was predominantly distributed in oromia region with 36.4% frequency value. Most of the genes possessed by the differentials were ineffective against one or more of the tested isolates except Sr24. Only stem rust resistance gene 24 was found to confer resistance to most of the races prevalent in Ethiopia. These, this gene could be used in combination with other genes through gene pyramiding in breeding for resistance to stem rust in Ethiopia.

Keywords: Stem rust race; *Puccinia graminis f. sp. Tritici;* Stem rust resistance 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.sl [1]. Despite the large area under wheat in Ethiopia the national average yield is 2.11 t/ha (CSA, 2013), 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), Fussarium head blight (FHB), Septoria blotch, *Helmenthosporium spp*., and tan spot are the dominant ones that were reported over time [6-9].

Among these rusts is the most important disease of wheat worldwide, in spite of great progress made in their control in many countries [5]. Rusts are the major disease of wheat since no other wheat disease could result in greater loss over large area in a given year [10]. 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.

The high virulence diversity and evolution rate of the pathogen makes a considerable proportion wheat germplasm at risk. According to Leppik, the highland of Ethiopia is considered as a hot spot for the development of stem rust diversity. Furthermore, studies that were carried out in Ethiopia showed that most previously identified races were virulent on most of varieties grown in the country and are among the most virulent in the world [11]. The past study indicated that race surveys help to generate information regarding the virulence of races and their frequency and distribution patterns across regions and over time . In addition, race survey is important to study evolvement of new races and determine virulence shifts in a population. This information help to detected new race before build inoculums and cause epidemic. Therefore, the currently study was proposed to determine the distribution and dominant virulent races of *Puccinia graminis* f. sp. *tritici* in Ethiopia in 2013.

Materials and Methods

Race analysis was conducted only for stem rust. Stem rust samples were collected from Oromia, Amhara and Tigray regions. A total 100 samples were collected and analyzed. Stems of wheat infected with stem rust were cut into small pieces of 5 to 10 cm in length using scissors and

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Received March 06, 2015; **Accepted** June 26, 2015; **Published** July 03, 2015

Citation: Hailu E, Woldaeb G, Danbali W, Alemu W, Abebe T (2015) Distribution of Stem Rust (*Puccinia graminis* f. sp. *tritici*) Races in Ethiopia. Adv Crop Sci Tech 3: 173. doi:10.4172/2329-8863.1000173

placed in paper bags after the leaf sheath was separated from the stem in order to keep the leaf sheath dry.

Spores were collected from samples using atomizer collector in capsule and suspension prepared by mixing spores with lightweight mineral oil (Soltrol). The prepared spore suspensions were inoculated using atomized inoculators on seven days old seedlings of the universally rust susceptible variety "Morocco" which does not carry known stem rust resistance to get enough amount of spore to inoculate on stem rust differentials. Greenhouse inoculations were done using the methods and procedures developed by Stakman et al. [10]. The mono-pustule was further multiplied to get enough spores for the differentials. The plants were then 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, the spores 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 a greenhouse to avoid contamination and produce infection.

Stem rust infection types were scored after 14 days of inoculations based on Stakman et al. [10]. Zero to four scales was used in which 0-2 stands for low infection where as 3-4 for high infection. Five latter race code nomenclatures were done based on Roelfs, Martens and Jin et al. [12,13] (Table 1).

Result and Discussions

Stem rust race in Ethiopia

Of 100 stem rust samples collected, 34 did not yield viable isolates at the time of inoculation in the laboratory. Hence, 66 isolates were used for the final race analysis. From 66 isolates studied, 9 races were identified. 57 *Puccinia graminis* f. sp. *tritici* isolates collected from Oromia region were assigned to 7 races. Similarly, the 8 and 1 isolates collected from Amhara and Tigray region belongs to 2 and 1 race, respectively (Table 2). The highly virulent race called Ug99 (TTKSK) was the most abundant and widely distributed race across the country with a frequency of 52%. The second abundant and virulent race was TKTTF (Digelu race) with frequency 36.4%. These two races accounted for almost 88.4% of the stem rust population. The remaining 8 races composed of the rest of the population (11.6%). Of these, the least abundant races were TTKTF, TTKTK, JRCQC, TTKSC, TRTTF, SRKSC and RRKSF, which were detected only at single location.The identification of 9 races from 66 samples is a clear indication of high virulence diversity within the *Puccinia graminis* f. sp. *tritici* population in Ethiopia. Admassu and Fekadu [14] reported that there is high *Puccinia graminis* f. sp. *tritici* population variability in Ethiopia.

Of the 57 isolates studied in Oromia region, race TTKSK (Ug99) was pre-dominant with frequency of 47% followed by race TKTTF (Digelu race) with 42%. In Oromia, the five races TTKTF, TTKTK, JRCQC, TTKSC and TRTTF were the least abundant, each with frequency of less than 5%. In Amhara region, two races TTKSK (Ug99) and SRKSC were identified in which Ug99 was again the most dominant (87.5%) race. Where as in Tigray, only one race RRKSF identified this may be due to viability of samples collected from the region.

Most of the races were virulent to one or more of the resistance genes (Table 2). For instance, the differential host carrying the resistance gene 5, 21, 6, 9g, 17, 9a, 9d and McNair were susceptible to all of the races. Similarly, four differential hosts carrying the resistance gene: 9e, 11,9b and 10 were susceptible to more than 88.8% of the races.

Puccinia graminis f. sp. tritici -code	Set 1	5	21	9e	7b
	Set 2	11	6	8a	9g
	Set 3	36	9b	30	17
	Set 4	9a	9d	10	Tmp
	Set 5	24	31	38	McN
B		Low	Low	Low	Low
C		Low	Low	Low	High
D		Low	Low	High	Low
F		Low	Low	High	High
G		Low	High	Low	Low
H		Low	High	Low	High
J		Low	High	High	Low
K		Low	High	High	High
L		High	Low	Low	Low
M		High	Low	Low	High
N		High	Low	High	Low
P		High	Low	High	High
Q		High	High	Low	Low
R		High	High	Low	High
S		High	High	High	Low
T		High	High	High	High

Source: Roelfs and Martens [12]; Jin et al. [13]; *Low: Infection types 0, ;, 1, and 2 and combinations of these values. **High: Infection types 3 and 4 and a combination of these values.

Table 1: Nomenclature of *Puccinia graminis* f. sp. *tritici* 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	27	47
TTKTF	5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10,TMP,38,MCN	1	2
TTKTK	5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10,TMP,31,38, MCN	1	2
JRCQC	21,9e,11,6,9g,17,9a,9d,MCN	1	2
TKTTF	5,21,9e,7b,6,8a,9g,36,9b,30,17,9a,9d,10,TMP,38,MCN	24	42
TTKSC	5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10,MCN	1	2
TRTTF	5,21,9e,7b,11,6,9g,36,9b,30,17,9a,9d,10,TMP,38,MCN	1	2
	Total	57	100
Amahara			
TTKSK	5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10, 31, 38,MCN	7	87.5
SRKSC	5,21,9e,11,6,9g,9b,30,17,9a,9d,10,MCN	1	12.5
Total		**8**	**100**
Tigray			
RRKSF	5,21,7b,11,6,9g,9b,30,17,9a,9d,10,38,MCN	1	100
G total		**66**	**100**

Table 2: Races of *P.graminis f.sp.tritici* identified and their virulence spectrum in Amhara, Oromiya and Tigray regions of Ethiopia in 2013.

Sr7b and Sr30 were susceptible to seven races where as Sr38, Sr8a and SrTmp were susceptible to six, five and four races respectively. Sr36 were susceptible only to TKTTF race and conform resistant to the rest eight races. On contrary, Sr24 resistance gene was found to be effective to all races detected in this study and hence can be considered as source of resistance.

Only three of the differential lines carrying resistance gene Sr36, SrTmp and Sr24, were effective against the most dominate race TTKSK (Ug99) whereas only Sr11, Sr24 and Sr31 were effective against the most virulent race TKTT (Table 2).

In general out of nine races identified the most dominant and virulent race were TTKSK and TKTTF. Most of the genes were ineffective except Sr36, SrTmp and Sr24 against TTKSK race. The discovery of the race Ug99 with Virulence to Sr31 in Uganda in 1999. Pretorius et al. [15] represented a real threat to wheat production in the world, including Ethiopia where stem rust epidemics had not occurred since the resistant cultivar lost its resistance in 1993. In Ethiopia Ug99 was first detected in 2003 at six dispersed sites. 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 [16].

Similarly, the second most dominant race TKTTF which is known as Digelu race was virulent on 17 stem rust resistant gene and widely distributed in the central and southern part of the country. This race is for the first time reported in Ethiopia and cause localized stem rust endemics in Bale and Arsi zone of Oromia region in 2013. Stem rust resistance gene in Digelu variety SrTmp gene was broken and most farmers grow Digelu variety were highly affected. The discovery of new race in the present study with earlier report [16,17] revealed some differences. The Stem rust resistance gene Tmp became in effective in Ethiopia due to the evolvement of new virulent race TKTTF.

Virulence frequency of *P. graminis f. sp. tritici* isolates on Stem rust resistant genes

The Stem rust differential host carrying the resistance gene 21, 6, 17, 9a, 9d and MCN were found to be ineffective against all stem rust races detected with frequency value 100%. Similarly, six differential stem rust differentials carrying resistance genes *Sr9d*, *Sr21*, *Sr6*, *Sr10*, *Sr9g*, and *Sr9b* were found to be ineffective against most of stem rust races detected, with virulence frequencies of 65.6, 78.1, 75, 81.2, 87.5, and 93.8% respectively (Table 3). In general, about 75% of the Stem rust resistance genes were ineffective to more than 60% of the isolates. McNair 701 (*SrMcN*) was ineffective to 96.9% of the isolates tested (Table 3).

On contrary, Stem rust resistance gene 24 was effective against all races detected. Of the 20 stem rust resistance genes, the differential hosts carrying *SrTmp*, *Sr31*, and *Sr17* were was resistant to 87.5, 75.0, and 78.1% of the isolates tested, respectively. Correspondingly, gene *Sr38* was effective against 62.5% of the isolates analyzed followed by *Sr36* which was effective against 59.4% (Table 3).

Stem rust resistance gene 24 was effective against most of the isolates tested in Ethiopia. [16] Admassu also indicated that no virulent race detected against Sr24 gene in Ethiopia. Use of this gene for breeding in Ethiopia is permanent [18] 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, incessant supply of resistance varieties unquestionably needed to avoid wheat rust epidemics.

Stem rust resistance gene (Sr gene)	Virulence frequency (%)	Stem rust resistance gene (Sr gene)	Virulence frequency (%)
5	96.97	*30*	98.5
21	100	*17*	100
9e	98.5	*9a*	100
7b	96.97	*9d*	100
11	63.64	*10*	98.5
6	100	*Tmp*	40.91
8a	93.94	*24*	0
9g	98.5	*31*	54.55
36	62.12	*38*	95.5
9b	98.5	*McN*	100

Table 3: Virulence frequency of *P. graminis f. sp. tritici isolates* (32 isolates) on 20 Stem rust resistance genes.

Acknowledgment

We would like to offer a great thanks to wheat rust research team of Ambo plant protection research Center for their valuable encouragement and technical support during the whole period of the study. Without the support of some individuals and institutions the successful completion of this experiment would have not been realized. Durable Rust Resistance in Wheat (DRRW) project and International maize and wheat improvement center (CIMMYT) is duly acknowledged for fully funding this work.

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