



SOURCES OF RESISTANCE TO STEM RUST (*Puccinia graminis* f. sp. *tritici*) IN IMPROVED DURUM WHEAT (*Triticum turgidum* L.) VARIETIES OF ETHIOPIA

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ABSTRACT

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Stem rust disease caused by *Puccinia graminis* f. sp. *tritici* (Eriks and E. Henn) possess the greatest threat to global wheat production due to continuously producing new races that can attack previously resistant varieties. This investigation was therefore conducted under greenhouse and field conditions by evaluating durum wheat (*Triticum turgidum* L.) cultivars along with a susceptible check for their resistance to stem rust during the 2017 main cropping season. Greenhouse evaluation was conducted at Ambo for race TTTTF. The field experiment was undertaken at Adet and Debre-Tabor in a randomized complete block design (RCBD) with three replications. The spreader row Morocco was inoculated with a virulent race (TTTTF) at stem elongation. Field resistance at the adult plant stage was assessed through final disease severity (FRS), coefficient of infection (CI), and area under disease progressive curve (AUDPC). Durum wheat cultivars Mettaya and Oda were found to be resistant both at the seedling and post seedling stages suggesting resistance confer by a major gene. Whereas Bakalcha, Lelisso, Ilani and Yerer showed low disease severities <30 with lower AUDPC values (<500) and CI (<20) and were identified to have a good level of field resistance for stem rust population present at Adet and Debre-Tabor. High correlation coefficients were observed between stem rust resistance parameters. Among the cultivars having a good level of resistance Lelisso, Bichena and Bakalcha produce high yields with heavy kernel weight in both locations. The resistance cultivars identified from the present study can be used for further wheat improvement programs.

Contribution/Originality: This study is one of the very few studies which have investigated the sources of resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) in improved durum wheat (*Triticum turgidum* L.) varieties of Ethiopia.

1. INTRODUCTION

Wheat is the most important food grain for billions of people worldwide, being cultivated on 17% of the world cultivated land [1]. It covers around 30% of global grain production and 44% of cereals used as food [2]. Of the wheat species, durum wheat (*Triticum turgidum* subsp. durum, 2n=4x=28, AABB) is the second most cultivated species after common wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD) [3]. It represents 12% of the total global

wheat production [4]. Globally, the total area covered by durum wheat is estimated at 17 million hectares (ha) [5]. In Ethiopia, two types of wheat (bread and durum wheat) are cultivated under rain-fed conditions [6]. Among cultivated land, 1.7 million hectares are covered by wheat with a production of 4.23 million tons [7]. In-country level there is no recent statistical data on the proportion of durum wheat to the total wheat area; however, it is estimated to constitute about 20% of the total wheat area [8].

In Ethiopia, the actual mean wheat yield is around 2.5 t/ha [7] which is by far below the world's average. The low wheat yield in Ethiopia could be attributed to biotic and abiotic factors [9]. Amongst biotic factor stem rust of wheat caused by the fungus *Puccinia graminis* f.sp. *tritici* (pgt) is the most destructive disease [10]; which can cause up to 100% yield loss if a susceptible cultivar is grown under a favorable environment [11].

Various management options are available to minimize losses caused by stem rust including cultural, biological, and chemical methods [11]. Fungicides remain an option for emergency control but, their large-scale use by small farmers is neither feasible nor economical. Recently stem rust management most likely lies in the cultivation of resistant wheat varieties [12] which is the most economical and environmentally friendly management strategy to control the disease. Starting from the middle of 1950s, stem rust resistance genes were identified within common wheat and wild relatives [13] and successfully deployed in commercial wheat cultivars worldwide. However, resistant varieties with acceptable levels of disease resistance often rapidly succumb to the disease soon after release due to the continuous evolution of new races [14]. For instance the evolution of race TTKSK (Ug99) and its variant in 1999 attack approximately 90 % of the world's contemporary wheat cultivars [15] with yield losses of more than 71% in experimental fields [16]. Similarly, a severe localized stem rust epidemic was reported in the southern parts of the country during 2013/2014 cropping season after large scales production of cultivar Digalu on farmer's field [17]. The primary cause of the epidemic was race TKTTF (Digelu race) [17].

The East African highlands in general and Ethiopian highlands, in particular, are considered as a hot spot area for the development of stem rust [18]. Studies in the country reported that most previously identified samples indicate high virulence diversity with different virulence profiles within the Pgt population present in Ethiopia [18-21]. According to Olivera, et al. [17] only 7 cultivars were resistant at the seedling stage for race TTKSK and TKTTF from all the released 66 Ethiopian bread wheat cultivars. Thus, all these may show that there was a potential danger of resistance breakdown in existed wheat cultivars. Therefore, evaluation of durum wheat varieties against stem rust races at both growth stages was mandatory to identify cultivars having durable resistance and simultaneously to remove susceptible cultivars from production. Hence the present study was designed to identify durum wheat cultivars resistant to wheat stem rust and draw possible recommendations for further wheat improvement research and development interventions related to stem rust resistance.

2. MATERIALS AND METHODS

2.1. Seedling Resistance Test

Twenty four improved durum wheat varieties Table 2 were evaluated against virulent stem rust races TTTTF (Ug99) for their resistance at the seedling stage at Ambo Plant Protection Research Center (APPRC) in 2017/18. Five seeds of each of the durum wheat cultivars, along with the susceptible check cultivar McNair were raised in 5 cm diameter plastic pots separately. A complete randomized design (CRD) with two replications was used. Seedlings were inoculated at 2-3 leaf stage with the spores of the virulent race adjusted to 4mg spores per 1ml lightweight mineral oil (Soltrrol 170) suspension using a spore inoculator. Thereafter inoculated plants were moistened with fine droplets of distilled water by using atomizer after twenty minutes of inoculation and placed in dew chamber for 18 hrs in darkness at 18-22 °C and 98-100% relative humidity. Upon removal from the dew chamber, seedlings were exposed to 3 hr of fluorescent light to dry dew on the leaves. Following this, inoculated plants were transferred to greenhouse benches where the temperatures were kept between 18 and 25°C and the

relative humidity at 60-70% for 14 days [22]. Data on infection types Table 1 were recorded 14 days after inoculation from leaves using 0-4 scale [23].

Table-1. Major infection type classes for stem rust.

Infection type	Host response	Symptoms
0	Immune	No visible uredia
0;	Very resistant	Hypersensitive flecks
1	Resistant	Small uredia with necrosis
2	Resistant to moderately Resistant	Small to medium sized uredia with green islands and surrounded by necrosis or chlorosis
3	Moderately resistant/moderately susceptible	Medium sized uredia with or without Chlorosis
4	Susceptible	Large uredia without chlorosis
X	Resistant	Heterogeneous, similarly distributed over the leaves

Note: Infection types based on Stakman, et al. [23] scale for seedling resistance.

2.2. Field Experiment

The experiment was conducted at Adet and Debre-Tabor agricultural research stations, where wheat is commonly grown. Adet is found at an altitude of 2240m above sea level. The station receives an annual mean rainfall of 869 mm with an average annual temperature of 18.56. Whereas another research site Debre -Tabore is found 2591m above sea level and receives an average annual rainfall of 1102.7mm with 15.48°C average annual temperature.

A total of twenty-four durum wheat cultivars Table 2 collected from different agricultural research centers along with susceptible check Arendeto were laid in a randomized complete block design (RCBD) with three replications. Each plot had a size of 1.5 m long and 1 m width containing five rows with a spacing of 20 cm. The spacing between plots and reps were 40 cm, and 1.5 m, respectively. The recommended seeding rate of 150kg/ha was used for both locations. Recommended fertilizer rate 138/46 kg, N/P₂O₅ for Debre-Tabor and 92/46 kg N/P₂O₅ for Adet were applied. Weeds were managed through hand weeding. Seeds of all genotypes were planted 15 days after the regular sowing date to expose plants to a suitable environment of rust incidence and development. Universally susceptible Morocco was planted as border rows of experimental plots 7 days before the test varieties to facilitate rust epidemic. Plants in the spreader rows were inoculated with urediniospores of virulent race maintained at the Ambo Plant Protection Research Center. A water suspension of these stem rust urediniospores was inoculated onto spreader rows using an ultralow volume sprayer to generate fine mist. This took place twice when the spreader row reaches stem elongation.

Ten plants per plot were randomly tagged from the central rows and disease severity was assessed four times at every 10 days interval on the tagged plants, based on modified Cobbs' scale where 0% = immune and 100% = completely susceptible [24]. Terminal severity was score at near maturity stage of the crop.

The disease severities recorded at different time were utilized for the calculation of AUDPC for each cultivar using the formula below [25, 26]:

$$AUDPC = \sum_{i=1}^{n-1} 0.5(x_{i+1} + x_i)(t_{i+1} - t_i)$$

Where, x_{i+1} is the cumulative disease severity expressed as a proportion at the i^{th} observation; t_i is the time (days after planting) at the i^{th} observation and n is total number of observations.

Coefficients of Infection (CI) were calculated by taking into account the severity of stem rust of the cultivars and their infection response [22]. The reaction type was record according to the description of Roelfs, et al. [27]. The scores were converted into coefficients of infection by multiplying the percentage severity and a constant assigned for host response: where immune = 0.0, R= 0.2, MR= 0.4, MR-MS = 0.6, MS = 0.8, MS-S = 0.9 and S= 1.0.

The disease severity observations recorded at 10 days interval were regressed over time and the apparent infection rates or the coefficient of the regression line for each plot was calculated [28].

$$\text{Inf-rate} = \ln [X / (100 - X)]$$

Where X is average coefficient infection plotted against time in days [28].

Table-2. Description of durum wheat cultivars used for the study.

S/N	Cultivar	Released by	Released year
1	Utuba	DZARC	2015
2	Bichena	DZARC	1995
3	Mangudo	SARC	2012
4	Mekuye	DZARC	2012
5	Toltu	SARC	2010
6	Hitosa	DZARC	2009
7	Denbi	DZARC	2009
8	Werer	DZARC	2009
9	Tati	SARC	2009
10	Filakit	SRARC	2007
11	Obsa	SARC	2006
12	Ejersa	SARC	2005
13	Bakalcha	SARC	2005
14	Kokate	AWARC	2005
15	Malefia	SRARC	2005
16	Oda	SARC	2004
17	Ilani	SARC	2004
18	Megenagna	ADARC	2004
19	Mosobo	ADARC	2004
20	Mettaya	ADARC	2004
21	Selam	ADARC	2004
22	Yerer	DZARC	2002
23	Lelisso	SARC	2002
24	Ude	DZARC	2002
25	Arendeto	Susceptible check	

Note: Ministry of Agriculture (MoA) [29].

2.3. Data Analysis

Green house evaluation was analyzed by using descriptive statistics. All the data generated from field experiments were subjected to ANOVA following the procedure described by Gomez and Gomez [30] for one factor complete randomized block design (RCBD) using the PROC GLM procedure of SAS 9.2 statistical software [31] to determine significant differences among cultivars. Duncan multiple range test was used to compare the significant means. Correlation was done using SPSS software [32] version 16 to determine the relationship between disease parameters.

3. RESULTS AND DISCUSSION

3.1. Results of Seedling Reaction

The greenhouse experiment revealed that the tested durum wheat cultivars differed in their reaction to the stem rust isolates of TTTTF Table 3. Of the 24 Ethiopian durum wheat cultivars, no complete resistance "0" was observed. However, 17 cultivars exhibiting resistant (IT's of ";", "1", "2" or combinations), to isolates of race TTTTF. Three cultivars (Mangudo, Obsa and Ejersa) showed susceptible infection types (3-) for the tested race. The susceptible infection types (3-, 3+) of the check Arendato and McNair indicates effectiveness of inoculation. While, some cultivars including Hitosa, Denbi, Tati and Mosobo were noted having a heterogeneous reaction "x".

The low infection types scored on most of the cultivars against these races could be due to the presence of one or more major Sr-genes. This result is in agreement with Ogutu, et al. [33] who reported cultivars that exhibited low infection types at seedling stage could be either due to one or more of the Sr-genes or a combination that had

similar infection type pattern towards the races. Major gene resistance/seedling resistance can offer complete protection and significant economic benefits to farmers [34]. Therefore these cultivars can be used as sources of stem rust resistance when the aim of the breeding program is for major gene. However, stem rust resistance at the seedling stage may not be indicative of the reaction at the adult plant stage because some genes are effective only at specific growth stages Ogutu, et al. [33] and cultivars that exhibited high infection type may display minor gene resistance at adult plant stage [34]. Therefore, combining seedling resistance with adult plant resistance in the field will provide valuable indications to select resistance cultivars.

3.2. Field Experiment

3.2.1. Categories of Cultivars Based on Seedling and Adult Plant Reaction

Based on infection types observed at seedling and adult plant stages cultivars were categorized into four groups according to Safavi and Afshari [35] as follows: The first group included two cultivars (Oda and Mettaya) that were resistant to stem rust both at the seedling and post seedling stages. This group most probably carried a major gene(s) that were effective against all the pathotypes present in the test site. Low disease development on the tested cultivars in each location presenting evidence that seedling resistance was effective under field conditions as these cultivars displayed significantly low FRS [36]. Cultivars with R genes are not durable in agriculture because of new virulent race would arise that overcame single resistance genes in race-specific resistance varieties [37].

The second group included five cultivars (Obsa, Ejersa, Mangudo, Denbi and Mosobo) which had susceptible and mixed reactions to stem rust at the seedling stage but moderately resistant to intermediate (MR or MRMS) or moderately susceptible (MS) at the adult plant stage on both locations. This result is in agreement with [36] cultivars susceptible at the seedling stage expressed different levels of slow rusting in field tests. This kind of resistance in some cultivars cannot alter with variability in climatic conditions coupled with pathogen population variability. Slow rusting through many gene resistances was assumed to be more durable as compared to resistance conditioned by single major resistance genes [37].

The third group included only susceptible check Arendeto that was susceptible to stem rust both at the seedling and adult plant stages (MSS or S). This group lacked effective race-specific and non-race-specific resistance gene(s) towards the race(s) populations present at the test site.

The fourth group comprises 17 cultivars (Kokate, Toltu, Lelisso, Ude, Yerer, Selam, Megenagna, Ilani, Bakalcha, Denbi, Werer, Tati, Filakit, Mekuye, Utuba, Malefia and Bichena) that were resistant at the seedling stage but MR/MRMS to MSS/S at the adult plant stage. Some of these cultivars including Malefia may lack adult plant resistance genes. In accordance with this Fekadu, et al. [38] reported durum wheat varieties Kilinto and Gerardo were resistant at the seedling stage but were susceptible at the adult stage. However, cultivars that exhibited resistance at the seedling stage but MR/MRMS field reaction with statistically low stem rust disease severities have a possibility of containing other stem rust resistance genes in their background that are responsible for reduced disease severity [39].

According to Singh, et al. [40] cultivars with compatible host-pathogen interaction may possess field resistance. Therefore, low disease development with high infection response on the tested cultivars in each location presenting evidence that the presence of partial resistance in addition to race specific resistance. In harmony with this Figlan, et al. [41] reported of the tested Kenyan cultivar (Paka) carry the APR *Sr2* gene with race specific resistance gene which exhibited resistance reaction at seedling stage but high infection response at adult stage. Generally, according to Chen, et al. [42] the resistance confers at field conditions may provide either due to race-specific or race nonspecific and /or combination of both resistance. However, it is difficult to distinguish race nonspecific resistance from the resistance conferred by genes of race-nonspecific nature based on the adult plant infection type. Therefore, evaluating cultivars by using parameters such as disease severity, area under disease progress curve or the measurement of apparent infection rates and coefficients of infection values is important.

3.2.2 Final Rust Severity

Results of ANOVA for final rust severity showed a highly significant difference at $p < 0.01$ among the tested Ethiopian commercial durum wheat cultivars evaluated at Adet and Debre-Tabor. The highest mean final stem rust severities were recorded on cultivar Malefia 72.5 % and susceptible check Arendeto (71.0%) at Debre-Tabor and Adet respectively but terminal severity did not exceed 30% on cultivar Bakalcha, Lelisso, Ilani, Mettaya, Oda, Yerer and Bichena at both locations. Diverse field reactions ranging from resistance (R) to moderately resistant (MR) and moderately resistance to moderately susceptible (MR-MS) to susceptible (S) responses were observed. According to Herrera-Foessel, et al. [43]; Hei [44] variation in levels of resistance could be attributed to differences in the number of resistance genes present and mode of gene action. Besides, variation in final stem rust severity suggested that the genotypes had diverse genetic backgrounds [45].

Final rust severity represents the cumulative result of all resistance factors during the progress of epidemics [46]. Terminal severity is a measure of the disease potential under existing environmental conditions Roelfs, et al. [27]. Previously, Ali, et al. [47]; Safavi, et al. [48]; Saleem, et al. [49] and Hei [50] also used final rust severity to assess partial resistance behavior of wheat cultivars and lines. Similarly, based on FRS the tested cultivars were grouped into three groups of high, moderate and low levels of partial resistance having 1-30%, 31-50% and 51-70% of FRS respectively.

In the present study, all the cultivars except malefia tested at Debre-Tabor showed disease severities of up to 30%; whereas at Adet only seven cultivars (Bakalcha, Lelisso, Ilani, Mettaya, Oda, Yerer and Bichena) displayed up to 30% FRS Table 3. Of these Yerer and Bichena showed resistance reaction at seedling stage but compatible host pathogen interaction at adult plant stage Table 3. According to Singh, et al. [40]; Nzuve, et al. [39] cultivars that display compatible final infection response may possess slow rusting. Therefore, the cultivars that displayed up to 30% FRS may possess highly effective race-nonspecific resistance genes with race specific resistance gene effective towards race TTTTF.

At Adet fourteen cultivars (Selam, Filakit, Mangudo, Ude, Megenagna, Ejersa, Mekuye, Werer, Kokate, Mosobo, Toltu, Obsa, Denbi and Utuba) displayed FRS of 31-50% with compatible host pathogen interaction at adult plant stage. Therefore, cultivars displayed a moderate level of severity with the absence of hypersensitive flecks at seedling stage may suggest that miner gene resistance confer a moderate level of field resistance. In the remaining four cultivars (Hitosa, Tati, Malefia and susceptible check Arendeto) the final disease severities (FRS) observed was more than 50%.

In fact, cultivars that grouped under a high level of field resistance at Debre-Tabor were grouped under moderate and low levels of field resistance at Adet. This is because varieties that are resistant to stem rust in one year or one location may be susceptible in another year or location depending upon the virulence of the pathogen [51]. Variability in the host response could be attributed to the variability in the resistance expression due to pathogen population variability, coupled with variability in climatic conditions Ali, et al. [47]. Strange [52] suggests that temperature may have significant effects on the expression of resistance genes. The gene *Sr6*, is effective at lower temperatures less than 20°C but ineffective at 24-27°C. Similarly, the gene *Sr10* and *Sr12* is effective at lower temperatures; whereas the gene *Sr14* is effective under high temperature and high light conditions [53]. In addition virulence capacity of pathogen affected by temperature changes [27].

Table-3. Seedling reaction, adult plant response, FRS, CI, AUDPC and Inf-rate of durum wheat cultivars to stem rust at both locations.

Variety	Seedling reaction	Adet					Debre-Tabore				
	TTTTF	FRS	FR	CI	AUDPC	Inf-rate	FRS	FR	CI	AUDPC	Inf-rate
Utuba	2 ⁺	49.33 ^{ab}	MS	39.47 ^{abcd}	791.5 ^{abc}	0.148 ^{abcd}	5.07 ^{fe}	MR	2.03 ^{ghij}	57.0 ^{hij}	0.048 ^{cdefg}
Bichena	;2 ⁻ c	21.30 ^{de}	S	21.33 ^{efgh}	318.7 ^{de}	0.114 ^{defg}	2.33 ^{hg}	MRMS	1.40 ^{hij}	33.3 ^{jk}	0.024 ^{gh}
Mangudo	3 ⁻	36.60 ^{abcd}	MRMS	22.00 ^{bcdefg}	529.5 ^{cd}	0.139 ^{bcde}	2.33 ^{hg}	MRMS	1.40 ^{hij}	31.7 ^{ik}	0.031 ^{gh}
Mekuye	2	40.30 ^{abcd}	MRMS	24.20 ^{abcd}	618.3 ^{cbd}	0.138 ^{bcde}	10.00 ^{cde}	MRMS	6.00 ^{ef}	166.0 ^{cdef}	0.047 ^{cdefg}
Toltu	2 ⁺	44.60 ^{abc}	MS	35.73 ^{abcde}	681.3 ^{abc}	0.155 ^{abc}	9.50 ^{cde}	MSS	8.55 ^{cde}	168.2 ^{cdef}	0.051 ^{bcdefg}
Hitosa	;2 ⁺ 3 ⁻	55.60 ^{abc}	MSS	50.10 ^{abc}	894.3 ^{abc}	0.159 ^{abc}	16.83 ^{bc}	MSS	15.15 ^c	263.2 ^{cd}	0.048 ^{cdefg}
Denbi	2 ⁺ 3 ⁻	48.00 ^{abc}	MRMS	28.80 ^{abcdef}	646.8 ^{bc}	0.151 ^{abcd}	11.20 ^{cd}	MS	8.96 ^{cde}	181.7 ^{cdef}	0.045 ^{cdefg}
Werer	2	40.30 ^{abcd}	MR	16.13 ^{defgh}	566.2 ^{bcd}	0.146 ^{abcd}	8.17 ^{cde}	MS	6.54 ^{efgh}	117.8 ^{fg}	0.077 ^{bcd}
Tati	2 ⁺ 3 ⁻	62.00 ^{ab}	MSS	55.80 ^{ab}	743 ^{abc}	0.180 ^a	11.50 ^{cd}	S	11.50 ^{dc}	224.2 ^{cde}	0.067 ^{bcde}
Filakit	2	32.60 ^{bcd}	MSS	29.40 ^{bcdefg}	505.7 ^{bcd}	0.127 ^{defg}	3.67 ^{fg}	MRMS	2.20 ^{fghij}	41.7 ^{ijk}	0.026 ^{gh}
Obsa	3 ⁻	46.30 ^{abc}	MRMS	27.80 ^{abcdef}	724.5 ^{abc}	0.158 ^{abc}	15.67 ^c	MS	12.54 ^{cd}	300.0 ^c	0.044 ^{defg}
Ejersa	3 ⁻	40.00 ^{abcd}	MRMS	16.00 ^{bcdefg}	567.5 ^{bcd}	0.143 ^{abcd}	9.33 ^{cde}	MS	7.46 ^{de}	161.0 ^{cdef}	0.065 ^{bcdef}
Bakalcha	;1 ⁺	8.40 ^g	MS	6.72 ⁱ	98.7 ^g	0.067 ^h	4.67 ^{ef}	MR	1.87 ^{fghij}	64.7 ^{ghi}	0.028 ^{gh}
Kokate	2 ⁺	41.00 ^{abcd}	MSS	36.90 ^{abcde}	649.3 ^b	0.137 ^{bcde}	8.33 ^{cde}	MS	6.66 ^{efghi}	137.3 ^{def}	0.048 ^{cdefg}
Malefia	2 ⁻	62.60 ^{efg}	S	62.67 ^a	1040.5 ^{ab}	0.167 ^{ab}	72.50 ^a	S	72.50 ^a	1275.8 ^a	0.113 ^a
Oda	;1 ⁺	14.60 ^{efg}	MR	11.73 ^{ghi}	235.5 ^{ef}	0.103 ^{efgh}	2.33 ^{hg}	R	2.33 ^j	31.7 ^{jk}	0.031 ^{gh}
Ilani	2	10.10 ^{fg}	MRMS	6.06 ⁱ	161.8 ^{ef}	0.096 ^{fgh}	2.33 ^{hg}	MR	0.93 ^{ij}	33.3 ^{jk}	0.024 ^{gh}
Megenagna	2c	39.30 ^{abcd}	MS	31.47 ^{abcde}	645.3 ^{bc}	0.135 ^{abcde}	7.07 ^{def}	MS	5.66 ^{efghi}	119.0 ^{fg}	0.054 ^{bcdefg}
Mosobo	2 ⁺ 3 ⁻	42.30 ^{abcd}	MS	33.87 ^{abcdef}	622.7 ^{bcd}	0.136 ^{bcde}	11.33 ^{dc}	MS	9.06 ^{cde}	136.7 ^{def}	0.077 ^{bc}
Mettaya	2 ⁻	14.00 ^{ef}	MR	8.40 ^{hi}	232.3 ^{ef}	0.097 ^{fgh}	2.33 ^{gh}	R	0.47 ^j	31.7 ^{jk}	0.031 ^{gh}
Selam	;1 ⁺	31.30 ^{dc}	MS	25.07 ^{bcdefgh}	455.7 ^{cd}	0.124 ^{cdef}	6.83 ^{edf}	MSS	6.15 ^{efg}	117.5 ^{efg}	0.042 ^{efg}
Yerer	2 ⁻	15.00 ^{ef}	MS	12.00 ^{fghi}	209 ^{ef}	0.103 ^{efgh}	6.40 ^{edf}	MS	5.12 ^{efghij}	116.3 ^{fgh}	0.032 ^{fgh}
Lelisso	;1 ⁺	9.30 ^{fg}	MRMS	5.60 ⁱ	142.2 ^{fg}	0.083 ^{hg}	1.00 ^h	MR	0.40 ^j	25.0 ^k	0.000 ^h
Ude	2	38.30 ^{abcd}	S	38.33 ^{abcdef}	506.8 ^{bcd}	0.146 ^{abcd}	12.83 ^{cd}	MS	10.26 ^{cde}	161.8 ^{def}	0.067 ^{bcde}
Arendeto	3 ⁻	71.00 ^a	S	56.80 ^{abc}	1369.7 ^a	0.172 ^{ab}	30.00 ^b	MSS	27.00 ^b	585.0 ^b	0.082 ^b
McNair	3 ⁺										
Sig. level		**		**	**	**	**		**	**	**
CV(%)		10.3		15.25	6.15	14.6	16.8		18.5	7.67	35.7

ITs of 1, 2 = resistant and 3, 4 = susceptible. Positive (+) = larger uredinia than the normal size; Negative (-) = smaller uredinia than the normal size; FR= Field reaction; MR= moderately resistant; MS = moderately susceptible; MRMS = moderately resistant to moderately susceptible; MS-S = moderately susceptible to susceptible; S= susceptible; FRS= final rust severity; CI= coefficient of infection; AUDPC = area under disease progress curve and Inf-rate = infection rate; ** represent highly significance difference at P < 0.01; Means with the same letter(s) within a column are statistically similar at p=0.01.

3.2.3. Coefficient of Infection (CI) Value

The data on disease severity and host reaction were combined to calculate the coefficient of infection (CI) [54]. Results of ANOVA for coefficient of infection showed a highly significant difference at $p < 0.01$ among the tested Ethiopian commercial durum wheat cultivars at both locations. This shows that the presence of diversity among tested cultivars along with the environment. Based on CI value [47] classified cultivars in three groups; i.e cultivars having 0–20, 21–40, and 41–60 CI values were considered as holding high, moderate, and low levels of partial resistance, respectively. In the present study, all the test cultivars except Arendeto and Malefia showed CI values between 0 and 20 at Debre-Tabor; whereas at Adet eight cultivars (Mettay, Oda, Lelisso, Ilani, Bakalcha, Yerer, Ejersa and Werer) exhibited CI values in between 0 and 20 Table 3 and were designated as having a high level of adult plant resistance for stem rust population present at prescribed area.

Thirteen cultivars (Bichena, Mangudo, Mekuye, Selam, Obsa, Denbi, Filakit, Megenagna, Mosobo, Toltu, Kokate, Ude and Utuba) displayed CI values of 21–40 and marked as possessing a moderate level of adult plant resistance for stem rust population present at Adet. While two cultivars Hitosa and Tati showed CI values ranged from 41 to 60 Table 3 and marked as the final group having a low level of adult plant resistance. However, cultivars Malefia and Arendeto were noted having CI values greater than 60 in both locations and were grouped as susceptible. As indicated by Safavi, et al. [48] CI values greater than 60 recorded on the susceptible check shows that disease pressure was considerably sufficient in the season. Previously, Draz, et al. [55] reported the presence of different level of partial resistance among tested cultivar. The CI data obtained from combination of final rust severity and field reaction recording on 25 cultivars are shown in Table 3.

3.2.4. Area under Disease Progress Curve (AUDPC)

Data pertaining AUDPC values of durum wheat cultivars is given in (Table 3). Analysis of variance result showed highly significance difference at $p < 0.01$ between cultivars in AUDPC value at both locations. At Adet the highest AUDPC value was recorded on Arendeto (1369.7); whereas at Debre-Tabor the highest AUDPC value was noted on Malefia (1275.8). However, cultivar Bakalcha (98.7) and Lelisso (25) were better in reducing mean AUDPC value at Adet and Debre-Tabor respectively. According to Ali, et al. [47] and Saleem, et al. [49] cultivars with AUDPC values of 1–500, 500–800, and 800–1100 were categorized as possessing high, moderate, and low levels of partial resistance respectively. The tested cultivars were categorized into three distinct groups for partial resistance, based on their AUDPC values. At Debre-Tabor all the cultivars except Arendato and Malefia showed AUDPC value less than 300; whereas at Adet eight cultivars (Bakalcha, Lelisso, Ilani, Yerer, Bichena, Oda, Mettaya and Selam) showed AUDPC value up to 500 Table 3 were grouped as having a high level of partial resistance.

Correspondingly, at Adet, fourteen durum wheat cultivars exhibiting AUDPC values ranging from 500 to 800 were grouped as moderately level of partial resistance, which included Filakit, Ude, Mangudo, Werer, Ejersa, Mekuye, Mosobo, Megenagna, Denbi, Kokate, Toltu, Obsa, Tati and Utuba. While cultivar Hitosa and Malefia showed 894.3 and 1040.5 Table 3 and marked as having a low level of adult plant resistance. Likewise cultivar Arendato showed AUDPC value greater than 1100 at Adet and considered as susceptible to stem rust population present in the area. Previously, Denbel, et al. [56] confirmed cultivar Arendato was susceptible to Ug99 variants.

AUDPC is a good indicator of adult plant resistance under field conditions [57]. It is directly related with level of resistance and yield loss [58]. In quantitative resistance, where differences in level of resistance are usually less distinct, measuring disease progress is important for understanding plant–pathogen interaction [59]. Furthermore, AUDPC in particular is the result of all factors that influence disease development such as differences in environmental conditions, varieties and population of the pathogen [40]. Varieties that had low AUDPC and FRS may have a good level of adult plant resistance [57]. Therefore, selection of cultivars having

low AUDPC with terminal disease scores is normally accepted for practical purposes where the aim is to utilize slow rusting resistance as one of the stem rust management mechanisms [60].

3.2.5. Apparent Infection Rate

Analysis of variance (ANOVA) for apparent infection rate showed a highly significant difference at $p < 0.01$ among tested cultivars at both environments. At Adet the fastest disease multiplication was noted on Tati (0.180) followed by Arendeto with 0.172 infection rates; whereas at Debre-Tabor the maximum rate of disease development (0.113) was noted on cultivar Malefia followed by Arendato (0.082). Cultivar Lelisso showed a constant disease severity, thus showed no disease increase per unit time with an inf-rate of 0 at Debre-Tabor. On the other hand, Bakalcha exhibited slow rate of disease development (0.067) at Adet Table 3.

Plants differ in their ability to either reduce or increase the rate of disease development for a particular pathogen [61]. Rate of disease development can be quantified through regressing several observations (logit x) of a disease symptom over time [62]. Meanwhile, the progress of disease symptoms is parallel to the progress of inoculum production. Therefore pathologists use “Inf-rate” to measure the aggressiveness of pathogen or the resistance/susceptibility range of the host [63]. However, there is no clear information to categorize cultivars based on infection rate.

The present study evaluate cultivars based on FRS, CI and AUDPC value results infection rate of less than 0.12 had a better level of partial resistance. Cultivars with a moderate level of partial resistance, with regard to other parameters, had infection rates ranging from 0.126 to 0.16. In contrast to this Safavi, et al. [48] reported cultivars having infection rate of (0-0.057) and (0.065-0.086) ranked as high and moderate level of partial resistance. Additionally, Safavi and Afshari [35] reported cultivars having infection rates less than 0.09 comprise better level of partial resistance. Therefore, it lacks consistency. In spite of its importance to quantify the rate of disease development on the cultivars tested using infection rate to estimate partial resistance seemed unreliable when compared with FRS, CI and AUDPC because it could not identify different levels of partial resistance among the tested cultivar. Similarly, Hei [50] and Ali, et al. [64] reported infection rate doesn't yield consistent estimation for their study.

3.2.6. Correlation between Slow Rusting Parameters of Wheat Stem Rust

During this investigation, attempts were made to elucidate the relationship between field-based slow rusting parameters. The Pearson correlation coefficient analysis showed highly significant and positive correlation coefficients between the disease parameters in both locations Table 4. A positive and highly significant correlations of FRS with AUDPC ($r = 0.959$), CI ($r = 0.896$) and Inf rate (0.909) were found at Adet. Significant and positive correlation coefficients of 0.986, 0.994 and 0.668 were also observed between FRS and AUDPC, CI and Inf rate at Debre-Tabor respectively.

The highest correlation coefficient was between final rust severity and AUDPC in both locations ($r = 0.959$) at Adet and ($r = 0.986$) Debre-Tabor. This indicating that cultivars that were severely infected showed higher AUDPC values [58]. This positive correlation was in agreement with the results of other researchers on cereal-rust pathosystems [35, 48, 50, 65]. Averagely the lowest r -value was obtained between infection rate and other parameters Table 4. This indicates that diminishing of disease progress rate over time with expanding of rust disease. This can be due to less availability of healthy plant tissue for additional infections [66].

Table-4. Pearson correlation coefficient of pair-wise relationships between slow rusting parameters for stem rust of wheat evaluated over two locations.

Parameter	Adet			Debre-Tabor		
	FRS	AUDPC	CI	FRS	AUDPC	CI
AUDPC	0.959**			0.986**		
CI	0.896**	0.881**		0.994**	0.975**	
Inf-rate	0.909**	0.837**	0.793**	0.668**	0.620**	0.649**

Note: FRS = final rust severity; AUDPC = relative area under disease progress curve; Inf-rate = infection rate; CI - coefficients of infection; **. Correlation is significant at the 0.01 level (2-tailed).

Furthermore, highly significant and positive correlation coefficients observed between FRS, CI and AUDPC suggested, these parameters were reliable estimators in assessing partial or slow rusting resistance of different cultivars to wheat rust diseases. These epidemiological parameters give a dependable rate of disease increase and are related with components of partial resistance like low receptivity, longer latent period and smaller pustules [67].

Therefore, selection of cultivars based on lower values of final disease score, coefficient of infection and AUDPC provides a sound basis for identifying cultivars having durable resistance. Accordingly, wheat cultivars Bakalcha, Lelisso, Ilani, Yerer, Mettaya, Oda and Bichena showed AUDPC values below 500, final disease scores of less than 30 and CI between 0-20 with varied field responses (R to MS) in both locations. All these cultivars were posed resistance at the time of seedling reaction test towards race TTTTF. Therefore, the cultivars that had seedling stage resistance with a lower value of epidemiological parameters suggesting a possibility to reflect adequate and durable resistance. Wang, et al. [57] explained that durable resistance achieve through combining major resistance genes and APR genes and added the adult plant resistance (APR) is of major importance in breeding for an efficient genetic control strategy.

3.2.7. Thousand Grain Weight

Thousand kernel weight is an important component of yield mostly affected by stem rust. The analysis of variance showed highly significant variability at $p < 0.01$ among cultivars evaluated for production of 1000 grain weight.

The heavier 1000 grain weight was obtained at Debre-Tabor by the cultivar Lelisso (39.8g) followed by Selam (37.9g) and Ude (34.4g). On the other hand at Adet Kokate yields a maximum 1000-grain weight of 35.9 g followed by Lelisso (34.5g).

In fact, cultivar Arendeto and Malefia displayed higher severity resulting in lowest 1000-grain weight production among tested cultivar on both locations Table 5. The reduction in TKW for susceptible Arendeto and Malefia might be due to the effect of the disease on the size and mass of the seed. Nzuve, et al. [39] also observed that the susceptible check "CACUKE" used in their study showed the least TKW. Asmmawy, et al. [68] reported an average loss in 1000 kernel weight by stem rust ranged from 2.03 % to 6.71 %.

3.2.8. Grain Yield

There was a highly significant difference at $P < 0.01$ between entries for grain yield. From the beginning, it should be underlined that the variances in grain yield among the entries could be clarified not only by differences in the levels of disease attack but also in the yield potential of the varieties.

At Adet, the highest grain yield (4.13 t ha⁻¹) was obtained from cultivar Selam. However, the yields obtained from Bichena (4.07 t ha⁻¹), Megenagna (4.04 t ha⁻¹), Kokate (4.03 t ha⁻¹) and Lelisso (3.96 t ha⁻¹) were not significantly different from Selam. The lowest grain yield was produced by cultivar Malefia (1.8 t ha⁻¹) followed by Arendeto, Ejersa, Yerer and Mettaya with 2.33, 2.72, 2.93 and 2.97 t ha⁻¹ grain yields respectively. Cultivars Lelisso and Selam produce highest yield at Debre-Tabor with 3.53 and 3.1 t ha⁻¹ respectively while the lowest grain yield was harvested from cultivar Arendeto (0.58) and Malefia (1.15) t ha⁻¹ Table 5.

The result revealed that the ranking of the cultivars for grain yield partially disagree with ranking for resistance specifically at Adet. For example, the disease severity recorded on cultivar Tati was 62% at Adet but the yield obtained from cultivar Tati was higher (3.82 t ha⁻¹) than some cultivar that had disease severities less than 15% such as Yerer, Oda and Ilani Table 5. This may confirm that stem rust severity difference alone could not cause variation in yield among the cultivars [44].

Cultivars might also differ in their genetic yield potential and may express differently in different environments. In the present study yields of the tested cultivars were lower at Debre-Tabor while their yields were high at Adet. This indicated that environment affected the expression of the cultivars' genetic potential. The study of Hamam and Khaled [69] and Mohammadi, et al. [70] indicated that grain yield of wheat varieties largely depend on climatic conditions. Cultivar Selam clearly demonstrates the effect of environment on the expression of the genetic yield potential of a cultivar as its grain yield declined from 4.13 t ha⁻¹ at Adet to 3.1 t ha⁻¹ at Debre-Tabor.

Table-5. Mean values of thousand kernel weights (TKW) and grain yield for 25 durum wheat cultivars at a different location.

Cultivar	Adet		Debre-Tabor	
	GY(t ha ⁻¹)	TKW(g)	GY(t ha ⁻¹)	TKW(g)
Utuba	3.58 ^{abcd}	29.5 ^{bcdef}	1.71 ^f	26.30 ^{efghi}
Bichena	4.07 ^a	33.4 ^{abc}	2.58 ^{bc}	35.17 ^{abc}
Mangudo	3.36 ^{abcde}	32.7 ^{abcd}	1.93 ^{def}	25.27 ^{fgjh}
Mekuye	3.34 ^{abcde}	31 ^{abcde}	2.59 ^{bc}	32.50 ^{bcde}
Toltu	3.09 ^{abcde}	23 ^{ghi}	1.78 ^{ef}	20.53 ^{jk}
Hitosa	3.56 ^{abcd}	26 ^{fgh}	2.36 ^{cde}	22.60 ^{hjk}
Denbi	3.10 ^{abcde}	26.3 ^{efgh}	2.69 ^{bc}	28.27 ^{defgh}
Werer	3.65 ^{abcd}	27.2 ^{efg}	2.55 ^{bed}	35.63 ^{abc}
Tati	3.82 ^{abc}	24.7 ^{fghi}	1.81 ^{ef}	24.90 ^{fgjh}
Filakit	3.62 ^{abcd}	30.4 ^{bcde}	2.32 ^{cdef}	23.27 ^{ghj}
Obsa	3.17 ^{abcde}	21.4 ^{hij}	1.82 ^{ef}	21.60 ^{ik}
Ejersa	2.72 ^{def}	23.7 ^{ghi}	2.17 ^{cdef}	25.27 ^{fgjh}
Bakalcha	3.63 ^{abcd}	29.6 ^{bcdef}	2.67 ^{bc}	32.43 ^{bcde}
Kokate	4.03 ^{ab}	35.9 ^a	2.74 ^{bc}	33.87 ^{abcd}
Malefia	1.80 ^f	20.6 ^{ij}	1.15 ^g	16.70 ^k
Oda	3.47 ^{abcd}	28 ^{defg}	2.33 ^{cdef}	27.67 ^{defgh}
Ilani	3.44 ^{abcd}	29.1 ^{cdef}	2.55 ^{bed}	27.10 ^{efgh}
Megenagna	4.04 ^{ab}	32.6 ^{abcd}	2.55 ^{bed}	30.07 ^{cdef}
Mosobo	3.79 ^{abcd}	33.6 ^{abc}	2.46 ^{cd}	31.53 ^{bcdef}
Mettaya	2.97 ^{bcde}	26.8 ^{efg}	2.50 ^{bed}	29.60 ^{cdefg}
Selam	4.13 ^a	31.3 ^{abcde}	3.10 ^{ab}	37.97 ^{ab}
Yerer	2.93 ^{cde}	25 ^{fghi}	2.27 ^{cdef}	27.33 ^{defgh}
Lelisso	3.96 ^{abc}	34.5 ^{ab}	3.53 ^a	39.80 ^a
Ude	3.53 ^{abcd}	34.4 ^{abc}	2.44 ^{cd}	32.13 ^{bcde}
Arendeto	2.33 ^{ef}	17.9 ^j	0.58 ^h	10.83 ^l
Level of significance	**	**	**	**
CV%	16.0	9.7	14.04	12.3

Note: ** represent highly significance difference at P < 0.01; Means within a column followed by the same letter are not significantly different at p=0.01; TKW= thousand kernels weights and GY=grain yield.

Among the identified resistance cultivars Lelisso, Bichena and Bakalcha had the highest yield in both locations Table 5. Their comparatively better yields make them recommended as a resistance cultivars and stem rust resistance donor parent in wheat breeding programs. Conversely, cultivars Malefia had lowest yield with poor genetic resistance in both locations. The combined extent of poor genetic resistance with low yield could probably suggest that stem rust disease reduced the grain yield of durum wheat cultivar. However, there was no protected check plot established for each genotype to obtain information to calculate yield loss.

4. CONCLUSION AND RECOMMENDATIONS

In the study, seventeen durum wheat cultivars were resistant and three cultivars were susceptible to stem rust race TTTTF at the seedling stage. Cultivar Mettaya and Oda were supposed to confer major gene resistance. Correspondingly, at Adet Bkalcha, Lelisso, Ilani, Yerer and Bichena identified as having high level of field resistance.

Durum wheat cultivars Selam, Filakit, Mangudo, Ude, Megegnagna, Ejersa, Mekuye, Werer, Kokate, Mosobo, Toltu, Obsa, Denbi and Utuba had moderate level of partial resistance while, Hitosa and Tati grouped under low level of partial resistance. However, all of the durum wheat cultivars except Malefia and Arendeto were grouped under a high level of partial resistance for the stem rust population present at Debre-Tabor. The correlations among the epidemiological parameters were highly significant. Among the identified resistance cultivars comparatively better TKW and grain yields were produced by Lelisso, Bichena and Bakalcha. Most of cultivars identified as having high and moderate level of field resistance also had resistant reaction at the seedling stage indicating that they possess major gene resistance towards race TTTTF.

Therefore they can be used as sources of stem rust resistance when the aim of the breeding program is for major gene. However, these cultivars should be assessed for stem rust population on different locations by including other growing seasons to account for stability over years and locations before approval. This could be followed by inheritance studies coupled with marker assisted selection to establish the identity of these genes conditioning the resistance among these cultivars.

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