Journal of Agricultural Science and Practice
Volume 4(6), pages 188-201, December 2019
Article Number: 52E878294
ISSN: 2536-7072
https://doi.org/10.31248/JASP2019.175
https://integrityresjournals.org/journal/JASP

Full Length Research

Identification of new sources of resistance to stem rust race Ug99 (TTKSK) in wheat genotypes

Fentaw Abate[1*], Patrick Rubaihayo[1], Thomas Odong[1], Seid Ahmed[2] and Hailay Mehari[1]

[1]Department of Agricultural production, school of Agriculture, college of Agricultural and environmental sciences, Makerere University, Kampala, Uganda.
[2]International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat-Institutes, Rabat, Morocco.

*Corresponding author. Email: fenti2004@gmail.com

Received 26th November, 2019; Accepted 30th December, 2019

ABSTRACT: Stem rust disease which caused by *Puccinia graminis* f. sp. *tritici* is one of the major wheat production constraints in the high lands of central, south eastern and north western part of Ethiopia. The disease had caused up to 100% yield loss on the unprotected wheat farms. This study was conducted to identify new sources of resistance to stem rust disease. After the removal of genotypes that showed a major gene (race specific) resistance expression at seedling test, 60 selected genotypes were evaluated for their adult plant resistance to stem rust and agronomic traits across three locations (Debrezeit, Adet and Kulmsa) using a 5 × 12 alpha lattice design with three replications. Genotypes showed a highly significant difference ($p = 0.001$) on the traits of final rust severity (FRS), area under disease progress curve (AUDPC), coefficient of infection (CI) and apparent infection rate (r) at different locations. Moreover, combined analysis of variance also showed the presence of highly significant effects of genotypes, environments and GE interaction on the magnitude of FRS, AUDPC, CI, r, days to maturity (DM), plant height (PH), hectoliter weight (HLW), thousands kernel weigh (TKW) and grain yield (GY) across testing locations. The correlation analysis revealed the presence of positive highly significant ($p<0.001$) relationship of FRS with both CI ($r=0.894$) and AUDPC ($r=0.877$). A positive and highly significant ($p<0.001$) correlation was also observed between CI and AUDPC ($r=0.996$) of the tested genotypes. Principal component analysis indicated that only the first four principal components (PCs) explained 86.58% of the total variation among the tested genotypes. Cluster analysis also confirmed the presence of variation among the tested genotypes by dividing them into five major groups. In this study, four bread wheat genotypes (G12, G60, G31 and G52) were found to be resistant to stem rust disease across the three locations and could be used as source of stem rust resistance in future wheat improvement program.

Keywords: AUDPC, coefficient of infection rate, cluster analysis, final rust severity, stem rust.

INTRODUCTION

Wheat stem rust disease which caused by the fungus *Puccinia graminis* f. sp. *Tritici* was first reported in Uganda in 1999 and has now spread to different wheat producing countries of the world (Singh et al., 2011). The disease appeared in Ethiopia in 2003 and became a major production constraint in wheat-growing areas of the country (Priyamvada et al., 2011). It has the potential of causing up to 100% yield losses in the unprotected wheat fields (Hailu et al., 2015). Tremendous efforts were done to control this devastating disease by spraying fungicides, though the method was not sustainable to small scale farmers due to the unaffordable chemical costs and unfriendly consequence of fungicides on the health of human being and their surrounding environments (Jaleta et al., 2019). Host plant resistance is the most economical feasible and ecologically safe method for controlling wheat stem rust disease (Gamalat and El-sawi, 2015). Accordingly, efforts have been put in looking for novel

sources of resistance to this destructive disease. There is, however, a potential of resistance breakdown among the deployed resistant wheat varieties as there is frequent appearance of new virulent pathotypes of Ug99 and its highly adapted long distance migration of uredospores through wind and rain deposition (Singh et al., 2011).

In Ethiopia, resistance breeding for wheat rust disease was conducted by various national and regional agricultural research centers and developed many improved varieties which were resistant to stem rust disease. While after the appearance of race Ug99 in Ethiopia, most of the improved wheat varieties such as 'Lacketch', 'Kubsa' and 'Enkoy' were removed from production (Beteselassie et al., 2007) and the national average productivity of wheat declined from 2.1 t/ha to 1.83 t/ha, which was 45% lower than the average wheat productivity of the world (Sahoo et al., 2016). Hence, in order to improve the national average wheat productivity, it was vital that undergoing extensive screening of various wheat genotypes to explore novel sources of resistance genes to Ug99 and incorporate those effective genes into new high yield commercial varieties. Thus, in this research, various bread wheat genotypes which were collected from International Centre for Maize and Wheat improvement (CIMMYT), International Centre for Agricultural Research in the Dry Areas (ICARDA), Debrezeit, Kulmsa and Adet Agricultural Research Centers were evaluated against stem rust disease that caused by race Ug99.

MATERIALS AND METHODS

The experiment was conducted across three stem rust hot spot locations: Debrezeit, Adet and Kulumsa Agricultural Research Centers in the main growing season of year 2016. Detail description of experimental sites is indicated in Table 1.

Experimental materials

A total of 120 bread wheat genotypes: 85 from International Centre for Maize and Wheat improvement (CIMMYT), 20 from International Centre for Agricultural Research in the Dry Areas (ICARDA) and 15 promising genotypes from Debrezeit, Kulmsa and Adet Agricultural Research Centers along with susceptible check (PBW343) were used for seedling resistance test against race Ug99 under controlled greenhouse conditions. After the removal of genotypes that showed a major gene resistance expression at seedlings resistance test, a panel of 60 wheat genotypes which exhibited mixed (intermediate and susceptible) and susceptible infection types were used to conduct adult plant resistance test against stem rust (*puccinia graminis)* disease at Debrezeit, Adet and Kulmsa Agricultural Research Centers which are internationally known as stem rust disease hot spot area. The description of the tested genotypes is presented in Table 2.

Experimental design and management

Seedling resistance test

Evaluation of 120 bread wheat genotypes against race TTKSK (Ug99) was carried out under controlled greenhouse conditions at their seedling stage. Five seedlings were grown per genotype in each 10 cm diameter plastic pot which filled with compost, light soil and sand at a 1:1:1 ratio (v/v/v) respectively. Until the seedlings were ready for inoculation, they were kept in microclimate room that had a temperature of 15 to 20^{o}C. Before inoculation, urediniospores of *Pgt* race TTKSK which maintained at liquid nitrogen tank were heat-shocked for 10 minutes in a water bath at 40°C and kept in a rehydration chamber maintained in a KOH solution for 2 to 4 hours with 80% relative humidity (Jin et al., 2007). Then spores were suspended in a light mineral oil, Soltrol 170 light oil (Chevron Phillips Chemical Company, The Woodlands, TX) and inoculation of seedlings with spore suspension adjusted to 4 X 10^6 spores ml^{-1} was conducted using spore inoculators when seedlings were reached at 2 to 3 leaf stage. Subsequently, the inoculated seedlings were placed in a dew chamber in darkness for 18 hours at 18 to 22^{o}C and 98 to 100% relative humidity. Then inoculated plants were transferred to glass compartments in greenhouse where the temperature and the relative humidity were kept in with a range of 18 to 25^{o}C and 60 to 70% respectively, for 12 hours photoperiod (Stubbs et al., 1986).

Adult plant resistance test

It was conducted using alpha lattice design (5 x12) with three replications. Each genotype was planted on two adjacent rows that had 1 m row length with inter-row spacing of 20 cm. The well-known most susceptible bread wheat variety (Morocco) was planted as spreader plant around the four sides of experimental area while variety PBW343 was used as susceptible check. To ensure uniform disease pressure and inoculums dissemination, seven-day-old seedlings of spreader plants were inoculated with the water-spores mixture (approximately 3 to 5 mg of freshly collected spores per 1 ml of distilled water suspension) and then the spreader plants were sprayed with urediniospores suspended in light mineral oil Soltrol 170 (Chevron Phillips Chemical Company, The Woodlands, TX) (Sikharulidze et al., 2015). The recommended fertilizer rate (50 kg/ha urea and 100 kg/ha DAP) and seed rate (150 kg/ha) were used in the experiment (Haile et al., 2012). The whole amount of DAP

Table 1. Description of study areas.

Locations	Altitude (masl)	Rainfall (mm)	Soil type	Global Position		Temperature (ºC)	
				Latitude	Longitude	Min	Max
Debrezeit	1900	851	clay loam	8°44 ' N	38°58 ' E	8.9	28.3
Adet	2216	1331	Nitosol	11°16 ' N	37°29 ' E	9.2	25.5
Kulmsa	2210	832	clay loam	8°00 ' N	39°07 ' E	9.9	23.1

Sources: (Denbel et al., 2013; Tamene et al., 2015; Zemede et al., 2019).

Table 2. Description of the tested bread wheat genotypes.

Geno	Code	Pedigree
G1	6003	KINGBIRD #1
G2	6006	MUTUS/DANPHE #1/4/C80.1/3*BATAVIA//2*WBLL1/3/C80.1/3* QT4522//2*PASTOR
G3	6008	BAJ #1/5/ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92
G4	56016	PAURAQ/4/MARCHOUCH*4/SAADA/3/2*FRET2/KUKUNA//FRET2
G5	41441	unknown
G6	6019	INQALAB 91*2/KUKUNA//PFAU/WEAVER/3/INQALAB 91*2/KUK UNA/4/TRCH/SRTU//KACHU
G7	6020	INQALAB 91*2/KUKUNA//PFAU/WEAVER/3/INQALAB 91*2/KUK UNA/4/TRCH/SRTU//KACHU
G8	6021	INQALAB 91*2/KUKUNA//PFAU/WEAVER/3/INQALAB 91*2/KUK UNA/4/TRCH/SRTU//KACHU
G9	6023	WHEAR/SOKOLL/3/TRCH/SRTU//KACHU
G10	6024	SW2148/2*ROLF07/3/HUW234+LR34/PRINIA*2//SNLG
G11	KAKABA	Unknown
G12	6027	WAXWING/7/TNMU/6/CEP80111/CEP81165/5/IAC5/4/YKT406/3/AG/ ASN//ATR/8/ATTILA/3*BCN//BAV92/3/TILHI/4/ SHA7/VEE#5//ARIV92
G13	127812	unknown
G14	6028	WAXWING/7/TNMU/6/CEP80111/CEP81165/5/IAC5/4/YKT406/3/AG/ ASN//ATR/8/ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92
G15	6036	KACHU/SAUAL/3/TRCH/SRTU//KACHU
G16	6049	KACHU/6/WHEAR/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/C80.1/ 3*BATAVIA//2*WBLL1/7/TRCH/SRTU//KACHU
G17	6062	ATTILA*2/PBW65//KIRITATI/3/QUELEA
G18	6066	PF74354//LD/ALD/4/2*BR12*2/3/JUP//PAR214*6/FB6631/5/NL750/ 6/PVN/7/TOBA97/PASTOR/8/UP2338*2/KKTS*2// YANAC
G19	6081	FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD # 1//INQALAB 91*2/TUKURU
G20	6086	MELON//FILIN/MILAN/3/FILIN/4/2*TRCH/SRTU//KACHU
G21	6091	ATTILA*2/PBW65//TAM200/TUI/5/2*SERI.1B//KAUZ/HEVO/3/ AMAD*2/4/KIRITATI
G22	6095	ATTILA*2/PBW65/5/CNO79//PF70354/MUS/3/PASTOR/4/BAV92/6/KI NGBIRD#1/7/CNO79//PF70354/MUS/3/PASTOR/4/BAV92*2/5/FH6-1-7
G23	6099	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/SAUAL/5/SERI.1B//KAUZ /HEVO/3/AMAD*2/4/KIRITATI/6/KACHU/SAUAL
G24	6101	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/SAUAL/5/SERI.1B //KAUZ/ HEVO/3/AMAD*2/4/KIRITATI/6/KACHU/SAUAL
G25	6103	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/SAUAL/5/PBW343*2/ KUKUNA//PARUS/3/PBW343*2/KUKUNA/6/KACHU/SAUAL
G26	6104	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/SAUAL/5/PBW343*2/ KUKUNA//PARUS/3/PBW343*2/KUKUNA/6/KACHU/SAUAL

Table 2. Contd.

G27	6107	ATTILA/3*BCN//BAV92/3/TILHI/5/BAV92/3/PRL/SARA//TSI/VEE #5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA*2/6/ HUW234+ LR34/PRINIA//UP2338*2/VIVITSI
G28	139463	unknown
G29	6113	ROLF07*2/KIRITATI*2/10/PFAU/WEAVER*2//BRAMBLING/9/RABE/6/WRM /4/FN/3*TH//K58/2*N/3/AUS-6869/5/ PELOTAS-ARTHUR/7/2* RABE/8/IRENA
G30	6114	FRET2/KUKUNA//FRET2/3/YANAC/4/FRET2/KIRITATI*2/5/WBLL1/ KUKUNA//TACUPETO F2001/3/UP2338*2/VIVITSI
G31	6115	WBLL1*2/KUKUNA*2//WHEAR*2/3/ATTILA*2/PBW65*2//YANAC
G32	6116	TRCH/SRTU//KACHU/3/WAXWING/PARUS//WAXWING/KIRITATI /4/TRCH/SRTU//KACHU
G33	6117	PRL/2*PASTOR//SRTU/3/PRINIA/PASTOR/5/2*SERI.1B//KAUZ/HEVO/3 /AMAD*2/4/KIRITATI
G34	6123	HW2045/3/WAXWING/SRTU//WAXWING/KIRITATI/4/KINGBIRD #1//INQALAB 91*2/TUKURU
G35	6124	HW2045/3/WAXWING/SRTU//WAXWING/KIRITATI/4/KINGBIRD #1//INQALAB 91*2/TUKURU
G36	41699	unknown
G37	6141	TC870344/GUI//TEMPORALERA M 87/AGR/3/2*WBLL1/5/ONIX /4/MILAN/KAUZ//PRINIA/3/BAV92
G38	6145	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1/5/SOKOLL/3/PASTOR //HXL7573/2*BAU
G39	6148	BECARD #1/BAVIS
G40	6149	BECARD #1/BAVIS #1
G41	6155	VEE/MJI//2*TUI/3/PASTOR/4/BERKUT/6/2*OASIS/5*BORL95/5 /CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI
G42	6165	BAVIS*2/4/PASTOR//HXL7573/2*BAU/3/SOKOLL/WBLL1
G43	80893	unknown
G44	6175	ETBW115 (Digelu)
G45	6180	KACHU*2/3/ND643//2*PRL/2*PASTOR
G46	84772	Unknown
G47	6187	SHA7/VEE#5//ARIV92/3/PBW343*2/KUKUNA/4/2*VARIS/MISR2, EGY/3/FRET2/KUKUNA//FRET2
G48	6188	KACHU/SAUAL/4/VARIS/MISR2, EGY/3/FRET2/KUKUNA//FRET2 /5/KACHU/SAUAL
G49	6189	VARIS/MISR2, EGY/3/FRET2/KUKUNA//FRET2*2/7/TUKURU// BAV92/RAYON/6/NG8201/KAUZ/4/SHA7//PRL/VEE#6/3/FASAN/ 5/MILAN/KAUZ
G50	6192	KRONSTAD F2004/3/TRCH/SRTU//KACHU/4/TRCH/SRTU//KACHU
G51	6194	BABAX/LR42//BABAX/3/ER2000*2/4/SRN/AE.SQUARROSA (358)/ / MILAN/SHA7
G52	6195	KRONSTAD F2004/3/TRCH/SRTU//KACHU/4/TRCH/SRTU//KACHU
G53	6199	KACHU/SAUAL/4/VARIS/MISR2, EGY/3/FRET2/KUKUNA//FRET2 /5/ KACHU/SAUAL
G54	6203	KACHU/SAUAL/4/VARIS/MISR2, EGY/3/FRET2/KUKUNA//FRET2 /5/KACHU/SAUAL
G55	120699	Unknown
G56	6207	FRET2/KUKUNA//FRET2/3/WHEAR/4/FRET2*2/KUKUNA/5/2* WBLL1/KUKUNA//TACUPETO F2001/3/UP2338*2/VIVITSI
G57	6208	FRET2/KUKUNA//FRET2/3/WHEAR/4/FRET2*2/KUKUNA/5/2* WBLL1/KUKUNA//TACUPETO F2001/3/UP2338*2/VIVITSI
G58	DANDAA	KIRITATI//2*PBW65/2*SERI.IB
G59	PBW343	Unknown
G60	WANE	SOKOLL/EXCALIBUR

was applied at planting while urea was split into half at planting and the remaining half at tillering stage. The plants were repeatedly irrigated to ensure optimum plant growth and development, create favourable environment for disease development and enable the plants to express their genetic resistance to stem rust (Kosgey et al., 2015; Nzuve et al., 2013).

Data collection

Seedling resistance test

Disease data was collected 14 days after inoculation using Stakman et al. (1962) scoring system (IT = 0 – 4) with McIntosh et al. (1995) modifications: where ITs "0" represented no visible uredinia, ";" (hypersensitive flecks without uredinia), "1" (small uredinia surrounded by necrosis), "2" (small to medium sized uredinia usually with green islands and surrounded by chlorosis or necrosis), "3" (moderate sized uredinia with or without chlorosis) and "4" (large uredinia without chlorosis). The plus and minus signs were used to indicate the occurrence of larger or smaller uredinia than the normal uredinia, respectively within a given Infection type. Genotypes with low infection types (ITs=0-2) were considered as resistant, and genotypes with infection type =2+ and 3- showed mixed reaction (resistance and susceptible) while genotypes with high infection types (ITs= 3-4) were considered as susceptible for stem rust disease.

Adult plant resistance test

Rust severity data collection was started when the spreader row plants showed maximum rust infection and continued at a weekly interval up to the plants attaining physiological maturity. Rust severity was determined as percentage using Peterson et al. (1948) modified Cobbs' scale method. The response of plants to stem rust infection was measured based on the pustule size and any associated necrotic and/or chlorotic lesions that occurred on plants at field conditions. According to Roelfs et al. (1992) scaling method, the response of plants to stem rust infection were classified as follows: R = resistant, RMR = resistant to moderately resistant, MR = moderately resistant, MRMS = moderately resistant to moderately susceptible, MSS= moderately susceptible to susceptible, MS = moderately susceptible, and S = susceptible. Selected quantitative traits such as Days to heading, Days to maturity, Thousands kernels weight (g), Hectoliter weight (g/hL) and grain yield (adjusted at moisture content 12.5%) were also collected by considering all plants of each plot as sample while data of Plant height (cm), Spike length (cm), Number of spikelets per spike and Number of kernels per spike were collected from five randomly selected plants from each plot.

Data analysis

The generated data were subjected to separate analysis of variance for each location using Genstat software 13th edition (Payne et al., 2011) to observe differences among genotypes on their resistance to stem rust. Bartlett's chi-square test and normality test were also used to check the homogeneity and the normal distribution of error variance between environments, respectively. Combined analysis of variance over locations was conducted using linear mixed model. The genotypes were considered as a fixed effect while blocks, replications and environments were random effects. The least significant difference (LSD) value was used to compare genotypic means at $p<0.05$ probability level. The ANOVA was fitted as the following linear Mathematical model:

$$\boldsymbol{y}_{ijklm} = \mu + \boldsymbol{\rho}_i + \boldsymbol{\iota}_j + \boldsymbol{b}_{m(\iota)} + \boldsymbol{\rho\iota}_{ji} + \boldsymbol{\varepsilon}_{ijklm}$$

Where, $\boldsymbol{y}_{ijklm}$ = the observed value for the i^{th} genotype from j^{th} location, m^{th} block nested within l^{th} replication; $\boldsymbol{\mu}$ = the general mean effect, $\boldsymbol{\rho}_i$ = the i^{th} genotype effect (considered as fixed effect), $\boldsymbol{\iota}_j$ = the j^{th} environment effect (considered as random effect), $\boldsymbol{b}_{m(l)}$ = the effect of m^{th} replication nested within the i^{th} environment, $\boldsymbol{\rho\iota}_{ji}$ = interaction effect of j^{th} environment and i^{th} genotype (considered as random effect) and $\boldsymbol{\varepsilon}_{ijklm}$ = the experimental error(considered as random). Besides, area under disease progress curve (AUPDC) of each genotype was also calculated by using Wilcox son et al. (1975) method.

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

Where x_i = stem rust severity on the i^{th} date, t_i = the time in days after appearance of the disease, and n = number of date on which stem rust will be recorded. Spearman rank correlation coefficient was also carried out to know the relationship between disease measuring parameters. Coefficient of infection (CI) was also calculated by multiplying the rust severity with constant value for field response where R = 0, RMR = 0.1, MR = 0.2, MRMS = 0.4, MS = 0.6, MSS = 0.8 and S = 1(Stubbs et al., 1986). Apparent infection rate (r) was calculated by using vander Plank's equation (vander Plank, 1963) as follows:

$$r = \frac{1}{(t_2 - t_1)}\left[log_e^{\frac{x_2}{1-x_1}} - log_e^{\frac{x_1}{1-x_1}}\right]$$

Where: "r" = Apparent infection rate; "t_1" = initial time of disease assessment; "t_2" = final time of disease assessment; "x_1" and "x_2" represent amounts of disease present at "t_1" and "t_2" respectively. The principal component analysis was calculated using disease measuring parameters and other agronomic traits through varimax rotation method that is generally considered

Table 3. Seedling infection type (IT) for seedling test against race TTKSK.

Infection type	Number of genotypes	Genotypes reaction to race TTKSK
2	32	Resistance reaction
2+3-	41	Mixed reaction
3-	17	Moderate Susceptible reaction
3	25	Susceptible reaction
3+	5	Very Susceptible reaction

Table 4. Analysis of variance for FRS, AUDPC and CI of genotypes at individual locations.

SOV	df	Debrezeit			Adet			Kulumsa		
		FRS	CI	AUDPC	FRS	CI	AUDPC	FRS	CI	AUDPC
Rep	2	342 ***	121.8***	18418***	1799.7***	275.47***	40981.3***	1830.1***	533.1***	89515***
Rep/Block	8	14.38 ns	8.273 ns	1390 ns	9.62 ns	1.56 ns	185.2 ns	67.5 ns	12.47 ns	2359 ns
Genotypes	59	665.8***	186.6***	34827.3***	287.96***	173.92***	40028.2***	446.7***	169.8***	33933.3***
Residual	110	23.78	8.64	1288	16.97	4.20	578.8	84.5	14.55	2285
LEE	84	23.14	8.62	1295.7	16.46	4.02	551.81	83.34	14.41	2289.7

*, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively; ns = non-significant, SOV= sources of variation, df = Degree of freedom, E=Environment, G=Genotype, LEE=Lattice effective error, FRS= Final rust severity, CI= coefficient infection, AUDPC= Area under Disease Progress Curve, r= Apparent infection rate.

superior to other orthogonal factor rotation methods in achieving a simplified factor structure (Hair et al., 2010). Hierarchical cluster analysis was carried out by using SAS software for windows 9.

RESULTS AND DISCUSION

Seedling reaction

Among 120 tested genotypes evaluated in greenhouse, 32 (26.67%) of the genotypes showed seedling infection type (IT = 2) with small to medium sized uredinia usually with green islands and surrounded by chlorosis or necrosis as shown Table 3. This indicated that those genotypes were resistant to race TTKSK due to the presence of race specific major resistant gene which was expressed at seedling stage of genotypes. While 41 (34.17 %) of the tested genotypes exhibited mixed reaction (2+ 3-) which suggesting that these genotypes would have minor non race specific resistance genes that able to stay for a longer period of time without losing their resistance. Majority 47 (39.16%) of the tested genotypes showed susceptible seedling reaction (IT=3- to 3+) with moderate sized uredinia and with or without chlorosis. Similarly, different authors (Hundie et al., 2018; Olivera, et al., 2018) reported the existence of variability among bread wheat genotypes on their response to different stem rust races at their seedling stage.

Adult plant resistance performance of genotypes at individual locations

Analysis of variance for FRS, AUDPC and CI at individual location are presented in Table 4. The result of ANOVA revealed the presence of highly significant ($p<0.001$) variation among genotypes on the traits of FRS, AUDPC and CI at each of the three individual testing locations. This indicated that the presence of a considerable amount of genetic variation among the tested genotypes. The mean values of final stem rust severity (FS), coefficient of infection (CI), area under disease progress curve (AUDPC), apparent infection rate (r) and host response (HR) of 60 bread wheat genotypes at Debrezeit, Adet and Kulmsa locations are presented in Table 5. There was variation in plants response to stem rust disease at field conditions. These responses were ranging from MRMS that exhibited as small/medium uredia coupled with either chlorotic or necrotic areas to susceptible reaction which display as large uredia present, generally with little or no chlorosis and necrosis. Significant differences were also observed among the bread wheat genotypes for FS, CI, AUDPC and r at individual location. This showed the presence of great variation among the tested bread wheat genotypes on their resistance potential against the race Ug99. The highest overall mean values of genotypes in FRS (29.61), CI (11.26) and AUDPC (149.4) were recorded at Debrezeit testing location. This is due to the combination of moderate temperature and wet environmental conditions at Deberezit (28.3^{o}C, 851 mm) was more favourable to rust development than the other two testing locations; thus, high rust disease pressure overcame the defense system of the plants in Debrezeit conditions as previously reported by Agrios (2005). G12 and G59 also scored the highest (65) and lowest (5) FRS values at both Adet (50, 5) and Kulmsa (65, 5) locations, respectively.

Table 5. Mean values of FRS, CI, AUDPC, r, HR and IT for the 60 Bread genotypes grown at individual locations.

Geno	Debrezeit					Adet					Kulmsa					Infection
	FRS	CI	AUDPC	r	HR	FRS	CI	AUDPC	r	HR	FRS	CI	AUDPC	r	HR	Type (IT)
G1	23.3	7.22	94.5	0.054	MR-MS	21.7	3.22	35.8	0.094	MRMS	18.3	2.78	31.8	0.072	MR-MS	2+3-
G2	69.3	31.00	408.0	0.165	S	47.7	27.00	405.0	0.155	S	61.0	28.00	409.0	0.153	S	3
G3	30.0	20.67	303.3	0.105	S	20	5.11	60.7	0.202	MS	36.7	23.89	327.8	0.046	S	3
G4	21.7	6.22	86.3	0.086	MR-MS	20	10.29	134.9	0.091	S	18.3	10.45	144.7	0.070	MS-S	2+3-
G5	20.0	6.33	79.3	0.187	MS-S	21.7	6.11	74.7	0.219	MS-S	23.3	9.67	131.8	0.251	MS-S	2+3-
G6	18.3	5.89	79.3	0.081	MS	15	5.56	78.2	0.110	MS	25.0	7.33	94.5	0.167	MS	3-
G7	35.0	10.75	133.9	0.152	MS	20	5.91	66.8	0.127	MS-S	28.3	9.11	122.5	0.233	MS	3-
G8	18.3	7.00	91.0	0.047	MS-S	21.7	7.02	87.7	0.116	MS	20.0	8.78	117.8	0.106	MS-S	2+3-
G9	15.0	5.56	75.8	0.058	MS	31.7	21.11	309.2	0.111	S	28.3	13.34	178.5	0.253	S	3-
G10	18.3	6.27	83.3	0.103	MS	16.7	2.78	35.0	0.033	MR-MS	13.3	4.11	56.0	0.027	MR-MS	3-
G11	53.0	27.00	375.0	0.105	MS-S	43.7	23.0	364.0	0.090	MS	48.3	25.00	367.2	0.101	MS-S	3+
G12	5.0	0.31	3.6	0.000	MR-MS	5.0	0.29	4.3	0.060	MR-MS	5.0	0.51	7.3	0.093	MR-MS	2+3-
G13	20.0	7.33	98.0	0.064	MS-S	18.3	5.69	77.2	0.136	MS	21.7	5.45	70.0	0.054	MS	2+3-
G14	20.0	6.00	79.3	0.085	MR-MS	16.7	3.41	46.3	0.123	MR-MS	21.7	4.58	65.6	0.134	MR-MS	2+3-
G15	71.7	30.00	432.0	0.172	S	48.3	27.0	408.0	0.164	S	63.0	30.00	412.0	0.168	S	3
G16	50.0	20.00	261.3	0.083	MS-S	26.7	9.22	121.3	0.226	MS-S	33.3	10.33	138.8	0.251	MS	3
G17	36.7	14.22	180.8	0.056	MS-S	20	7.15	89.1	0.086	MS-S	23.3	8.45	119.0	0.234	MS	2+3-
G18	30.0	8.67	107.3	0.123	MS	15	3.33	46.7	0.177	MR-MS	23.3	5.61	82.3	0.184	MR-MS	3-
G19	33.3	10.78	134.2	0.119	MS	6.7	1.32	15.4	0.017	MS	20.0	7.22	102.7	0.145	MS	3-
G20	20.0	7.64	101.3	0.152	MS-S	21.7	7.35	91.2	0.133	MS-S	30.0	8.67	102.7	0.177	MS-S	2+3-
G21	38.3	9.86	124.6	0.169	MS	30	15.00	213.5	0.094	MS-S	26.7	10.89	151.7	0.235	MS-S	3-
G22	43.3	14.07	172.2	0.077	MS-S	30	13.29	162.9	0.078	S	38.3	18.78	252.0	0.167	S	3
G23	40.0	22.22	326.7	0.106	MS-S	26.7	10.00	133.0	0.240	MS-S	41.7	16.67	221.7	0.174	MS-S	2+3-
G24	30.0	13.07	179.2	0.126	MS-S	30	7.81	99.5	0.208	MS	28.3	8.45	119.0	0.251	MS	2+3-
G25	20.0	10.11	141.2	0.066	MS-S	16.7	6.00	84.0	0.075	MS	23.3	9.78	136.5	0.073	MS	2+3-
G26	38.3	18.67	270.7	0.137	MS-S	26.7	7.44	92.2	0.207	MS	33.3	11.89	150.5	0.161	MS-S	3
G27	23.3	7.67	99.2	0.088	MS-S	16.7	3.70	52.9	0.188	MRMS	16.7	4.39	60.1	0.094	MR-MS	2+3-
G28	26.7	8.11	110.8	0.094	MS	18.3	4.15	52.9	0.161	MR-MS	11.7	2.28	33.3	0.122	MR-MS	3-
G29	28.3	9.02	108.7	0.163	MS-S	20	6.10	75.3	0.152	MS-S	30.0	11.44	157.5	0.207	MS-S	2+3-
G30	23.3	6.34	87.5	0.132	MR-MS	15	3.69	48.1	0.133	MR-MS	15.0	1.47	19.20	0.027	MR-MS	2+3-
G31	6.7	0.87	11.6	0.055	MR-MS	6.7	0.50	7.3	0.110	MR-MS	10.0	1.47	19.2	0.027	MR-MS	2+3-
G32	23.3	7.78	105.0	0.067	MS-S	21.7	5.72	74.1	0.226	MS	25.0	8.44	107.3	0.287	MS-S	2+3-
G33	20.0	5.34	70.1	0.106	MS	21.7	5.00	68.8	0.078	MRMS	21.7	4.67	60.7	0.084	MS	3-
G34	38.3	13.49	166.1	0.103	MS-S	30	10.73	133.7	0.084	MS-S	35.0	24.33	337.2	0.046	MS-S	3
G35	43.3	14.23	174.0	0.171	MS	30	9.41	112.9	0.224	MS-S	33.3	11.17	142.9	0.195	MS-S	2+3-
G36	26.7	9.31	116.4	0.139	MS-S	23.3	8.20	100.8	0.146	MS-S	33.3	10.73	127.9	0.056	MS-S	2+3-
G37	36.7	13.98	178.3	0.140	MS-S	31.7	20.56	291.7	0.082	S	23.3	12.00	166.8	0.037	MS-S	3
G38	26.7	9.44	130.7	0.325	MS	21.7	6.86	93.1	0.135	MS	28.3	19.22	273.0	0.086	S	3-
G39	20.0	8.22	107.3	0.034	MS-S	21.7	7.02	95.9	0.168	MS	23.3	6.33	87.5	0.095	MR-MS	2+3-

Table 5. Contd.

G40	30.0	10.76	137.4	0.160	MS-S	41.7	26.67	373.3	0.072	S	30.0	13.45	178.5	0.214	S	2+3-
G41	23.3	4.57	60.8	0.140	MR-MS	20	3.63	47.5	0.194	MR-MS	28.3	13.33	164.5	0.034	S	2+3-
G42	20.0	7.98	108.3	0.170	MS-S	21.7	5.74	74.4	0.224	MS	23.3	8.82	123.0	0.136	MS	2+3-
G43	30.0	7.78	100.3	0.071	MR-MS	20	4.22	56.0	0.067	MR-MS	26.7	14.11	186.7	0.072	S	2+3-
G44	65.0	30.00	403.0	0.135	MS-S	40	25	396.0	0.125	MS-S	51.7	26.00	399.0	0.128	S	3-
G45	38.3	14.89	191.3	0.071	MS-S	21.7	9.56	131.8	0.014	MS	30.0	19.56	277.7	0.070	S	3+
G46	15.0	6.44	85.2	0.048	MS-S	18.3	5.93	79.8	0.191	MSS	26.7	10.11	129.5	0.123	MS	2+3-
G47	20.0	6.33	89.8	0.230	MS-S	18.3	3.89	54.8	0.204	MR-MS	26.7	12.89	177.3	0.085	MS-S	2+3-
G48	30.0	10.22	130.7	0.067	MS-S	15	3.36	44.6	0.216	MR-MS	16.7	3.44	47.8	0.194	MR-MS	2+3-
G49	36.7	17.75	241.3	0.164	MS-S	30	9.44	116.7	0.208	MS-S	40.0	17.85	228.2	0.102	S	2+3-
G50	30.0	8.57	115.6	0.166	MS	30	5.27	67.4	0.166	MR-MS	28.3	5.33	72.3	0.276	MR-MS	3-
G51	26.7	8.11	101.5	0.116	MS	30	8.44	102.7	0.187	MS	26.7	5.60	66.3	0.193	MS	3-
G52	8.3	1.59	20.8	0.110	MR-MS	8.3	0.69	10.2	0.110	MR-MS	10.0	2.17	28.6	0.050	MR-MS	2+3-
G53	25.0	9.09	116.4	0.142	MS-S	26.7	7.56	93.3	0.190	MS-S	33.3	12.00	151.7	0.144	MS-S	2+3-
G54	26.7	8.68	105.1	0.149	MS-S	16.7	5.00	60.0	0.144	MS-S	26.7	7.93	90.8	0.138	MS-S	2+3-
G55	20.0	7.27	90.3	0.076	MS-S	18.3	6.15	85.6	0.257	MS-S	23.3	8.17	109.1	0.211	MS-S	2+3-
G56	21.7	4.28	54.3	0.044	MR-MS	18.3	4.05	49.6	0.067	MR-MS	28.3	4.67	61.8	0.094	MR-MS	2+3-
G57	21.7	5.89	82.8	0.095	MR-MS	18.3	4.67	65.3	0.068	MR-MS	20.0	5.33	74.7	0.088	MR-MS	2+3-
G58	48.0	28.00	380.0	0.128	MS	35.0	24.00	372.0	0.116	MS	41.7	8.71	376.0	0.120	MS	3
G59	73.0	36.33	456.0	0.173	S	50.0	31.33	416.0	0.168	S	65.0	33.0	420.0	0.175	MR-MS	3+
G60	5.0	0.51	7.4	0.055	MR-MS	6.7	0.38	4.9	0.055	MR-MS	8.3	0.58	8.1	0.072	MR-MS	2+3-
Mean	29.61	11.26	149.4	0.114		23.36	8.80	120.8	0.139		28.01	10.68	148.68	0.142		
LSD	7.77	4.75	58.2	0.096		6.56	3.25	37.9	0.095		14.92	6.14	77.37	0.111		
CV(%)	16.2	26.0	24.1	52.3		17.38	22.82	19.45	42.16		33.0	34.6	32.19	48.3		

Where: Geno = Genotypes, FRS= Final rust severity, AUDPC= Area under disease progress curve, CI = coefficient of infection DH= Days to heading, DM= Days to maturity, PH=Plant height, SL= Spike length, NSPS=Number of Spikelet per spike, HLW= Hectoliter weight, TKW = Thousands kernel weight, GY= Grain yield, LSD= Least significant difference, CV=Coefficient of variation, MR-MS = moderately resistant to moderately susceptible, MS = moderately susceptible, MS-S = moderately susceptible to susceptible and S= susceptible.

In the case of AUDPC values, the maximum (456) and minimum (3.6) values across the three locations were scored by G59 and G12, respectively. Moreover, the magnitude apparent infection rate of the genotypes ranged between 0.000 to 0.325 at Debrezeit; and also ranged from 0.017 to 0.257 and 0.027 to 0.287 at Adet and Kulmsa locations, respectively. The smaller range value of coefficient of infection (r) recorded at Adet indicated the occurrence of a more steady lower disease infection progression than the other two locations. Generally, identification of susceptible or resistant genotypes based on “r” values alone may not lead to useful results. Hence, it is paramount to use two or more disease index parameters to delineate the genotypes whether they are susceptible or resistance to stem rust disease. Genotypes such as G59, G15, G2, G44, G11 and G58 had scores (FRS ≥ 35, AUDPC >360, CI >20 and r > 0.10) with susceptible plant response across the three locations and considered as very susceptible genotypes; whereas G12, G60, G31 and G52 were found with (FRS $\leq$ 10, AUDPC <30, CI<3 and r<0.2) values and considered as resistant genotypes. This indicated that these selected genotypes would have gene Sr13 which is effective against pathotype TTKSK and its derivatives; and could be used as source of slow rusting resistance gene in wheat breeding research program. In their previous studies, different authors (Safavi et al., 2013; Draz et al., 2015) used FRS, CI and AUDPC for measuring the slow rusting resistance potential of wheat genotypes under field conditions.

Table 6. Mean sum of square for ACI, AUDPC and Agronomic traits of 60 wheat genotypes across locations.

SOV	df	FRS	CI	AUDPC	r	DH	DM	PH	SL	NSPS	HLW	TKW	GY
Genotypes	59	1252.85***	468.48 ***	94816.30 ***	0.0179***	90.70***	368.96***	320.16***	4.12***	152.07***	94.94***	176.10***	1.90**
Environment	2	1886.12 ns	930.66 *	148914.3 *	0.0431ns	488.41ns	6621.80*	212.33ns	0.459 ns	5461.78**	1106.49***	529.14ns	51.38***
Env.Rep	6	1323.93***	108.29 *	16014.45NS	0.0326**	221.89***	892.87***	334.98***	3.72*	19.03***	14.48ns	328.28***	3.05***
Env.Rep.Block	36	109.52***	43.56***	9835.87***	0.0094***	11.344***	44.72***	67.69***	0.95***	152.06***	12.27***	42.36***	0.30**
GXE	118	82.66***	31.84***	6567.17***	0.0084***	7.00***	29.89***	81.52***	0.24 ns	9.00***	8.13***	18.99***	0.26***
Error	318	33.23	5.12	421.62	0.0033	0.0154	0.287	13.28	0.194	3.098	3.171	11.59	0.159

*, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively; ns = non-significant, SOV= sources of variation, df = Degree of freedom, E=Environment, G=Genotype, GXE = Genotype by Environment Interaction, FRS= Final rust severity, CI= coefficient infection, AUDPC= Area under Disease Progress Curve, r= Apparent infection rate, DH= Days to heading, DM= Days to maturity, PH=Plant height, SL= Spike length, NSPS=Number of Spikelet per spike, HLW= Hectoliter weight, TKW = Thousands kernel weight, GY= Grain Yield.

Adult plant resistance performance of bread genotypes over the three locations

The combined analysis of variance for FRS, CI, AUDPC, r and other agronomic traits across the three locations are presented in Table 6. The results showed highly significant differences ($p < 0.001$) among genotypes in FRS, CI, AUDPC, r and in all other agronomic traits. This indicated the existence of sufficient variability among genotypes on their response to stem rust disease which caused by race Ug99. Moreover, highly significant differences ($p < 0.001$) on GY and HLW; and significant differences on CI, AUDPC, DM and NSPS were also observed among locations. This suggested that testing locations had considerable effects on rust disease resistance and agronomic performance of genotypes. It also indicated the existence of different stem rust disease pressures across the testing locations. The existence of highly significant ($p < 0.001$) genotype by location (G×E) interaction effects on FRS, CI, AUDPC, r, DH, DM, PH, HLW, TKW and GY performances of genotypes indicated that inconsistent performance of genotypes across the three locations. This explained the extent of challenges that farmers would face when they try to manage stem rust disease by using improved variety that has race specific resistance gene since this gene might not work in areas where a virulent race does not existed. Furthermore, this result also implying the importance of location specific breeding program; different varieties have to be developed for different environments (Acquaah, 2007).

The combined mean performances of both disease measuring and agronomic traits across locations indicated that the highest (62.5) and lowest (5.0) scores of FRS were recorded by G59 and G12 respectively as shown in Table 7. Besides, G59 and G12 had also scored the maximum (430.7) and minimum (5.1) AUDPC values of genotypes across locations, respectively. This implied that the two genotypes have consistent stem rust resistance performance across the testing locations, therefore, farmers can use G12 for commercial production of wheat across the three locations. In the case of yield and yield related traits, G39 showed the highest scores of NSPS (41.4 spikelet/spike), TKW (47.3g) and GY (4.7t/ha) across the three locations. Therefore, G39 can be used as a parent material for future breeding program to improve NSPS, TKW and GY of bread wheat genotypes.

Interrelationships among stem rust disease traits

The results of correlation analysis among the stem rust disease measuring parameters are presented in Table 8. A positive and highly significant correlation of FRS with CI ($r^2 = 0.894$) and AUDPC ($r^2 = 0.877$) implied that severity of stem rust disease increased with increasing of CI and AUDPC. This significant positive correlation could have resulted from the effect of strong coupling linkage between genes or due to pleiotropic genes that control these traits in the same direction. The results also indicated that the FRS values of genotypes at adult plant test could, therefore, be used to extrapolate information about the CI and AUDPC values of the tested genotypes with a better accuracy. Numerous authors (Safavi et al., 2010; Duncan et al., 2015) used FRS, CI and AUDPC to identify bread wheat genotypes that have partial resistance to stem rust disease. Weak and non-significant positive correlations were observed between apparent infection rate (r) and

Table 7. Mean performances of genotypes on FRS, CI, AUDPC and selected agronomic traits across locations.

Geno	FRS	CI	AUDPC	NSPS	TKW	GY
G1	21.1	4.4	54.0	34.3	40.4	4.1
G2	59.3	28.7	407.3	22.0	28.5	2.6
G3	28.9	16.6	230.6	28.2	34.7	3.5
G4	20.0	9.0	122.0	31.6	38.1	3.8
G5	21.7	7.4	95.3	35.4	40.3	4.0
G6	19.4	6.3	84.0	37.0	41.7	4.1
G7	27.8	8.6	107.7	39.4	45.6	4.5
G8	20.0	7.6	98.9	36.0	47.2	4.7
G9	25.0	13.3	187.8	38.3	44.2	4.4
G10	16.1	4.4	58.1	34.0	37.7	3.7
G11	48.3	25.0	368.7	32.2	35.6	3.3
G12	5.00	0.4	5.1	35.8	46.2	4.0
G13	20.0	6.2	81.7	34.2	40.5	4.0
G14	19.4	4.7	63.7	38.7	46.0	4.6
G15	61.0	29.0	417.3	32.8	34.2	3.0
G16	36.7	13.2	173.8	35.6	41.1	4.1
G17	26.7	9.9	129.7	29.7	35.3	3.5
G18	22.8	5.9	78.8	32.4	38.2	3.8
G19	20.0	6.4	84.1	36.0	40.1	4.0
G20	23.9	7.9	98.4	39.7	45.6	4.5
G21	31.7	11.9	163.3	27.7	35.2	3.5
G22	37.2	15.4	195.7	30.6	35.3	3.5
G23	36.1	16.3	227.1	38.2	42.7	4.3
G24	29.4	9.8	132.6	35.9	40.8	4.0
G25	20.0	8.6	120.6	38.1	29.4	2.9
G26	32.8	12.7	171.1	35.9	40.7	4.0
G27	18.9	5.3	70.7	35.2	40.2	4.0
G28	18.9	4.9	65.7	34.3	39.8	4.0
G29	26.1	8.9	113.8	36.5	40.9	4.1
G30	17.8	4.3	58.4	31.7	37.0	3.7
G31	7.8	0.95	12.7	38.5	40.5	4.1
G32	23.3	7.3	95.5	31.5	38.0	3.8
G33	21.1	5.0	66.5	29.6	35.7	3.6
G34	37.2	12.5	153.2	25.4	31.1	3.1
G35	35.6	11.6	143.3	25.0	30.9	3.1
G36	27.8	9.4	115.0	38.2	42.8	4.3
G37	30.6	15.5	212.3	32.0	38.3	3.8
G38	25.6	11.8	165.6	37.6	43.4	4.3
G39	21.7	7.2	96.9	36.2	40.0	4.0
G40	33.9	17.0	229.8	25.1	30.0	3.0
G41	23.9	7.2	90.9	40.7	45.6	4.5
G42	21.7	7.5	101.9	36.6	40.1	4.0
G43	25.6	8.7	114.3	35.8	39.7	3.9
G44	52.2	27.0	399.3	36.4	39.7	3.7
G45	30.0	14.7	200.3	35.1	38.6	3.8
G46	20.0	7.5	98.2	32.0	37.7	3.8
G47	21.7	7.7	107.3	36.7	41.7	4.2
G48	20.6	5.7	74.4	31.3	37.0	3.7
G49	35.6	15.0	195.4	33.7	38.3	3.8
G50	29.4	6.4	85.1	38.8	44.7	4.5
G51	27.8	7.4	90.1	32.9	37.8	3.8

Table 7. Contd.

G52	8.9	1.5	19.9	38.4	47.3	4.7
G53	28.3	9.6	120.5	33.3	38.8	3.9
G54	23.3	7.2	85.3	38.0	42.1	4.2
G55	20.6	7.2	95.0	39.5	43.9	4.3
G56	21.1	4.3	55.2	32.9	38.0	3.8
G57	20.0	5.3	74.3	41.3	46.2	4.5
G58	41.6	25.8	376.0	35.8	42.9	3.7
G59	62.7	33.6	430.7	32.0	36.9	3.1
G60	6.7	0.5	6.8	37.6	43.3	4.3
Mean	26.9	10.3	139.6	34.4	39.6	3.89
LSD	7.32	3.47	43.3	2.98	4.14	0.44
CV	29.3	36.2	33.5	9.33	11.3	12.1

*, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively; ns = non-significant, SOV= sources of variation, Df = Degree of freedom, E=Environment, G=Genotype, GXE = Genotype by Environment Interaction, FRS= Final rust severity, CI= coefficient infection, AUDPC= Area under Disease Progress Curve, r= Apparent infection rate, DH= Days to heading, DM= Days to maturity, PH=Plant height, SL= Spike length, NSPS=Number of Spikelet per spike, HLW= Hectoliter weight, TKW = Thousands kernel weight, GY= Grain Yield.

Table 8. Correlation of stem rust disease traits on wheat genotypes.

	FRS	**CI**	**AUDPC**	**r**
FRS	1			
CI	0.894 ***	1		
AUDPC	0.877 ***	0.996 ***	1	
r	0.447	0.330	0.324	1

*** Significant at 0.001 probability level; FRS= Final rust severity; CI= Coefficient of infection; AUDPC= Area under Disease Progress Curve; r= Apparent infection rate.

the other three stem rust disease measuring traits (FRS, CI and AUDPC) with correlation (r^2) values of 0.447, 0.330 and 0.324, respectively. This implied that FRS or AUDPC was increasing with the reduction of infection rate over time. i.e. as the epidemic of stem rust disease progressed, only small healthy plant tissue existed for further infections and therefore the rate of epidemic development would be slow. Similar research output was obtained on wheat stem rust resistance studies by Ali et al. (2008). Moreover, a positive and significant correlation of CI with AUDPC (r^2 = 0.996) indicated that these two disease traits were related to each other and either of them could be used for selection of stem rust resistant genotypes.

Principal component analysis (PCA)

The results of principal component analysis designated the contribution of each trait on individual genotypes and thus to the overall genetic variability observed among the tested genotypes. In this study, the first four principal components (PCs) had captured 86.58% of the total variation among genotypes as indicated in Table 9. PC1 explained 42.61% of the total variation which mainly was contributed by GY, TKW, CI, AUDPC, and FRS.

The high positive and negative effects of GY, TKW, CI, AUDPC and FRS on PC1; and the high positive effect of DH, DM and PH on PC2 indicated that these traits are the major contributors to the significant variation among the tested genotypes. Similarly, Alebachew (2012) reported positive and direct attribution of DM and TKW on the grain yield performance of bread wheat genotypes. Mollasadeghi et al. (2011) also ratified that the grain yield of bread wheat genotypes was significantly influenced by number of NSPS, TKW and biological yield of genotypes. Moreover, Nzuve et al. (2013) reported the existence of a highly significant variation ($p<0.001$) in FRS, CI and AUDPC values of bread wheat genotypes. This therefore indicates that the bread wheat genotypes used in this study were genetically diverse in their stem rust resistance potential and performance of other agronomic traits. Moreover, PC4 showed that the largest share variation was contributed by apparent infection rate of the tested genotypes. This indicated that the redundancy of the trait associated to PC4 and, therefore, the apparent infection rate (r) could be described as redundant descriptor in the description and characterization of the tested genotypes. Similar results were reported by Afutu et al. (2016) on the evaluation of Uganda cowpea germplasm for yield and resistance to scab disease.

Table 9. Principal components, Eigen values, percentage of total variance, cumulative percentage of variance and eigenvector loadings of twelve traits.

Traits	PC1	PC2	PC3	PC4
FRS	-0.3804	0.1599	0.2575	0.2131
CI	-0.3728	0.1455	0.3377	0.1344
AUDPC	-0.3666	0.1429	0.3558	0.1212
r	-0.1178	0.2759	-0.1393	0.6568
DH	-0.1847	0.4627	-0.1148	-0.4140
DM	-0.1787	0.4615	-0.1218	-0.4287
PH	0.1222	0.4325	-0.3018	0.0882
SL	0.1717	0.2771	-0.4133	0.3292
NSPS	0.3293	0.2236	0.3316	0.0118
HLW	0.2812	0.2262	0.3453	-0.0902
TKW	0.3499	0.2009	0.3344	0.0634
GY	0.3844	0.1560	0.2069	0.0829
Eigen values (Explained variance)	5.11	2.83	1.49	1.04
Percentage of total variance	42.61	23.59	12.38	8.00
Cumulative percentage of variance	42.61	66.20	78.58	86.58

Where FRS=final rust severity, CI= Coefficient of infection, AUDPC=Area under disease progress curve, r =Apparent infection rate, DH=Days to heading, DM=Days to maturity, PH=Plant height, SL=Spike length, NSPS=Number of spikelet per spike, HLW= Hectoliter weight, TKW=Thousands kernel weight, GY=Grain yield.

Cluster analysis (CA)

The results of cluster analysis which constructed using the mean values of FRS, CI, AUDPC and r are presented in Figure 1. Bread wheat genotypes that showed the same susceptibility or resistance reaction are presented with the same font color. The genotypes that are displayed in green, orange and red colours were identified as resistant, susceptible and very susceptible genotypes to stem rust disease, respectively. The genotypes with blue and black colours were considered as moderately resistant and moderately susceptible genotypes, respectively. This cluster analysis grouped the tested bread genotypes into five major groups: Cluster-I which accounted for the largest weight consist of 36 moderately stem rust resistant genotypes with AUDPC (54-132.6) and CI (4.31-9.94) values. Cluster-II comprised four stem rust resistant genotypes which had small mean values of AUDPC (5.1-19.9) and CI (0.37-1.48). Cluster –IV had 6 moderately stem rust susceptible genotypes with a magnitude of (143.3-173.8) and (11.6-13.19) for AUDPC and CI, respectively. While Clusters III and V consisted of 8 and 6 genotypes which were susceptible and very susceptible to stem rust disease, respectively. This indicated the existence of genetic divergence in bread wheat genotypes in response to stem rust disease particularly race TTKSK. Earlier studies of Beteselassie et al. (2007) and Ali et al. (2008) reported the existence of genetic variability in stem rust resistance among wheat germplasms. The existed genetic diversity can be exploited during introgression of stem rust resistance genes into susceptible adapted varieties using conventional and genetic engineering approaches.

Conclusions

This study revealed the existence of significant variations among the tested 60 bread wheat genotypes in terms of their response to Ug99 and performance of their yield and agronomic traits that could be used to selecting parental materials for improving yield and stem rust (Ug99) resistance performance of bread genotypes. Four bread wheat genotypes (G12, G60, G31 and G52) were found to be resistant to Ug99 (race TTKSK) across the three locations and could be used as source of stem rust resistance in future wheat improvement programs.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENTS

Sincere gratitude to DAAD-RUFORUM (Regional Universities Forum for Capacity Building in Agriculture) Scholarship Program for financial support of this study. We would like to also thank the wheat research teams of Debrezeit, Adet and Kulmsa Agricultural Research Centers for providing wheat germplasms, experimental land and helping us during planting and harvesting of the experimental trials.

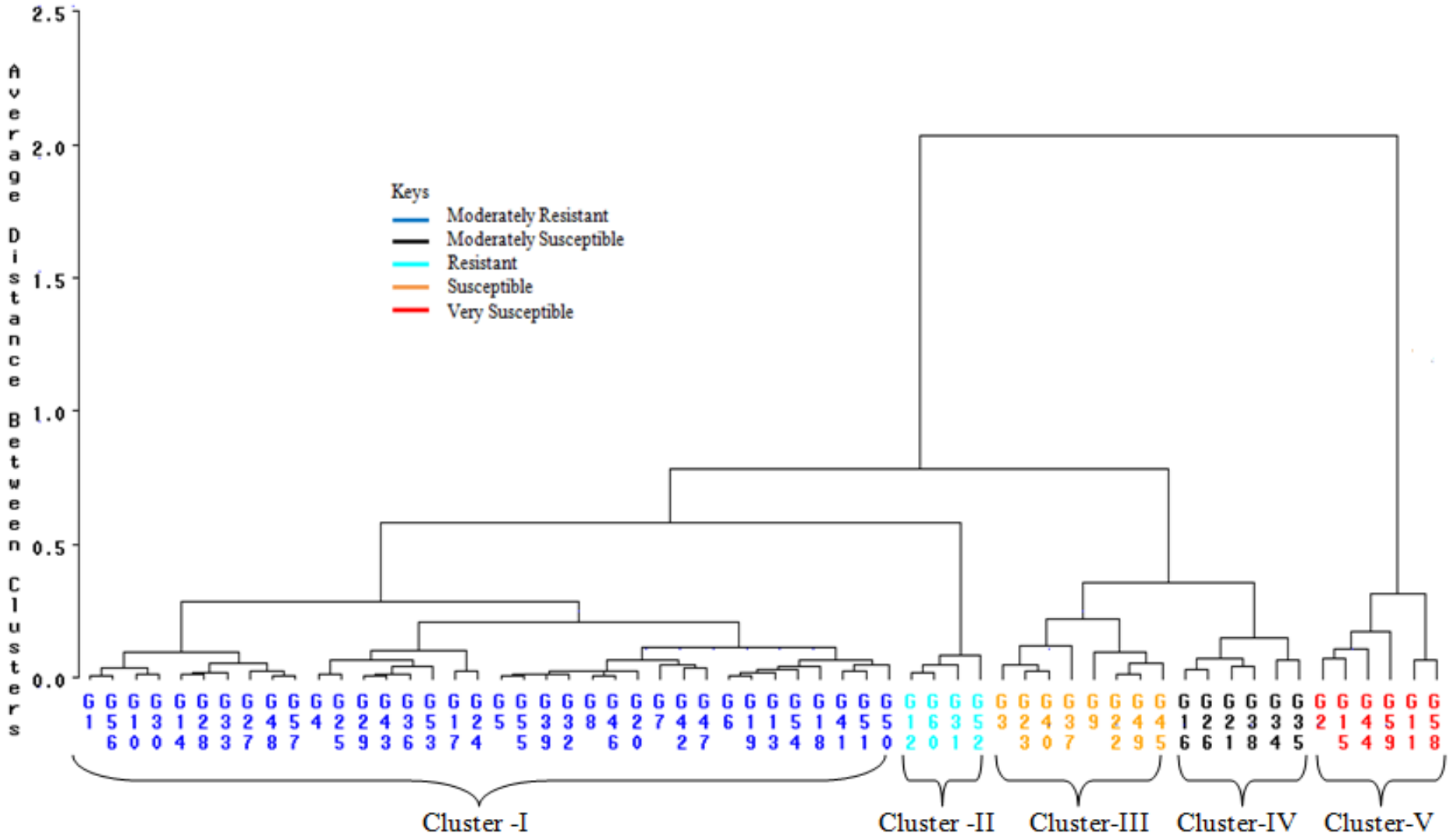

Figure 1. Cluster dendogram of the 60 bread wheat genotypes based on four stem rust disease measuring traits.

REFRENCES

Acquaah, G. (2007). *Principles of Plant Genetics and Breeding*. Blackwell Publishing Limited. Oxford, UK. 569p.

Afutu, E., Mohammed, K. E., Odong, T. L., Biruma, M., & Rubaihayo, P. R. (2016). Evaluation of Ugandan cowpea germplasm for yield and resistance to scab disease. *American Journal of Experimental Agriculture*, 12(2), 1-18.

Agrios, G. N. (2005). *Plant Pathology*, 5th edition. Elsevier Academic Press. Burlington, USA. p. 922.

Alebachew, H. (2012). Participatory and performance evaluation of improved bread wheat (*Triticum aestivum* L.) varieties in degua tibien and ofla woredas of tigray region, Ethiopia M.Sc. Thesis, Haramaya University, Haramaya, Ethiopia.

Ali, S., Shah, S. J. A., & Maqbool, K. (2008). Field-based assessment of partial resistance to yellow rust in wheat germplasm. *Journal of Agriculture & Rural Development*, 6(1), 99-106.

Beteselassie, N., Fininsa, C., & Badebo, A. (2007). Sources of stem rust resistance in Ethiopian tetraploid wheat accessions. *African journal of Crop Science,* 15(1), 51-57.

Denbel, W., Badebo, A., & Alemu, T. (2013). Evaluation of Ethiopian commercial wheat cultivars for resistance to stem rust of wheat race Ug99. *International Journal of Agronomy and Plant Production*, 4(1), 15-24.

Draz, I. S., Abou-Elseoud, M., Kamara, A., Alaa-Eldein, O. A., & El-Bebany, A. (2015). Screening of wheat genotypes for leaf rust resistance along with grain yield. *Annuals of Agricultural Sciences,* 60(1), 29-39.

Duncan, C., Pascal, P., Okwiri, O., Peter, N., Peter, F., & Sridhar, B. (2015). Evaluation of advanced wheat (Triticum aestivum L.) lines for stem rust (*Puccinia graminis* f. sp. tritici) resistance and yield. *International Journal of Agronomy and Agricultural Research*, 6(3), 57-70.

Gamalat, A., & Elsawi, S. (2015). Inheritance of stem rust resistance and some yield components in crosses from five Egyptian wheat cultivars. *Egypt Journal of Plant Breeding,* 19(1), 71-87.

Haile, D., Nigussie, D., & Ayana, A. (2012). Nitrogen use efficiency of bread wheat: Effects of nitrogen *rate* and time of application, *Journal of Soil Science and Plant Nutrition,* 12(3), 389-409.

Hailu, E., Woldaeb, G., Danbali, W., Alemu, W and Abebe, T.2015. Distribution of stem rust (*Puccinia graminis f. sp. tritici*) races in Ethiopia. *Advances in crop science and technology*, 3(3),171-173.

Hair, J. F., Jr Black, W. C., Babin, B. J., & Anderson, R. E. (2010). *Multivariate Data Analysis* (7th edition). Pearson Prentice Hall.

Hundie, B., Yirga, F., Kassa, D., Hailu, E., Negash, T., Tesfaye, T., Bacha, N., Shewaye, Y., Woldeab, G., Zegaye, H., & Tadesse, Z. (2018). Evaluation of Advanced Bread Wheat Lines for Field and Seedling Resistance to Stem Rust (Puccinia graminis f. sp. tritici). *American Journal of Biological and Environmental Statistics*, 4(2), 74-82.

Jaleta, M., Hodson, D., Abeyo, B., Yirga, C., & Erenstein, O. (2019). Smallholders' coping mechanisms with wheat rust epidemics: Lessons from Ethiopia. *PLoS ONE*, 14(7), e0219327.

Kosgey, Z., Owuoche, J. O., Okiror, M. A., & Njau, P. N. (2015). Inheritance of stem rust (*Puccinia graminis Pers.F. Sp. Tritici ericks* and *E. Hen)* resistance in bread wheat (*Triticum aestivum* L.) lines to TTKST race. *International Journal of Agronomy and Agricultural Research*, 7(4),1-13.

McIntosh*,* R. A., Wellings, C. R., & Park, R. F. (1995). *Wheat Rusts*: An atlas of resistance genes. Plant Breeding Institute*,* the University of Sydney, CSIRO Australia.

Mollasadeghi, M. V., Imani A. A., Shahryari, R., & Khayatnezhad, M. (2011). Correlation and path analysis of morphological triats in different wheat genotypes under end drought stress condition. *Middle East Journal of Scientific Research*, 7(2), 221-224.

Nzuve, F., Tusiime, G., Bhavani, S., Njau, P., Wanyera, R. (2013). Studies of the genetics of inheritance of stem rust resistance in bread wheat. *African Journal of Biotechnology*, 12(21), 3153-3159.

Olivera, P. D., Rouse, M., & Jin, Y. (2018). Identification of new sources of resistance to wheat stem rust in Aegilops spp. in the tertiary gene pool of Wheat. *Frontiers in Plant Science*, 9, 1719.

Payne, R. W., Murray, D. A., Harding, S. A., Baird, D. H., & Soutar, D. M. (2013). Genstat for Windows Introduction. 16th Edition, VSN International, Hemel Hempstead.

Peterson, R. F., Campbell, A., & Hannah, A. E. (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research,* 26(5), 496-500.

Priyamvada, Saharan, M. S., & Ratan, T. (2011). Durable resistance in wheat. *International Journal of Genetics and Molecular Biology*, 3(8), 108-114.

Roelfs, A. P., Singh, R. P., & Saari, E. E. (1992). Rust diseases of wheat: Concepts and methods of disease management. CIMMYT, Mexico, D.F. ISBN: 968-6127-70-4, p. 81.

Safavi, S. A., Ahari, A. B., Afshari, F., & Arzanlou, M. (2010). Slow rusting resistance in 19 promising wheat lines to yellow rust in Ardabil, Iran. *Pakistan Journal of Biological Sciences*, 13(5), 240-244.

Safavi, S. A., Ahari, A. B., 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.

Sahoo, A., Beyene, L. M., Shiferaw, B., & Sika, G. (2016). Economy-wide impacts of technological change in food staples in Ethiopia: A macro-micro approach. MPIA working paper serie 2016-17.

Sikharulidze, Z., Mgeladze, L., Rusudan, D. R, Natsarishvili, K., & Chkhutiashvili, N. (2015). Reaction of wheat germplasm to stem rust in Georgia. *Ekin Journal of Crop Breeding and Genetics*, 1(1), 63-68.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S., Singh, P.K., 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, 465-481.

Stakman, E. C., Steward, D. M., & Loegering, W. Q. (1962). Identification of physiological races of *Puccinia graminis var. tritici*. *USDA: Agricultural research service*, E-617,1-53.

Stubbs, R. W., Prescott, J. M., Sarrri, E. E., & Dubinm, H. J. (1986). Cereal disease methodology manual. CIMMYT, El Batan, Mexico.

Tamene, T., Gemechu, K., & Hussein, M. (2015). Genetic progresses from over three decades of faba bean (*Vicia faba* L.) breeding in Ethiopia. *Australian Journal of Crop Science*, 9(1), 41-48.

Van der Plank, J. E. (1963). *Plant diseases: Epidemic and control*. Academic Press, New York, 349p.

Wilcox son, R. D., Skovmand, B., & Atif, A. A. (1975). Evaluation of wheat cultivars for the ability to retard development of stem rust. *Annals of Applied Biology*, 80(3), 275-281.

Zemede, A., Mekbib, F., Assefa, K., & Bishaw, Z. (2019). Variability in Ethiopian Durum Wheat under Rainfed Environment Subjected to Drought at Anthesis. *Ethiopian Journal of Agricultural Sciences*, 29(2), 17-29.