American Journal of Agriculture and Forestry
2022; 10(2): 72-76
http://www.sciencepublishinggroup.com/j/ajaf
doi: 10.11648/j.ajaf.20221002.15
ISSN: 2330-8583 (Print); ISSN: 2330-8591 (Online)

Physiological Races of *Puccinia graminis* f. sp. *tritici* in Ethiopia in 2019/2020

Kitessa Gutu[1], Tsegaab Tesfaye[1], Netsanet Bacha[1], Tamrat Negash[2], Daniel Kassa[2], Fikirte Yirga[2], Megersa Debela[3], Gudeta Bedada[3], Tesfaye Dessale[4], Ashenafi Gemechu[5], Tilahun Bayisa[6], Zerihun Eshetu[6], Zerihun Tomas[7], Girma Teshome[8], Mitiku Kebede[9], Bilal Temam[9], Misgana Mitiku[9], Bekele Kassa[10], Yitagesu Tadesse[10], Mequnanint Andualem[11], Tsehaye Brhane[12], Wulita Wondwosen[13], Nurhussen Seid[14], Zemedkun Alemu[14]

[1]Ethiopian Institute of Agricultural Research, Ambo Agricultural Research Center, Ambo, Ethiopia
[2]Ethiopian Institute of Agricultural Research, Kulumsa Agricultural Research Center, Assela, Ethiopia
[3]Oromia Agricultural Research Institute, Bako Agricultural Research Center, Bako, Ethiopia
[4]Amhara Region Agricultural Research Institute, Sirinka Agricultural Research Center, Wollo, Ethiopia
[5]Ethiopian Institute of Agricultural Research, Debre Zeit Agricultural Research Center, Bishoftu, Ethiopia
[6]Oromia Agricultural Research Institute, Sinana Agricultural Research Center, Bale Robe, Ethiopia
[7]Southern Agricultural Research Institute, Areka Agricultural Research Center, Areka, Ethiopia
[8]Oromia Agricultural Research Institute, Bore Agricultural Research Center, Bore, Ethiopia
[9]Southern Agricultural Research Institute, Werabe Agricultural Research Center, Werabe, Ethiopia
[10]Ethiopian Institute of Agricultural Research, Holetta Agricultural Research Center, Holeta, Ethiopia
[11]Amhara Region Agricultural Research Institute, Adet Agricultural Research Center, Adet, Ethiopia
[12]Tigray Agricultural Research Institute, Alamata Agricultural Research Center, Alamata, Ethiopia
[13]Amhara Region Agricultural Research Institute, Debrebirhan Agricultural Research Center, Debrebirhan, Ethiopia
[14]Ethiopian Institute of Agricultural Research, Worer Agricultural Research Center, Worer, Ethiopia

Email address:

kitessagutu@gmail.com (K. Gutu)

To cite this article:

Kitessa Gutu, Tsegaab Tesfaye, Netsanet Bacha, Tamrat Negash, Daniel Kassa, Fikirte Yirga, Megersa Debela, Gudeta Bedada, Tesfaye Dessale, Ashenafi Gemechu, Tilahun Bayisa, Zerihun Eshetu, Zerihun Tomas, Girma Teshome, Mitiku Kebede, Bilal Temam, Misgana Mitiku, Bekele Kassa, Yitagesu Tadesse, Mequnanint Andualem, Tsehaye Brhane, Wulita Wondwosen, Nurhussen Seid, Zemedkun Alemu. Physiological Races of *Puccinia graminis* f. sp. *tritici* in Ethiopia in 2019/2020. *American Journal of Agriculture and Forestry*. Vol. 10, No. 2, 2022, pp. 72-76. doi: 10.11648/j.ajaf.20221002.15

Received: February 14, 2022; **Accepted**: March 3, 2022; **Published**: April 9, 2022

Abstract: Wheat stem rust caused by *Puccinia graminis* f.sp *tritici* is constant biotic constraint of wheat production across the world. Because of quick alteration of genetic makeup of wheat stem rust pathogen; monitoring shift in virulence within pathogen is crucial to avoid sudden occurrence of epidemics due race change. This study was to identify physiological races stem rust pathogen in Ethiopia during 2019 cropping season. Wheat stem rust samples were collected during 2019 main cropping season from major wheat growing areas of Oromia, Amhara, Tigray and Southern Nations Nationalities and Peoples regions of Ethiopia. Besides, samples were also collected from Afar region where wheat was grown by irrigation during off season. The samples where isolated on universally wheat stem rust susceptible line (McNair) for the sake of mono pustule isolation and multiplication to have sufficient spores. Each isolates where inoculated on twenty standard differential lines and each line was evaluated after fourteen days to determine the races. Eight stem rust races namely, TKKTF, TKTTF, TTTTF, TKKTT, TTKTT, TTRTF, TKPTF and TTKTF were identified from samples analyzed; TKKTF was identified from 175 (44.1%) stem rust isolates, while TTTTF was detected from 73 (18.4) samples analyzed. In addition, TKTTF was isolated from 70 (17.8%) samples; however, TTKTF, TTKTT, TTRTF, TKPTF and TKKTT were recorded from 48 (12.1%), 21 (5.3%), 4 (1%), 1 (0.25%) and 1 (0.25%) samples analyzed in the season. TTKTT races have 95% virulence spectra to stem rust resistance genes with in differential lines. Resistance genes (Sr24) that is available in most of commercial varieties worldwide became ineffective with these races. Therefore, breeding program should focus on searching for more sources of resistance to virulent races of the pathogen.

Keywords: Genes, *Puccinia graminis*, Races, Virulence

1. Introduction

Wheat stem rust disease is cosmopolitan disease which has adapted to different agro ecologies throughout the world. This is due to the nature of rust spore (uridiniospores) that are very tough and adapt to various environmental and have ability to travel long distance even intercontinental being carried by air following the wind direction [11]. It is quite important at low and mid altitudes (< 2400 m). However, Stem rust could be important at higher altitudes on late sown and/or late maturing wheat varieties, especially grown on vertisols [15]. Stem rust caused by *Puccinia graminis* f.sp. *tritici* (*Pgt*) is one of the major production constraints in most wheat growing areas Ethiopia, causing yield losses of up to 100% during epidemic years [7]. Wheat stem rust causing pathogen is constantly changing enemy of wheat production. The diversity could be created due to mutation and sexual recombination [8]. Studies showed that races identified in Ethiopia were virulent on most of the varieties grown in the country and they are among the most virulent in the world [1]. For this reason, continuous monitoring is paramount important to monitor virulence pattern within pathogen population and prepare ahead to reduce possible damage. To this end, regular race identification can offer baseline information about the occurrence and distribution of pre dominant races as well as the appearance of the new virulent pathotypes of the disease. Having the knowledge of prevalent races can assist to develop wheat cultivars with durable stem rust resistance [1]. Besides, due to rapid changes in stem rust race patterns, wheat varieties were vulnerable to the disease shortly after release. Therefore, regular wheat stem rust physiological races identification is important to detect new races and to know the frequency of pre dominant races. Therefore, this study was aimed to identify stem rust races prevalent in major wheat growing areas in 2019/20 cropping season.

2. Materials and Methods

2.1. Sample Collection

Wheat stem rust samples were collected at 5-10 km interval from wheat fields in major wheat growing areas of Ethiopia. Stem of wheat plants infected with stem rust was cut into pieces of 5 to 10 cm in length using scissors. Leaf sheat was removed from the core tissue and placed in paper bag to dry the sample sooner [14]. The samples collected in the paper bags were labeled with required information including crop variety, growth stage and the location from where the samples were collected.

2.2. Isolation and Multiplication of Single-Pustule Isolates

Five seeds of universally susceptible wheat line (McNair) were sown in 10 cm diameter pots filled with a mixture of sterilized soil, sand, and manure with the volume ratio of 2:1:1, respectively. Seedlings were grown in the greenhouse with a temperature and relative humidity of 18-25°C and 98-100% relative humidity. Urediniospores from each field was suspended in lightweight mineral oil, Soltrol 170 (Chevron Phillips Chemical Company, The woodlands, Texsas, United States) and sprayed onto 7-day-old seedlings of variety McNair [11]. Seven days after inoculation, leaves containing a single fleck that produces a single pustule was selected from the base of the leaves and the remaining seedlings within the pots were removed using scissors. Only leaves containing single pustules from each location were covered with cellophane bags and tied up at the base with a rubber band [2]. After two weeks, spores from each pustule was collected in gelatin capsule and suspension was made by mixing urediospores with Soltrol 170. Then after, it was inoculated on seven-day-old seedlings of the susceptible variety McNair for the sake of multiplication in separate pots. The seedlings were placed in the dew chamber in the dark condition at 18-22°C for 18 hours and exposed to light for four hours and transferred to the greenhouse. Fourteen days after inoculation, the spores were collected in gelatin capsule aided with power driven motor pump and inoculated on differential lines.

2.3. Inoculation of Differential Lines

The seeds of 20 wheat stem rust differential lines and universally susceptible line (McNair) were sown in 10 cm diameter pots. Seven days after planting, when the first leaf is fully expanded and the second leaf emerged, the spores each isolate was suspended in Soltrol 170 and inoculated on the seedlings. Inoculated seedlings were put in a dew chamber for 18 hours at 18-25°C and 98-100% relative humidity. Then, plants were exposed to fluorescent light for four hours to facilitate infection and were allowed to dry for about 1-2 hours. Then, inoculated seedlings were transferred to greenhouse where the temperature and relative humidity is adjusted at 18 - 25°C and 98-100% [13], respectively.

2.4. Determination of Races

Seedling infection type was evaluated 14 days after inoculation using a 0 to 4 scale [12]. The IT readings of 3 (medium-size uredia with/without chlorosis) and 4 (large uredia without chlorosis or necrosis) were regarded as susceptible. Other readings, i.e. 0 (immune or fleck), 1 (small uredia with necrosis), and 2 (small to medium uredia with chlorosis or necrosis) were regarded as low infection type or resistance reaction. The variations were refined by modifying characters like -, uredinia slightly smaller than normal for the infection type; +, uredinia slightly larger than normal for the infection type [12]. Race designation was done by grouping the differential lines into five subsets as indicated in Table 3. Each isolate was assigned using a five-letter designation based on its reaction on the differential lines [10, 4].

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

		Infection types produced on near-isogenic Sr lines			
	Set 1	5	21	9e	7b
	Set 2	11	6	8a	9g
Pgt- code	Set 3	36	9b	30	17
	Set 4	9a	9d	10	Tmp
	Set 5	24	31	38	McN
B		Low[a]	Low	Low	Low
C		Low	Low	Low	High[b]
D		Low	Low	High	Low
F		Low	Low	High	High
G		Low	High	Low	Low
H		Low	High	Low	High
J		Low	High	High	Low
K		Low	High	High	High
L		High	Low	Low	Low
M		High	Low	Low	High
N		High	Low	High	Low
P		High	Low	High	High
Q		High	High	Low	Low
R		High	High	Low	High
S		High	High	High	Low
T		High	High	High	High

Source: (Roelfs and Martens, 1988); (Jin et al., 2008)
[a]Low = Infection types 0,;, 1, and 2 and combinations of these values
[b]High = Infection types 3 and 4 and a combination of these values.

3. Results and Discussion

A total of 599 stem rust samples were collected and received from Oromia, Amhara, SNNP, Beneshangul Gumuz and Tigray regions during 2019/2020 cropping season. Of these, 393 samples were analyzed using the International Nomenclature System of *P. graminis f. sp. tritici* [10]. Eight stem rust races namely TKKTF, TKTTF, TTTTF, TKKTT, TTKTT, TTRTF, TKPTF and TTKTF were identified from samples analyzed; TKKTF was identified from 175 (44.1%) stem rust isolates, while TTTTF was detected from 73 (18.4) samples analyzed. In addition, TKTTF was isolated from 70 (17.8%) samples; however, TTKTF, TTKTT, TTRTF, TKPTF and TKKTT were recorded from 48 (12.1%), 21 (5.3%), 4 (1%), 1 (0.25%) and 1 (0.25%) samples analyzed in the season (Table 2).

Table 2. *Samples collected and analyzed in 2019/2020 cropping season.*

Region	Samples collected	Samples analyzed	Races
Oromia	326	253	TKKTF (124), TKTTF (48), TTTTF (35), TTKTT (16), TTKTF (26), TTRTF (3) and TKKTT (1)
Amhara	122	42	TKKTF (25), TKTTF (6), TTKTT (5), TTTTF (4) and TTKTF (2)
SNNP	29	14	TKTTF (4), TTTTF (7), TKKTF (2) and TTKTF (1)
Beneshangul Gumuz	47	33	TTKTF (13), TKKTF (8), TKTTF (3) and TTTTF (9)
Tigray	22	11	TKKTF (8), TTKTF (2), and TTTTF (1)
Afar	52	40	TKKTF (8), TTTTF (17), TKTTF (9), TKPTF (1), TTKTF (4) and TTRTF (1)
Total	599	393	TKKTF (175), TTTTF (73), TKTTF (70), TTKTT (21), TTKTF (48), TTRTF (4), TKKTT (1) and TKPTF (1)

The wheat cultivars; Ogolcho, Kubsa, Hidase, Lakech, Digelu, Denda'a, ETBW 9553, Lemu, Ogolcho, Kingbird, Honqoltu, Hidase, Wane, Kakaba, and unknown varieties were infected by one or more of these races. Race TKKTF was the dominant race and is virulent to all of differential lines except Sr11, Sr36, Sr24 and Sr31. Race TTTTF was the second dominant race followed by TKTTF (Digelu race) and they wide virulence spectrum. TTTTF is virulent to all resistant genes, except Sr24 and Sr31. Similarly, TKTTF is virulent to all resistant genes, except Sr11, Sr24 and Sr31. On the other side, TKKTT and TKPTF were the least dominant races that were identified from one sample each.

Table 3. *Wheat varieties from which the races were identified.*

Race	Variety
TKKTF	Unknown, Kekeba, Kingbird, Digelu, Denda'a, Hidase and Ogolcho,
TKTTF	Unknown, ETBW 9553, Denda'a, Kekeba, Kubsa and Digelu
TTTTF	Denda'a, Kekeba, Digelu and Unknown varieties
TTKTT	ETBW 9553, Honqoltu, Lakech and Ogolcho
TTKTF	Denda'a, Wane and ETBW 9553
TKKTT	Lemu
TTRTF	Danda'a, Unknown
TKPTF	Unknown

TKTTF was first detected in 2012 in Southeastern parts of Ethiopia and caused wheat stem rust epidemics in 2013/14 cropping season by attacking the popular variety Digelu [7]. TTKTT is virulent on all resistance genes in differential lines except *Sr36* (Table 4). TKKTF was reported in Germany and was among the races that caused an unusual wheat stem rust outbreak in 2013/14 cropping season [6]. TTKTT race was first reported in Ethiopia in 2018 from commercial wheat cultivars Shorima, Huluka, Ogolcho, Hidase, and Danda'a [3]. It has the most virulence combination of all Ug99 (TTKSK) race groups. TTTTF race was reported from samples collected in 2009 from the Eastern Shoa zone of central Ethiopia [5]. It was also detected in Iran in 2010 (Patpour et al., 2014). Moreover, TTTTF race caused a wheat stem rust outbreak in Italy hitting several thousands of hectares of durum wheat [9].

Table 4. *Virulence spectrum of the Pgt races identified in Ethiopia in 2019/20 main cropping season.*

Races	Virulence	Avirulence
TTTTF	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*	*24, 31,*
TKTTF	*5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*	*11, 24, 31*
TKKTF	*5, 21, 7b, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Tmp, 9e, 38, McN*	*11, 36, 24, 31*
TTKTF	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*	*36, 24, 31*
TTKTT	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Tmp, 24, 31, 38, McN*	*36*
TTRTF	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, 38, McN*	*30, 24, 31*
TKPTF	*5, 21, 9e, 7b, 6, 8a, 9g, 36, 30, 17, 9a, 9d, 10, Tmp, 38, McN*	*11, 9b, 24, 31*

4. Conclusion

Wheat stem rust races identified in this study have more virulence combination than predominant races in Ethiopia. TTKTT have 95% virulence spectra to stem rust resistance genes with in differential lines. Resistance genes (Sr24) that is available in most of commercial varieties worldwide became ineffective with these races. The rapid evolution of new races within rust population is bottleneck to rust management worldwide. Once a new strain of the pathogen occurred in a particular area; it can spread over the long distance being aided by air within a short period. Therefore, breeding program should focus on searching for more sources of resistance to virulent races of the pathogen.

Acknowledgements

We are thankful Biotechnology and Biological Sciences Research Council (BBSRC, UK Research and Innovation) and the Delivering Genetic Gains in Wheat (DGGW) Project for their to financial support.

References

[1] Admassu, B., Lind, V., Friedt, W. and Ordon, F. 2009. Virulence analysis of *Puccinia graminis* f. sp. tritici populations in Ethiopia with special consideration of Ug99. Plant Pathology 58 (2): 362 - 369.

[2] Fetch Jr, T. G. and Dunsmore, K. M., 2004. Physiologic specialization of *Puccinia graminis* on wheat, barley, and oat in Canada in 2001. *Canadian Journal of Plant Pathology*, *26* (2), 148-155.

[3] Hei, N. B., Tsegaab, T., Getaneh, W., Girma, T., Obsa, C., Seyoum, A., Zerihun, E., Nazari, K., Kurtulus, E., Kavaz, H. and Ozseven, I., 2020. First Report of *Puccinia graminis* f. sp. tritici Race TTKTT in Ethiopia. Plant Disease, 104 (3), 982.

[4] Jin, Y., Szabo, L. J., Pretorius, Z. A., Singh, R. P., Ward, R. and Fetch Jr, T., 2008. Detection of virulence to resistance gene Sr24 within race TTKS of *Puccinia graminis* f. sp. *tritici*. *Plant Disease*, *92* (6), 923-926.

[5] Lemma, A., Woldeab, G., Semahegn, Y. and Dilnesaw, Z., 2014. Survey and virulence distribution of wheat stem rust (*Puccinia graminis* f. sp. tritici) in the major wheat growing areas of central Ethiopia. Sci-Afric Journal of Scientific Issues, Research and Essays, 2 (10), 474-478.

[6] Olivera Firpo, P. D., Newcomb, M., Flath, K., Sommerfeldt-Impe, N., Szabo, L. J., Carter, M., Luster, D. G. and Jin, Y., 2017. Characterization of *Puccinia graminis* f. sp. tritici isolates derived from an unusual wheat stem rust outbreak in Germany in 2013. Plant pathology, 66 (8): 1258-1266.

[7] Olivera, P., Newcomb, M., Szabo, L. J., Rouse, M., Johnson, J., Gale, S., Luster, D. G., Hodson, D., Cox, J. A., Burgin, L., Hort, M., Gilligan, C. A., Patpour, m., Juatesen, A. F., Hovmoller, M. S., Woldeab, G., Hailu, E., Hundie, B., Tadese, K., Pumphrey, M., Sigh, R. P., and Jin, Y., 2015. Phenotypic and Genotypic Characterization of Race TKTTF of *Puccinia graminis* f. sp. tritici that Caused a Wheat Stem Rust Epidemic in Southern Ethiopia in 2013 – 14. Phythopathology 105 (7): 917–928.

[8] Pathan, A. K. and Park, R. F. 2007. Evaluation of seedling and adult resistance to stem rust in European wheat cultivars. *Euphytica* 155: 87-105.

[9] Patpour, M., Hovmøller, M. S., Hansen, J. G., Justesen, A. F., Thach, T., Algaba, J. R., Hodson, D. and Randazzo, B., 2018. Epidemics of yellow and stem rust in Southern Italy 2016-2017. In BGRI 2018. Retrieved on April, 2021 (https://www.globalrust.org).

[10] Roelfs AP, Martens JW, 1988. An international system of nomenclature for *Puccinia graminis* f.sp. tritici. Phytopathology 78, 526-33.

[11] Roelfs, A. P., R. P. Singh & E. E. Saari 1992. Rust Disesases of Wheat: Concept andMethods ofDisease Management. Mexico, D. F.: CIMMYT. 81 pages.

[12] Stakman, E. C., D. M. Steward, W. Q. Loegering. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. US Dep. Agric. Agric. Res. Serv. E-617.

[13] Stubbs RW, Prescott JM, Sarrri EE, Dubin H J, 1986. Cereal Disease Methodology Manual. CIMMYT, El Batan, Mexico, p. 51.

[14] Woldeab G., Hailu E., and Bacha N., 2017. Protocols for Race Analysis of Wheat Stem Rust (*Puccinia graminis* f. sp. *tritici*). Ethiopian Institute of Agricultural Research, Ambo Plant Protection Research Center, Ethiopia. pp: 27.

[15] Ayele Badebo and Bekele Hundie, 2016. Incidence and Challenges of Rust Diseases in Wheat Production. *In:* Containing the Menace of Wheat Rusts: Institutionalized Interventions and Impacts. Ethiopian Institute of Agricultural Research. pp: 153, Addis Abeba, Ethiopia.