

Screening of Some Ethiopian Wheat Lines Against Stem Rust Races at Seedling Stage

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To cite this article:

Mekonnen Assefa, Kitessa Gutu. Screening of Some Ethiopian Wheat Lines Against Stem Rust Races at Seedling Stage. *Bioprocess Engineering*. Vol. 6, No. 1, 2022, pp. 5-9. doi: 10.11648/j.be.20220601.12

Received: May 18, 2022; Accepted: June 22, 2022; Published: June 30, 2022

Abstract: One of the most harmful wheat diseases in Ethiopia is stem rust caused by *Puccinia graminis f. sp. tritici* (Pgt). It has the capacity to result in yield losses of up to 100% on susceptible cultivars. In a study, forty-two wheat lines along with one susceptible check were tested for their ability to fight off eleven isolates of the stem rust races TTKSK (Ug99 race), TTRTF, TKTF (Digalu race), JRCQC, TKKTF, TRTF, TTTTF, TTKTF, TTKTT, RRTTF, and TKPTF. In the Ambo Plant Protection Research Center in 2021, wheat lines were experimentally infected with urediniospores of the various races under controlled circumstances. Different seedling resistance responses were seen against the Pgt races that were investigated. Thirteen wheat lines (WL-1, WL-4, WL-21, WL-35, WL-37, WL-38, WL-41, WL-43, WL-45, WL-48, WL-49, WL-61, WL-64), which demonstrated low infection types (ITs of 2 or lower) against all the races, resulted in twenty-three lines having both resistant and susceptible infection types (ITs 0 to 3). In contrast, susceptible checks and six wheat lines (WL-26, WL-31, WL-33, WL-47, WL-51, WL-66) consistently showed susceptible infection types (ITs 2+ to 3+) to all races. The study's found resistant wheat lines can be employed as sources of resistance in the breeding program for wheat.

Keywords: Infection Types, Pathotypes, Uredinia, Virulent, A Virulent, Race-Specific

1. Introduction

Wheat is grown on about 1.6 million hectares of land in Ethiopia, accounting for 13.33% of total grain crop area and producing 4.2 million tons per year, contributing roughly 15.81 percent of overall grain production and ranking third behind teff and maize. [1]. The crop is primarily farmed in Ethiopia's mid- and highlands, at elevations ranging from 1500 to 3000 meters above sea level. It is, however, mostly grown between 1800 and 2500 meters above sea level. [2, 3]. Even though Ethiopia's wheat productivity has increased in the last ten years, it remains poor when compared to other wheat-producing countries. Biotic (diseases, insect pests, and weeds) and abiotic (moisture, soil fertility, frost, excess or low fertilizer) factors are all blamed for the low yield. Rusts are the most common wheat disease, with varying degrees of destruction in the field. Stem rust, caused by *Puccinia graminis f. sp. tritici* (Pgt), is the most well-known and catastrophic, causing up to 100% loss of wheat crops, compared

to 60% for leaf (brown) (*Puccinia recondita* Roberge ex Desm.) or stripe (yellow) (*Puccinia striiformis* Westend. f.sp. tritici) rust [4, 5]. The treatment is used to protect the crop from being afflicted by rust, which causes 50 percent to 100 percent of the crop to be destroyed. [6]. The value of grain production, disease resistance, straw yield, seed color, baking quality, and other relevant social values are all favorites among Ethiopian farmers' wheat crop varieties. Genetic variation in pathogen makes its control difficult. Later, identifying key gene sources is a crucial step in breeding for long-term rust resistance. The current study aimed to test forty-two wheat lines for stem rust resistance at the seedling stage under control circumstance in green house adult plant resistance (APR) infield level.

2. Materials and Methods

2.1. The Study Area's Description

The Ambo Plant Protection Research Center evaluated the

greenhouse (APPRC). It is situated at an altitude of 2147 meters at 08° 96' 885" N latitude and 37° 85' 923" E longitude. The average annual temperature is 27.540°C, and the average annual rainfall is 1077.68mm.

2.2. Plant Materials

The planting materials include 42 wheat lines generated from

farmer varieties preserved at the Ethiopian Biodiversity Institute. (Figure 1). Because many landrace accessions are heterogeneous, single plant selections were made from resistant plants identified during preliminary characterization at Debre Zeit (known hot spot area for stem rust) based on grain yield, seed size, rust resistance (*Puccinia graminis* f. sp. tritici), field establishment (germination, seedling emergence, and growth), and crop stand.

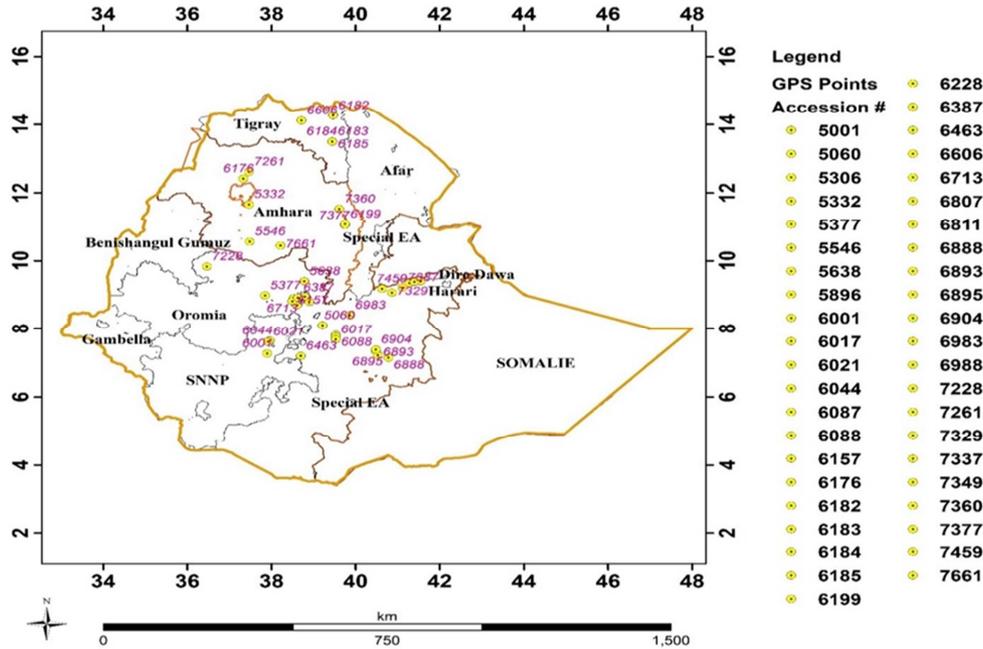


Figure 1. The mother accession collection area is depicted on a map.

2.3. Inoculation and Seedling Phenotyping

In 2021, the Ambo Plant Protection Research Center conducted a greenhouse experiment to determine seedling resistance in a controlled environment. 42 wheat lines were tested against 11 (TTKSK, TTRTF, TKTTF, JRCQC, TKKTF, TRTTF, TTTTF, TTKTF, TTKTT, RRTTF, and TKPTF) *Pgt* races were identified and maintained at Ambo Plant Protection Research Center. To get adequate inoculum, the spores were inoculated onto a universally susceptible to stem rust host McNair (without the Sr gene). Seedlings of the wheat lines, as well as the susceptible check, McNair, were raised in 7 cm x 7 cm x 6 cm plastic pots filled 1:1:1 (v/v/v) with compost, light soil, and sand. McNair was used to test the vitality of spores

that had been introduced into the lines. Each race's spores were suspended in Soltrol 170, a light mineral oil, and adjusted to 1x10⁵ spores ml⁻¹. Using atomized inoculators, each race's spore suspensions were then injected on 7-day-old seedlings. To establish ideal circumstances for infection, seedlings were moistened with fine drops of distilled water 30 minutes after inoculation and transported to a dew chamber for 18 hours of darkness at 18 to 22°C followed by 4 hours of light. The seedlings were then relocated to glass compartments in the greenhouse, where conditions were managed at a 12 hour photoperiod, a temperature of 18 to 25°C, and a relative humidity of 60 to 70%. Infection types (IT) were scored 14 days after inoculations, based on a 0–4 scale [14]. Table 1 shows the virulence/a virulence formulae for different races.

Table 1. Virulence or a virulence formula of *Puccinia graminis* f. sp. tritici isolates.

Races	Virulence	A virulence
TKTTF	5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	11, 24, 31
TTKSK	5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38, McN	36, Tmp, 24
TRTTF	5, 21, 9e, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	8a, 24, 31,
JRCQC	21, 9e, 11, 6, 17, 9g, 9a, 9d, McN	5, 7b, 8a, 36, 9b, 30, 10, mp, 24, 31, 38
RRTTF	5, 21, 7b, 11, 6, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	9e, 8a, 24, 31
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
TKKTF	5, 21, 9e, 7b, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, Tmp, 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, N	36
TTTTF	5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN	24, 31

Table 2. To each of eleven isolate races of *pgt*, forty-two Ethiopian farmers varieties wheat lines were tested, coupled with one susceptible check (Mac Nair).

No	Genotype		Selection History			
	code	Cultivar	Latitude	Longitude	Altitude	
1	WL1	Wheat Line	14-07-00-N	38-43-00-E	2000.00	
2	WL2	Wheat Line	13-30-00-N	39-27-00-E	2062.00	
3	WL4	Wheat Line	09-24-00-N	38-47-00-E	2160.00	
4	WL6	Wheat Line	08-55-00-N	38-38-00-E	2100.00	
5	WL8	Wheat Line	09-24-00-N	41-33-00-E	2150.00	
6	WL9	Wheat Line	11-40-00-N	37-28-00-E	2080.00	
7	WL10	Wheat Line	12-25-00-N	37-20-00-E	1750.00	
8	WL13	Wheat Line	08-24-00-N	39-52-00-E	2040.00	
9	WL18	Wheat Line	07-49-00-N	39-32-00-E	2110.00	
10	WL19	Wheat Line	12-37-00-N	37-28-00-E	2140.00	
11	WL20	Wheat Line	07-09-00-N	40-47-00-E	1630.00	
12	WL21	Wheat Line	11-32-00-N	39-37-00-E	2090.00	
13	WL23	Wheat Line	09-50-00-N	36-28-00-E	2090.00	
14	WL26	Wheat Line	09-04-00-N	40-52-00-E	1990.00	
15	WL28	Wheat Line	11-03-00-N	39-45-00-E	2160.00	
16	WL31	Wheat Line	07-39-00-N	37-58-00-E	2190.00	
17	WL33	Wheat Line	09-11-00-N	40-38-00-E	1800.00	
18	WL34	Wheat Line	10-27-00-N	38-13-00-E	2490.00	
19	WL35	Wheat Line	08-54-00-N	38-32-00-E	2090.00	
20	WL37	Wheat Line	11-05-00-N	39-45-00-E	1850.00	
21	WL38	Wheat Line	08-57-00-N	38-48-00-E	2100.00	
22	WL39	Wheat Line	08-49-00-N	38-30-00-E	2150.00	
23	WL41	Wheat Line	08-06-00-N	39-13-00-E	2033.00	
24	WL43	Wheat Line	07-41-00-N	39-32-00-E	2080.00	
25	WL45	Wheat Line	09-21-00-N	41-17-00-E	2180.00	
26	WL47	Wheat Line	09-23-00-N	41-24-00-E	2150.00	
27	WL48	Wheat Line	07-16-00-N	37-54-00-E	2135.00	
28	WL49	Wheat Line	07-12-00-N	38-42-00-E	1910.00	
29	WL50	Wheat Line	07-20-00-N	40-29-00-E	2040.00	
30	WL51	Wheat Line	07-13-00-N	40-32-00-E	2180.00	
31	WL55	Wheat Line	07-46-00-N	39-32-00-E	2100.00	
32	WL57	Wheat Line	08-42-00-N	38-37-00-E	2070.00	
33	WL60	Wheat Line	08-51-00-N	38-49-00-E		
34	WL61	Wheat Line	08-48-00-N	38-54-00-E	2080.00	
35	WL63	Wheat Line	13-30-00-N	39-27-00-E	2062.00	
36	WL64	Wheat Line	10-34-00-N	37-29-00-E	2145.00	
37	WL66	Wheat Line	09-13-00-N	41-07-00-E	2110.00	
38	WL67	Wheat Line	08-59-00-N	37-51-00-E	1772.00	
39	WL69	Wheat Line	07-37-00-N	37-55-00-E	2190.00	
40	WL71	Wheat Line	14-16-00-N	39-28-00-E	2060.00	
41	WL73	Wheat Line	13-30-00-N	39-27-00-E	2061.00	
42	WL75	Wheat Line	07-23-00-N	40-29-00-E	2080.00	
43	Check		McNair APPS	3	3	

Table 2. Continued.

No	Infection response to <i>pgt</i> races										
	TTKSK	TTRTF	TKTTF	JRCQC	TKKTF	TRTTF	TTTTF	TKKTF	TKKTT	RRTTF	TKPTF
1	2-	1+	,1	2-	,1	2-	;1	;1	;1	;1	;1
2	2-	2-	,2-	,1	2	2-	2+	;1	;1	2-	;1
3	1+	,1	,1	,1	,1	,1	2	;1	;1	;1	;1
4	2-	2-	1+	2-	2	,1+	2+	;1	;1+	;1	;1+
5	,1+	2-	1+	2-	,1	,1	2+	;1	;1+	;1+	;1
6	2+	2	2	2+	,1	2-	2+	;1+	2+	2-	2
7	2+	2-	2-	2	2	2-	2+	;1+	2	2+	2
8	1+	2+	2-	2-	,1+	2-	2	;1	;1	;1+	;1
9	,1	2-	,1	2-	,1	2-	2+	;1	;1	2-	;1
10	2-	2-	,1	1+	,1	,1+	3-	;1	;1	;1	2
11	2-	2-	1+	2	,1	2-	2+	2	2+	2	2+
12	2	2-	1+	,1	,1	,1	2_	;1	;1	;1	;1
13	2	2	2-	2-	2-	2-	2+	2-	;1	;1	;1
14	2+	2+	2+	3-	3-	3-	2+	3-	3-	2+	2+
15	2-	2	2	2	,1	2	2+	2	;1	2-	;1
16	3-	2+	2+	2+	2+	2+	3-	3	3-	3-	3-
17	3-	2+	2+	2+	3-	3-	3-	3-	3-	3-	3-

No	Infection response to <i>Pgt</i> races										
	TTKSK	TTRTF	TKTTF	JRCQC	TKKTF	TRTTF	TTTTF	TKKTF	TKKTT	RRTTF	TKPTF
18	,1	2+	2	,1+	,1	3-	2+	;1	;1	2+	2+
19	1+	,1	,1	,1	,1	,1	;1+	;1	;1	;1	;1
20	2-	2	,1	;1+	,1+	2-	2	;1	;1	;1	;1
21	0	,1+	,1+	,1+	,1	,1	;1	;1	;1	;1	;1
22	2-	2	2-	2-	2-	2-	3-	;1	;1	2-	2-
23	2-	,1	,1	,1	,1	2-	;1	;1	;1	;1	;1
24	,1+	1+	,1	,1	,1	2-	;1	;1	;1	;1	;1
25	,1	2	,1+	,1+	,1	,1	;1	0	;1	;1	;1
26	3-	3+	3-	2+	3-	2+	3-	3-	3-	3-	3-
27	2-	,1	1-	,1+	2-	2-	;1	;1	;1	;1	;1
28	2-	,1	2	,1+	2-	2-	;1	;1	2-	;1	;1
29	3-	2-	2+	2	2+	2+	3-	3-	2+	3-	3
30	3-	2+	2+	3-	3-	3	3	3-	3-	3-	3
31	2	2+	2-	,1+	2-	,1	2	2+	2	2	2
32	2	2	2-	,1+	2-	2	2	2-	;1	2+	;1+
33	2+	2+	2	2+	2+	2+	2+	2+	2	2+	2+
34	2-	2-	,1	,1	2-	,1	2-	;1	;1	;1	;1
35	2+	2+	2-	2+	2+	2+	2+	;1	3-	3-	2+
36	,1	2	,1+	,1	,1+	,1	2-	;1	;1	;1	;1
37	2+	2+	2+	2+	3	2+	3-	3-	3-	2+	2+
38	2+	3-	3-	3-	3	2+	3-	3-	3-	2+	2-
39	3-	2-	3-	2-	2	,1+	1+	;1	2+	;1+	;1+
40	2-	2	2+	2	2	2	3-	3-	2+	2+	;1+
41	3-	2+	2+	2	,1+	2+	3-	3-	2+	2+	2-
42	,1	2+	2	,1+	2-	2-	2+	2+	2	2+	2-
43	3	3	3	3	3	3	3	3	3	3	3

APPR= (Ambo Plant Protection Research Center), WL=Wheat Lines; *Pgt* = *Puccinia graminis f. sp. triticia*
 IT readings of 3 (medium-size uredinia with/without chlorosis) and 4 (large uredinia without chlorosis or necrosis) were considered compatible, while readings of 0 (immune or fleck), 1 (small uredinia with necrosis), and 2 (small to medium uredinia with chlorosis or necrosis) were considered incompatible [14]. The variations were fine-tuned by changing the characters in the following way: +, uredinia somewhat larger than normal for infection type; -, uredinia slightly smaller than normal for infection type

3. Results and Discussion

A close association between the host and an obligatory pathogen results in rust diseases. As a result of the interaction, the contact may cause differential selection in either the host or the pathogen [10]. High levels of resistance can stop the rust fungus from surviving, whereas high levels of virulence can prevent a host plant from producing; so, it is not unexpected that the host and its rusts have established such potent genetic ties [7]. According to the gene-for-gene theory, race-specific resistance is regulated by the interaction between the resistance gene(s) in the host and the associated virulence gene(s) in the pathogen, making resistance in wheat lines visible through testing with particular *Pgt* pathotypes or genes. In seedlings, resistance is widespread, monogenic, and active during the whole host's growth cycle, uses the McNair cultivar as a weak reference and shows the typical responses of forty-two wheat lines to eleven *Pgt* races (Table 2). All of the lines revealed different infection types relative to the stem rust races used. Given that 13 wheat lines showed low infection types (ITs of 2 or lower) against all of the races investigated, it is likely that these lines carry the Sr24 resistance gene or other resistance genes; also, the results of the current investigation revealed that low ITs against the TTKSK (Ug99 race) and TKTTF (Digalu race), were present in 70% and 72%, respectively, of the tested wheat lines; these two races account for over 88.4% of the stem rust population in Ethiopia [13] and results suggest

that stem rust resistance comes from good sources in Ethiopian durum wheat accessions [12]. Twenty-three lines had both resistant and susceptible infection types s(ITs 0 to 3), according to the confirmed races, and six wheat lines, in addition to the susceptible check, consistently showed susceptible infection types (ITs 2+ to 3+). The sensitivity lines indicate that they either lack strong gene resistance or possess fewer efficient genes that shield them from the races. High IT values on tested lines suggest the absence of virulent test isolate (race resistance) genes [8-10] (Table 1). These lines, however, may be resistant to the races not covered in this study or possess adult plant resistance (APR), as they were first chosen based on their high level of resistance in the field. According to [11], the seedling stage of Ethiopian wheat lines exhibits susceptible infection types, however the severity of stem rust in the field is negligible. High ITs throughout the trial for wheat lines (WL-26, WL-31, WL-33, WL-47, WL-51, and WL-66) and susceptible host (McNair) showed that they lacked any significant genes that imparted race-specific resistance gene-for-gene. Similar results were seen for wheat line WL-67, which showed high ITs for all races except TKPTF, indicating that it is likely to carry the sr11, sr9b, sr24, and sr31 resistance genes. The two lines have the same gene in reaction case for TKTTF, however WL-63 is responsible for carrying sr24, sr31, and sr36 independently. WL-60 demonstrated low infection types for TKTTF and TTKTT, which signifies committed for sr11, sr24, and sr36. Table 1.

Wheat line WL-73 demonstrated low ITs against (JRCQC,

TKKTF, and TKPTF), indicating that it may contain the sr24 and sr31 resistance genes. Similarly, wheat lines WL-50 and WL-34 had high ITs to all pathotypes except TTRTF and JRCQC, WL-34 had high ITs to TTRTF, TRTF, TTTTF, RRTTF, and TKPTF, while WL-71 had high ITs to TKTF, TTTTF, TTKTF, TTKTT, and RRTTF. These lines may lack the resistance gene sr24, which confers virulence. Furthermore, wheat lines WL-9, WL-10, WL-20, WL-73, and WL-75 had high ITs to the TTTF races; and WL-9, WL-10, WL-69, and WL-73 had high ITs against TTKSK; WL-10 and WL-20 combined had high ITs to TTTTF; and WL-73 and WL-75 had high ITs against TTRTF, TTTTF, TTKTF, and RRTTF. Wheat lines WL-2, WL-6, WL-8, WL-18, WL-19, WL-23, WL-28, WL-39, had high ITs only against TTRTF and TTKTF, while wheat lines WL-13 and WL-57 had high ITs only against (TTRTF) and (RRTTF), respectively, while WL-55 had high ITs against TTRTF and TTKTF indicating the presence of an effective major R-gene against multiple races or a hint for multiple (horizontal) disease resistance due to the presence of two or more minor genes; as a result, using these lines in breeding programs can aid in the development of new resistant wheat cultivars.

4. Conclusion

In conclusion, wheat lines, Infection types resistant to all races were regularly found in WL-1, WL-4, WL-21, WL-35, WL-37, WL-38, WL-41, WL-43, WL-45, WL-48, WL-49, WL-61, and WL-640 (ITs of 0 to 2); the Sr24 resistance gene, which is effective against all tested races, and/or unidentified resistance genes may thus be present in these lines [12]. In the aftermath of recent stem rust outbreaks, locating such resistance sources and creating resistant cultivars are essential. The current analysis suggested that several of the wheat lines examined may have Pgt resistance genes. The actual number and identity of the genes, however, are outside the scope of this study. In order to create stem rust resistant cultivars, it is crucial to map the resistance genes, use molecular techniques to create diagnostic markers for the genes, and take use of these genes.

Acknowledgements

We are grateful to the Ethiopian Biodiversity Institute for their financial support of the project. We gratefully acknowledge the Wheat Rust Research Team of the Ambo National Plant Protection Research Center for providing the greenhouse and technical assistance during the project.

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