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Evaluation of triticale (*X. Triticosecale* Wittmack) genotypes for adult plant resistance to stem rust (*Puccinia graminis* f. sp. *tritici*)

Isaiah Aleri, James O. Owuoche* and Pascal P. Okwiri Ojwang

Department of Crops, Horticulture and Soils, Faculty of Agriculture (FOA), P. O. Box 536-20115, Egerton Kenya.

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Stem rust (*Puccinia graminis* f. sp. *tritici*) is one of the three rust diseases that infect triticale (*X. Triticosecale* Wittmack). Twenty-six triticale genotypes were evaluated for adult plant resistance (APR) to stem rust (*Sr*) infection in the greenhouse in a Randomized Complete Block Design (RCBD). *R-TR* (resistant –trace or minimal *uredinia*) reactions were observed on 6 genotypes EUT046, EUT59, EUT090, EUT093, 118 and EUT139 at adult plant stage. Reactions of 5*RMR* (5% disease severity with resistant-moderately resistant reactions) were observed on EUT001, EUT034, EUT035, EUT123 and EUT124. Four of the genotypes evaluated showed moderately resistant (*MR*) reactions with severity ranging from *10 MR* to 15 *MR*. Effects due to genotype, stage and genotype × stage interactions were significant ($p<0.001$) for length, width and area of spore. The mean length of spore increased from 0.75 mm at 17 days to 2.65 mm at 35 days after inoculation (DAI). The mean area of spore ranged from 0.27 to 1.33 mm^2 between the 17 and 35 DAI. The slowest development (0.003 mm^2 day^{-1}, 0.006 mm^2 day^{-1} and 0.009 mm^2 day^{-1}) of *Sr* spore was observed between 17-20 DAI for EUT035, EUT123 and EUT034, respectively. Triticale genotypes were also significantly ($p<0.001$) different for the latent period, AUDPC, number and weight of grains per spike. The mean latent period ranged from 10.00 days for EUT004 to 22.66 days for EUT001 and EUT087. Triticale genotypes that showed high level of APR were identified and can be used as sources of resistance in triticale breeding programs against stem rust.

Key words: Adult Plant Resistance, Stem rust, Resistant, Triticale genotypes.

INTRODUCTION

Adult plant resistance (APR) to rusts plays a major role in the management of rust diseases in triticale (*X. Triticosecale* Wittmack), wheat (*Triticum aestivum*) oats (*Avena sativa)* and barley (*Hordeum vulgare*). Minor and major genes which confer resistance to rust at adult and seedling plant stages, respectively are considered when breeding for resistance to rust diseases (Ellis et al., 2014). Although seedling resistance extends into adult

*Corresponding author. E-mail: owuoche@yahoo.com. Tel: +254 720 710 040.

stages in some genotypes, genes involved in this type of resistance are vulnerable to breakdown whenever they are exposed to new virulent races of stem rust (*Puccinia graminis* f sp. *tritici*) (McIntosh et al., 1995; Burdon et al., 2014). APR is controlled by minor genes and it provides durable resistance to stem rust (McIntosh 1992; Navabi et al., 2005). In addition, APR genes confer phenotypes with varying degree of partial resistance but under circumstances where several minor genes with additive effect are combined a near complete resistance of adult plants may be exhibited (Singh et al., 2014). The partial resistance that is expressed by adult plants is mainly characterized by slow pathogen growth and low disease severity on the host plant.

Genetic resistance, cultural techniques and use of fungicides are key strategies that are used to reduce the effect of stem rust infection on triticale, wheat and rye (*Secale cereale*) (Roelfs et al., 1992; Singh et al., 2008). Among these techniques, the development and deployment of resistance genotypes is the most economical and environmentally sound means of controlling stem rust (Singh et al., 2012). McIntosh (1988) recognized genetic resistance in wheat and triticale, that is conferred at seedling stage and that effectively expressed at post seedling growth stages (APR). On triticale genes, *Sr27* and *SrSatu* are resistance genes that were postulated to confer resistance to stem rust at seedling stage and are also effective at adult plant stage (McIntosh 1988; Adhikari, 1996; Zhang et al., 2010). Initially, McIntosh (1983) concluded that there is a narrow genetic base for stem rust resistance genes in the triticale germplasm that were available and evaluated at that moment. However, high frequencies of genes *SrBj*, *SrNin, SrLa1*, *SrLa2* and *SrVen* were detected in CIMMYT triticale population for resistance to stem rust (Adhikari, 1996). Adhikari (1996) also found that, among these additional genes *SrBj*, and *SrVen* were found to confer moderately susceptible reactions in the field while *SrLa1* and *SrLa2* were effective against stem rust at seedling stage in the greenhouse and under field conditions.

Although adult plant resistance is often identified under field conditions (McIntosh, 1988; Ellis et al., 2014), greenhouse experiments have been used successfully to evaluate the components of APR to stripe rust (*Puccinia striiformis* f. sp. *tritici*) (Pretorius et al., 2007) and leaf rust (*Puccinia triticina*) (Herrera-Foessel et al., 2007) among wheat genotypes. Kolmer et al. (2018) also showed that evaluation of wheat for APR to leaf rust (*Puccinia triticina*) can be conducted in the greenhouse since there was a significant correlation between the results obtained in the greenhouse and those observed in the field for the same test genotypes. When plants are evaluated for APR to rusts, extended (long) latent period, low values of disease severity and AUDPC have been used as resistant components of adult plants (Pretorius et al., 2007; Aktas and Zencirci, 2016; Kolmer et al., 2018). The latent period would show the ability of the host genotype to initiate late infection and appearance of disease symptoms while the AUDPC would generally show the development of disease over time after infection. The objective was to assess the response of adult triticale plants to infection by stem rust.

MATERIALS AND METHODS

Experimental site description and genotypes

Triticale genotypes were evaluated for adult plant resistance in the greenhouse at the Kenya Agricultural and Livestock Research Organization (KALRO), (0º 20′ S, 35º 56′ E), Njoro station. A total of 26 triticale genotypes with different parental backgrounds were obtained from CIMMYT nursery.

Experimental procedure

Seeds were planted in 11.6 cm diameter plastic pots tapered to a height of 11.5 cm. Each pot was filled with approximately 808 cm^3 of growth medium made of top soil and sand mixed in a ratio of 3:1. Sand was included in the potting mix to improve drainage and facilitate seepage of water by capillarity. The top soil was sterilized by steam-heating in a closed metallic bin for 3 h. The experiment was set up in a Randomized Complete Block Design (RCBD) with three replicates. Approximately 5-10 seeds were planted in each pot and later thinned to maintain a plant density of 4 plants per pot. Fertilizer was applied as a solution by dissolving an equivalent rate of 125 kg ha^{-1} of Diammonium phosphate at planting time and 100 kg ha^{-1} of Calcium Ammonium Nitrate at tillering stage based on the top surface area of the planting pots. The pots were placed on an Aluminium tray partially filled with granite gravel and water to allow watering by capillarity. Plants in each pot were supported with metallic grids and fastened to avoid lodging. During the experimental period, temperature and humidity of the greenhouse were maintained at 20-25°C and 80-100%, respectively.

Inoculation of triticale genotypes

Stem rust urediniospores were collected from a susceptible wheat cultivar Robin planted at the KALRO-Njoro stem rust trap nursery. Fresh samples of single pustules were scrapped from the stems and leaves of infected plants into gelatin capsules. Inoculum was prepared by suspending approximately 2.00 g of urediniospores in 100 mL of distilled water enhanced with approximately 1 mL of surfactant (Tween20). In order to increase and purify the inocula, wheat plants of variety Robin were inoculated in the greenhouse with samples collected from KALRO-Njoro stem rust trap nursery. Wheat plants were adequately sprayed with urediniospore suspension as fine mist using an atomized sprayer. Samples of infected stems and leaves were then collected and single spore collection was done as described above. Thereafter, all triticale plants in each pot were inoculated by injecting the stems at booting stage with equal quantities of urediniospores suspension using a hypodermic syringe. Inoculation was done late in the evening when conditions were conducive for germination of spores to induce stem rust epiphytotic. The pots were placed in a dew chamber maintained at RH 80-100% for 24 hours. The pots were transferred to the greenhouse where misting was done during the day at an interval of 4 hours to provide moisture and humidity conducive for

development of the disease.

Data collection in the greenhouse

The latent period was computed from the date of inoculation to the first date on which stem rust pustules or flecks were observed on plants for each experimental unit. Development of stem rust pustules on individual plant stems were assessed based on the length and width of spore and the *uredium* area was calculated. The length and width were measured on three randomly selected *uredinium*. Four plants in each pot were used to take subsequent measurements of length and width of spore, five times (days after inoculation) from the 17th day after inoculation up to 35th day post inoculation. The length and width of the spore were estimated using a graduated ruler. The shape of the pustule was assumed to be elliptical. Therefore, the area was calculated as suggested by Lee and Shaner (1985).

$$\textit{Uredinium}\ \text{Area} = \pi \times r_a \times r_b$$

Where, r_a is the semi major axis and r_b is the semi minor axis.

Triticale genotypes were assessed for stem rust severity based on a modified Cobb scale as described by Peterson et al. (1948) and the infection types (Roelfs et al., 1992). The percent disease severity readings taken over time were used to calculate the Area under Disease Progress Curve (AUDPC) according to Wilcoxson et al. (1975). Plants were considered headed when 50% of the plants had fully emerged heads plants and considered flowered when 50% of the heads in the pot had anthers extruded from the florets. The numbers of days to physiological maturity were calculated from the day of planting to the time when triticale stems turned golden brown colour. Height of plant and length of spike were measured at physiological maturity from a sample of three plants per pot. Plants were measured from the base to the tip of the plant excluding the awns while the length of spike was from the proximal to the distal end of the spike excluding the awns. At physiological maturity all the plants were harvested separately to estimate the biomass, grain yield, number and weight of grains per spike. Weight of kernels for each entry was estimated from a random sample of 50 kernels. Three heads were randomly sampled from each pot to determine the number and weight of grains per spike. Biomass was determined by taking the dry weight of the plants and adjusting to 12% moisture content.

Data analyses

Analysis of variance of length, width and area of spore was done using the PROC GLM in Statistical Analysis System (SAS Institute, 2002) using the following statistical equation:

$$Y_{ijkl} = \mu + R_i + G_j + S_k + GS_{ik} + \varepsilon_{ijkl}$$

Where, Y_{ijkl} = observation made on j^{th} triticale genotype within i^{th} replicate and at k^{th} stage; μ = overall mean; R_i = effect due to i^{th} replicate; G_j = effect due to j^{th} genotype; S_k = effect due to k^{th} triticale stage, GS_{ik} = effect due to interaction between j^{th} genotype and k^{th} stage, ε_{ijkl} = random error component. Stage as a factor was used to designate the number of days after inoculation when subsequent measurements of spore size were done. Analysis of Variance for AUDPC, grain yield and yield components was done based on the following statistical model:

$$Y_{ij} = \mu + R_i + G_j + \varepsilon_{ij}$$

Where, Y_{ij}= observation made in i^{th} replicate and on j^{th} triticale genotype; μ = overall mean; R_i = effect due to i^{th} replicate; G_j = effect due to j^{th} triticale genotype, ε_{ij} = random error component. Means of stage and genotypes were separated using the Tukey Honestly Significant Difference (HSD) procedure at $p \leq 0.05$. The relationship between latent period, AUDPC and spore size of triticale genotypes was determined using the SAS PROC CORR analysis. The average rate of change in the area of spore for each genotype was estimated using 2nd or 3rd order polynomial expressions obtained after plotting a graph of spore size against the number of days after inoculation. The choice of 2nd or 3rd order polynomials was based on the maximum (close to but less than 1) R^2 that was obtained for each genotype.

RESULTS

Adult plant reaction of triticale genotypes to stem rust infection

During the experimental period, adult triticale plants showed a range of reactions from *R* to *S* types with severity ranging from *R* to 30*S* (30% severity and plants showing a susceptible reaction) (Table 1). Triticale genotypes EUT046, EUT059, EUT090, EUT093, EUT118 and EUT0139 were resistant to stem rust infection. Among these resistant genotypes EUT046, EUT090, EUT093 and EUT139 showed *R* reaction with no visible stem rust pustules on the plants while EUT059 and EUT118 had minimal flecks (*TR*) on the leaf blades. Triticale genotypes EUT001, EUT034, EUT035, EUT123 and EUT124 showed disease severity of 5*RMR* (5% disease severity with plants showing resistant-moderately resistant responses) (Table 1). Four of the genotypes evaluated were moderately resistant with severity ranging from 10*MR* for three genotypes to 15*MR* for EUT078. For the moderately susceptible genotypes stem rust severity of 5*MS* for EUT030 to 20*MS* for EUT137 was observed. Two triticale genotypes EUT107 and EUT108 were moderately susceptible-susceptible with severity range of 10*MSS* and 30*MSS*, respectively. Based on the percent area of spikes infected by stem rust, 19 of 26 triticale genotypes were resistant (*R*, *TR* and 5*R* responses) to ear rust at adult plant. The rest of triticale genotypes exhibited *S* and *MS* reactions with severity of 5*MS* to 10*S* (Table 1).

Analysis of variance for spore size, AUDPC and agronomic traits

Effects due to genotype, stage of measurement, genotype × stage interactions were significant ($p \leq 0.001$) for length, width and area of spore (Table 2). The effects due to genotypes were significant ($p \leq 0.001$) for the latent period (LP), AUDPC, height of plant, number of grains per spike and weight of grains per spike (Table 3). The mean squares due to genotypes were also significant ($p \leq$

Table 1. Adult plant resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) for 26 triticale (*X. Triticosecale* Wittmack) genotypes.

Genotype	Pedigree and selection history	LP	FDS	ER	AUDPC
EUT001	DAHBI_6/3/ARDI_1TOPO 1419//ERIZO_9.. CTSS93B00368S-9Y-0M-0Y-0B-2Y-0B-IB-0Y	22.67	5RM5	TR	32.17
EUT004	BULL_10/MANATI_1//FARAS/CMH84.4414 CTSS99Y00415S-5Y-0M-0Y-5B-2Y-0B	10.00	20S	TR	81.50
EUT007	AR/SNP6//TARASCA 87_3/C, S10/3/URON_5/ TATU_1/4/POLLMER_4//2*ERIZO_10/… CTSS02B00009T-3Y-6M-3Y-4M-1Y-0M	14.33	5S	TR	21.50
EUT026	HX87-244/HX87-255/5/PRESTO//2*TESMO_1/MUSX 603/4/ARDI_1TOPO 1419//ERIZO_9/3/… CTSS03H00028S-25Y-2M-3Y-1M-1Y-0M	10.33	30S	10S	145.29
EUT030	BULL_10/MANATI_1//FARAS/CMH84.4414/3/SONNI_3*2//FARAS/CMH84/4414 CTSS04Y00003S-16Y-06M-06Y-5M-4Y-0M	14.33	5MS	TR	35.00
EUT034	POLLMER_2.2.1*2//FARAS/CMH84.4414/4/DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9 CTSSO4Y00066S-60Y-06M-06Y-1M-1Y-0M	17.60	5RMR	5R	35.00
EUT035	POLLMER_2.2.1*2//FARAS/CMH84.4414/4/DAHBI-6/3/ARDI-1/TOPO 1419//ERIZO_9 CTSS04Y00066S-60Y-06M-06Y-2M-1M-0M	16.67	5RMR	R	12.50
EUT046	BAT*2/BCN//CAAL/3/3RIZO_7/BAGAL_2//FARAS_1/9/LIRON_2/5/DIS B5/3/SPHD/PVN//… CTSS05Y00236T-0TOPB-020Y-16M-3Y-0M04Y-0M	-	R	R	0.00
EUT059	T1505_WG//ERIZO_10/BULL_1-1/3/ERIZO_10/BULL_1-1/4/COPI_1/5/ARDI-1/TOPO 1419// CTSS07Y00001S-17Y-010M-6Y-3M-3Y-0B	17.33	0; TR	R	7.67
EUT072	DAGRO/IBEX//CIVET#2/3/F3 IND.PCZ/5/HX87-244/HX87-265/4/DAHBI-6/3/ARDI-1/… CTSS07Y00105S-18Y-010M-3Y-2M-3Y-0B	15.00	15MS	TR	60.24
EUT078	ARDI_1/ TOPO1419//ERIZO_9/3/LIRON_1-1/4/FAHAD_4/FARAS-1/5/CT775.81/ARDI_1//… CTSS07Y00158S-1Y-010M-20Y-4M-3Y-0B	15.00	15MR	TR	55.00
EUT084	NILEX/3/BULL_10/MANATI_1//FARAS/CMH84.4414/6.HX87-244/HX87-255/5/PRESTO//… CTSS08Y00155T-099Y-016M-17Y-099M-4Y-4BMX-4Y-0B	13.33	10MS	5MS	46.33
EUT085	NILEX/3/BULL_10/MANATI_1//FARAS/CMH84.4414/6.HX87-244/HX87-255/5/PRESTO//… CTSS08Y00155T-099Y-016M-17Y-099M-19Y-2BMX-3Y-0B	18.67	10MR	TR	38.67
EUT087	95T159/4/T1502_WG/MOLOC_4//RHINO_3/BULL_1-1/3/POLLMER_3/FOCA_2-1/5/CAAL/3/… CTSS08Y00168T-099Y-024M-5Y-099M-4Y-3BMX-1Y-0B	22.66	10MR	TR	18.00
EUT090	TURACO/CENT.SARDEV/7/LIRON_2/5/DIS B5/3/SPHD/PVN//YOGUI_6/4/KER_3/6/BULL_10/… CTSS08Y00035S-099Y-026M-4Y-099M-9Y-1BMX-4Y-0B	-	R	R	0.00
EUT93	TURACO/CENT.SARDEV/7/LIRON_2/5/DIS B5/3/SPHD/PVN//YOGUI_6/4/KER_3/6/BULL_10/… CTSS08Y00035S-099Y-026M-4Y-099M-10Y-4BMX-2Y-0B	-	R	R	0.00
EUT107	08L128 F5 11.033132-6Y-0B	14.67	10MSS	TR	69.00

Table 1. Contd.

Genotype	Pedigree and selection history	LP	FDS	ER	AUDPC
EUT108	09L166 F4 11.033111-11Y-0B	11.00	30MSS	5MS	100.50
EUT118	12P175 14-200013	21.67	0; TR	R	4.34
EUT123	10L005-INT-13 12.033004-F4-6M-2Y-3M-04Y-0B	15.00	5RMR	TR	19.17
EUT124	10L005-INT-13 12.033004-F4-6M-3Y-1M-04Y-0B	15.67	5RMR	5R	36.50
EUT128	PRESTO//2*TESMO_1/MUSX 603/4/ARDI_1/TOPO 1419//ERIZO_9/3/SUSI_2/5/AR/SNP6//… CTSS12Y00106S-2Y-2M-3Y-1M-04Y-0B	10.67	30S	15MS	211.17
EUT129	PRESTO//2*TESMO_1/MUSX 603/4/ARDI_1/TOPO 1419//ERIZO_9/3/SUSI_2/5/AR/SNP6//… CTSS12Y00106S-2Y-6M-1Y-1M-04Y-0B	13.67	10M	5MS	48.83
EUT136	WIR 46058/GNU_1//ERIZO_11/3/SVHT 02/STIER_3/4/WIR 46058/GNU_1/ERIZO_11/5/… CTSS12Y00125S-11Y-1M-3Y-2M-04Y-0B	12.67	15MS	10MS	63.50
EUT137	WIR 46058/GNU_1//ERIZO_11/3/SVHT 02/STIER_3/4/WIR 46058/GNU_1/ERIZO_11/5/… CTSS12Y00125S-16Y-3M-4Y-2M-04Y-0B	14.67	20MS	10MS	74.58
EUT139	BICEN/8/GAUR_2/HARE_3//JLO 97/CIVET/5/DIS B5/3/SPHD/PVN/YOGUI_6/4/KER_3/6/… CTSS12Y00279S-23Y-3M-2Y-2M-04Y-0B	-	R	R	R
$HSD_{(0.05)}$		6.63			26.88
Mean		12.99			46.38

LP, latent period; FDS, Final disease score; ER, Ear rust; AUDPC, Area under disease progress curve. R, resistant (no visible spores on the stem or leaf surface); TR, trace (flecks or minimal uredinia); MR, moderately resistant or small uredinia; MS, moderately susceptible, moderate sized uredinia, S- susceptible, dense and large sized uredinia. Comparison of means for AUDPC: Any two means in the same column whose difference is less than the $HSD_{(0.05)}$ value are not significantly different.

0.01) for all growth stages of heading, flowering and physiological maturity as well as the length of spike but significant at $p \leq 0.05$ for biomass. On the other hand, the genotypes were not significantly different ($p > 0.05$) for grain yield and weight of 50 seeds (Table 3).

Apart from the width of spore of which the mean at 30 and 35 days after inoculation (DAI) were not significantly different ($HSD_{(0.05)}$), all stages were significantly different for the length and area of spore (Table 4). The mean length of spore increased from 0.75 mm to 2.65 mm between the 17 and 35 (Stage 5) DAI while the mean area of spore increased from 0.27 mm^2 to 1.33 mm^2 between 17 and 35 DAI, respectively (Table 4).

Among the 26 genotypes EUT046, EUT090, EUT093, EUTI39, EUT118 and EUT059 had the lowest mean AUDPC of 0.00-7.67. Triticale genotypes EUT026 and EUT128 had the highest mean AUDPC of 145.29 and 211.17, respectively and were significantly different from each other and the rest of the genotypes (Table 1). With the exception of EUT046, EUT090, EUT093 and EUT139 that showed *R* reaction to stem rust, EUT004 and EUT026 took the shortest period of 10.00-10.33 days for symptoms to appear while the longest latent period (LP) of 22.66 and 22. 67 days was observed on EUT087 and EUT001, respectively (Table 1).

The ranking of 26 triticale genotypes based on the mean performance for different agronomic traits varied (Table 5). Triticale plants attained 50% heading from 42.67-57.00 days with a mean of 48.44 days. The duration from planting to 50% anthesis was between 47.67-67.67 days with a mean of 54.82 days. The plants were mature within 88.83-105.67 days with a mean of 97.33 days. The plants grew to a height of between 56.15-72.67 cm while the length of spikes were between 5.91-8.09 cm (Table 5).

The mean biomass yield obtained ranged from 1.45 g per plant for EUT026 to 2.49 g per plant for

Table 2. Analysis of variance for spore size of 26 triticale (*X. Triticosecale* Wittmack) genotypes evaluated in the greenhouse for adult plant resistance to stem rust (*Puccinia graminis* f. sp. *tritici*).

Source of variation	Df	Length of spore	Width of spore	Area of spore
Replicate	2	20.88	0.19	5.66
Genotype	25	31.73***	1.11***	14.44***
Stage	4	43.53***	0.41***	14.05***
Stage × Genotype	100	1.57***	0.02***	0.97***
Error	389	0.06	0.002	0.04
C.V (%)		15.51	14.87	26.52
R^2		0.99	0.98	0.98

***- significant at $p \leq 0.001$.

Table 3. Analysis of variance for the latent period, grain yield and yield components of 26 triticale (*X. Triticosecale* Wittmack) genotypes evaluated in the greenhouse for adult plant resistance to stem rust (*Puccinia graminis* f. sp. *tritici*).

Source of variation	df	Latent period	AUDPC	No. of days to heading	No. of days to flowering	No. of days to Maturity	Height of plant	Length of spike	Biomass	No. grains per spike	Weight of grains per spike	Grain yield	Weight of 50 kernels
Replicate	2	2.94	3447.81	15.55	12.01	53.84	12.31	0.19	7.03	135.09	0.12	3.20	0.06
Genotype	25	129.43***	7094.92***	36.44**	68.67**	55.21**	69.05***	0.96**	3.27*	42.24***	0.05***	0.61	0.10
Error	50	4.34	71.56	16.22	27.29	20.01	21.11	0.36	1.80	3.92	0.02	0.47	0.07
C.V (%)		16.06	18.24	8.31	9.65	4.69	7.13	8.97	16.88	9.58	18.43	22.02	15.28
R^2		0.94	0.98	0.54	0.55	0.59	0.62	0.58	0.52	0.87	0.73	0.48	0.65

*, **, ***- significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

EUT123. The mean values of grain yield and weight of 50 seeds ranged from 0.59 for EUT026 to 0.98 g per plant for EUT093 and from 1.37 for EUT004 to 2.13 g for EUT034, respectively (Table 5). EUT108 and EUT001 had the highest (26.69 and 26.22) mean number of grains per spike while EUT090 and EUT72 had the heaviest grains per spike with mean of 0.91 and 0.89 g, respectively. On the other hand, the lowest mean number of grains per spike (13.54) and weight of grains per spike (0.36 g) was observed for EUT124 and EUT085, respectively.

Correlation between latent period, AUDPC and spore size

The latent period was negatively correlated with AUDPC (r = -0.63**), length of spore (r = -0.65***), width of spore (r = -0.70***) and area of spore (r = -0.64***) (Table 6).

On the other hand, positive and significant ($p \leq$ 0.001) correlation was observed between AUDPC and length of spore (r = 0.84), width of spore (r = 0.91) and area of spore (r = 0.91).

Development of stem rust spores on triticale genotypes

Among the 10 selected triticale genotypes, the slowest (0.003 mm^2 day^{-1}, 0.006 mm^2 day^{-1} and 0.009 mm^2 day^{-1}) development of stem rust spore

Table 4. Means of width, length and area of spore at five stages after inoculation for 26 triticale genotypes evaluated in the greenhouse for adult plant resistance to stem rust (*Puccinia graminis* f. sp. *tritici*).

Number of days after inoculation (stage)	Width of spore	Length of spore	Area of spore
	mm		(mm^2)
17 (1)	0.23[d]	0.75[e]	0.27[e]
20 (2)	0.29[c]	1.15[d]	0.48[d]
25 (3)	0.36[b]	1.55[c]	0.75[c]
30 (4)	0.39[a]	2.07[b]	1.03[b]
35 (5)	0.40[a]	2.65[a]	1.33[a]
$HSD_{(0.05)}$	0.02	0.11	0.09
Mean	0.34	1.63	0.77

Means in the same column followed by the same letter are not significantly ($p \leq 0.05$) different.

was observed between 17- 20 days after inoculation (DAI) for EUT035, EUT123 and EUT034, respectively (Table 7). The development of spores depicted significant genotype × stage interactions revealed from the ANOVA (Table 2) and hence triticale genotypes attained different sizes of spore at different stages when they were measured (Figure 1). The fastest increase (0.337 mm^2 day^{-1} for EUT026 and 0.387 mm^2 day^{-1} for EUT128) in spore size was observed between 30-35 DAI and from 17-20 DAI, respectively. Among the triticale genotypes with *RMR* or *MR* reactions, the average rate of change in spore size was increasing for EUT034, EUT085, EUT087 and EUT123 but was fluctuating for EUT035 (Figure 1). The average rate of change in spore size increased from 0.006 mm^2 day^{-1} for EU123 to 0.205 mm^2 day^{-1} for EUT085 between 17-20 and 30-35 DAI, respectively (Table 7). The development of stem rust spore on EUT035 was fluctuating, that is, the development was slowest (0.003 mm^2 day^{-1}) between 17-20 DAI but fastest (0.009 mm^2 day^{-1}) between 25-30 days post inoculation. Although the rate of change in spore size was fluctuating for genotypes with *MS* to *S* reactions, the development of the stem rust was fastest on these genotypes with a minimum of 0.040 mm^2 day^{-1} and a maximum of 0.387 mm^2 day^{-1} for EUT128, between 25-30 and 17-20 DAI. The development of spores was slowest on the genotypes with *RMR* or *MR* responses, and the minimum and maximum average rate of change in spore size was 0.003 mm^2 day^{-1} and 0.205 mm^2 day^{-1} for EUT085, respectively (Table 7).

DISCUSSION

Genetic variations for virulence of stem rust infection on triticale genotypes were observed and this could be due to diversity and their genetic background in which genes were placed. The variability was depicted by a range of reactions from *R* to *S* responses that were observed. Determination of specific genes present in the genotypes was beyond the scope of this study. Although developing and distribution of resistant genotypes has to some extent controlled the damage of stem rust in triticale and wheat production areas, there exist significant genetic vulnerability (Ellis et al., 2014). Mishra et al. (2015) suggested that promising genotypes that show resistance to rust diseases should be identified and recommended for use as sources of resistance for breeding programs. It is important to note that four genotypes EUT046, EUT090, EUT093 and EUT139 exhibited high level of resistance with no visible stem rust pustules. In particular, adult plant resistance to rust is effective and durable resistance especially when it is conferred by a combination of minor genes with additive effects (Singh et al., 2005; Singh et al., 2014).

Significant effects due to genotypes for disease parameters and yield components suggest that there exists genetic variability among the genotypes. These genetic variations amongst genotypes could be attributed to the different parental backgrounds and selection procedure used to develop Recombinant Inbred Lines (RILs). All genotypes that were evaluated have different pedigrees implying that they have different parental background except for EUT090 and EUT093 as well as EUT0128 and EUT129 that have common parents but with different selection histories. The significant genotype × stage interactions for the *uredinium* size suggest that during spore development after the latent period, spores attained different width, length and area. The differential attainment of size and areas of spores could be due to resistance genes.

Determination of the spore area depended on the magnitude of virulence of stem rust at the end of the latent period (LP). The size and areas of spores on genotypes EUT046, EUT090, EUT093, EUT059, EUT118 and EUT139 could not be determined because they expressed severity of 0 with no reaction or rather *TR* reaction with minimal flecks that did develop into visible

Table 5. Grain yield and yield components of 26 triticale genotypes (*X. Triticosecale* Wittmack) evaluated in the greenhouse for adult plant resistance to stem rust (*Puccinia graminis* f. sp. *tritici*).

Genotype	No. of days to heading	No. of days to anthesis	No. of days to PM	Height of plant	Length of spike	Biomass	Grain yield	Weight of 50 seeds	Weight of grains per spike	No. of grains per spike
				cm		g plant^{-1}			g	
EUT001	49.67	57.33	99.67	69.83	7.12	2.35	0.82	1.62	0.87	26.22
EUT004	54.67	57.33	100.33	66.56	6.30	1.81	0.62	1.37	0.58	20.53
EUT007	49.67	56.00	96.00	70.61	7.00	1.88	0.76	1.64	0.75	21.36
EUT026	45.00	50.67	93.67	56.15	6.47	1.45	0.59	1.66	0.57	17.78
EUT030	57.00	67.67	105.67	70.58	7.71	2.20	0.79	1.49	0.80	25.22
EUT034	45.00	51	92.00	64.45	7.33	2.07	0.76	2.13	0.76	15.68
EUT035	44.00	49.33	98.67	63.31	6.82	1.74	0.63	1.98	0.56	15.22
EUT046	48.33	54.00	95.67	60.37	5.96	1.90	0.96	1.51	0.71	23.66
EUT059	45.67	51.33	93.67	59.78	6.28	1.73	0.68	1.90	0.68	17.44
EUT072	47.67	50.33	97.33	64.50	6.86	1.71	0.89	1.64	0.89	23.73
EUT078	47.33	54.67	97.67	58.00	6.83	1.96	0.84	1.67	0.80	21.36
EUT084	42.67	47.67	97.33	60.00	6.41	1.95	0.67	1.74	0.68	17.67
EUT085	44.33	49.33	95.67	59.33	6.27	1.71	0.65	1.71	0.36	14.55
EUT087	45.00	50.67	92.67	65.17	6.14	1.98	0.62	1.66	0.64	18.44
EUT090	47.00	53.00	96.00	64.00	5.91	1.98	0.92	1.92	0.92	24.98
EUT093	49.00	57.33	98.67	66.28	6.19	2.19	0.98	1.74	0.87	22.43
EUT107	51.00	58.00	101.00	67.46	7.24	2.10	0.87	1.81	0.80	12.33
EUT108	46.00	51.67	88.33	62.83	8.09	1.96	0.81	1.49	0.83	26.69
EUT118	52.33	55.33	100.33	70.67	7.19	2.41	0.89	1.70	0.80	22.33
EUT123	51.00	61.33	104.00	72.67	6.01	2.49	0.82	1.71	0.67	18.33
EUT124	49.33	57.67	101.67	66.11	6.12	2.41	0.79	1.47	0.65	13.54
EUT128	50.33	54.67	96.67	56.87	6.02	1.70	0.61	1.42	0.61	18.76
EUT129	53.00	60.00	99.67	59.89	6.39	1.90	0.78	1.48	0.86	25.55
EUT136	49.33	57.33	98.67	71.72	6.76	2.03	0.75	1.94	0.74	21.70
EUT137	49.33	54.67	103.33	66.72	6.37	2.18	0.86	1.65	0.67	20.78
EUT139	45.30	51.33	95.70	61.43	6.92	2.10	0.83	1.67	0.87	23.65
$HSD_{(0.05)}$	12.80	16.82	14.49	14.60	1.89	1.07	0.54	0.82	0.43	6.29
Mean	48.44	54.82	97.31	64.43	6.64	1.99	0.78	1.68	0.73	20.65

PM, physiological maturity. Comparison of grain yield and agronomic traits: Any two means in the same column whose difference is less than the $HSD_{(0.05)}$ value are not significantly different

Table 6. Pearson correlation coefficients (*r*) showing relationship between latent period, stem rust AUDPC and spore size traits of triticale genotypes evaluated in the greenhouse.

Parameter	Latent period	Length of spore	Width of spore	Area of spore
AUDPC	- 0.63**	0.84***	0.91***	0.91***
Latent period		- 0.65***	- 0.70***	- 0.64**

, *- significant at $p \leq 0.01$ and $p \leq 0.001$, respectively.

Table 7. Average rate of change in the mean area of spore (mm^2 day^{-1}) of selected 10 triticale genotypes evaluated in the greenhouse for adult plant resistance to stem rust (*Puccinia graminis* f sp. *tritici*).

Genotype	Reaction	Average rate of change in the mean area of spore (mm^2 day^{-1})			
		17-20	20-25	25-30	30-35
EUT026	30S	0.291	0.139	0.133	0.337
EUT128	30S	0.387	0.152	0.040	0.138
EUT108	30MSS	0.059	0.215	0.280	0.195
EUT004	20S	0.319	0.289	0.225	0.131
EUT129	10MS	0.136	0.090	0.081	0.121
EUT087	10MR	0.047	0.050	0.053	0.056
EUT085	10MR	0.100	0.131	0.168	0.205
EUT034	5RMR	0.009	0.013	0.021	0.032
EUT123	5RMR	0.006	0.013	0.029	0.052
EUT035	5RMR	0.003	0.004	0.009	0.008

spores. As a result, these genotypes had the lowest mean value for the width, length and area of spore. The LP was taken for 22 of 26 genotypes because stem rust pustules or flecks were not observed on triticale genotypes EUT046, EUT090, EUT090 and EUT139. It was evident that the latent period was longest on genotypes that showed *R-MR* reactions suggesting that these genotypes possess APR qualities which are in agreement with the findings of Singh et al. (1991) and Azzimonti et al. (2013). According to Draz et al. (2015), short and long latent periods are a characteristic of fast and slow rusting genotypes, respectively.

The obtained results revealed that with the exception of genotypes that showed *R* responses, lowest AUDPC was attained on EUT118, EUT059 and EUT035 among others that showed low disease severity of *TR*-5*RMR* while highest AUDPC was attained on genotypes that exhibited highest disease severity. These results are in agreement with findings of Aktas and Zencirci (2016) who found that wheat genotypes that showed APR to stripe rust were characterized with low disease severity and AUDPC values. In a different study on leaf rust, Draz et al. (2015) demonstrated that late infection and slow growth of the pathogen on infected plants led to low values of AUDPC. According to Herrera-Foessel et al. (2007) such traits of disease resistance are ideal qualities of a slow rusting genotype.

The negative correlation of latent period with the AUDPC, length, width and area of spore suggest that triticale genotypes that were earliest to show symptoms of stem rust (pustules) enable the pustules to develop to a large size hence high AUDPC and size of spore are realized. For instance, EUT059 and EUT118 with the lowest mean AUDPC values were among genotypes that had the longest LP while EUT128, EUT026 and EUT108 had the highest mean AUDPC and shortest LP. These results are in accordance with the findings of Singh *et al*. (1991) who found that the fast-rusting wheat cultivar *Morocco* had the shortest latent period, largest spore size and highest AUDPC for leaf rust. Similar results were obtained regarding the relationship of *uredinium* size with the latent period and AUDPC of leaf rust (Herrera-Foessel et al., 2007). The high AUDPC observed for these genotypes indicates that it took short time for stem rust spores to show virulence on these genotypes. Therefore, this provided more time for the stem rust spores to develop on the leaves or stem of these genotypes.

Although variation in spore size on different genotypes may be observed as a result of differences in latent period and continuous growth of the pustule after rupture of epidermal tissue (Singh et al., 1991), measurement of spore size on plants was done to assess the rate of increase in spore size on different triticale genotypes. The results obtained revealed that the development of spores on *R-MR* genotypes was slower than on the

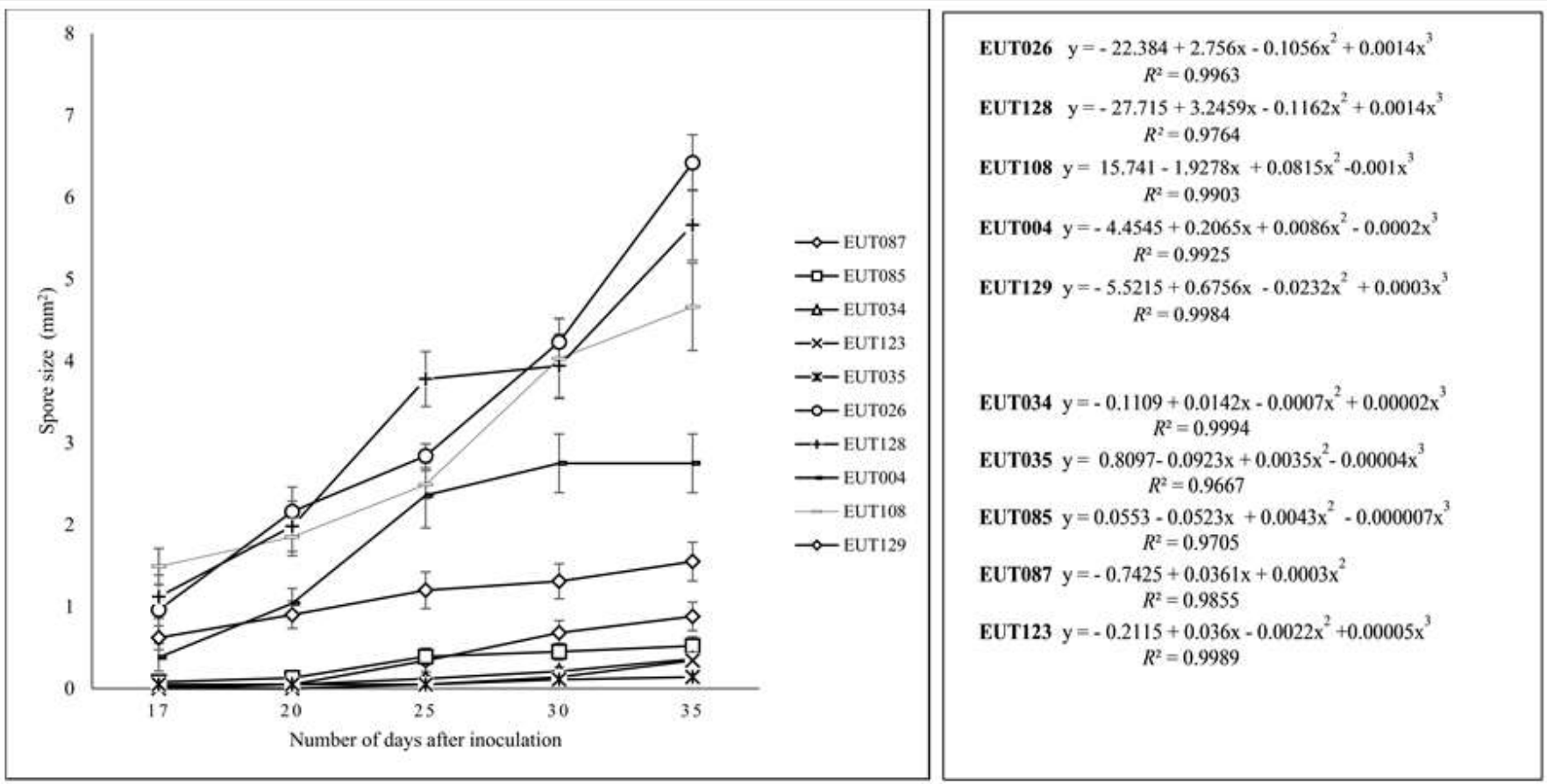

Figure 1. Change in the area of spore over time after inoculation for selected 10 triticale (X. Triticosecale Wittmack) genotypes evaluated in the greenhouse for Adult Plant Resistance (APR) to stem rust. Plotting was done to compare spore development on selected genotypes that showed MR, RMR, MS, MSS and S (Table 7) responses to stem rust infection. MR, moderately resistan;, RMR, resistant to moderately resistant; MS, moderately susceptible, MSS-moderately susceptible to susceptible; S, susceptible

MS-S triticale genotypes and this could be attributed to their genetic differences. The slow change in the size of spore that was exhibited by triticale genotypes with *TR*, *RMR* and *MR* responses could be because of their genetic constitution that slow down the development of spores. Despite the infection by stem rust, triticale genotypes with the slowest rate of increase in spore size could be postulated to possess traits for APR because APR ranges from immunity to partial resistance which is characterized by slow development of disease (Singh et al., 2012; Azzimonti et al., 2013; Draz et al., 2015). This reduced development is encouraged by extended latent period and may translate into low values of

AUDPC.

Among the genotypes that were evaluated 6 genotypes EUT046, EUT059, EU090, EUT093, EUT118 and EUT139 exhibited high level of APR to stem rust which can be used as sources of resistance genes to stem rust. Further step in investigation will be postulation and characterization of genes that confer resistance to stem rust.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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