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Screening of Kenyan Bread Wheat Varieties for Resistance to the Emerging Strains of Stem rust Fungi (*Puccinia graminis* f. sp. *tritici*) Race Ug99

Kimani N. C[1,2,*], Onguso J.[1], Njau P.[2]

[1]Jomo Kenyatta University of Agriculture and Technology P.O Box 62000-00200 Nairobi, Kenya,
[2]Kenya Agricultural and Livestock Research Organisation, Food Crops Research Centre-Njoro P.O Box PRIVATE BAG-2107 Njoro, Kenya
*Corresponding author: nkimanicyrus@gmail.com

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Abstract Stem rust disease of wheat caused by *Puccinia graminis* f. sp. *tritici* is of major concern because of its devastating effects on wheat. It can cause yield loss of up to 100% in susceptible varieties. East Africa has been designated as a "hot spot" of the stem rust pathogen as evidenced by the emergence of a new race of stem rust designated as TTKSK or better known as Ug99 and several of its variants. This pathogen therefore poses a threat to wheat production and hence to food security in Kenya. The frequent use of fungicides to control the disease also poses a potential adverse effect on the environment. The objective of this study was to screen a core collection of Kenyan bread wheat varieties to determine those with natural resistance to stem rust disease hence reduce the risk posed to food security and the environment. Twenty Kenyan commercial bread wheat varieties were screened for stem rust resistance under artificial disease epidemic simulation in the International Stem Rust Screening Field at Kenya Agricultural and Livestock Research Organisation, Food Crops Research Centre-Njoro, Kenya. The disease notes were taken using the Modified Cobb's Scale and the Area Under Disease Progress Curve (AUDPC) values computed. Thirteen random samples of stem rust fungi were collected from the trial plot and analyzed using Ug99 race group-specific Single Nucleotide Polymorphism (SNP) markers. The varieties fell in three disease categories of resistant, intermediate and susceptible, with the most susceptible being Pasa and Kenya Swara being the most resistant. The mean AUDPC computed showed that there was variation in the AUDPC values among the varieties with the variety K.Swara having the lowest AUDPC value of 78.33 and variety Pasa having the highest value of 478.67. Analysis of Variance (ANOVA) showed that both AUDPC and disease scores had significant variation ($P<0.0001$) among the varieties. From the analysis of stem rust fungi samples two genotypes of stem rust race TTKSK (AF-001ad and AF-001aa) were detected indicating mutations within the same race variant. In conclusion there are Ug99 resistant Kenyan bread wheat varieties which hold a promise for food security. There is also evidence of further mutation within the TTKSK race variant and hence a possible increased virulence on the wheat genotypes.

Keywords: *Stem rust, AUDPC of stem rust, Puccinia graminis f. sp. tritici, Emerging strains of Race Ug99, SNP markers*

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1. Introduction

Kenya is a net importer of wheat, with consumption of about 900,000 tons per year against an average production of 350,000 tons per year, implying it usually imports close to two-thirds of its requirements [18]. This is a worrying trend for a country where agriculture contributes nearly two thirds (63 percent) of the Gross Domestic Product (GDP) [6]. The limitations on production are usually due to abiotic and biotic constraints but of recent the latter, specifically rust diseases have contributed to heavy crop loss. Of these, stem rust has been the most destructive especially the highly virulent Ug99 race and its variants. Majority of Kenyan germplasm are known to be susceptible or partially susceptible to Ug99 **[9]**.

The race Ug99, first identified in Uganda in 1998 [11], is the only known race of *P. graminis tritici* that has virulence for gene *Sr31* known to be located in the translocation 1BL.1RS from rye (*Secale cereale*) [11]. This race was later designated as TTKS [19] using the North American nomenclature system [13]. A number of stem rust resistance genes have been identified in wheat. Some of them were discovered in bread wheat (e.g. *Sr5* and *Sr6*), while others have including *Sr21*, *Sr31*, and *Sr44* were bred from other wheat species. None of the *Sr* genes provide resistance to all races of stem rust [14]. For instance many of them are ineffective against the Ug99

lineage. Notably Ug99 has virulence against *Sr31*, which was effective against all previous stem rust races. Singh *et al*., [14] provide a list of known *Sr* genes and their effectiveness against Ug99. Unfortunately, race Ug99 not only carries virulence for gene *Sr31* but also for most of the genes of wheat origin. The only gene that provides broad spectrum resistance is *Sr2*. However this gene only reduces the severity of stem rust disease by slowing the latent period, a phenomenon referred to as slow rusting [16]. Currently there are eight Ug99 variants (TTKSK, TTKST, TTTSK, TTKSF, TTKSF+, TTKSP, PTKSK, and PTKST) [5,12]. This shows that the pathogen is evolving at an alarming rate. A two step Ug99 race group-specific Single Nucleotide Polymorphism (SNP) marker assay was recently developed by Les Szabo and Crouch Joanne [17].

Stem rust resistance follows the gene for gene hypothesis proposed by Harold Flor [2] where there is interaction of the avirulence gene product from the host and the virulence gene product from the pathogen. A disease response is only observed if both gene products are from dominant genes, otherwise there's no disease reaction. Race specific resistance means the gene is effective only to some races of the pathogen and is based on genes that are effective at seedling as well as throughout the life stages of the plant [8]. This resistance is also referred to as monogenic or vertical resistance, it's simply inherited and follows the gene for gene hypothesis. It is usually characterized by hypersensitive reaction and prone to a boom and bust cycle [1]. Race-nonspecific, polygenic, horizontal or adult plant resistance (APR) is effective in the post-seedling stage and adult stage. It provides a broad spectrum resistance and is observed in the field conferring the slow rusting effect [4]. Race non-specific resistance doesn't follow the gene for gene hypothesis.

Ug99 threat in most countries can be reduced to low levels by urgently identifying, releasing and providing seed of new high yielding, resistant varieties [15]. Towards breeding for resistance to the predominant local stem rust races, the objective of this study was firstly to profile the resistance in commercially popular varieties and secondly to discover genes from these materials based on field assays.

2. Materials and Methods

2.1. Materials

Twenty selected Kenyan bread wheat varieties namely Ngamia, Mbega, Chozi, Kwale, Pasa, Yombi, Duma, Kulungu, Mbuni Njoro BWII, Kenya Kongoni, Kenya Chiriku, Kenya Tembo, Eagle10, Kenya Fahari, Robin, Kenya Ibis, Kenya Nyangumi, Kenya Swara and Kenya Kingbird were used in this study. Three international varieties Danphe, PBW343 and Cacuke were used as checks.

2.2. Method

2.2.1. Stem Rust Screening

Twenty Kenyan wheat varieties and three international check varieties (controls) were planted in the International Stem Rust Screening Field at Kenya Agricultural and Livestock Research Organisation, Food Crops Research Centre-Njoro (°20′S, 35°56′E at 2,185 m in elevation) using Randomized Complete Block Design (RCBD) with three replicates.

Land was prepared conventionally by harrowing and making furrows. Di-Ammonium Phosphate (DAP) was applied at planting at the recommended rate of 150 kg/ha, principally as the source of both Nitrogen and Phosphorus (18:46:0). Fifteen grams of seeds for each of the varieties were planted in 2 rows, 0.75 m long with alley spacing of 0.2 m between the rows. Moreover, a spacing of 0.5 m was provided between adjacent replicates.

A mixture of stem rust susceptible varieties was planted as disease spreaders in between the replicates as a single row perpendicular to the entries. To ensure sufficient disease pressure, three spreader rows were also planted to surround the experiment. Broad leaved weeds and cereal aphids particularly the 'Russian Wheat Aphid' were controlled by applying the post emergence herbicide Buctril MC (bromoxynil+ MCPA) at the rate of 1.25 l/ha and insecticide, Bulldock star 262.5 EC (beta-cyfluthrin and chlorpyrifos) at the rate of 0.6 l/ha respectively.

To initiate stem rust epidemic bulk stem rut fungi spores were collected from rust increase plots and spore suspensions prepared both in Soltrol oil and Tween 20 detergent. The disease spreader rows were then inoculated with the spores by simultaneously spraying them with the Soltrol suspension using a hand sprayer and injecting them with the Tween 20 suspension using a syringe. The first stem rust disease scores were taken when the susceptible check (Cacuke) showed a disease severity of 50% based on the Modified Cobb's scale [10]. Two more scores were then taken at intervals of seven days in between.

2.2.2. Stem Rust Fungi Race Analysis

Thirteen stem rust samples were collected for race analysis. The procedure involved cutting out the wheat plant stem section with distinct single pustule of the stem rust spores with an ethanol disinfected pair of scissors, observing maximum asceptic techniques. Excess tissue was carefully removed leaving only the epidermal layer containing the single pustule spore. This layer was then immersed in a screw cap cryovial tube containing 70% ethanol in order to kill the spores and avoid any biosafety bleach while transporting the samples. The procedure was applied in collecting all the thirteen samples. To avoid cross contamination of samples hands and the scissors were disinfected with 70% ethanol in-between collecting different samples. The tubes were labeled with unique sample numbers. Information on the genotype from which each sample was collected, collection site and the GPS information of the area were also recorded. The collected samples were left in ethanol for five days to allow complete killing of the stem rust fungi spores. After five days the ethanol was poured out and tubes left open to completely dry off the samples. The sample tubes were recapped, placed in polythene zip lock bags and packed in a carton box. The samples were then shipped to the USDA-ARS Cereal Disease Laboratory at University of Minnesota-St. Paul, USA for analysis with the Ug99 SNP marker assay.

2.2.3. Data Analysis

The data collected was entered in excel worksheets. The AUDPC values were generated on an excel worksheet using the formula below [20]:

$$AUDPC = \sum_{I=1}^{n-i} 0.5(Xi+1+Xi)(ti+1-ti)$$

Where, Xi is the cumulative disease severity expressed as a proportion at the *i*th observation; ti is the time (days after planting) at the *i*th observation and n is total number of observations.

One way-ANOVA computed using SAS program was used to analyse variance of the infection scores and AUDPC means among the wheat varieties and replicates.

Least Significant Difference (LSD 0.05) was employed to compare treatment means using SAS software (SAS Version 9.1, SAS Inc.).

3. Results and Discussion

3.1. Disease Scores

Eleven varieties Ngamia, Mbega, Chozi, Kwale, Pasa, Yombi, Duma, Kulungu, Mbuni Njoro BWII and K.Kongoni showed susceptibility of varying degree ranging from Moderately Susceptible (MS), Moderately Susceptible-Susceptible (MSS) to Susceptible(S) with the most susceptible being Pasa which had a score of 60S (Table I). These are some of the old varieties that were bred up to the late 90s and were part of the first varieties that were seen to be susceptible to the new Ug99 stem rust race [9].

This might have been due to the fact that most have stem rust resistance genes ineffective against Ug99 e.g. *Sr31* gene which was present in most of the world's wheat germplasm at that time before it was broken down by the new Ug99 stem rust race in the late 90s. However the varieties Mbega, Pasa and Duma were less susceptible than the susceptible check variety Cacuke which had a final score of 100S. The moderately susceptible to susceptible (MS and MSS) varieties Ngamia, Chozi, Kwale, Yombi, Kulungu, Mbuni Njoro BWII and K.Kongoni showed lower disease scores than the moderately susceptible check PBW343 (Table 1).

Two varieties K.Chiriku and K.Tembo showed intermediate infection type (M) with a score of 10M and 20M respectively. This indicates a possibility that these varieties carry several stem rust resistance genes some effective while others ineffective against Ug99. There is also a possibility that they may have effective stem rust resistance genes but the expression level of the genes is still low to provide sufficient resistance (Table 1).

Seven varieties Eagle10, K.Fahari, Robin, K.Ibis, K.Nyangumi, K.Swara and K.Kingbird showed varying levels of resistance with the most resistant being K.Swara with a score of 5 Resistant to Moderately Resistant (RMR). Most of these are recent varieties and are known to carry major (Race-specific Resistance) and minor (Adult Plant Resistance) genes or a combination of both. An example is Robin which has been postulated to carry the major gene *SrTmp* and minor gene *Sr2* [14] while K.Kingbird has been postulated to carry the APR gene *Sr2* based on expression of the PBC phenotype. These genes provide varying resistance to Ug99 stem rust.

However, among those varieties which showed resistance were some old varieties K.Fahari, K.Nyangumi and K. Swara (Table 1). This resistance can be attributed to the presence of *Sr2* gene in these varieties as they all showed expression of Pseudo-Black Chaff (PBC) which is a phenotypic marker for the *Sr2* gene, characterized by black pigmentation on the stem internodes and glumes of wheat [3]. *Sr2* is the only well characterized or known APR gene and it provides a broad-spectrum resistance to all the known variants of the Ug99 race.

The varieties K.Fahari, K.Ibis, and K.Kingbird performed as well as the resistant check variety Danphe. Robin and Eagle10 showed less resistance than the check while K.Nyangumi and K.Swara showed better resistance than the check (Table 1). It is worth noting that from the above table, the disease severity in some cases did not necessarily reflect on the infection type. A good example is Kulungu which had a severity of 15 and an infection type of MS compared to Robin and Eagle10 which had severities of 30 and an infection type of Moderately Resistant (MR). The severity in Kulungu might be an indicator of some level of resistance in spite of the susceptible infection type, whereas the high severities in Robin and Eagle10 might be an indicator of a near future breakdown of the gene(s) responsible for resistance in these two varieties.

Table 1. Kenyan bread wheat commercial varieties reactions to stem rust race Ug99 in the field

Variety	Highest disease severity (%)	Highest Infection type
Ngamia	50	MSS
Mbega	40	S
Chozi	50	MSS
Kwale	30	MSS
Pasa	60	S
Yombi	40	MSS
Duma	50	S
Kulungu	15	MS
Mbuni	25	MSS
Njoro BWII	30	MSS
K.Kongoni	20	MSS
*Cacuke	100	S
**PBW343	60	MSS
K.Chiriku	10	M
K.Tembo	20	M
Eagle10	30	MR
K.Fahari	15	MR
Robin	30	MR
K.Ibis	10	MR
Nyangumi	10	RMR
K.Swara	5	RMR
Kingbird	15	RMR
***Danphe	15	MR

*Ug99 stem rust susceptible check variety
**Ug99 stem rust moderately susceptible check variety
***Ug99 stem rust resistant check variety

Analysis of Variance (ANOVA) computation showed that there was significant variation in disease scores among the varieties ($P<0.0001$) but no significant variation from one replicate to another ($P>0.1$) as shown in Table 2.

Table 2. ANOVA table for disease scores of the twenty Kenyan bread wheat varieties

Source	df	Sum of Squares	Mean Square	F Value	Pr>F
Rep	2	3.33333	1.66667	0.07	0.9365
Variety	19	10576.66667	556.66667	21.96	<.0001

Figure 1. Photo of a resistant Kenyan variety, K.Swara (A) and a susceptible variety Pasa (B)

Figure 2. Photo of the resistant check variety, Danphe (C) and the susceptible check, Cacuke (D)

3.2. AUDPC

Table 3. Mean AUDPC Values of the twenty Kenyan bread wheat commercial varieties

Genotype	N	Mean	t Grouping
Pasa	3	478.67	A
			A
Duma	3	461.00	B A
			B A
Chozi	3	455.00	B A
			B A
Yombi	3	391.00	B A C
			B C
Ngamia	3	385.33	B C
			C
Mbega	3	344.33	D C
			D C
Robin	3	344.33	D C
			D C
Eagle10	3	326.67	D C
			D
Kwale	3	262.67	E D
			E
Mbuni	3	227.67	E F
			E F
Njoro BWII	3	192.67	E F G
			F G
K.Tembo	3	158.00	H F G
			H F G
K.Kongoni	3	153.00	H F G
			H F G
K.Fahari	3	143.67	H F G
			H G
Kulungu	3	135.67	H G
			H G
K.Chiriku	3	113.67	H G
			H G
K.Kingbird	3	112.33	H G
			H G
K.Ibis	3	111.33	H G
			H
K.Nyangumi	3	95.00	H
			H
K.Swara	3	78.33	H

The AUDPC computed showed that there was variation in the mean AUDPC values among the varieties, with the variety K.Swara having the lowest mean AUDPC value of 78.33 and Pasa the highest value at 478.67 (Table 3). However, the values for Duma and Pasa, which were susceptible, were lower than that of the susceptible check Cacuke which was 1085. AUDPC values of K.Chiriku, K.Tembo, K.Fahari, K.Ibis, K.Nyangumi, K.Swara and K.Kingbird were lower than that of the resistant check Danphe which was 175 (Table 3). One interesting observation is that most of the AUDPC values seemed not to depend on whether a variety was susceptible or resistant, for example the moderately susceptible variety Kulungu had its highest value as 175, same as the resistant check Danphe. AUDPC values can be used to measure the phenomenon of slow rusting which is majorly attributed to the slow rusting or APR gene *Sr2*, which seems not to follow the gene for gene hypothesis. From the results above all the varieties which showed expression of PBC, which is linked with the *Sr2* gene, had mean AUDPC values below 150. These varieties were K.Nyangumi, K.Kingbird, K.Swara and K.Fahari. The variety Kwale which showed low expression of PBC was an exception since it had a mean AUDPC value of 262.7, which was a bit higher than the other varieties which had higher PBC expression levels.

This observation agrees with previous findings that the PBC expression has a true linkage to the *Sr2* gene and that the gene confers slow rusting effect as a form of broad spectrum resistance to stem rust disease [3,4,7].

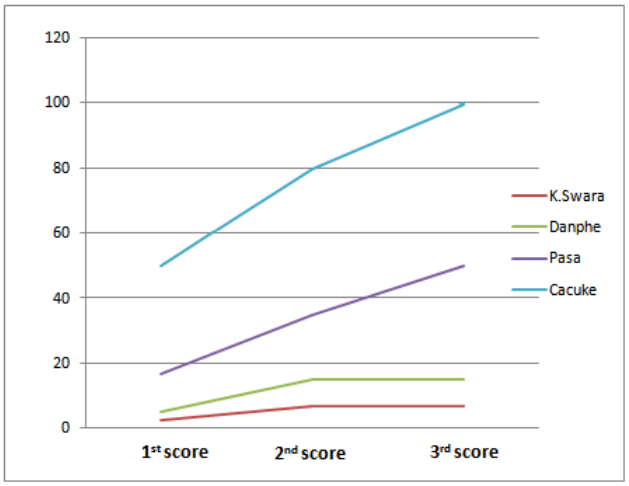

Figure 3. A graph of disease scoring intervals against mean disease severities for selected varieties

The above graph is an illustration of the disease progress of the most resistant and the most resistant Kenyan bread wheat varieties, K.Swara and Pasa respectively. The two are compared with the stem rust susceptible wheat check variety Cacuke and the stem rust resistant check variety Danphe. From the graph it is clear that Pasa is susceptible judging by the exponential increase in the mean disease severities from the first score up to the third score. This shows that the variety offers very low resistance to the stem rust fungi probably due to compatible interaction between dominant avirulence gene and virulence gene products as hypothesized in the gene for gene hypothesis.

However Pasa a less exponential increase in the mean disease severity than the susceptible check variety Cacuke indicating that it had some levels of resistance compared

to Cacuke which reached 100% disease severity, indicating an almost zero resistance level. From the graph the variety K.Swara showed slow progress in mean disease severity from the first score up to the third score. This may be due to the slow rusting effect conferred by *Sr2* gene as discussed earlier. K.Swara shows better resistance than the resistant check Danphe.

Table 4. ANOVA table for AUDPC values of the twenty Kenyan bread wheat varieties

Source	df	Sum of Squares	Mean Square	F Value	Pr>F
Rep	2	616.033	308.017	0.11	0.8972
Variety	19	1071365.650	56387.666	19.92	<.0001

Analysis of Variance (ANOVA) computation showed that there was significant (P<0.0001) difference in AUDPC values among the varieties but no significant difference from one replicate to another (Table 4).

3.3. Stem Rust Fungi Race Analysis

In the analysis of stem rust fungi using the Ug99 qPCR assay two genotypes of the Ug99 variant TTKSK were detected. The two genotypes were AF-001ad and AF-001aa and varied at SNP marker positions (A023) and (A030) with genotype AF-001ad having Adenine and Cytosine (AC) bases at both positions and genotype AF-00aa having two Adenine (AA) bases at both positions (Table 5). This shows that even within the race TTKSK there are different strains produced through mutation and which might probably mutate further in the future to form new Ug99 race variants.

Table 5. Table showing the Ug99 race group SNP analysis results

	RACE GROUP MARKERS								GENOTYPE MARKERS						
AF-ooaa	A003	A005	A007	A010	A012	A021	A022	A031	A013	A014	A017	A020	A023	A026	A030
13KEN08	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CT	AC	GG	AC
13KEN09	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CT	AC	GG	AC
13KEN010	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CT	AC	GG	AC
13KEN011	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CT	AC	GG	AC
13KEN013	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CT	AC	GG	AC
13KEN014	CC	GG	GG	TT	TT	rr	CT	GG	CC	AA	CC	CT	AC	GG	AC
13KEN015	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CT	AC	GG	AC
13KEN017	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CT	AC	GG	AC
13KEN018	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CT	AC	GG	AC
13KEN19	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CT	AC	GG	AC
13KEN12B	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CT	AC	GG	AC
AF-ooiad															
13KEN12A	CC	GG	GG	rr	TT	TT	CT	GG	CC	AA	CC	CC	AA	GG	AA
13KEN16	CC	GG	GG	TT	TT	TT	CT	GG	CC	AA	CC	CC	AA	GG	AA

4. Conclusion

From this study the Kenyan commercial wheat varieties can be categorized as those having resistance to stem rust race Ug99 and those that are susceptible to this race, with a majority of the old varieties being susceptible or moderately susceptible except K.Swara, K.Ibis, K.Fahari and K.Nyangumi. This is in agreement with the findings of Njau *et al*., 2009 [9]. All the recent varieties Robin, Eagle10 and K.Kingbird have varying levels of resistance to Ug99 indicating that we have resistant varieties that can withstand the threat posed by the pathogen. There is also an observation, from the AUDPC values, that the presence of *Sr2* gene indicated by expression of PBC on some wheat varieties may be conferring a slow rusting effect described by Sunderwirth and Roelfs [16]. This might be the mechanism of broad spectrum resistance to stem rust, including that caused by the Ug99 race. From the results of race analysis it's evident that there are different genotypes even within the original Ug99 variant TTKSK. This is a clear indicator that the stem rust pathogen is in a constant mutation state in order to be able to infect new wheat genotypes that are an output of the continuous breeding programs. In summary there are Ug99 resistant Kenyan bread wheat varieties which hold a promise for food security. Despite this, we must keep in mind that the stem rust pathogen is also evolving as evidenced by occurrence of different genotypes of the stem rust race TTKSK, so we must keep on breeding for durable stem rust resistance in wheat.

A recommendation would be for farmers to cultivate the stem rust resistant wheat varieties, which can also be used in breeding programs to improve the susceptible ones.

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Statement of Competing Interests

The authors have no competing interests.

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