Vol. 18(9), pp. 770-775, September, 2022
DOI: 10.5897/AJAR2022.16136
Article Number: 6904B9769709
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

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African Journal of Agricultural Research

Full Length Research Paper

Status of Ethiopian durum wheat varieties for the stem rust disease resistance under field condition

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Received 5 July, 2022; Accepted 2 September, 2022

Production of wheat in Ethiopia is under danger from wheat stem rust, which is brought on by the disease *Puccinia graminis* f.sp. *tritici* and has resulted in a yield loss of 100%. An investigation was conducted in a hotspot area (Debere Zeit, Ethiopia) to evaluate the status of Ethiopian durum wheat cultivars for their level of stem rust resistance in the field. During the main seasons of 2017, 2020, and 2021, 34 durum wheat varieties with three susceptibility checks were examined. Terminal Rust Severity (TRS), Coefficient of Infection (CI) and Area under Disease Progress Curve (AUDPC) were used to measure stem rust field resistance. Analysis of all disease parameters was carried out using R studio. Due to their low levels of AUDPC, ACI, and TRS values combined with MR infection type, two commercial varieties (Oda and Tob-66) may have a significant resistance gene(s) and seven varieties (Boohie, Kilinto, Ginchi, Robe, Toltu, Lelisso, and Bekelcha) showed a moderate level of field resistance with MR to MRMS responses and may have adult plant resistance mediated by minor resistance genes. Stem rust was found to affect 73.5% of the Ethiopian durum cultivars. Therefore, this study suggests that the national breeding effort should focus on local durum cultivars rather than the ones that were imported.

Key words: Field resistance, durum wheat, stem rust, *Puccinia graminis* f.sp *tritici.*

INTRODUCTION

Wheat is Ethiopia's second-most significant food after maize, accounting for 14% of the country's total calorie intake (FAO, 2015; Wageningen, 2016). On 1.6 million hectares, 4.5 million tons of wheat are produced each year. However, wheat productivity is still poor at 2.67 t/ha (CSA, 2017). The productivity of wheat is substantially below the global average. Abiotic and biotic restrictions are to blame for low wheat productivity.

A bottleneck issue for Ethiopian wheat production is stem or black rust of wheat, yield loss up to 100% (Figueroa et al., 2018), which is caused by the fungus *Puccinia graminis* Pers. f. sp*. tritici* Eriks. & E. Henn. The population of this pathogen varies significantly for pathogenicity to resistance genes due to its high level of specialization. In the fungi that cause wheat stem rust, new virulence has evolved more frequently through migration, mutation, recombination, and their selection.

Ethiopia, a major wheat producer in sub-Saharan Africa, was among the nations on all continents where wheat stem rust was most dreaded due to its propensity

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to periodically bring devastating destruction (Singh et al., 2015; Newcomb et al., 2016; Lewis et al., 2018; Shaman et al., 2020). Three significant outbreaks, in 1974, 1993, and 2013, respectively, in Ethiopia caused the cultivation of three popular varieties: Lakech, Enkoy, and Digalu, to cease completely (Kebede et al., 1995; Olivera et al., 2015).

The frequency, size, and impact of epidemics have periodically increased dramatically. More recently, severe stem rust epidemics in Italy destroyed thousands of hectares of durum wheat. The concern of stem rust is justified given that, three weeks before harvest, a crop of a vulnerable cultivar that appeared to be in good health could turn into a black tangle of broken stems and shriveled grains.

Because of the nature of the disease and the erratic temperature and rainfall patterns, there is a potential that new stem rust races could evolve that will outperform the resistance of widely used wheat types and continue to be a continual problem for newly released cultivars (Ambika and Meenakshi, 2018).

Rusts have evolved independently in the past due to human-influenced redistribution of the primary host or the secondary host (Martinez, 2019). A key contributing factor to the harm that rust infections cause is the long-distance transmission of rust spores (Nagarajan and Singh, 1990). Many *P. graminis* Pers. f. sp. *tritici* uredio-spores may be transported by the prevailing winds and their directions. Given that this fungus can grow more than a million uredio-spores on a single wheat stem (Zadoks and Schein, 1979). Every year, this long-distance transportation covers 2,000 km (Luig, 1985).

Selection from native wheat landraces, selection from introductions, hybridization, and evaluation of selected lines for commercial production are the breeding techniques used to create new varieties (Bechere et al., 1994).

The main tactic utilized in the release of high-yielding disease-resistant varieties has been the testing of varieties at multiple locations throughout various seasons. It typically takes at least five years to identify a new variety for a region. In most cases, hybridization takes up to 10 years or more to produce a new variety (Efrem et al., 1994). But in recent years, irrigation has made it possible to cut the time between variety releases in half.

The simple fact that a variety is released does not ensure that it will continue to be produced for a long time. A newly released high-yielding rust-resistant variety may be attacked by a new race of rust due to the nature of rusts and the variability of the country's agro-climatic conditions, which could cause it to go out of production before its effects are felt by the farming community for which it is intended.

Most of the durum wheat cultivars in Ethiopia have been made available from imported resources. The stem rust resistance of these newly introduced materials was not observed to be as varied as that of Ethiopian landraces. Many of the 40 durum wheat varieties that have been released for production thus far have gone out of production due to the frequent release of new varieties, so it is unclear how resistant those varieties are to stem rust. As a result, the goal of this paper is to determine the field resistance status of Ethiopian durum wheats that have been released thus far.

MATERIALS AND METHODS

Descriptions of the study area

The experiment was carried out in Ethiopia's Debre Zeit Agricultural Research Center on vertosol. Debre Zeit is in the Oromia National Regional State's East Shewa Administrative Zone, 47 km south of Addis Abeba, at 38°57' E longitude and 08°44' N latitude, at an elevation of 1900 m above sea level (Bemnet et al., 2003). It has an annual average rainfall of 851 mm, an annual temperature range of 8.9 to 28.3°C, and a mean annual relative humidity of 61.3% (WRB, 2006).

Planting materials

A total of 34 durum wheat varieties, including 3 susceptible checks, were employed in Debre Zeit's main seasons of 2017, 2020, and 2021 for field resistance under natural infection against wheat stem rust. The planting materials were received from the Debre Zeit Agricultural Research Center's national breeding program for durum wheat.

Plot size and design

The experiment was set up in an augmented design, with a plot size of 1 m × 0.2 m, a plot spacing of 0.4 m between plots, and a block spacing of 1 m. According to the guidelines for the area, the seeding rate, fertilization, weeding, and other management methods were applied. The susceptible wheat cultivar "Local Red" was planted a week earlier around the experimental sites to guarantee equal dispersion of inoculum and sufficient disease development during the trial periods.

Data collection and analysis

Using a modified Cobb scale (Peterson et al., 1948), disease severity was determined by estimating the approximate percentage of green area affected. Beginning with the development of stem rust on the susceptible checks, disease severity was recorded from every plot six times in a 10-day period. According to Roelf et al. (1992), the host plant responses (infection types) were recorded. Coefficient of infection is determined by multiplying the percentage severity of an infection by the constant value allocated to each type of reaction (Saari and Wilcoxson, 1974). R (Resistant) = 0.2, MR (Moderately Resistant) = 0.4, M (Intermediate) = 0.6, MS (Moderately Susceptible) = 0.8, and S (Susceptible) = 1 were taken into consideration as the constant values.

The area under the disease progress curve (AUDPC) for each plot was calculated using the severity scores for stem rust. The area under the disease progression curve (AUDPC) was calculated using the formula proposed by Saari and Wilcoxson (1974). For the field resistance evaluation, the average coefficient of infection (ACI), terminal rust severity (TRS), and relative area under disease progress curve (r.AUDPC) were used as parameters. Using R studio, one-way ANOVA was used to test the significance of the difference in the TRS, ACI, and r.AUDPC.

RESULTS AND DISCUSSION

Analysis of variance

Table 1 displays the analysis of variance for several stem rust resistance factors. The test materials and susceptible checks for ACI and r.AUDPC differed significantly ($p<0.01$), and the difference in TRS differed significantly ($p<0.05$). For all criteria, there were no discernible differences between the test cultivars. For all field resistance measures, there was no statistically significant difference ($p>0.05$) between the check cultivars.

Terminal rust severity

The typical terminal rust severity for stem rust varied, ranging from 21 to 60%. Additionally, a variety of host responses were seen, from resistance (R) to susceptibility (S). The combined impact of all resistance factors as epidemics spread determines the severity of terminal rust (Parlevliet and Ommeren, 1975). The examined durum wheat cultivars were divided into three categories of field resistance levels based on the severity of terminal rust, with resistance, moderate resistance, and moderate susceptibility having 1-30, 31-40, and over 40%, respectively (Safavi, 2012).

LD-357, Boohie, Ude, Assasa, Bichena, Tob-66, Quamy, Kilinto, Ginchi, Cocorit-71, Selam, Arendeto, and *Lelisso* were among 13 evaluated durum wheat varieties that showed good levels of slow rusting resistance with a moderate resistance response in 2017. Specifically, *Ude, Kilinto, Utuba*, and *Oda* were four durum wheat varieties that had good field resistance to stem rust with *MR* to *MRMS* host pathogen reactions in 2020. In contrast, *LD-357*, *Kilinto, Robe, Oda*, and *Illani* were five durum wheat varieties that had better field resistance with RMR to MRMS responses in 2021.

To achieve effective breeding for long-lasting resistance to stem rust, slow rusting-resistant genotypes are crucial (Nzuv et al., 2012; Parlevliet, 1988). Despite the compatible host pathogen reactions, Nzuv et al. (2012) claim that the presence of resistance genes in the genotypes enabled them to outcompete the dominantly virulent stem rust pathogens in the field and resulted in statistically low disease severity. Previously, Ali et al. (2007), Li et al. (2010), Safavi (2012) and Tabassum (2011) assessed the field resistance of rust in wheat cultivars using the severity of terminal rust.

As opposed to this, eight varieties: *Hitosa, Tob-66, Selam, Filakit, Mossobo, Kokatie, Mukuye*, and *Mangudo,* will be available in 2020, including six in 2017: *Cocorit, Metaya, Ejersa, Tate*, *Illani,* and *Mangudo*. While in 2021 twelve cultivars, including Gerardo, *Hitosa, Worer, Bichena, Quamy, Cocorit, Selam, Tate, Kokate, Illani, Mukuye*, and *Utuba*, displayed terminal rust severity ranging from 31 to 40% with *MR* to *MS* responses and were thought to have a reasonable level of field resistance. *Kilinto* did not exhibit susceptibility among the three susceptible checks, whereas *Digalu* and *Local Red* showed the highest disease severities of 50 to 60% with susceptible responses, showing that a suitable epidemic pressure had been developed.

Since the wheat rust pathogen's virulent pathotype is highly variable due to mutation, recombination, and transit from other places, cultivars' levels of resistance have likewise changed through time. For instance, some cultivars (*Cocorit, Leliso, Kilinto, Ginchi, Bichena, Selam, Robe*, and *Boohie*) are resistant in 2017 but somewhat susceptible in 2020 and 2021, whilst in some cultivars like Ld-357, the opposite is true. Very few varieties namely, *Oda, Tob-66* and *Illani* consistently displayed resistance reaction over the course of all years.

Coefficient of infection

To determine the coefficient of infection, the information on disease severity and host response was pooled (CI). According to Ali et al. (2009), cultivars are considered to have a high level of field resistance if their coefficient of infection values range from 0 to 20. The average coefficient of infection (ACI) values for 17 durum wheat cultivars (Cocorit-71, Gerardo, LD-357, Boohie, Hitossa, Quamy, Kilinto, Tob-66, Robe, Toltu, Leliso, Tate, Oda, Illani, Bekelcha, Mukuye, and Utuba) in this study ranged from 0 to 20, with MR to MRMS responses. These genotypes are therefore regarded as having good field resistance. Pathon and Park (2006) and Draz et al. (2015) evaluated the coefficient of infection for slow rusting resistance to wheat stem rust and reported the presence of various adult plant resistance-contributing genes in wheat cultivars.

Area under disease progress curve

A better indicator of how a disease will manifest itself over time is the disease progress curve (Van der Plank, 2006). Therefore, choosing cultivars with a lower AUDPC score is appropriate in practice. The examined wheat cultivars for slow rusting resistance were divided into three different groups based on the AUDPC score. The cultivars with AUDPC values of up to 30% of the check were classified as having a high level of field resistance, while those with values of up to 70% of the check were classified as having a moderate level of resistance, and cultivars with values of more than 70% of the check were classified as susceptible cultivars (Ali et al., 2009).

Only the wheat varieties *Oda* and *Tob-66* provided AUDPC values up to 30% higher than the check cultivars

Table 1. ANOVA, block-Adjusted.

Source	Df	ACI	r.AUDPC	TRS
Treatment (ignoring Blocks)	33	0.02^{ns}	0.01^{ns}	0.01^{ns}
Treatment: Check	2	0.004^{ns}	0.0035^{ns}	0.00027^{ns}
Treatment: Test vs. Check	1	0.47**	0.2**	0.11*
Treatment: Test	30	0.01^{ns}	0.01^{ns}	0.0045^{ns}
Block (eliminating Treatments)	2	0.02^{ns}	0.01^{ns}	0.01^{ns}
Residuals	4	0.01	0.0042	0.01
CV		7.71	3.83	5.35

ns P >= 0.05; * P <= 0.05; ** P <= 0.01, ACI= average coefficient of infection, r.AUDPC= relative area under disease progress curve, TRS= terminal rust severity.

Table 2. Correlations among stem rust resistance parameters in durum wheat genotypes.

Parameter	TRS	AUDPC	ACI
TRS	1		
AUDPC	0.88**	1	
ACI	0.93**	0.94**	1

**Significant level at P<0.01. TRS=Terminal rust severity, ACI= average coefficient of infection, AUDPC= area under disease progress curve.

with MR and MS responses, respectively, in this investigation. Genes granting long-lasting resistance may be present in genotypes with the MS infection type and low AUDPC value, according to Brown et al. (2001), Kaur and Bariana (2010) and Singh et al. (2005). These genotypes initially manifested rust infection with chlorotic and necrotic lesions; later, the disease progression remained sluggish and severely delayed. Because multiple point mutations are so uncommon in this situation, partially resistant cultivars like these could significantly slow the evolution of new virulent disease races (Ali et al., 2008; Schafer and Roelfs, 1985).

Conversely, cultivars exhibited MR responses; may have resulted from hypersensitive reactions; this sort of resistance frequently fails as a result of the emergence of new disease races. In the breeding effort for durum wheat, appropriate breeding techniques, such as the direct transfer of these resistance genes through back-crosses, were employed to generate resistance types.

Out of the examined durum wheat varieties, 16 of them, including Gerardo, LD-357, Boohie, Hitossa, Quamy, Kilinto, Robe, Cocorit, Toltu, Leliso, Tate, Bekelcha, Kokate, Illani, Mukuye and Utuba, showed AUDPC values of up to 70% of the check with median responses of MR to MRMS, and these genotypes are considered good level of field resistance. The remaining 11 genotypes were deemed vulnerable because they had an AUDPC score exceeding 70% of the checks. The AUDPC of several wheat genotypes and cultivars has also been recorded by other studies (Habtamu, 2019). Lower AUDPC values are thought to confer a resistance to stem rusting caused by slow rusting on wheat.

Correlations between stem rust's field resistance metrics

Table 2 displays the correlations of stem rust resistance measuring parameters. TRS exhibited a high and positive association with AUDPC and ACI, with r values of 0.88 (p=0.004) and 0.93 (p=0.002), respectively. Similarly, AUDPC and ACI showed a strong link with r values of 0.94 (P=0.002). The findings of Ali et al. (2008) and Shah et al. (2016) agreed with a substantial positive correlation between all slow rusting indices.

Since there were significant positive connections between TRS, ACI, and AUDPC, choosing cultivars with terminal rust severity less than 30%, ACI between 0 and 20, and relative AUDPC less than 30% is typically acceptable for practical purposes (Ali et al., 2009; Savavi et al., 2013). It is possible to measure slow rusting resistance in the field, ideally by a low terminal rust severity and infection coefficient, according to Safavi et al. (2013) and Singh (2007) also mentioned field selection for slow rusting resistant with a preference for low AUDPC value.

As a result, the only two durum wheat cultivars, *Oda* and *Tob-66*, were identified for resistance breeding as a parent cultivar with field resistance characteristics, having values of TRS 22 and 30, ACI 8 and 12, and relative

AUDPC 30 and 28%, respectively, with MR responses.

Boohie, Kilinto, Ginchi, Robe, Toltu, Lelisso, and *Bekelcha* cultivars were recognized as relatively slow rusting resistance cultivars because they displayed median response of MR-MRMS with TRS up to 30, ACI values ranging from 0 to 20, and rAUDPC between 31 and 50%. The remaining 22 durum wheat cultivars lacked any field resistance against stem rust resistance, though.

It is anticipated that the slow rusting and moderately slow rusting durum wheat genotypes discovered in this study will contain the genes for different levels of slow rusting, and they may prove useful in future durum wheat improvement initiatives.

Furthermore, genotypes with high and moderate slow rusting resistance (Singh, 2004) have suggested that these genotypes may possess persistent resistance that is controlled by more than one gene and might act as ideal parents for resistance breeding.

In this study, 34 durum varieties were tested for field resistance at a hotspot for stem rust over a three-year period, and only nine varieties showed significantly better field resistance, while the remaining varieties were all susceptible to the prevailed stem rust isolates. Since Ethiopian durum wheat varieties are available from foreign sources, these introduced materials do not have as diverse a genetic background as Ethiopian landraces in terms of stem rust resistance.

Recently, durum wheat accessions collections from around the world were tested for stem rust at hot spot locations, including here in Debre-zeit, Ethiopia, and Minnesota, USA. Surprisingly, the results showed that 98% of Ethiopian landraces were found to be resistant (Olivera et al., 2021). As a result, taking advantage of our natural resources is significantly better for the national breeding program for stem rust resistance. The findings of this study showed that the commercially available durum wheat varieties in Ethiopia did not offer a reliable and varied source of stem rust resistance. After a three-year field evaluation in Debre-zeit, only two varieties were found to be resistant, and seven varieties were moderately resistant. Oda and Tob-66, two resistance cultivars, could be used to improve stem rust resistance in both durum and common wheat. The findings that 73.5% of Ethiopian durum wheat varieties are susceptible to moderately susceptible to stem rust suggest that the national breeding program's methods need to be altered and should place more emphasis on native durum cultivars than on imported ones.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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