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Research Article

# Response of Ethiopia Durum Wheat Accessions for Resistance to Stem Rust (*Puccinia graminis f.sp. Tritici*) Disease

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## ABSTRACT

The horizontal resistance is effective against broad range of pathogen races and even reduces the costs of fungicides for controlling. The objective of present study is based on the field assessment of adult resistance genes in Ethiopia durum wheat accessions for resistance to stem rust (*Puccinia graminis f.sp. tritici*). The 142 durum wheat accessions were obtained from the Ethiopian Biodiversity Institute and screening for stem rust in Debrezeit agricultural research experimental fields using alpha lattice design. The bulk of races (TTKSK (*Ug*99), TTTTF, TTRTF, JRCQC, TKTTF) inoculated during stem elongation stage. The disease assessment started the first symptom of seen in infector rows. In the field, durum accessions were examined utilizing for slow rust parameters. Accordingly, to that the 23 accessions were identified having low value of terminal rust resistance, low average coefficient of infection and low area under disease progress curve. The grain yield is negative and highly significant associated with slow rusting parameters. These accessions considered as having adult resistance genes with high partial resistance genes and important for further resistance breeding.

**Keywords:** Accessions; Terminal rust resistance; Wheat; Breeding; Infection

## INTRODUCTION

Wheat is the most important cereal crops to guarantee food security program in the world population [1]. Many African countries are producing wheat for the purpose of both home consumption and marketing. The leading wheat producing countries in Sub-Saharan Africa are Ethiopia, South Africa, Sudan, Kenya, Tanzania, Nigeria, Zimbabwe, and Zambia respectively [2]. However, the production is limited both biotic and abiotic factors. The estimated yield under stress is 2.7 t/ha, which is far less than the world average 3.4 t/ha [3]. The low productivity due to lack of resistant varieties to the prevalent wheat rusts namely the stem rust (*Puccinia graminis f.sp. tritici Eriks*. and *E. Henn*), leaf rust (*P. triticina Eriks*) and stripe rust (*P. striiformis Westend. f. sp. tritici Eriks*) are the major important diseases. Among the three rust diseases in wheat, stem rust can cause 100% yield loses when cultivars become susceptible with favorable environment for mass uridiospore production [4-6].

Wheat producers in Ethiopia require disease resistant varieties since they are environmentally safe, farmer friendly and economically feasible. Therefore, it is important to identify sources of resistance genes in order to develop disease resistant wheat cultivars. One of the rich sources of stress resistance germplasm are landraces, which are also known to be reservoirs of genetic resources like resistance genes for several plant diseases including wheat rusts [7-9]. The Adult plant résistance is Race-nonspecific which were effective against multiple races of a pathogen species (effective against broad ranges of pathogens), quantitative, exhibiting partial or incomplete resistance typically triggered at later stages of development. The genes usually exhibit slower disease progress through an increased latency period, reduced infection points, lower levels of sporulation and increased rate of removal of infectious tissue (reducing the infectious period). The phenotypic effect of such genes is relatively minor to moderate, however, additive effects of multiple *APR* genes in combinations can result in very high levels of resistance [10]. Therefore, the present study is based on evaluation of the adult resistance genes of durum wheat accessions grown in Ethiopia for resistance to stem rust (*Puccinia graminis f.sp. tritici*).

## MATERIALS AND METHODS

### Description of study areas

Field study was conducted at the research facility farm of Debrezeit Agricultural Research Center (DZARC), during 2021 main cropping season. The center is located at geographic coordinates of 08°46'N and 39°00'E latitude and longitude respectively. The

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research farm is situated at an altitude of 1900 m.a.s.l [11]. The area receives annual average rainfall of 851 mm with 61.3% mean annual relative humidity. The annual average temperature ranges from 8.9°C to 28.3°C. The soil type is characterized by pellicvertisol [12].

## Experimental materials

One hundred forty two durum wheat accessions were collected from the Ethiopian Biodiversity Institute and four additional cultivars namely, Boohai, Tob66, Arendato and Digalu were obtained from DZARC. Boohai and Tob66 were used as resistant control because they exhibit low severity percentage on field evaluation of stem rust pathogen races whereas, both Arendato and Digalu were equally mixed together and used as planting material for spreading the disease and bulk of stem rust races which are currently dominating the field infection were used for field evaluation; namely TTKSK (*Ug*99), TTTTF, TTRTF, JRCQC, TKTTF. These Pgt races were harvested from Debrezeit Agricultural Research experimental fields.

## Experimental design and treatments

One hundred forty two durum wheat accessions and two additional cultivars (Tob66 and the Boohai) were planted in alpha lattice design with two replications. The field trial was arranged in 12 blocks per replication and 12 plots per block (12 × 12=144 plots). Each plot has 50 cm row length and 20 cm width. Distance between blocks and plots are 15 cm and 10 cm, respectively. Planting was carried out by drilling and inserting twenty seeds per plot with spacing of 2 cm × 30 cm. additionally, two susceptible cultivars namely, Digalu and Arendato were planted in mixture at equal ratio on borders and also at 50 cm intervals between two blocks of each replication as spreader row of Pgt [13]. Fertilizers were applied as side dress at rate of 41 kg/ha N (applied in splits, the first half during planting time and remaining half a 30 days after planting) and 46 kg/ha $P_2O_5$ during planting [14]. All other recommended agronomic practices such as cultivation, weeding, etc. were adopted during the growing season.

## Inocula preparation and inoculation

Urediniospores were collected from infected durum wheat and bread wheat nursery fields using cyclone collector and were stored in refrigerator at 4°C [15]. Inoculum increase was carried out using universal susceptible cultivar Morocco in greenhouse and harvesting viable urediospore for field inoculation according to the protocol described by Roelfs [15]. Inoculum was prepared with a mixture of 0.6 mg urediospores of five stem rust races (JRCQC, TRTTF TKTTF, TTTTF, TTKSK) and suspending in distilled water plus one drop of Tween 20 per 0.5 liters of suspension [16]. In the field stem rust epidemic was initiated by inoculating spreader rows with the inoculum mixtures of 0.6 mg Urediniospores [16]. A total of three inoculations were carried out at weekly interval to ensure disease development. The first two inoculations were done through injection during stem elongation stage using 10 ml syringe and the last inoculation was carried out at booting growth stage using ultra low volume sprayer [17]. Inoculation at field was done late in the evening when conditions were conducive for germination of spores and establish infection [15].

## Data collection

The data recording was started when first symptom of disease was observed in the infector rows. This was continued afterwards until disease severity reached 100% in the infector rows and the data were collected at weekly interval during the course of disease progress. Disease severity was estimated as percentage of diseased plant parts (portion of stems, leaves) from twenty plants within each experimental plot using modified Cobb's scale [18]. This scale has a rate of score between 0 and 9. Where, 0%=immune and 100%=completely susceptible. Host plant response to infection was scored according to the description by Roelfs (Table 1) [15]. The Coefficient of infection was calculated by taking the product of percent disease severity (modified Cobb scales) and a constant value of host response [15]. Average Coefficient of Infection (ACI) was derived from the sum of Chlorophyll Index (CI) values of each entry divided by the number of observation. Terminal Rust Severity (TRS) is the final record of stem rust severity when the susceptible check/spreader line displayed maximum disease severity (Ma and Singh). The Grain yield in gram/plot at 12.5% moisture content (determined by high performance moisture analyzer) was recorded using sensitive balance and transformed into kg/ha.

**Table 1:** Baseline characteristic of patients with ALT>/=1000 IU/L.

| Field response | Symbol | Constant value | Infection type |
|---|---|---|---|
| Immune | 0 | 0 | No visible infection |
| Resistant | R | 0.2 | Necrotic areas with or without small pustules. |
| Moderately resistant | MR | 0.4 | Small pustules surrounded by necrotic areas |
| Intermediate or Moderate | M | 0.6 | Pustules of variable size, some necrosis or chlorosis. |
| Moderately susceptible | MS | 0.8 | Medium sized pustules, no necrosis, but some chlorosis |
| Susceptible | S | 1 | Large pustules no necrosis or chlorosis. |

## Data analysis

The stem rust severity data were summarized to produce, Average Coefficient of Infection (ACI), Area under Disease Progress Curve (AUDPC), disease progress rate (r) across different genotypes. The AUDPC values were produced by taking the weekly disease severity data using trapezoidal method in Microsoft Excel as described by Wilcoxson, using the following formula per accession lines per replication

$$AUDPC = \sum_{i=1}^{n-1} \frac{(X_{i+1} + X_i)}{2}(t_{i+1} - t_i)$$

Where, $X_i$ is the cumulative disease severity expressed as a proportion at the $i^{th}$ observation; ti is the time (days after planting) at the $i^{th}$ observation and n is total number of observations. The apparent infection rate (r) of disease progress curve was estimated for each accession line per replication over successive disease severity recording periods using the lme4R statistical package [19]. The rates of stem rust increase (r-value) as a function of time were estimated based on proportional measures of the extent of infection at different times by taking the coefficient of the slope of the regression line [20].

The residual (restricted) maximum likelihood estimation method to fit the alpha lattice design model with the different disease parameters (indicated below) was carried using the Agricola package as implement in R package [21]. The estimation method produced the Anova table, the standardize and fitted value of the model, F-value, means and other relevant statistics to check model adequacy and the mean comparison using the Least Significance Difference (LSD) method.

The model of alpha lattice design:

$$y_{ijl} = \mu + \tau_i + \gamma_j + \rho_{l(j)} + \in_{ijl},$$

Where, $\tau_i$=treatment effect (wheat accessions), i=1,2,...t, $\gamma$=replication effect, j=1,2....r, $\rho l_{(j)}$=block within replication effect, l=1,2...s, $\in_{ijl}$=random error. The relationship between grain yield and slow rust parameters were computed using SAS version 9.0 [22].

## RESULTS AND DISCUSSION

Slow rusting genotypes were identified in the field considering their Terminal Rust Severity (TRS), Average Coefficient of Infection (ACI), Area Under Disease Progress Curve (AUDPC) and rate of stem rust progress. The analysis of variance showed highly significant variation among durum wheat lines for the stated disease parameters.

### Infection response and terminal disease severity

The distribution of field responses to infection by the durum lines is indicated on Figure 1. The majority of the tested lines were in the category of susceptible and moderately susceptible with frequency of 59 and 75 respectively. Although, none of the lines examined have exhibited immune or resistance reaction, the two reference lines (Boohai and Tob66) showed a moderately resistance response. The remaining 23 lines were moderate in their response to field infection by *P. graminisis f.sp. tritici* at DZAR. According to Nzuve, the available resistance genes in the wheat landraces overcame the stem rust virulence in the field and led to statistically low disease severities despite the compatible host-pathogen reactions [23].

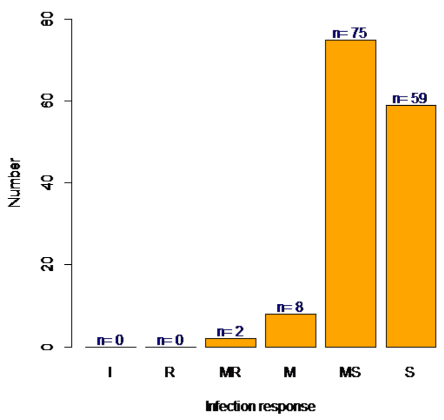


**Figure 1:** Frequency distribution of infection response by durum wheat accession lines from Ethiopia. **Note:** I: Immune; R: Resistance; MR: Moderately resistance; M: Medium; MS: Moderately susceptible; S: Susceptible.

The Terminal Disease Severity (TDS) ranges between 15% and 100% and most of the durum wheat accession lines investigated in this study produced variable results (Table 2). Accordingly, they were classified into three groups of slow rusting resistance based on the level of severity as having high, moderate and low partial resistance for genotypes showing 1-30%, 31-50% and >50% TRS, respectively [24]. In the first case, a considerable number of wheat lines (25 in total) falls under a high partial resistance groups indicating presence of potentially diverse group of durum wheat lines conferring some degree of resistance against the rust disease in Ethiopia as previously reported [25]. Durum wheat with a moderately partial resistance terminal disease severity constitutes 49 lines which may also be important for exploring stem rust resistance. The remaining lines were not promising to harbor resistance according to the level of disease severity observed.

### Coefficient of infection

The coefficient of infection values for wheat genotypes showed significance difference ($p<0.001$). The maximum value was recorded on accession 238127 and the lowest value was on the reference cultivars Bohai and Tob66 (Table 3 and Supplementary Table 1). The values of coefficient of infection are regarded as indicative of the presence of stem resistance in adult plant study. According to Ali wheat lines with coefficient of infection values of 0-20, 21-40, and 41-60 considered as possessing high, moderate, and low level of slow rusting resistance respectively. In this study a total 19 lines were found with CI values to satisfy the assumption of indicative resistance genes in the Ethiopian durum wheat lines. In addition, 44 lines were found to show a moderate level of slow rusting resistance according to the description by Ali [26]. These accessions might be low level of slowing stem rust development. The earlier findings reported that the slow rusting resistance in wheat stem rust was associated with low coefficient of infection indicating the presence of different partial resistance conferring genes as reported for the different durum wheat lines in this study [27-29]. The remaining lines were found to show low level of slow rusting resistance indicating their limitation for use in stem rust management [29,30]

### Disease progress rate (Infection rate)

Slow rusting resistance is characterized by a reduced rate of epidemic development despite a compatible host pathogen interaction [31,32]. The genotypes having lower disease progress rate are acceptable for practical purpose. As expected the accession lines analyzed in this study produced significantly variable infection rate ($p<0.0001$). The maximum mean disease progress rate (2.52) was observed on accession number 238127 and lowest disease infection rate from Boohai (Table 3). The result also indicated that a considerable number of accession lines (28%) having infection rate of less than one. In order to successful reduce the amount of disease, these genotypes can provide effective protection against the spread of the pathogens. The genotypes assigned in first group using slow rusting parameters of TRS and CI have generally low infection rate than the genotypes categorized in second group and third groups. However, mismatches were also observed for some genotypes between infection rate and the other slow rusting parameters such as TRS, CI and AUDPC. A report of such cases was demonstrated in other studies where estimate of infection rate was not in line with results for TRS, CI, and AUDPC [32-34].

Table 2: Analysis of variance table for adult resistance parameters.

| APR | Sum square | | Mean square | | CV (%) | F value | Pr(>F) |
|---|---|---|---|---|---|---|---|
| Parameters | Genotypes | Residuals | Genotypes | Residuals | | | |
| AUDPC | 35,555,589.00 | 9,027,042.00 | 248640.0 | 68387.0 | 30 | 3.6 | *** |
| ACI | 158,818.00 | 24235.0 | 1110.6 | 183.6 | 28 | 6.0 | ** |
| TRS | 119921.00 | 23887.00 | 838.6 | 181.0 | 25 | 4.6 | *** |
| rate (r) | 73.10 | 14.40 | 0.5 | 0.1 | 25 | 4.7 | *** |

**Note:** ***:0; **: 0.001; *:0.01.

Table 3: Analysis of variance table for adult resistance parameters.

| Disease parameters | GY |
|---|---|
| TRS | -0.54** |
| CI | -0.57** |
| AUDPC | -0.53** |

**Note:** **: Highly significant at P<0.001.

### Area under disease progress curve

The area under the disease progress curve AUDPC) is a good indicator of partial resistance under field condition and directly related with yield loss [20,35]. In the present study, significant (p<0.001) variation was observed in the level of AUDPC across wheat genotypes. The range of the AUDPC value recorded was 241.5 and 1788.5 for accession 214606 and 238127 (Table 2). In total, 13 significance groups of accession lines were detected based on the mean comparison results at alpha level of 5% (Table 2). The majority of the accession lines (68.75%) were clustered in one significant group (abcdefg) which was not significantly different from the reference cultivars (Tob66 and Bohai) which were grouped under different significance groups. The drum wheat accession line with the lowest AUDPC score formed its own significance groups and was significantly different from the majority of the genotypes tested. Different reports indicated that genotypes with low AUDPC values and Moderately Susceptible (MS) response carried genes for conferring durable resistance [36,37].

### The relationship between disease parameter and grain yield

The disease parameters (TRS, CI, AUDPC) were negative and highly significant (P<0.001) associated with grain yield. This might be an indication that the amount of stem rust severity increased resulted in the highly significant reduction on the yield. The damage of stem rust disease was not only grain yield rather than several yield components. However, the sum of negative effect resides on final yield. Several previous studies showed that stem rust attacks or interferes with the normal physiological activities of the plant and results reduced number of tiller, small number of kernel per spike, reduced grain yield have the mechanism of limited transportation of water, inadequate nutrient flow to the plant [38,39].

## CONCLUSION

Stem rust is the most yields reducing in wheat over all epidemics in the world and devastating now. For this problem, 142 durum wheat accessions screening in the field and evaluated using slow rusting parameters. The 23 durum genotypes selected based on TRS and CI<30%, the AUDPC ranges 241.5-619.5. On the other hand, 49 Durum genotypes having TRS (31%-50%), CI (21-50), AUDPC ranges 458.5-1092 were might be the moderately slow rusting resistance genotypes and the rest 70 genotypes were no slow rusting resistance. The Durum wheat genotypes having the slow rusting and moderately slow rusting from present study were assumed to be having genes for varying degree of slow rusting and this genes useful for further durum wheat resistance breeding program.

## DATA AVAILABILITY STATEMENT

The basic important data related for this study is included in this manuscript. If, additional is needed we can provide it up on request.

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