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Research Article

Identification of Stripe Rust (*Puccinia striiformis* F. sp. *Tritici*) Resistance in the Ethiopian Wheat Landraces

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ABSTRACT

Stripe rust is caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is threatening wheat production in Ethiopia. Wheat varieties succumb to new *Pst* race (s) soon after their release from research centers. The objective of this study was to determine stripe rust resistance in selected Ethiopian wheat landraces obtained from the Ethiopian Institute of Biodiversity (IBCE). In 2017, a total of 197 accessions (152 bread and 45 durum wheat land-races) were exposed to the prevailing stripe rust races in hot spot areas (Kulumsa and Meraro) in Arsi zone of Oromia region. In the second year 2018, only promising landraces 103 (69 bread and 34 durum)) were evaluated both at seedling and adult plant growth stages. The seedling test was conducted in the greenhouse at Kulumsa research center using three *Pst* races. In field evaluations, terminal severity (TRS), coefficient of infection (CI), area under disease progress curve (AUDPC), disease progress rate (DPR) and spike infection (SI) were considered. High disease pressure was noted with 100% severity on susceptible entries at both locations and seasons. Highly significant (P<0.001) differences were noted among the landraces for all disease parameters indicated above. Of the 103 landraces, 57 (55%) exhibited lower or equal disease reaction compared to the resistant check (Enkoy) across locations and seasons. Thirty two landraces showed both adult plant and seedling resistance. The 103 Ethiopian wheat landraces that showed field resistance further exposed to three *Pst* races at seedling stage and 61 exhibited seedling resistance to all races. This study has identified potential sources of seedling and adult plant resistance in the Ethiopian wheat landraces to the prevailing *Pst* races. Future wheat improvement should focus on utilization of these genetic resources to minimize the re-current outbreak of rust diseases.

Keywords: Wheat; Land-race; *Pst*; *Race*; Resistance; Stripe rust; Isolates

INTRODUCTION

Ethiopia is the largest wheat producers in Sub-Saharan Africa (SSA). The crop ranks fourth in area coverage next to tef, maize and sorghum, respectively [1]. The area under wheat production is estimated to be about 1.7 million hectares of land with average productivity of 2.7 tons/ha. Wheat production is constrained by several biotic, a biotic and socio-economic factor in the country. The Ethiopian highlands are suitable for the wheat plant as well as for perpetuations wheat rust diseases [2]. Stripe rust caused by *P. striiformis* f. sp. *tritici*. Westend *(Pst)* is the most threatening wheat disease in the highlands of Ethiopia [1]. The disease was first reported in the early 1940's, but it gained importance with the commercialization of wheat in the early 1980s [3]. Recurrent stripe rust epidemics have occurred since then; however, the 2010 epidemics was the most damaging. Most of the commercial bread wheat cultivars, including *Kubsa* and *Galama* scummbed to the new *Yr27+* virulent race and caused 70 to 100% yield loss in major wheat producing areas of the country [4].

In the depletion of rust resistance in commercial cultivars, new sources often sought from landraces and close relatives of wheat. Landraces are grown by farm communities in remote villages using centuries'- old technologies, including hand planting and harvesting [5]. The main reasons for farmers to maintain these historic wheat landraces could be due to their specific adaptability and preferred quality for home use. Wheat landraces contribute important traits including disease resistance [6], tolerance to a biotic stresses [7], and protein content and gluten quality [8]. Wheat landraces have been successfully used by wheat breeders to improve agronomic traits, particularly disease and pest resistance [9]. Such genetic resources have been successfully used by plant breeders to improve agronomic traits, particularly disease and pest résistance. Landraces of wheat have resulted from selection within field populations by traditional agriculturists. Because they are agronomically adapted to field conditions, landraces may be more easily utilized as sources of novel genes or gene combinations than wild relatives [10]. Tetraploid and hexaploid wheat landraces have been evaluated to a limited extent for their potential use in modern

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plant breeding for disease resistance and landraces collections in general show a much higher level of genetic diversity than elite varieties.

In Ethiopia, a large number of wheat genotypes have been preserved in the Institute of Biodiversity Conservation (IBC), which could potentially serve as sources to stripe rust resistance. In this study a set of Ethiopian wheat landraces from IBCE have been characterized for their resistance to the prevailing *Pst* races under field and greenhouse conditions.

MATERIALS AND METHODS

Description of the study area

The field study was conducted at Kulumsa and Meraro research stations in Arsi Zone of Oromia Region, Ethiopia. The greenhouse experiment was conducted at Kulumsa Agricultural Research Center. Meraro and Kulumsa are known to be hot spot areas for wheat stripe rust, although the disease pressure increases with altitude. Kulumsa represents mid highlands (2200 m.a.s.l.) and located at 39º 09'11''E l and 08º 01'10''N. It has average maximum 22.8ºC and minimum 10.5ºC temperatures and receives 832 mm rainfall annually. Meraro represents extreme highlands (2960 m.a.s.l.) and located at 39º 14'56''E and 07º 24' 27''N. Meraro receives 1196 mm annual rainfall with maximum and minimum temperatures of 18.1ºC and 5.7ºC, respectively.

Planting materials

A total of 197 bread wheat landrace accessions plus 3 checks were used in this study. The accessions and checks were obtained from the institute of biodiversity of Ethiopia (IBCE) and the wheat breeding program at Kulumsa research center, respectively. In 2017, all of the accessions were tested under field conditions whereas only selected entries were exposed to the prevailing races of *Pst* in the field and greenhouse during 2018.

Field experimental design and testing procedures

The experiment was laid out in augmented design where the three check varieties; Digelu (susceptible), Pavon-76 (moderately resistant) and Enkoy (resistant) were replicated in each block. Each plot consisted of 2 rows of 1 m length with 20 cm between rows. To ensure uniform spread of inoculum and sufficient disease development, infector plants consisted of mixtures of different susceptible bread wheat varieties (Morocco, Digelu and Kubsa) bordered the plots in all direction. Fertilizer application and other agronomic practices were applied according to the recommendations for each location.

Field disease assessments

Different epidemiological parameters which include terminal rust severity (TRS), coefficient of infection (CI), area under disease progress curve (AUDPC) and disease progress rate (DPR) were used to determine stripe rust resistance in the wheat landraces.

Disease severity

Disease severity notes were taken five times on plot bases starting from the onset of rust within 10 days intervals. Stripe rust severity was estimated visually as a proportion leaf area affected by stripe rust using the modified Cobb'sscale [11] and the host plant response (infection type) was noted according to Peterson, et al. [12]. The CI was calculated by multiplying the level of disease severity and the constant value of infection type. The constant values for infection types were used based on; R = 0.2, MR = 0.4, M= 0.6, MS = 0.8, S = 1 [11]. Head infection was noted using 0-5 scale and then converted to percentages for analysis: 0 = no infection, 1 = 20%, 3 = 60%, 4 = 80% and 5 = 100% severity.

Area under Disease Progress Curve (AUDPC)

AUDPC is an indicator of disease expression over time [13], and it was calculated for each experimental unit according to Wilcoxson, et al. [14]:

$$AUDPC = \sum_{i=1}^{n} [0.5\left(xi + x_{i+1}\right)\left[t_{i+1} - ti\right]$$

Where,

xi = The average coefficient of infection of i th record

x_{i+1} = The average coefficient of infection of i+1th record

$t_{i+1} - t_i$ = Number of days between the ith record

i+1th record and

n = Number of observations.

Disease Progress Rate (DPR)

Disease progress rate was estimated from the logistic model [12]: ln (Y/ (1-Y)) and Gompertz model: ln [-ln (Y)]; where y = the percent of severity divided by 100; t = time measured in days. In this study, Gompertz model was used for all assessment dates because of the higher values of coefficient of determination (R^2) compared to the logistic model.

Field data analysis

Data on different epidemiological parameters were generated for each test material. The data have been transformed using $\sqrt{\text{üü}}$ before analysis. A standard analysis of variance was conducted to identify significance differences among the wheat landarces for each disease parameter. Correlation analysis was done among disease parameters and thousand kernel weight (TKW) using Minitab Software . The data were analyzed using SPAD and SPSS software's [15,16].

Seedling test

One hundred three accessions were selected for seedling resistance tests based on their field performances in 2017. The experiment was conducted according to standard procedures [17] in the greenhouse at Kulumsa research center in 2018. Five to six seeds of each entry plus three checks were grown in 7×7×6 cm plastic pots filled with compost, soil and sand at a ratio of 1:2:1 (v/v/v) in spore-free greenhouse compartment under supplemental light. The first leaves of seven to eight days old seedlings were inoculated with spores of three *Pst* (*PstS2* (v32), *PstS11* and *PstS11 (v25)*) isolates suspended in light weight mineral oil (Soltrol 170) using atomizer. Inoculation was carried in an enclosed cage that was rinsed with water subsequently to avoid spore contamination. Inoculated seedlings were allowed to dry for 20 minutes and then incubated in a dew chamber for about 24 hrs at 9-10ºC with 100% relative humidity. Seedlings were kept in semi open plastic cubicles in a greenhouse compartment at 18-22°C and 70 to 80% relative humidity. The seedlings were supplemented with 12 hrs light using florescent lamps. The experiment was replicated twice and repeated based on the infection type (IT) on susceptible variety (*Morocco*). Seedlings were evaluated 16-20 days post-inoculation using a 0-9 scale [18]. Entries with ITs 0-6 were considered as resistant and 7-9 susceptible [19].

RESULTS AND DISCUSSION

Terminal rust severity on wheat leaves (TRS)

Stripe rust disease pressure was high in the study areas during 2017 and 2018. The disease severity went up to 100% on susceptible entries across locations and seasons. The landraces exhibited various reactions to stripe rust on wheat leaves and spikes. The disease pressure was more at Meraro (2900 masl) than at Kulumsa (2200 masl). In 2017, the mean severity scores on leaves of resistant, moderately resistant and susceptible checks were, 9.6%, 40% and 78% at Kulumsa and 20%, 59% and 94% at Meraro, respectively whereas the mean severity on spikes for the respective checks were 18%, 82% and 62% at Meraro (Table 1). The frequency distribution of landrace accession under different disease severity (TRS) classes at Kulumsa and Meraro during 2017 is depicted in Figure 1. The frequency of susceptible entries were more at Meraro than at Kulumsa. A total of 97 (49%) landraces at kulumsa and 75 (38.1%) and 97 (49%) at Meraro exhibited low (0-20%) severity on leaves

Table 1: The reaction of selected Ethiopian wheat landrace's to stripe rust at seedling and adult plant growth stages.

No	Accession no.	Seedling ITs (0-9)			Severity %				SI%	AUDPC % days			
	variety	Isolate			Kulumsa		Meraro			Kulumsa		Meraro	
		PstS2(v32)	*PstS11*	PstS11(v25)	2017	2018	2017	2018	2017	2017	2018	2017	2018
1	7451-1	2	2_3	4_5	0	0	0	10	0	0	0	0	42
2	214318	2	5_6	2	0	0	0	1	0	0	0	0	3
3	7259-1	2	2	2_3	10	5	30	10	20	84	62	460	60
4	222388-1	2	0	5_6	0	0	30	1	0	0	0	500	2
5	5011-1	4_5	2	4	1	1	5	20	0	5	6	54	102
6	222192-1	2	2	2	0	0	0	1	0	0	0	0	3
7	206689-1	0	5	5_6	0	5	0	10	0	0	62	0	52.5
8	222493-1	2	2	2	5	1	10	20	30	54	6	64	130
9	7251-1	2_3	3	3_4	0	0	0	5	0	0	0	0	22
10	7143-2	2	2	2	10	1	15	20	0	44	8	180	140
11	222550-2	2	2	2	1	1	5	5	30	5	5	24	30
12	6991-1	2	2_3	2_3	0	0	0	0	0	0	0	0	0
13	7145-1	2	5_6	5_6	0	1	0	1	0	0	4	0	4
14	206657-1	1_2	4_5	3_4	0	1	0	20	0	0	7	0	150
15	5435-1	2_3	3_4	5_6	0	0	0	5	0	0	0	0	22
16	222389-1	2	2	3	0	0	0	0	0	0	0	0	0
17	222545-1	2	2	2	0	5	0	30	0	0	52	0	165
18	7038-1	2	2	2	0	1	0	0	20	0	6	0	0
19	6885-1	2	3	2	10	1	20	20	20	84	8	322	150
20	214116-1	2_3	3_4	2_3	0	1	0	5	0	0	26	0	22
21	222758-1	5_6	3_4	3	0	1	0	20	0	0	24	0	150
22	7991-1	3_4	6	3_4	0	1	20	20	0	0	24	252	160
23	7292-1	2	2	2	0	1	0	10	0	0	24	0	42
24	7028-1	3	3	2	0	0	10	20	0	0	0	64	120
25	6930-1	2	2	2	0	0	0	0	0	0	0	0	0
26	7407-1	4	3_4	5_6	1	1	10	15	10	2	5	64	62
27	7178-1	2_3	6	2	0	1	0	5	0	0	6	0	12
28	7177-1	3_4	3_4	2	0	0	0	20	0	0	0	0	125
29	222405-1	2	2	2	0	0	0	0	0	0	0	0	0
30	6934-2	5_6	2_3	3_4	0	0	0	0	0	0	0	0	0
31	206622-2	2	3_4	2	4	1	4	10	3	20	5	68	60
32	7298-2	0	5_6	5_6	0	0	0	10	0	0	0	0	42
	Check												
1	Enkoy	7	7	7	9.6	1.5	20.4	14.2	18	138.1	11.5	292	98.7
2	Pavon -76	5-6	6-7	5-6	40	28	59.2	35	82	490.5	282	1481	271
3	Digelu	8	9	5-6	78	52	94.2	60	62	1655	680	2438	454

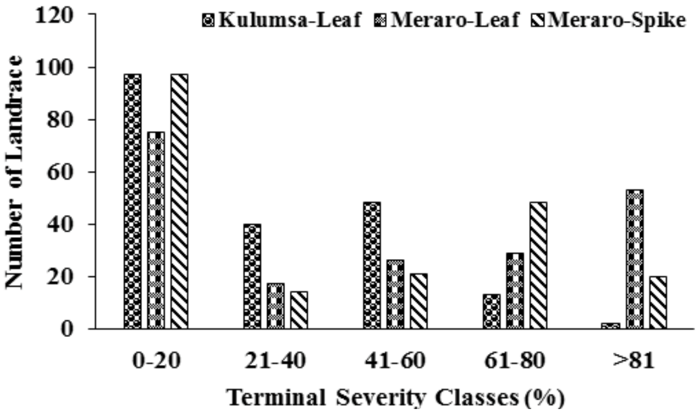

Figure 1: Frequency distribution of landraces under different terminal rust severity classes at Kulumsa and Meraro in 2017.

and spikes, respectively whereas 40 (20%) landraces at Kulumsa and 17 (9%) and 14 (7%) at Meraro, respectively had moderate TRS (21-40%. A total of 43 (22%) and 69 (35%) exhibited immune reaction to stripe rust on leaves and spikes, respectively across locations (data not shown).

The frequency of landraces under different stripe rust severity classes at Kulumsa and Meraro during 2018 is shown in Figure 2. Of the total 100 (50.8%) landraces, at Kulumsa and 57 (29%) and 89 (45%) at Meraro exhibited low (0-20) TRS on leaves and spikes, respectively.

On highly infected wheat genotypes, the grains were shriveled and resulted in losses in yield, quality and thousand kernel weights (TKW). This result is consistent with the findings of Purdy LH and Allan RE [20] reported the negative effect of spike infection on yield and yield components. Trace to low levels of spike infection on the moderately resistant and resistant varieties did not affect thousand kernel weights (TKW), yield and yield components. Spike infection of wheat by stripe rust at higher altitudes result in 100% losses [1].

The present study identified considerable variations among the landraces in terminal rust severity (TRS) at both locations and seasons. The higher disease severity level was observed at Meraro compared to Kulumsa. This may be attributed mainly due to more favorable environmental condition. In this study, 100 (50.8%) and 57 (29%) accessions exhibited high level of slow rusting resistance at Kulumsa and Meraro, respectively. Resistance in these landrace accessions might be controlled by several minor genes which give long-lasting resistance.

Area under disease progress curve

The distribution of landraces under AUDPC values across locations in 2017 and 2018 seasons is shown in Figure 3. In 2017, of the total number of landraces, 77 (39%) and 28 (14%) showed lower AUDPC values than the resistant (*Enkoy*) and moderately resistant (*Pavon-76*) checks at Kulumsa and 68 (35%) and 37 (19%) at Meraro, respectively.

The landrace accession with lower AUDPC values than the resistant check (*Enkoy*) is considered to possess slow rusting resistance. AUDPC is a good indicator of adult plant resistance under field condition [21]. It is directly related with grain yield loss [22] and provides critical information for designing effective disease management practices for lines with different types and levels of resistance.

Disease Progress Rate (DPR)

The frequency of landraces under different disease progress rate (DPR) classes at Kulumsa and Meraro during 2017 is shown in Figure 4. The DPR values ranged from 0 to 0.187 per unit time at Kulumsa and 0 to 0.159 at Meraro. Of the tested entries, 47 (24%) and 57 (29%) landraces exhibited no disease progress at Kulumsa and Meraro, respectively. However, 47 (24%) and 9 (5%) landraces had lower DPR value per unit time than the resistant check and moderately resistant checks each at Kulumsa and 58 (29%) and 2 (1%) at Meraro, respectively.

Of the total 103 landraces, 23 (12%) and 31 (16%) showed no progress in disease severity in 2018. However, 30 (15%) and 13 (7%) had lower DPR value per unit time than the resistant and

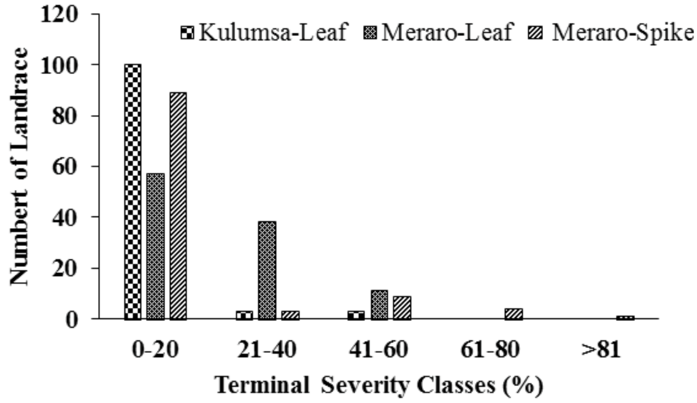

Figure 2: Frequency distribution of landraces under different terminal stripe rust severity classes at Kulumsa and Meraro in 2018.

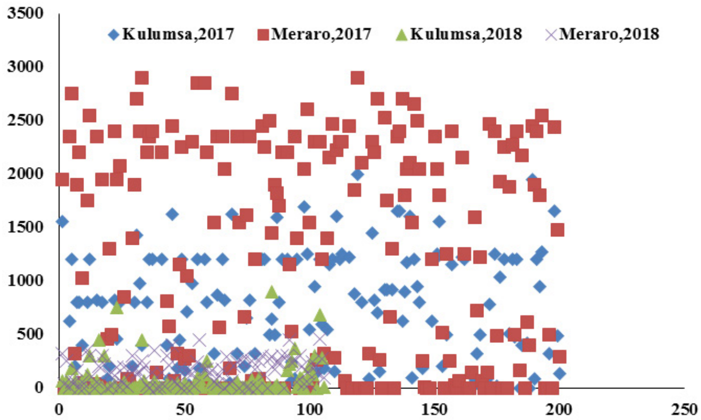

Figure 3: The distribution of AUDPC values of landraces across locations in 2017 and 2018.

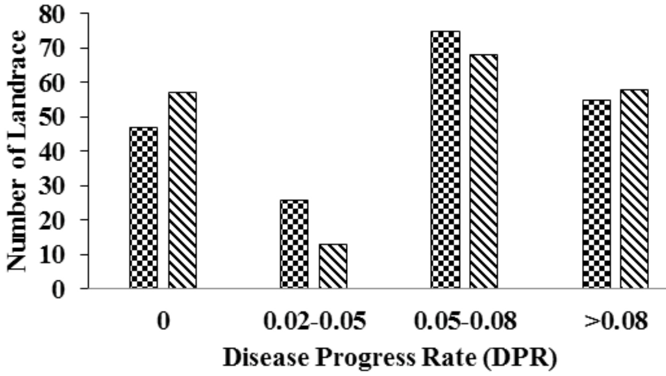

Figure 4: The frequency of wheat landraces under different disease progress rate classes at Kulumsa and Meraro, 2017.

moderately resistant checks at Kulumsa and 39 (20%), 9 (5%) at Meraro, respectively (data not shown). Disease progress rate in terms of disease spread was lower in resistant varieties compared to the susceptible ones. In general, a low AUDPC value in an accession might not ensure low in DPR. Once the pathogen reaches its maximum infection level, the DPR value may decrease or remain constant as disease progresses [23]. Ethiopian wheat landrace accessions with low DPR value per unit time could be potential sources to be exploited in breeding program.

Correlation among disease parameters and thousand kernel weight

Highly significant (P<0.001) and positive correlations observed among the disease parameters across locations and seasons (Table 2). There was high correlations between terminal rust severity (TRS) with area under disease progress curve (AUDPC) and disease progress rate (DPR) at Kulumsa (r = 0.986 and 0.969) and Meraro (r = 0.985 and 0.965), respectively. Relatively lower correlations were noted between area under disease progress curve (AUDPC) and disease progress rate (DPR) compared to the other slow rusting parameters at Kulumsa (r = 0.870) and Meraro (r = 0.886). Despite the severity and AUDPC increase, the rate of infection could be slowed down over time because as the epidemics progresses less plant tissue could be available for further infection of the pathogen [24]. The thousand kernel weight (TKW) has negatively correlated at P<0.001 with all the disease parameters at both locations (Table 3).

Highly significant and positive correlation observed between SI and the major disease parameters; however, the correlation with thousand kernel weight (TKW) was negative. The positive correlations among the disease parameters observed in this study are in agreement with the results of Safavi and Ashfari [25]. In the present study, all disease parameters were highly correlated and affected thousand kernel weight (TKW). Safavi, et al. [26] reported higher selection gains of slow rusting resistance using low terminal rating, area under disease progress curve (AUDPC) and coefficient of infection (CI) under field condition.

Seedling resistance

A total of 103 landrace accession were exposed to the three most virulent stripe rust isolates (*YR28WAB16*, *YR80NWA17* and *YR39AD17)* which were later designated as *PstS2 (v32)*, (*PstS11*) and *PstS11* (*v25*), respectively. Of these landraces, 79 (77%), 81 (79%) and 71 (69%) showed resistance reaction to *PstS2* (v32), (*PstS11*)

Table 2: Mean square variance of coefficient of infection, terminal rust severity, area under disease progress curve, disease progress rate and spike infection of stripe rust on Ethiopian wheat landraces at Kulumsa and Meraro, 2017.

Locations	Source						
	Disease parameter	Block (Adj)	Entry (Adj)	Check	Landrace	Landrace × check	Error
Kulumsa	DF	4	200	2	197	1	8
	CI	0.036^{NS}	3.897***	51.1***	3.37***	33.68***	0.09
	TRS	2.2^{NS}	313.8***	6,033.9***	251.0***	3,602.6***	1.8
	AUDPC	4.1^{NS}	93.5***	1,113.4***	82.1***	721.0***	2.17
	DPR	0.0001^{NS}	0.0003***	0.004***	0.0001***	0.0043***	0.002
Meraro	CI	0.123^{NS}	5.72***	48.4***	5.29***	73.2***	0.33
	TRS	19.3^{NS}	633.2***	6,918.9***	5,734.2***	2,106.4***	3.56
	AUDPC	7.79^{NS}	172.5***	1,410.4***	159.6***	715.8***	1.78
	DPR	0.00003^{NS}	0.0003***	0.0011***	0.0003***	0.0007***	0.003
	HI	110.1^{NS}	498.3***	5,350.8***	505.8***	2,040.2***	7.5

Key: DF=Degree of Freedom, CI=Coefficient of Infection, TRS=Terminal Severity, AUDPC=Area Under Disease Progress Curve, DPR=Disease Progress Rate, SI=Spike Infection, NS=Non Significant, *** Significant at P<0.001

Table 3: Pearson linear correlation coefficients among stripe rust disease parameters and thousand kernel weight in Ethiopian wheat landraces at Kulumsa and Meraro in 2017.

Location	Parameter				
	Parameter	CI	TRS	AUDPC	DPR
Kulumsa	TRS	0.989***			
	AUDPC	0.987***	0.986***		
	DPR	0.949***	0.969***	0.870***	
	TKW	-0.504***	-0.403***	-0.410***	-0.375***
Meraro	TRS	0.996***			
	AUDPC	0.999***	0.980***		
	DPR	0.903***	0.965***	0.886***	
	TKW	-0.643***	-0.641***	-0.662***	-0.590***
	SI	0.669***	0.608***	0.601***	0.546***

Key: CI=Coefficient of Infection, TRS=Terminal Rust Severity, AUDPC=Area Under Disease Progress Curve, DPR=Disease Progress Rate, TKW=1000 Kernel Weight, SI=Spike Infection of Wheat by Stripe Rust, *** Significant at P<0.001.

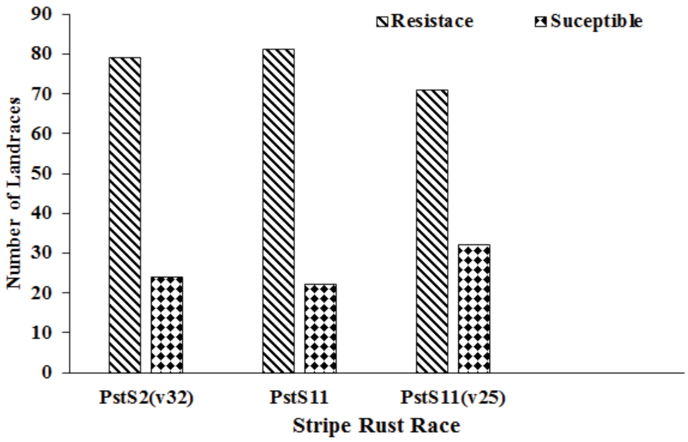

Figure 5: Frequency distribution of resistant and susceptible wheat landrace accessions to three stripe rust races at seedling stage.

and (*PstS11 v25*), respectively (Figure 5). Sixty one (59%) and 11 (11%) landraces showed resistance and susceptible reaction to all of the three isolates, respectively whereas 31 (30%) reacted differently.

A total of 103 wheat landraces evaluated at adult plant growth stages and of these, 57 (29%) exhibited lower values for all disease parameters under field conditions. Thirty-two (16%) landraces exhibited resistance at seedling (to all isolates) and adult plant growth stage whereas 14 (7%) showed susceptible/intermediate reaction at seedling stage but had low disease level for all parameters under field conditions.

Wheat genotypes could be susceptible at seedling tests but exhibit moderate resistance to moderate susceptible reaction at adult plant stage and these lines with show slow rusting resistance parameters at adult plant stage could have durable resistance [27]. This kind of resistance can be kept for a long time even if pathogen changes its genotype, it is controlled by more than one gene [28]. Generally, the seedling resistance genes are also active during the adult plant stage and they are classified into race-specific resistance types [29].

CONCLUSION

Most of the commercial wheat cultivars in Ethiopia succumb to new races of stripe rust in hot spot areas. In the depletion of resistance in the cultivated wheat, new sources are sought from landraces. In this study, a total of 197 wheat landrace accessions (152 bread and 45 durum) obtained from the Ethiopian institute of biodiversity (IBC) have been tested to stripe rust during 2017 and 2018 whereas only selected 103 accessions were exposed to the prevailing *Pst* races both in the field and at seedling stage in the greenhouse 2018. Overall, 57 accessions exhibited resistance in the field across locations and seasons. Of these, 32 and 9 accessions showed resistant and susceptible reaction to the three *PSt* races at seedling stage, respectively. This study has shown that Ethiopian wheat landraces are potential sources of overall (seedling) and adult plant resistances (APR) to stripe rust.

AUTHOR CONTRIBUTIONS

The 2nd authors have been engaged in initiation and supervision of the study. They were also involved in writing of this manuscript.

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CONFLICTS OF INTEREST

No conflict of interest to be declared.

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