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Evaluation of Stem Rust (*Puccinia graminis* f.sp *tritici*) Seedling Resistance in Kenyan Bread Wheat (*Triticum aestivum* L.) Mutant Lines

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Abstract Race TTKSK (*Ug99*) of stem rust is a serious threat to wheat production worldwide because of its wide virulence on many cultivars and its rapid spread over countries. The objective of this study was to determine resistance to *Puccinia graminis* f.sp. *tritici* races of TTKTK and TTKSK at seedling stage among the wheat mutant lines. Duma, Kwale and NJBWII mutant lines were used since the parents are susceptible. Sixty three mutant lines along with six checks of NJBWII, Kwale, Duma, Cacuke, Robin and Kingbird were evaluated in the greenhouse at Kenya Agriculture and Livestock Organization, Njoro. A high frequency of mutant lines, 53.6% and 88.4% were resistant to TTKTK and TTKSK respectively, with low infection types ranging from ";" to "2+". In addition, frequency of susceptibility was 46.38% for TTKTK and 11.59% for TTKSK on the evaluated genotypes. Resistance in these genotypes may be due to uncharacterized resistance genes or gene combinations that could not be resolved with the collection of races used. The information presented, when combined with the previous characterization of stem rust resistance genes will be useful for plant breeders in rationalizing germplasm enhancement programs.

Keywords: *stem rust, seedling stage resistance, mutation*

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1. Introduction

Wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* historically has been one of the most important diseases in the world. In 1999, a new race known as Ug99 or TTKSK, was able to cause disease on previously resistant wheat cultivars carrying stem rust resistance gene *Sr31* in Uganda. In 2006 and 2007, TTKSK and two of its variants including TTKST and TTTSK emerged in Kenya and infected wheat varieties carrying *Sr31*, *Sr24* and *Sr36* respectively [1]. Until recently, these genes were used in wheat breeding programs to confer major resistance for control of stem rust. Due to possibility of movement and spreading of new races of stem rust in wheat production regions worldwide, there is need for identification and transfer of novel resistance genes to high yield potential wheat cultivars.

Mutation breeding is one of the tools being used to study the nature and function of genes which are building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops [2]. Mutation induction offers significant increase in crop production [3] and possibility of inducing desired traits that either cannot be found in nature or have been lost during evolution.

Seedling resistance which is controlled by race-specific major genes, protects a plant against virulent pathogen isolate for its entire growing period. However, when a race-specific major gene used extensively over a long period of time, new races of the pathogen usually overcomes it leading to susceptibility of the released germplasm [4]. Race specific resistance is usually expressed by a hypersensitive response, controlled by major genes which are often led to a boom and bust cycle [5]. Through different studies, fifty five stem rust race-specific resistance genes based on seedling resistance test have been identified [6,7]. However, the seedling resistance genes are often broken down due to new and various races of the rusts pathogen that are evolving and mutating in the wheat field areas [8]. The aim of the study was to determine resistance to *Puccinia graminis* f.sp. *tritici* races of TTTSK and TTKTK at seedling stage among the wheat mutant lines.

2. Materials and Method

2.1. Wheat Genotypes

Sixty three mutant lines used in this experiment were developed from three selected Kenyan wheat cultivars including NjoroBWII/NJBWII, Kwale and Duma. The

three parental cultivars had been previously screened for stem rust resistance in the International Screening Nursery at Kenya Agricultural and Livestock Research Organization (KALRO), Njoro, Kenya. The three parental wheat cultivar seeds were sent to International Atomic Energy Agency in Vienna, Austria and subjected to gamma irradiation at three levels of 100, 200 and 400 Gry to get the sixty three mutant lines. Three parental cultivars of NjoroBWII/NJBWII, Kwale, and Duma, the resistant wheat cultivar Kingbird [9], and the two susceptible cultivars Robin and Cacuke were also included in the experiments.

2.2. Pathogen Isolates

Two *Puccinia graminis* f.sp *tritici* races of TTKTK and TTKSK were used to test the 69 wheat genotypes in the greenhouse. The races were derived from stem rust samples collected at trap nursery of stem rust resistance screening nursery (SRRSN), KALRO Njoro, Kenya.

2.3. Seedling Resistance Test

Ten seeds of each genotype were planted in 5 cm wide square pots filled with a vermiculite potting mix and placed in a plastic tray with each pot in a fixed position. Seedlings were applied upon emergence with CAN fertilizer. Nine days old seedlings were inoculated with the single pustuled urediniospores of TTKTK and TTKSK separately. The spores were suspended in Tween 20 liquid oil at a concentration of 6×10^6 spores/ml of oil. The inoculated seedlings were kept in a dew chamber for 16-20hr darkness at 18-22°C and relative humidity of 95% before transferring to a growth and sporulation chamber in the greenhouse adjusted at 18-24°C. The procedure above was also applied to the other experimental set using TTKSK urediniospores separately. Disease infection type (IT) was observed 15 days after inoculation following the procedure [10], where "0" is no disease and the genotype is resistant while "4" shows the highly susceptible genotype. The infection types "0", ";", "1", "1+", "2", or combinations indicated low infection types (resistance), while, the infection types "3-", "3", "3+" and "4" indicated susceptibility. The experiment was repeated thrice per the races tested and only the genotypes that produced similar infection types in the three experiments were considered for the data analysis.

3. Results

Of the total 63 genotypes assessed, 37 were resistant to TTKTK race while 61 were resistant to TTKSK races. In general the infection type coding resistance was ranging from (; - flecks) to (2+ (resistant) whereby the maximum infection type mostly displayed was 2+ on the primary leaves of the seedling. Thirty two genotypes were susceptible, 28 had a maximum infection type of 3 and the remaining four mostly the checks genotypes were noted with score of 4 for TTKTK race. With respect to TTKSK eight genotypes were susceptible, six genotypes had infection type of 3 and two had infection type of 4 (Table 1). However, some genotypes were noted having two combinations of disease scores, NJBWII 200gry (641) had a combination infection type (1, 2) to TTKTK race. Kwale 200gry(1777) and Kwale 200gry(1875) had infection type (1+, 2) while NJBWII 100gry(288), Duma 200gry(1099) and Duma 200gry(1124) had infection type (1+ 2) to TTKSK race (Table 1). A high frequency (53.6% and 88.4%) of mutant lines were resistance to the TTKTK and TTKSK races respectively at seedling stage with low infection types ranging from (flecks) to (2+). Additionally, frequency for susceptibility was (46.38%) for TTKTK and (11.59%) for TTKSK on the evaluated genotypes (Figure 1).

Table 1. Infection types of Bread wheat (Triticum aestivum) mutant lines exhibiting resistant and susceptible reaction to *Puccinia graminis* f. sp. *tritici* races, TTKTK and TTKSK at the seedling stage

	Puccinia graminis f.sp tritici races	
Genotypes	**TTKTK**	**TTKSK**
NJBWII Parent and the mutant lines		
NJBWII PARENT	3	2+
NJBWII 100 GRY(50)	3	1+
NJBWII 100GRY(57)	3	2
NJBWII 100 GRY(140)	3	2+
NJBWII 100 GRY(288)	3	1+ 2
NJBWII 100 GRY(382)	;	1+
NJBWII 100GRY(404)	2	1+
NJBWII 100GRY(415)	3	; , 1
NJBWII 200GRY(602)	2+	1+
NJBWII 200GRY(608)	2+	2
NJBWII 200 GRY(612)	3	1+
NJBWII 200 GRY(634)	2+	1+
NJBWII 200 GRY(641)	1,2	1+
NJBWII 200 GRY(660)	2	2+
NJBWII 200 GRY(662)	2+	1
NJBWII 400 GRY(675)	3	2+
NJBWII 400 GRY(776)	2	1+
NJBWII 400 GRY(798)	3	3
NJBWII 400 GRY(849)	2+	2
NJBWII 400 GRY(908)	2+	1+
NJBWII 400 GRY(915)	3	1
NJBWII 400 GRY(930)	3	1

	Puccinia graminis f.sp tritici races	
Genotypes	**TTKTK**	**TTKSK**
Duma parent and the mutant lines		
DUMA PARENT	4	4
DUMA 100 GRY(987)	;	1+
DUMA 100 GRY(992)	1+	1
DUMA 100 GRY(993)	3	2+
DUMA 100 GRY(995)	1	1+
DUMA 100 GRY(996)	2	3
DUMA 100 GRY(997)	3	2+
DUMA 100 GRY(1010)	3	2+
DUMA 200 GRY(1026)	3	2+
DUMA 200 GRY(1030)	2+	1+
DUMA 200 GRY(1033)	2	1+
DUMA 200 GRY(1099)	3	1+ 2
DUMA 200 GRY(1103)	1+	1+
DUMA 200 GRY(1124)	;	1+ 2
DUMA 200 GRY(1145)	2	1+
DUMA 400 GRY(1299)	3	2+
DUMA 400 GRY(1295)	2+	3
DUMA 400 GRY(1304)	2+	2
DUMA 400 GRY(1437)	4	3
DUMA 400 GRY(1349)	3	3
DUMA 400 GRY(1368)	2	1+
DUMA 400 GRY(1403)	2+	1+
Kwale parent and the mutant lines		
KWALE PARENT	3	2+
KWALE 100 GRY(1468)	1	1+
KWALE 100 GRY(1470)	3	1
KWALE 100 GRY(1483)	3	1
KWALE 100 GRY(1492)	3	2
KWALE 100 GRY(1499)	2+	1
KWALE 100 GRY(1502)	; , 1	1+
KWALE 100 GRY(1556)	3	2
KWALE 200 GRY(1621)	3	1
KWALE 200 GRY(1715)	3	1
KWALE 200 GRY(1731)	2	1
KWALE 200 GRY(1750)	; , 1	1
KWALE 200 GRY(1768)	3	2+
KWALE 200 GRY(1777)	3	1 2
KWALE 200 GRY(1818)	3	2
KWALE 400 GRY(1875)	2+	1 2
KWALE 400 GRY(1877)	2	1+
KWALE 400 GRY(1895)	1+	2+
KWALE 400 GRY(1907)	1+	1
KWALE 400 GRY(1949)	2+	1
KWALE 400 GRY(1961)	2+	1+
KWALE 400 GRY(1964)	2+	1
Resistant and susceptible* checks**		
KINGBIRD**	;	; , 1
CACUKE*	4	4
ROBIN*	4	3

Infection type (IT) was based on the scale described by Stakman *et al*. [10] with ITs; 1, 2 considered resistant and 3, 4 considered susceptible. Positive (+) = larger uredinia than the normal size.

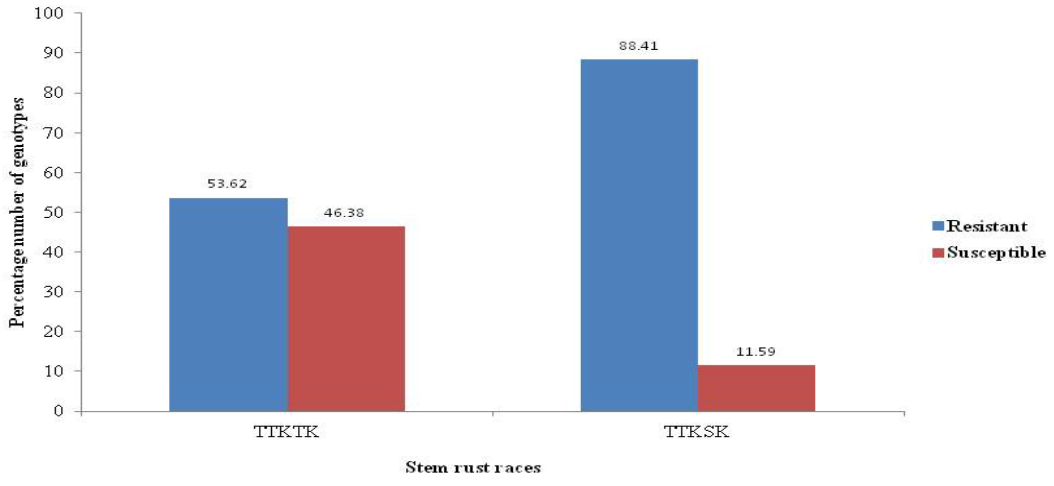

Figure 1. Percentage of bread wheat (*Triticum aestivum*) mutant lines exhibiting resistant and susceptible reaction to *Puccinia graminis* f. sp. *tritici* races TTKTK and TTKSK.

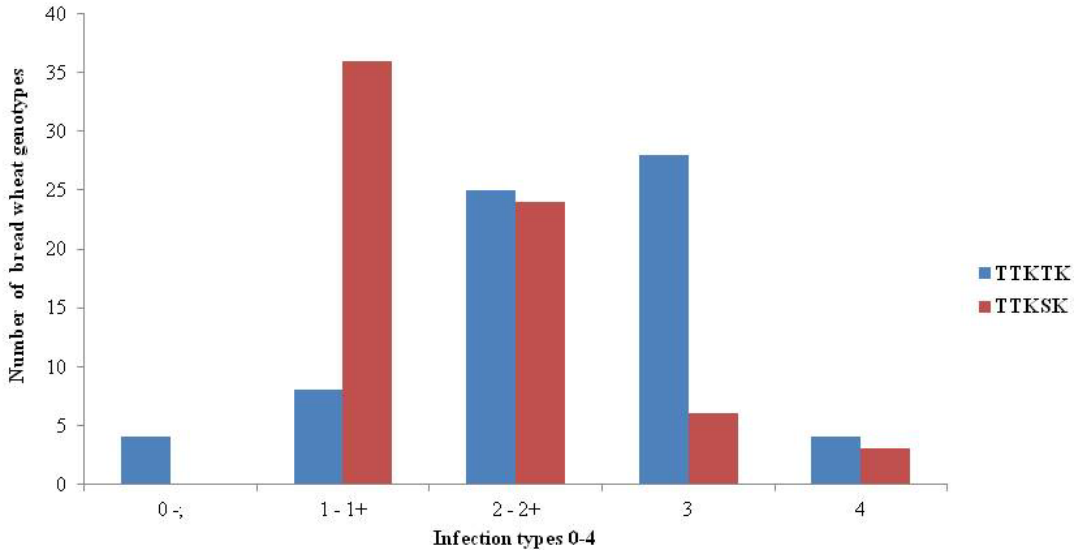

Figure 2. Frequency distribution of infection types (ITs) of 69 wheat (*Triticum aestivum*) genotypes evaluated at the seedling stage with two stem rust races.

The infection types frequency distribution presented in (Figure 2) depicts a continuous variation for the two races. The frequencies of the genotypes categorized as resistant and susceptible in their reaction to the two races varied markedly depending on the race (Figure 2). The results presented in (Figure 2) shows that 36 genotypes had resistance "1" to "1+" when inoculated with TTKSK while 8 genotypes were resistant to TTKTK. Out of the total genotypes, twenty eight and six genotypes were susceptible to TTKTK and TTKSK races respectively. This shows that TTKTK was most virulent to the genotypes tested in the study (Figure 2).

4. Discussion

Two virulent races of TTKTK and TTKSK were inoculated on the test genotypes at seedling stage in the greenhouse in which no complete resistance "0" was observed. Moreover, most of the mutant lines in the current study evaluated against the two races of stem rust were noted having infection types from ";" to "2+". A great protection against stem rust is conferred by specific genes which are usually expressed at seedling stage. In contrast, non-specific genes, they are usually assumed to be quantitative.

According to this study, the mutant lines evaluated showed a broader resistance spectrum compared to their parents of Duma, Kwale and NJBWII. This implies that after irradiation, some genes in the chromosomes of the mutant lines may have been successfully altered during the process. Duma, Kwale and NJBWII were among the old cultivars bred in the late 90s and were part of the first wheat cultivars noted to be susceptible to the new *Ug99* stem rust race [11]. Therefore, these wheat cultivars carry stem rust resistance genes which are ineffective against *Ug99* e.g. *Sr31* gene was broken down by the new *Ug99* stem rust race in the late 90s.

Olivera *et al*., [12] found sources of resistance to TTKSK from emmer wheat and the infection types ranged from "2" to "2+". Similarly, the current test genotypes showed results of infection types "2" to "2+" and 34.78% of the genotypes exhibited that range of infection type to TTKSK. Nevertheless, the present study result was also in agreement with Worku, [13] who did a study on resistance to TTKSK and TTTSK races in Ethiopian tetraploid wheat accessions and found most of the accessions resistant to TTKSK.

With respect to TTKTK, Robin carries *Sr Tmp* gene and *Sr 24* [14] which recently became highly ineffective to this race. Current study proven *Sr Tmp* gene from Robin is ineffective to the TTKTK race since this cultivar was highly susceptible when tested in the greenhouse. With the evaluated genotypes 46.38% were susceptible to the race while 53.62% were resistant. For the susceptible genotypes, the race was virulent to the genes in the test genotypes. This showed that the genes were ineffective.

The high percentage of resistance showed that most lines have major genes. The low infection types scored on these genotypes could be either due to one or more of the *Sr*-genes or a combination that had similar infection type patterns. On the other hand, the significant proportion of the tested genotypes showing resistance or susceptibility may have been altered during irradiation thus carrying unknown resistance or susceptible genes. As a result, this requires additional molecular work and races analysis with a wider virulence spectrum than the present races to determine the genes that are responsible for the low or high infection types displayed by the genotypes against some of the races.

5. Conclusion

The results of this study demonstrated that the lines that showed resistance could serve as a source of resistance to race TTKTK and TTKSK. This could be attained through a broad spectrum resistance at adult plant stage for the release of novel wheat genotypes because stem rust susceptibility is not high until heading. However, stem rust resistance at the seedling stage may not be indicative of the reaction at the adult plant stage because some genes are effective only at specific growth stages. Combining the results of this study with those in adult plant resistance

in the field where races such as TTKSK and TTKTK are prevalent will provide valuable indications to select suitable parental lines for further improving stem rust resistance of wheat. Nevertheless, the lines could be exposed to extra races analysis to ascertain their level of resistance to the evolving races. Further genetic analysis is required to also confirm presence of effective genes in the resistant lines after gamma-rays radiation since alteration may have occurred to the chromosomes. This could bring a background on which genes can be incorporated to the susceptible varieties that had been broken down by the present or existing races.

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Competing Interest

There is no competing interest among the Authors.

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