

Evaluation of Ethiopian Durum and Bread Wheat Genotypes for Stem Rust Adult Plant Resistance

Netsanet Bacha¹, Kitessa Gutu², and Ashenafi Gemechu³

¹Ethiopian Institute of Agricultural Research, Plant Protection Research Directorate, POB 2003 Addis Ababa, Ethiopia. Email: netsanetbacha@gmail.com; ²Ethiopian Institute of Agricultural Research, Ambo Agricultural Research Center, POB 37, Ambo Ethiopia; ³Ethiopian Institute of Agricultural Research, DebreZeit Agricultural Research Center, POB 32, Ethiopia

Abstract

Stem rust (*Puccinia graminis* Pers. f. sp. tritici Eriks. and Henn) incurs significant yield losses in wheat in Ethiopia, as most commercial varieties lack sufficient resistance to the rust. New resistance sources identification and incorporation of resistance has continued. Combining adult plant and seedling resistances based Sr-resistance genes is foundation for durable stem rust resistant variety providing effective rust protection. Thirty two durum wheat lines (DW) and 30 bread wheat genotypes (BW) were evaluated for stem rust seedling resistance. Twenty nine DW and 15 BW with heterogeneous seedling infection (2+3-) and ≥ 3 - were further evaluated for stem rust adult plant field resistance. Seedling infection types (ITs), host plant responses, terminal disease severity (TDS), coefficient of infection (CI) and relative area under disease progress curve (r-AUDPC) were used for evaluating adult plant resistance. Four DW lines 7974-2, 203680-2, 7974-1, 203831-2 and one advanced BW line ETB9550 expressed low TDS/field response of 15MS-25MS, CI of 12-20 and r-AUDPC of 12-22%. These lines had susceptible seedling infection types and were regarded as highly slow rusting. Likewise, DW lines 5454-1, 6112-1, 203726-2, 203855-2 and 203899-1 and BW lines ETBW9652 and ETBW9313 with ≥ 3 - ITs showed r-AUDPC, CI and TDS/response of 31-70%, 28-36 and 30S-45MSS, respectively, were grouped to genotypes with moderate slow rusting resistance. Slow rusting resistant wheat genotypes identified from the present study can be used for developing durable stem rust resistant wheat cultivars once they are postulated for their inherent resistance Sr-genes.

Keywords: bread wheat, durum wheat, landrace, slow rusting

Introduction

Wheat (*Triticum aestivum* L) is the most widely grown cereal crop in the world, encompassing more than 218

million hectares of land. It is the second most important food crop in the developing world after rice (Giraldo *et al.*, 2019). Ethiopia is the largest wheat producer in sub-Saharan Africa (Adugnaw and Dagninet, 2020)

with the productivity of 3 t/ha and production of 5.78 million tonnes harvested from over 1.8 million hectares of land [Central Statistical Agency (CSA), 2021]. Both bread (*T. aestivum* L.) and durum (*T. turgidum* var. *durum*) wheat varieties are grown in the country. Bread wheat is an introduced crop whereas durum wheat is an indigenous crop. The crop is an important staple food used in a wide variety of products in the country. However, its productivity is threatened by various biotic and abiotic constraints.

Stem rust disease caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks and Henn (*Pgt*) is one of the widespread biotic disease factors that severely affect wheat productivity in Ethiopia (Singh *et al.*, 2016). It often causes yield losses reaching up to 100% on susceptible cultivars (Meyer *et al.*, 2021). Disease control is possible with fungicides, but they are unaffordable for resource poor farmers in developing countries like Ethiopia (Rehman *et al.*, 2013). Use of wheat cultivars with durable resistance genes also called slow rusting, adult plant resistance or partial resistance is the most economic, effective, and ecologically sustainable method of stem rust control.

More than 60 resistance genes have been described for stem rust. Most of these genes are race-specific and function in a gene-for-gene fashion (McIntosh *et al.*, 2017). Virulence in the pathogen population has been

evolving rapidly following the deployment of race-specific resistance genes, often associated with a boom-and-bust cycle (Burdon *et al.*, 2014). Use of race non-specific resistance genes is the best strategy for breeding towards durable stem rust resistance. This form of resistance is effective against a broad range of stem rust races with an optimal level of expression at the adult plant stages (Parlevliet, 1985; McIntosh *et al.*, 1995). Race non-specific resistance is governed by polygenes each with minor effect. The stem rust resistance gene *Sr2*, *Sr55*, *Sr56*, *Sr57* and *Sr58* are examples of genes contributing to adult plant resistance, based up on partial resistance (Yu *et al.*, 2014; McIntosh *et al.*, 2017). The gene *Sr2* has provided durable resistance against stem rust worldwide for more than 50 years (Bhardwaj *et al.*, 2014). It is a recessive gene closely linked to pseudo-black chaff ('*pbcb*'), characterized by stem and head melanism in wheat.

Several race-specific stem rust resistance inherent wheat varieties grown in Ethiopia have been more frequently succumbed to stem rust epidemics and periodically dropped out of production despite sustained desirable agronomic attributes (Admassu *et al.*, 2012; Bekele *et al.*, 2019). The failures of many promising cultivars which were reportedly resistant to stem rust indicate the importance of breeding for durable resistance using non-race specific or adult plant resistance genes. However,

presence of a single or couple of adult plant resistance genes in a cultivar may not provide sufficient resistance levels in a high disease pressure area. Besides, it lacks stability as it is influenced by changing weather conditions. Therefore, cultivating commercial cultivars with pyramided minor resistance genes in addition to combining race-non-specific and seedling resistances could be the best stem rust control strategy (Singh *et al.*, 2011; Zhang *et al.*, 2019). Hence, searching and identifying new sources of resistance to stem rust is a continuous process in stem rust breeding programs. The present study was thus conducted to detect the presence of slow rusting resistance to stem rust in selected Ethiopian durum wheat landrace lines and bread wheat genotypes.

Materials and Methods

Experimental sites description

Pure line development and seedling evaluation of wheat genotypes were carried out at Ambo Agricultural Research Center. It is located at 08° 96' 885" N latitude and 37° 85' 923" E longitude and at an altitude of 2147 m.a.s.l. The annual average

temperature and rainfall are 27.54°C and 1077.68 mm, respectively.

The Debre Zeit Research Center is found at an altitude of 1900 masl. Its geographic location is 08° 44' N latitude and 38° 58' E longitude. The center receives mean annual rainfall of 851 mm. The average annual minimum and maximum temperatures are 8.9 and 28.3°C, respectively. The center is a reputed 'hotspot' site for field evaluation of wheat genotypes against stem rust.

Plant materials

A total of 62 wheat genotypes including 32 durum wheat pure lines selected from 25 original accessions acquired from Ethiopian Biodiversity Institute (EBI), 14 advanced bread wheat lines and 16 bread wheat varieties obtained from Kulumsa Agricultural Research Center were studied (Tables 1 and 2). The durum pure lines were obtained by selecting and planting five single heads representing the most frequent genotype within an accession. This was done over two selection cycles at Ambo Agricultural Research Center during the 2018 main season (June–October) and 2019 off-season (January–May). Malefia and Digalu were used as susceptible checks for durum and bread wheat genotypes, respectively.

Table 1. Passport description of the test durum wheat landraces

Accession	Region	Zone	Latitude	Longitude	Altitude
5454	Oromiya	Mirab Shewa	08-59-00-N	37-51-00-E	1772.00
7974	Oromiya	Mirab Shewa	08-50-00-N	38-28-00-E	2080.00
6112	Oromiya	Semen Shewa	09-46-00-N	38-31-00-E	2705.00
203680	Oromiya	Misrak Shewa	08-52-00-N	39-01-00-E	2600.00
7156	Oromiya	Semen Shewa	09-16-00-N	38-35-00-E	2735.00
203676	Oromiya	Misrak Shewa	08-52-00-N	38-47-00-E	NA
203733	Oromiya	Misrak Shewa	09-47-00-N	39-16-00-E	2200.00
203709	Oromiya	Mirab Shewa	08-51-00-N	38-30-00-E	2330.00
203818	Oromiya	Semen Shewa	09-03-00-N	39-04-00-E	2510.00
203826	Oromiya	Misrak Harerge	09-16-00-N	41-48-00-E	NA
203726	Oromiya	Misrak Shewa	08-50-00-N	39-19-00-E	2260.00
203882	Oromiya	Mirab Shewa	08-59-00-N	37-51-00-E	1772.00
203831	Oromiya	Misrak Harerge	NA	NA	NA
7169	Oromiya	Mirab Shewa	NA	NA	NA
203855	Oromiya	Misrak Harerge	NA	NA	NA
7972	Oromiya	Mirab Shewa	08-50-00-N	38-28-00-E	2080.00
203854	Oromiya	Misrak Harerge	09-26-00-N	41-48-00-E	
5768	Oromiya	Misrak Shewa	08-47-00-N	39-15-00-E	2300.00
7855	NA	NA	NA	NA	NA
203886	Oromiya	Mirab Harerge	09-00-00-N	40-53-00-E	NA
203899	Oromiya	Semen Shewa	09-16-00-N	38-40-00-E	2853.00
203695	Oromiya	Mirab Harerge	09-00-00-N	41-53-00-E	NA

NA: not available

Table 2. List of wheat genotypes used for field evaluation

Line/Variety	Pedigree	Breeder Institute/Origin	Year of release
ETBW9550	KFA/2*KACHU*2//WAXBI	EIAR	-
ETBW9548	REEDLING #1//KFA/2*KACHU	EIAR	-
ETBW9543	KFA//PBW343/PASTOR/3/PBW343*2/KUKUNA/4/ PBW343*2/KUKUNA*2//FRTL/PIFED/5/PBW343*2/ KUKUNA*2//FRTL/PIFED	EIAR	-
BW173472	NA	EIAR	-
ETBW9652	PFUNYE #1/KINGBIRD #1	EIAR	-
ETBW9313	ROLF07/YANAC//TACUPETOF2001/BRAMBLING* 2PASTOR	EIAR	-
Shina	GOV9/AZ//MUS"S"/3/R37GHL/21//KAL/BB/4/ ANI"S"	ARARI/CIMMYT	1999
Kubsa	NORDDESPRESZ/VG9144//KALYANSONA/BLUEBI RD/3/YACO/4/VEERY	EIAR/CIMMYT	1995
KBG 01	(300/SM+501M)/HAR-1709	EIAR/Germany	2001
Menze	MILAN/SHANGHAI#7	ARARI/CIMMYT	2007
Sirbo	V573.600/MRL/3/BOW//YR/TRF	EIAR/CIMMYT	2001
Kakaba	KIRITATI/SERI/RAYON	EIAR/CIMMYT	2010
Bolo	VEE/LIRA//BOW/3/BCN/4/KAUZ	ARARICIMMYT	2009
Hidase	YANAC/3/PRL/SARA//TSI/VEE#5/4/CROC- 1/AE.SQUAROSA(224)//OPATTA	ARARI/CIMMYT	2012
Tsehay	VEERY-5//ICTA-SARA-82//DUCULA	ARARI/NA	2011
Digalu(BW Check)	SHANGHAI-7/KAUZ,MEX	EIAR/CIMMYT	2005
Malefia (DW Check)	Altar84/Stn/Lahn	ARARI /ICARDA	2006

NA: Not available; EIAR: Ethiopian Institute of Agricultural Research; ARARI: Amhara Regional Agricultural Research Institute

Seedling resistance evaluation

The seedling evaluation experiment was conducted following the procedure described by Rouse *et al.* (2011). Five seeds each from 62 wheat test genotypes, a susceptible check (MacNair) and two field standard checks (Digalu and Malefia) were planted at Ambo Agricultural Research Center greenhouse separately in 5cm diameter plastic pots filled with growing medium composed of soil, sand and manure in the ratio of 2:1:1, respectively, and arranged in completely randomized design. The urediniospores (spores) of races TTTTF suspended in Soltrol 170 and adjusted at approximately 4×10^6 spores per 1 ml lightweight mineral oil was sprayed onto leaves of 7-day old seedlings when the first leaf was fully expanded and the second leaf was just emerged to grow. Inoculated plants were moistened with fine droplets of distilled water using atomizer after 30 minutes of inoculation and seedlings were incubated in the dark for 18 hours at 18°C and 95% relative humidity in a dew chamber. Thereafter, the seedlings were exposed to fluorescent light for four hours to provide favorable condition for stem rust infection. Seedlings were then allowed to dry for about two hrs and then, transferred from dew chamber to glass compartments in the greenhouse where conditions were regulated at 12 hrs photoperiod, and a temperature range of 18- 25°C and RH of 60-70%. Seedling infection type (IT) was noted

14 days post inoculation using the 0 to 4 infection scoring scale in which “0”, “;”, “;1”; “1”, “1+”, “2-”, “2”, “2+” stands for resistance and “3-”, “3”, “3+”, and “4” represent susceptibility infection (Stakman *et al.*, 1962). The experiment was repeated three times to exclude the possibility of disease escape.

Adult plant field resistance evaluation

Forty four genotypes that showed susceptible (≥ 3 -) and heterogeneous (2+3-) infection types in seedling test were retained and planted at a reputed ‘hotspot’ site, DebreZeit Agricultural Research Center, in 2020 main cropping season and 2021 off-season. Test materials along the two check varieties were planted in plots consisting of two rows of 1 m long with 20 cm intra-row spacing. The spacing between plots and blocks were maintained at 40 cm and 1m, respectively. Each genotype was planted manually at a seed rate of two grams per two rows. Experiments were established using an alpha lattice design (9 x 5) with two replicates. A mixture of two susceptible checks, Morocco and a standard cultivar PBW343 was planted around the experimental blocks a week earlier to serve as spreader rows. A water suspension of bulk urediniospores of TTKSK, TRTTF, TKTTF, TTTTF, TTRTF and JRCQC races approximately adjusted at 10 gm/ 5000 ml of water was applied using ultralow volume sprayer (Bromyard Industrial

Estate, HR7 4HS United Kingdom) twice on the spreader rows when most plants were at the stem elongation. Fertilizer was applied at the rate of 50/100 N/PKS ha⁻¹. In addition, other relevant field trial management practices were carried out as per the recommendations.

Adult plant field rust assessment

Disease severity

Disease assessment was started when 50% of the spreader rows were infected with stem rust. Stem rust severity was estimated visually as a proportion of the plant stem affected following a modified Cobb scale (Peterson *et al.*, 1948). Severity was assessed three times at twenty days interval from ten randomly pre-tagged plants of each plot and the mean of all stems was considered as the value for a plot.

Adult plant field response

Adult plant field response was scored using the description of Roelfs *et al.* (1992) as R = resistant (flecks and small uredinia), MR = moderately resistant (flecks and small to moderate uredinia), MS = moderately susceptible (moderate to large uredinia) and S = susceptible (large uredinia). If a line displayed multiple infection responses to stem rust, they were all recorded as RMR, MRMS and MSS.

Coefficient of infection

Coefficient of infection (CI) was calculated for each wheat genotype by multiplying the percentage disease severity by constant values of 0.2, 0.4, 0.6, 0.8, 0.9 and 1 assigned to R, MR, MR-MS, MS and MS-S and S responses, respectively (Stubbs *et al.*, 1986) and the average was used for differentiating the genotypes to various resistance categories.

Area under disease progress curve (AUDPC)

The computed CI value was used for calculating AUDPC for each genotype. AUDPC values were generated using the formula of Wilcoxon *et al.* (1975).

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

Where, x_i = the average coefficient of infection of i^{th} record, x_{i+1} = the average coefficient of infection of $i+1^{\text{th}}$ record and $t_{i+1} - t_i$ = Number of days between the i^{th} record and $i+1^{\text{th}}$ record, and n = number of observations.

Relative AUDPC (r-AUDPC) for each wheat genotype was calculated using the following formula:

$$r\text{-AUDPC} = \frac{\text{Line AUDPC}}{\text{Susceptible AUDPC}} \times 100$$

Data analysis

Seedling resistance evaluation

Of seedling infection types noted for each test genotype, the most frequent was retained to compare test materials for their seedling resistance (Stackman *et al.*, 1962).

Adult plant field resistance evaluation

Durum and bread wheat test genotypes and their respective susceptible checks were compared for their stem rust resistance and then assembled to different slow rusting genotype classes using the averages of TDS, CI and r-AUDPC following the same way followed by several authors (Parlevliet and van Ommeren, 1975; Ali *et al.*, 2007; Priyamvada *et al.*, 2011; Tabassum, 2011; Nzube *et al.*, 2012; Safavi, 2012; Mabrouk *et al.*, 2019; Abebech *et al.*, 2020; Alemu *et al.*, 2021). Correlation coefficients of disease parameters were determined using SPSS software (SPSS, 2016) version 24.

Results and Discussion

Seedling reaction of wheat genotypes to stem rust races

The greenhouse test revealed that both durum and bread wheat genotypes tested differed in their seedling reaction to the stem rust. None of the test genotypes were stem rust immune at seedling, however, 18 (29%), 27 (44%) and 17 (27%) genotypes had resistance (0; to 2), susceptible (≥ 3 -) and heterogeneous resistance (2+3-) infection types, respectively (Table 3). The susceptible checks used for the field evaluations, Malefia and Digalu, had susceptible (3-) infection type. The susceptible check, MacNair, used for seedling evaluation displayed infection type 3+ at the seedling stage. As reported by Nzube *et al.* (2012), wheat plants carrying adult plant resistance show disease susceptibility at seedling plant stage and varying levels of resistance to stem rust at post-seedling crop growth stages. Accordingly, 44 wheat genotypes which showed susceptible and heterogeneous infection types at seedling stage were selected and characterized for adult plant type of resistance to stem rust under field condition.

Table 3. Proportion of durum and bread wheat genotypes exhibiting resistant, heterogeneous and susceptible reaction to *Pgt* races TTTTF

Wheat types	Resistant(0; to 2+)	Susceptible(≥ 3 -)	Heterogeneous (2+3-)
Durum wheat lines	3	24	5
Bread wheat lines	8	-	6
Bread wheat varieties	7	3	6
DW+BW	18 (29)*	27 (44)	17 (27)
Susceptible checks	-	3	-

* Numbers in parentheses represent the percentage of genotypes in a given IT category

Adult plant field resistance evaluation

Field repose, terminal severity (TDS), coefficient of infection (CI) and relative area under disease progress curve (r-AUDPC) were used in classifying test genotypes into various adult plant resistance categories. Authors such as Tabassum (2011), Hei *et al.* (2015), Mabrouk *et al.* (2019) had also used these disease parameters to successfully identify slow rusting resistance sources in many cereal-rust pathosystems.

Adult plant field response

Disease symptom/host infection response can be used to quantify the level of host pathogen interactions. Adult plants exhibit field infection responses that vary from immune to highest expression of infection which is designated as susceptible field response type (S). In this study, the wheat genotypes tested had variable field responses that ranged from resistant to moderately resistant (R-MR) to susceptible (S) (Tables 4 and 5). The durum wheat genotypes 203726-2 and 203882-1 showed resistant to moderately resistant (R-MR) response in 2020, whereas another group of nine durum wheat lines 7974-2, 203680-2, 203676-1, 203733-1, 6112-2, 203831-2, 7169-1, 203855-2 and 203854-1 had MR field infection type in 2020, although none of these lines sustained reactions less than MS in the off-season of 2021 (Table 4). Field responses of durum lines 5454-1, 203818-1, 203826-1,

203899-1, 203855-1 and 203695-1 did not vary with years. The field response of the ten bread wheat genotypes was not found stable except for six genotypes ETBW9550 with MS, ETBW9548, BW173472, ETBW9652, Menze and Tsehay with MSS (Table 5). Overall field response results revealed the compatible interaction between test genotypes and six stem rust races used in mix. Likewise, several authors such as Parlevliet and van Ommeren (1975), Rubiales and Niks (1995), Priyamvada *et al.* (2011) and Nzuve *et al.* (2012) reported that high field response types reflect the occurrence of slow rusting resistance that are measured by slow epidemic build up or low disease severity. Hence, the wheat lines with compatible host pathogen interaction and low disease severity are expected to have resistance genes contributing to slow rusting that cannot be easily overcome by the virulent stem rust races.

Terminal disease severity

The terminal stem rust severity is a very useful parameter, measuring the overall cumulative resistances achieved at the end of the epidemics (Parlevliet and van Omeren, 1975). As indicated with TDS of the susceptible durum wheat variety Malefia (45% in 2020; 85% in 2021) and bread wheat check variety Digaelu (60% in 2020; 85% in 2021) the stem rust pressure was higher in 2021 than in 2020. Most of the wheat genotypes investigated in this study produced variable TDS

ranging from trace to 45% in 2020 and 15 to 75% in 2021 for durum wheat lines and 5 to 60% in 2020 and 25 to 75% in 2021 for bread wheat genotypes indicating that TDS recorded in both study years has successfully differentiated test genotypes to various slow rusting categories as reported by many authors (Ali *et al.*, 2007; Safavi, 2012; Mabrouk *et al.*, 2019; Abebech *et al.*, 2020; Alemu *et al.*, 2021). These authors grouped slow rusting resistance to high, moderate and low partial resistances with TDS values maintained within 1-30%, 31 - 50% and exceeding 50%, respectively. Accordingly, 36 (82%), 6 (13.6%) and 2 (4.5%) genotypes formed resistant, intermediates and susceptible resistance groups as measured by TDS noted in 2020.

During the off-season of 2021, only five durum wheat genotypes represented with numbers 7974-2, 6112-1, 203680-2, 7974-1, 203831-2 and bread wheat, ETBW9550 had 15

to 30% TDS with MS and S field responses (Tables 4 and 5). According to Parlevliet (1988) and Nzuve *et al.* (2012) such lines with low severity levels and compatible reaction not only reflect high levels of slow rusting but may possess resistance genes that can contain virulent rust races and avoid disease epidemics and minimize losses. These genotypes have great importance in achieving effective breeding for durable resistant variety development. On the other hand, 15 (34%) and 23 (52.3%) genotypes had intermediate (35-50%) and susceptible (55-75%) TDS as shown in Tables 4 and 5. The wheat lines with moderate levels of TDS had field stem rust responses ranging between MS and S. These genotypes may also be important for exploring stem rust resistance in wheat genotypes while the remaining lines under susceptible or low partial resistance were not promising to harbor resistance according to the level of disease severity observed.

Table 4. Seedling infection type (IT), terminal disease severity (TDS), coefficient of infection(CI) and relative area under disease curve(r-AUDPC) of durum wheat genotypes tested under field condition in 2020 main and 2021 off-season cropping seasons

Lines	2020 main cropping season					2021 Off-season			
	IT	TDS	CI	AUDPC	r-AUDPC	TDS	CI	AUDPC	r-AUDPC
5454-1	3-	TMS	0.8	10	1.30	35MS	28	329.5	35.68
7974-2	3-	2.5 MR	1	9	1.17	20MS	16	152.5	16.52
6112-1	3	2.5 MS	2	40	5.21	30S	30	317	34.33
203680-2	3-	5 MR	2	13.5	1.76	15MS	12	120	13.00
7974-1	3-	5 MRMS	3	26.5	3.45	15MS	12	114	12.35
7156-1	2+3-	5 MSS	4.5	32.5	4.23	50S	50	330	35.74
203676-1	3	7.5 MR	3	33	4.30	35MSS	31.5	129	13.97
203733-1	3-	7.5 MR	3	31	4.04	55MSS	45	492	53.28
6112-2	3	7.5 MR	3	53	6.91	55S	55	555	60.10
203709-1	3	7.5 MRMS	4.5	25	3.26	70S	70	550	59.56
203818-1	2+3-	7.5 MSS	6.75	64.5	8.40	50MSS	45	399	43.21
203826-1	2+3-	7.5 MSS	6.75	56.5	7.36	50MSS	45	485	52.52
7156-2	2+3-	7.5 MSS	6.75	94.5	12.31	60S	60	482.5	52.25
203726-2	3-	7.5 RMR	2.25	26.25	3.42	40MS	32	445	48.19
203882-1	3-	7.5 RMR	2.25	41.25	5.37	35MS	28	115	12.45
203831-2	3-	10 MR	4	55	7.17	20MS	16	202	21.88
7169-1	3-	10 MR	4	36	4.69	50S	50	515	55.77
203855-2	3	12.5 MR	5	75	9.77	45MS	32	410	44.40
203882-2	3-	12.5 MSS	11.25	110.75	14.43	75S	75	612.5	66.33
7972-2	3	12.5 MSS	11.25	96.25	12.54	45S	45	532.5	57.67
203854-1	3-	15 MR	6	60	7.82	40MSS	36	129.5	14.02
5768-1	3	17.5 MSS	15.75	233.3	30.40	70MRMS	42	402	43.53
7855-1	3-	20 MSS	18	155	20.20	55S	55	535	57.94
203886-2	2+3-	20MSS	18	155	20.20	65S	65	842.5	91.24
203899-1	3	22.5 MSS	20.25	205	26.71	40MSS	36	372.5	40.34
203899-2	3	22.5 MSS	20.25	256.25	33.39	55S	55	523.75	56.72
203886-1	3-	25 MSS	22.5	262.5	34.20	70S	70	740	80.14
203855-1	3	27.5 S	27.5	485	63.19	70S	70	937.5	101.53
203695-1	3	40 S	40	667.5	86.97	75S	75	1037.5	112.36
Malefia	3-	45 S	45	767.5	100.00	85S	85	923.4	100.00

MR, moderately resistant; MRMS, moderately resistant to moderately susceptible; MS, moderately susceptible; MSS, moderately susceptible to susceptible; S, susceptible; TDS, terminal disease severity; CI, coefficient of infection; AUDPC, area under disease progress curve; r-AUDPC, relative area under disease progress curve

Table 5. Seedling infection type (IT), terminal disease severity(TDS), coefficient of infection(CI) and relative area under disease curve(r-AUDPC) of bread wheat genotypes tested under field condition in 2020 main and 2021 off-season cropping seasons

Genotypes	IT	2020 main season				2021 Off-season			
		TDS	CI	AUDPC	r-AUDPC	TDS	CI	AUDPC	r-AUDPC
ETBW9550	3-	5 MS	4	29	3.27	25MS	20	202.5	21.57
ETBW9548	3-	7.5 MSS	6.75	80.25	9.04	65MSS	58.5	620	66.05
ETBW9543	3-	20 MRMS	12	153.25	17.27	55MSS	49.5	580.75	61.86
BW173472	3-	20 MSS	18	177.5	20.00	60MSS	54	745	79.36
ETBW9652	3-	20 MSS	18	187.25	21.10	35MSS	31.5	371.25	39.55
ETBW9313	3-	40 S	40	478.75	53.94	45MS	36	445	47.40
Shina	2+3-	20 MSS	18	247	27.83	75S	75	847.5	90.28
Kubsa	3-	27.5 MRMS	16.5	170	19.15	60S	60	616.25	65.65
KBG 01	2+3-	30 MSS	27	342.5	38.59	35MS	28	257.5	27.43
Menze	2+3-	35 MSS	31.5	500	56.34	55MSS	49.5	524.5	55.87
Sirbo	3-	35 MSS	31.5	290	32.68	65S	65	545	58.06
Kakaba	3-	45 MSS	40.5	597.5	67.32	75S	75	400	42.61
Bolo	2+3-	45 S	45	650	73.24	55MSS	49.5	528.75	56.32
Hidase	2+3-	55 S	55	942.5	106.20	75MSS	67.5	727.5	77.50
Tsehay	2+3-	60 MSS	54	817.5	92.11	65MSS	52	825	87.88
Digalu	3-	60 MSS	54	887.5	100.00	85S	85	938.75	100.00

MR, moderately resistant; MRMS, moderately resistant to moderately susceptible; MS, moderately susceptible; MSS, moderately susceptible to susceptible; S, susceptible; TDS, terminal disease severity; CI, coefficient of infection; AUDPC, area under disease progress curve; r-AUDPC, relative area under disease progress curve

Coefficient of Infection

Low values of coefficient of infection (CI) are an indicator for the presence of stem rust adult plant resistance. Many authors such as Draz *et al.* (2015); Sallam *et al.* (2016); Mitiku *et al.* (2018); Kokhmetova *et al.* (2021) proved that CI is instrumental for slow rusting genotypes classification into different genotypes with partial resistance. Lines with CI values of 0-20, 21-40, 41-60 were regarded as possessing high, moderate, and low levels of slow rusting resistance, respectively (Ali *et al.*, 2009). In this study, only five genotypes showed CI values between 0 and 20 in both seasons. Of these four (7974-2, 203680-2, 7974-1, 203831-2) were durum while ETBW9550 was bread wheat (Tables 4 and 5). These lines

also recorded low TDS of 15 to 25% and compatible field response (MS). Hence, they were grouped to highly slow rusting genotypes that probably have adult plant resistance genes and used as a resistance source in stem rust resistance breeding. In 2021, the disease pressure was considerably high as indicated by TDS and CI of check genotypes. Hence, most genotypes which had CI values of 0 to 20 in 2020 main season and categorized under highly slow rusting group had moved to moderately slow rusting with CI values of 21 to 40. In 2021 offseason, eleven wheat genotypes showed moderate level of CI ranging between 28 and 36. Of these, eight durum wheat lines represented with numbers 5454-1, 6112-1, 203676-1, 203726-2, 203882-1, 203855-2, 203854-1,

203899-1 and three bread wheat genotypes ETBW9652, ETBW 9313 and KBG 01 had moderate level of terminal severities (35-45%) with MS to S host responses and were categorized under moderately slow rusting genotypes.

In both main season 2020 and off-season 2021, bread wheat varieties Bolo, Hidase and Tsehay were as susceptible as susceptible checks, varieties Malefia and Digalu, having CI values exceeding 40, thus hardly contributing to stem rust resistance breeding.

Relative Area under Disease Progress Curve (r-AUDPC)

Disease progress curve is a better indicator of the extent of disease expression over time (van der Plank, 1963), thus, it is another important disease parameter routinely used for evaluating adult plant resistance in wheat. Several researchers including Safavi *et al.* (2013); Mabrouk *et al.* (2019) and Abebech *et al.* (2020) used relative area under disease progress curve (r-AUDPC) for classification of wheat varieties to resistant (r-AUDPC not exceeding 30% of the checks) and moderately resistant (r-AUDPC, 30-70%) groups. Hei *et al.* (2015) and Mitiku *et al.* (2018) also successfully classified wheat varieties to slow rusting genotypes using r-AUDPC. Thus, slow rusting behavior of the test materials in this study was assessed through r-AUDPC and the genotypes

were categorized into two distinct groups of partial resistance.

Results of the two years revealed that seven durum wheat lines represented by numbers 7974-2, 203680-2, 7974-1, 203676-1, 203882-1, 203831-2, 203854-1 and bread wheat ETBW9550 and KBG 01 exhibited r-AUDPC values not exceeding 30% of the respective susceptible checks. Of these genotypes, lines 7974-2, 203680-2, 7974-1, 203831-2 and ETBW9550 sustained low TDS not exceeding 25% and r-AUDPC values of 12-22% with MS field response and compatible ITs. These genotypes were considered as better slow rusting lines as the rust development in these lines was slower compared to the remaining lines. Wheat lines with low r-AUDPC and TDS values and high slow rusting traits are expected to possess genes that confer partial resistance, a more durable type of resistance as suggested by several researchers (Singh *et al.*, 2004; Draz *et al.*, 2015; Mabrouk *et al.*, 2019; EI-Orabey *et al.*, 2020).

Sustaining r-AUDPC values ranging between 31% and 70% of the respective checks, 8 and 27 wheat genotypes were grouped to moderately resistant genotypes in 2020 and 2021, respectively (Tables 4 and 5). Of the 27 moderately resistant genotypes detected in 2021, five durum wheat lines represented by numbers 5454-1, 6112-1, 203726-2, 203855-2, 203899-1 and two bread wheat lines, ETBW9652 and ETBW9313 had CI values of 28-36 and TDS/field

response values of 30S-45MSS in addition to r-AUDPC ranging between 31 and 70%. In these lines, the rust progress and development remained slower and restricted despite the ultimate expression of high infection types (Priyamvada *et al.*, 2011). According to Singh *et al.* (2004), genotypes with r-AUDPC values not exceeding 70% of the checks belong to partial resistance group which is a more durable resistance sought. Such lines with acceptable levels of partial resistance restrict the evolution of new virulent races of rust pathogen because multiple point mutations are extremely rare in nature (Ali *et al.*, 2007). Durum wheat lines represented by numbers 203886-2, 203886-1, 203855-1, 203695-1 and bread wheat genotypes, BW173472, Shina, Hidase and Tsehay were marked susceptible or highly

susceptible for showing r-AUDPC, CI and TDS exceeding 70%, 40% and 50%, respectively in 2021, the year with high stem rust pressure.

Association between disease parameters

TDS, CI and r-AUDPC were highly and significantly correlated invariably with years (Table 6). Strong positive correlation between these three slow rusting rating disease parameters (Table 6) indicates that either of these disease parameters can be used for measuring the slow rusting resistance of the wheat test materials. Such strong correlation between disease parameters were also reported by several authors (Safavi, 2012; Shah *et al.*, 2010; Attri and Dey, 2021).

Table 6. Linear correlation coefficients(r) of pair-wise relationships between terminal disease severity (TDS), coefficient of infection (CI) and relative area under disease progress curve (r-AUDPC) during 2020 main season and 2021 off-season

	2020 Main cropping season		2021 Offseason	
	TDS	CI	TDS	CI
CI	0.97**	1.00	0.96**	1.00
r-AUDPC	0.93**	0.97**	0.85**	0.85**

**Significance level at $P \leq 0.01$

Conclusion and Recommendation

This research demonstrated that durum wheat landraces can be a source for slow rusting resistance. Resistance categories including highly slow rusting, moderately slow rusting and susceptible were observed in the

results of the current wheat genotypes evaluation trials. Durum wheat lines represented by numbers 7974-2, 203680-2, 7974-1, 203831-2 and bread wheat line ETB9550 exhibited low frequency of TDS (15-25%) with MS field response, low CI values of 12-20 and r-AUDPC values of 12-22% with susceptible seedling ITs in 2021, the year with high stem rust pressure.

These wheat lines were supposed to have genes contributing to durable stem rust resistant, being sometimes expressed as highly slow rusting cultivars. Five durum wheat lines, 5454-1, 6112-1, 203726-2, 203855-2, 203899-1 and two bread wheat lines, ETBW9652 and ETBW9313 had moderate level of adult plant resistance, sustaining r-AUDPC of 31-70%, CI values of 28-36 and TDS/host responses of 30S-45MSS with susceptible seedling ITs. The highly and moderately slow rusting genotypes identified from this study can be confidently used as donor parents in efforts toward durable stem rust resistant cultivar development but after being postulated for inherent Sr-genes including the known adult plant resistance genes such as *Sr2*, *Sr55*, *Sr56*, *Sr57* and *Sr58*.

Acknowledgements

The authors are thankful to Ethiopian Biodiversity Institute, Kulumsa Agricultural Research Center and Adet Agricultural Research Center for providing the wheat seeds. Ambo and Debre-Zeit agricultural Research Centers are recognized for hosting the greenhouse and field experiments. Ethiopian Institute of Agricultural Research is highly acknowledged for financial support.

References

Abebech DA, Mesfin KG, Gobeze LY. 2020. Assessment of sources of adult

plant resistance genes to stem rust in Ethiopian durum wheat genotypes. Asian Journal of Applied Sciences, 13: 76-83.

Admassu B, Friedt W, Ordon F. 2012. Stem rust seedling resistance genes in Ethiopian wheat cultivars and breeding lines. African Crop Science Journal, 20:149-161.

Adugnaw A, Dagninet A. 2020. Wheat production and marketing in Ethiopia: Review study, Cogent Food and Agriculture, 6(1): 1778893.

Alemu AZ, Getnet MA, Lidiya TH, Tamirat NG, Daniel KT, Fikrte Y, Hawila T, Shumi RG. 2021. Evaluation of stripe rust (*Puccinia striiformis* f. sp. *Tritici*) resistance in bread wheat (*Triticum aestivum* L.) genotypes in Ethiopia. Advances in Bioscience and Bioengineering, 9: (2):25-31.

Ali S, Jawad S, Shah A, Ibrahim M. 2007. Assessment of wheat breeding lines for slow yellow rusting (*Puccinia striiformis* west. *tritici*). Pakistan Journal of Biological Sciences, 10:3440-3444.

Ali S, Shah SJA, Khalil IH, Raman H, Maqbool K, Ullah W. 2009. Partial resistance to yellow rust in introduced winter wheat germplasm at the north of Pakistan. Australian Journal of Crop Science, 3:37-43.

Attri H, Dey T. 2021. Screening of stripe rust resistance in bread wheat (*Triticum aestivum* L.) genotypes. International Journal of Current Microbiology and Applied Sciences, 10 (1): 1236-1244.

Bekele H, Bedada G, Zerihun T, Erena E, Pablo Olivera, Endale Hailu Abera, Worku Denbel Bulbula, Bekele Abeyo, Ayele Badebo, Gordon Cisar, Gina Brown-Guedira,

- Sam Gale, Yue Jin, and Matthew N. Rouse. 2019. Characterization of Ethiopian wheat germplasm for resistance to four *Puccinia graminis* f. sp. *tritici* races facilitated by single-race nurseries. *Plant Disease*, 103:2359-2366.
- Bhardwaj SC, Mohinder P, Pramod P. 2014. Ug99-Future challenges. p.231-247. In: Goyal, A. and Manoharachary, C. (ed.) *Future Challenges in Crop Protection against Fungal Pathogens*. Fungal Biology. Springer, New York.
- Burdon JJ, Barrett LG, Rebetzke G, Thrall PH. 2014. Guiding deployment of resistance in cereals using evolutionary principles. *Evolutionary Applications*, 7: 609-624.
- CSA (Central Statistics Agency). 2021. *Agricultural sample survey 2020/21 (2013 E.C.): Report on area and production of major crops (Private peasant holdings, Meher Season)*. Volume I. Statistical Bulletins 590, Addis Ababa, Ethiopia.
- Draz IS, Abou-Elseoud MS, Kamara AM, Alaa-Eldein OA, El-Bebany AF. 2015. Screening of wheat genotypes for leaf rust resistance along with grain yield. *Annals of Agricultural Science*, 60:29-39.
- El-Orabey WM, Mostafa FA, Abdel-Wahed GA, Selim ME. 2020. Assessment of some Sources of adult plant resistance wheat stem rust in Egypt. *Journal of Microbiology and Biochemistry Technology*, 12:451.
- Giraldo P, Benavente E, Manzano-Agugliaro, Gimenez E. 2019. World research trends on wheat and barley: A bibliometric comparative analysis. *Agronomy*, 9: 352.
- Hei N, Hussein AS, Laing M, Admassu B. 2015. Assessment of Ethiopian wheat lines for slow rusting resistance to stem rust of wheat caused by *Puccinia graminis* f.sp. *tritici*. *Journal of Phytopathology*, 163: 353–363.
- Kokhmetova A, Rsaliyev A, Malysheva A, Atishova M, Kumarbayeva M, Keishilov Z. 2021 Identification of stripe rust resistance genes in common wheat cultivars and breeding lines from Kazakhstan. *Plants*, 10(11): 2303.
- Mabrouk OI, El-Orabey WM, Esmail S. 2019. Evaluation of wheat cultivars for slow rusting resistance to leaf and stem rust diseases in Egypt. *Egypt. Journal of Phytopathology*, 47(2): 1-19.
- McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Appels R, Xia XC. 2017. *Catalogue of Gene Symbols for Wheat: 2017 Supplement*. [(Accessed on 20 June 2022)]; Available online: <http://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2017.pdf>
- McIntosh RA, Wellings CR, Park RF. 1995. *Wheat Rusts: An Atlas of Resistance Genes*. London: Kluwer Academic Publishers. Pp.200.
- Meyer M, Bacha N, Tesfaye T, Alemayehu Y, Abera E, Hundie B, Woldeab G, Girma B, Gemechu A, Negash T, Mideksa T, Smith J, Jaleta M, Hodson D, Gilligan CA. 2021. Wheat rust epidemics damage Ethiopian wheat production: A decade of field disease surveillance reveals national-scale trends in past outbreaks. *PLoS ONE*, 16(2): e0245697
- Mitiku M, Hei NB, Abera M. 2018. Characterization of slow rusting resistance against stem rust (*Puccinia graminis* f. sp. *tritici*) in selected

- bread wheat cultivars of Ethiopia. *Advances in Crop Science and Technology*, 6: 389.
- Nzuve FM, Bhavani S, Tusiime G, Njau P, Wanyera R. 2012. Evaluation of bread wheat for both seedling and adult plant resistance to stem rust. *African Journal of Plant Science*, 6:426–432.
- Parlevliet J. E. 1988. Strategies for the utilization of partial resistance for the control of cereal rusts. Pp. 48-62. In: Simmonds NW, Rajaram S (eds.), *Breeding Strategies for Resistance to the Rusts of Wheat*. CIMMYT, Mexico, D.F.
- Parlevliet JE, van Ommeren A. 1975. Partial resistance of barely to leaf rust, *Puccinia hordei*, II Relationship between field trials, micro plot tests and latent period. *Euphytica*, 24:293-303
- Parlevliet JE. 1985. Resistance of the non-specific type. *The Cereal Rusts*. Academic, Orlando. FL, 2. Pp. 501-525.
- Peterson RF, Campbell A, Hannah AE. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research*, 26:496–500.
- Priyamvada, Saharan MS, Tiwari R. 2011. Durable resistance in wheat. *International Journal of Genetics and Molecular Biology*, 3:108-114.
- Rehman, AU, Sajjad M, Khan SH, Ahmad N. 2013. Prospects of wheat breeding for durable resistance against brown, yellow and black rust fungi. *International Journal of Agriculture and Biology*, 15: 1209-1220.
- Roelfs AP, Singh RP, Saari EE. 1992. *Rust Diseases of Wheat: Concepts and Methods of Disease Management*. CIMMYT, Mexico, D.F.Pp.81.
- Rouse MN, Wanyera R, Njau P, Jin Y. 2011. Sources of resistance to stem rust race Ug99 in spring wheat germplasm. *Plant Disease* 95:762–766.
- Rubiales D, Niks RE. 1995. Characterization of Lr34, a major gene conferring nonhypersensitive resistance to wheat leaf rust. *Plant Disease* 79:1208-1212.
- Safavi SA. 2012. Evaluation of slow rusting parameters in thirty seven promising wheat lines to yellow rust. *Technical Journal of Engineering and Applied Sciences*, 2: 324-329.
- Safavi SA, Ahari AB, Afshari F, Arzanlou M. 2013. Slow rusting resistance in Iranian barley cultivars to *Puccinia striiformis* f.sp. *hordei*. *Journal of Plant Protection Research*, 53(1): 5-11.
- Sallam ME, El-Orabey WM, Omara RI. 2016. Seedling and adult plant resistance to leaf rust in some Egyptian wheat genotypes. *African Journal of Agricultural Research*, 11:(4), 247-258.
- Shah SJA, Imtiaz M, Hussain S. 2010. Phenotypic and molecular characterization of wheat for slow rusting resistance against *Puccinia striiformis* Westend. f.sp. *tritici*. *Journal of Phytopathology*, 158:393-402.
- Singh RP, William HM, Huerta-Espino J, Rosewame G. 2004. Wheat rust in Asia: meeting the challenges with old and new technologies. New directions for a diverse planet. Pp. 1-13. In: Fischer T, Turner N, Angus J, McIntyre L, Robertson M, Borrell A, Lloyd D. (eds.), *Proceeding of 4th International Crop Science Congress*.

- Brisbane, Australia, 26 September–1 October 2004. Brisbane, Australia.
- Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel S, Singh PK, Singh S, Govindan V. 2011. The Emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annual Review of phytopathology*, 49 (1):465–481.
- Singh RP, Singh PK, Rutkoski J, Hodson DP, He X, Jorgensen LN, Hovmoller MS, Huerta-Espino J. 2016. Disease impact on wheat yield potential and prospects of genetic control. *Annual Review of Phytopathology*, 54: 303–322.
- SPSS. 2016. *Statistical Package for Social Sciences*, 24ed users guide, Chicago.
- Stakman EC, Stewart DM, Loeggering WQ. 1962. Identification of physiological races of *Puccinia graminis* var. *tritici*. *Agricultural Research Services E617*: US Department of Agriculture, Washington, DC. Pp.53.
- Stubbs RW, Prescott JM, Sarrri EE, Dubin HJ. 1986. *Cereal Disease Methodology Manual*. CIMMYT, El Batan, Mexico. Pp.51.
- Tabassum S. 2011. Evaluation of advance wheat lines for slow yellow rusting (*Puccinia striiformis* f.sp. *tritici*). *Journal of Agricultural Science*, 3: 239-249.
- van der Plank JE. 1963. *Plant diseases: Epidemic and Control*. Academic Press, New York. Pp. 349.
- Wilcoxson RD, Skovmand B, Atif AH. 1975. Evaluation of wheat cultivars ability to retard development of stem rust. *Annals of Applied Biology*, 80: 275-281.
- Yu L, Barbier H, Matthew NR, Singh S, Singh RP, Bhavani S, Huerta-Espino J, Sorrells ME, 2014. A consensus map for Ug99 stem rust resistance loci in wheat. *Theoretical and Applied Genetics*, 127: 1561-1581.
- Zhang Bo, Chi D, Hiebert C, Fetch T, McCallum B, Xue A, Cao W, Depauw R, Fedak G. 2019. Pyramiding stem rust resistance genes to race TTKSK (Ug99) in wheat. *Canadian Journal of Plant Pathology*, 41(3): 443-449.