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Comparative Yield Analysis of Stem Rust Resistant Wheat Mutant Lines and Their Parent Varieties

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Authors' contributions

This work was carried out in collaboration between all three authors. Author PKC contributed substantially to the conception and design of the study, performed the statistical analysis and interpretation of data and wrote the first draft of the manuscript. Author MGK provided critical revision of the article. Author OKK managed the analyses of the study and literature review. All authors read and approved the final manuscript.

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ABSTRACT

Wheat (*Trticum aestivum* L.) farmers in Kenya are faced with a major threat on their wheat farming from stem rust (*Puccinia graminis* f. sp *tritici*). Smallholder's farmers are earning low income from their wheat farming but there's high demand for wheat products. The interaction between wheat and stem rust in a favourable environment causes complete crop failure. This study aimed to assess yield potential of stem rust resistant mutant's in comparison with their parent varieties. The objective was to select sample of high yielding stem rust resistant mutant lines to be introgressed into adaptable Kenyan wheat background. Seventeen wheat genotypes comprising eight mutant lines, two parents and seven commercial checks were evaluated. The experiments were conducted in the years 2012 and 2013 at University of Eldoret, KALRO Njoro and Kitale. Randomized Complete Block Design with three replications was used to conduct the experiement. Yield data and stem rust response were collected and analysed. Response to stem rust was recorded based on modified Cobb's scale while a severity was recorded on a scale of 0-

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100%. Grain yield ranged from 0.69-4.35 t ha^{-1} and disease response range from moderately resistant to susceptible. Genotype, location and genotype x location interaction for yield and disease response were significant at P $\leq$ 0.05. Results showed two mutant lines SP-21 and SP-26 were high yielding and moderately resistant to stem rust. Considering high yields and stem rust resistance they were superior to their parents and can undergo further tests for future release.

Keywords: Mutant lines; stem rust; resistance; wheat yields.

ACRONYMS

ACI : Average Coefficient of Infection
KALRO : Kenya Agricultural & Livestock Organozation
ANOVA : Analysis of variance
AUDPC : Area under Disease Progressive Curve
FDS : Final Disease Severity

1. INTRODUCTION

Wheat (*Triticum aestivum.* L) is an important cereal crop worldwide produced for domestic and commercial baking [1]. Wheat contributes to food security in Kenya and his ranked second important cereal crop after maize. But wheat production in Kenya is low compared to increasing demand. The small scale producers complain of prohibitive production expenses and low production output caused by use of non-certified seeds and low use of inputs**.** Wheat production in Kenya is constrained by unstable weather conditions, continued use of recycled seed, prevalence of stem rust race (Ug99) and sub-division of family-owned farms into smaller units Higher yield in wheat is a factor of disease resistant varieties, use of correct varieties, good crop husbandry and stable weather conditions[2]. Genetic variability as a result of induced mutation by various mutagens has contributed to modern plant breeding in the development of superior plant varieties with increased yield, early maturity, disease resistance, lodging resistance, improved quality among others. Area under wheat cultivation in Kenya is estimated at 170,000 ha with an annual production estimate of 400,000 tons while consumption is estimated at 1,700,000 tons per year. Kenya's local wheat production deficit is increasing over the years, with local production accounting for less than 20% of total wheat consumption necessitating over 80% imports (about 1,300,000 tons per year) while Kenya has good soils and vast land suitable for growing wheat [3].

Stem rust is one of the important diseases of wheat worldwide and a major concern to wheat farmers in Kenya. Yield losses associated with stem rust is estimated at 70%, but up to 100% yield loss has been recorded in Kenya [4]. Stem rust had been contained worldwide through utilisation of resistant genes but it was not until 1999 when a new race (Ug99) occurred in Uganda and overcame most resistant genes. The new race has enhanced the status of Kenya to a net wheat exporter [1]. Development of resistant varieties will reduce the cost of wheat production, increase farmer's income and enhance Kenya's food security. Farmers will access resistant varieties at no extra cost and will not need to use expensive fungicides to protect their crops [4]. Smallholder's farmers are the most affected because they recycled their seed due to high cost of certified wheat seed. Wheat breeders in Kenya have continued to develop resistant varieties, but virulence has been reported in most of these new varieties [5] Development of resistant varieties will be of less value if they are also not high yielding. An interaction between high yields and stem rust resistance is a prerequisite to staple genotypes. Yields are greatly influenced by genotype, environment and genotype x environment interactions [6] Efforts to tackle stem rust in this study were initiated through joint a collaborations between University of Eldoret, Kenya and Seibersdorf laboratories, Vienna Austria. The overall objective was to generate stem rust resistant mutant lines using mutation induction. Wheat seeds of Kenyan well adapted but susceptible varieties were used. Kinyua et al. (2008) used mutation induction to develop drought resistant wheat mutant varieties. The objective of this study was to evaluate yield potential of elite stem rust resistant mutant lines in comparison with their parents and commercial checks across stem rust 'hotspots' in Kenya [3].

2. MATERIALS AND METHODS

2.1 Experimental Sites

The experiment was carried out in three sites; Eldoret, Kitale and Njoro in Kenya. The first site was at University of Eldoret, which lies on 0°34'N; 35° 18 'E, at 2,153 m above sea level. The average temperature is 18°C and a mean annual rainfall of 1,100 mm. The second site was at KALRO-Kitale which lies on 0°33'S; 35° 55'E, at 2,900 m above sea level with a mean annual temperatures of 15°C and average rainfall of 1,800 mm. The third site was KALRO-Njoro, which lies on 0°20'S; 35° 56'E, at 2,185 m above sea level with a mean average temperatures of 20°C and the average annual rainfall of 900 mm. The three areas represent key wheat growing regions in Kenya and were selected on the basis of their significance in natural population of stem rust. The experiments were conducted in the year 2012 and 2013.

2.2 Plant Materials

Seventeen wheat genotypes were used comprising of eight pre-selected mutant lines from University of Eldoret; SP-9, SP-16, SP-20, SP-21, SP-26, SP-29, SP-31 and SP-34. Two parent varieties; Njoro II (SP-N) and Kwale (SP-K) and seven commercial checks; Duma (SP-D), Pasa (SP-P), Simba (SP-S), Farasi (SP-F), Robin (SP-R), KS Mwamba (SP-M) and Chozi (SP-C) sourced from KALRO Njoro Seed Unit. The two parents and commercial check varieties used are popular commercial wheat varieties grown in Kenya.

2.3 Field Experimental Procedures

The seventeen genotypes were planted a Complete Randomized Block Design with three replications per location. Experimental plots were 2 m by 6 rows in length with 20 cm inter-row by 5 cm intra-row spacing. A susceptible wheat cultivar was planted along border lines of the plots to facilitate inoculums build up. Seeds were hand planted head to row with Di-ammonium Phosphate at a rate of 125 Kg/ha, followed by an application of Urea at 75 kg/ha at tillering and booting stages. Irrigation was done depending on soil moisture. Wheat agronomic practices except fungicides use were carried out [7].

2.4 Data Collection and Analysis

Grain yield and host response to stem rust data were separately recorded for each entry in all the three sites. For grain yield, a paddock measuring one meter square for each entry was selected as a representative area, harvested, threshed, winnowed and dried to 13.5% moisture content, weighed and converted into to t ha^{-1} for statistical analysis. Disease response assessment was done from dough stage (Zadok's growth stage 65, 75 to 85) to grain development. Plant disease responses to stem rust were recorded based on modified Cobb's scale. Disease response to infections combines several infection type scales; immune (I), resistant (R), moderately resistant (MR), moderately resistant to moderately susceptible (M), moderately susceptible (MS) and susceptible (S). Stem rust severity was recorded using modified Cobb's scale in a range 0–100% scale where, 0% is immune while 100% is susceptible. Combined analysis of yield and coefficient of infection was performed using linear mixed model following restricted maximum likelihood procedure (REML). REML was used as a method for fitting linear mixed models because it produces unbiased estimates for variance components of a linear model. Host response and disease severity data were converted to average coefficient of infection (ACI) by multiplying disease severity with an arbitrary constant value for plant response (Roelfs et al., 1992). Where I=0.1, R=0.2, MR=0.4, M=0.6, MS=0.8 and S=1. Genotypes, location, replicate and Genotype x location were considered as fixed effects while incomplete blocks nested in replicates (Replicate x Block) were considered as random for coefficient of infection while blocks were fixed for yields.

The following statistical model was used; $Y_{ijkl} = \mu + G_i + L_i + R_i + B_{)k} + GL_{il} + \varepsilon_{ijk}$ Where: Y_{ijkl} = observations; μ = mean of the experiment; G_i = effect of the i^{th} genotype; L_i = effect of the l^{th} location; R_i = effect of the j^{th} replicate; $B_{)k}$ = effect of k^{th} block nested in the j^{th} replicate; GL_{ij} = effect of the interaction of the i^{th} genotype with l^{th} location and ε_{ijk} = the experimental error. For analysis purposes, the relationship between yield and disease response was done using simple linear regression using Genstat (Genstat 15th Edition, 2012). Genotypic means were separated based on Fishers protected least significance difference (LSD) at $P \leq 0.05$. The least square difference was calculated using the formula: LSD = average standard error of difference (REML output) x t/device degree of freedom (t-table). Correlation coefficient test was done to determine the relationship between yields and different disease parameters. The mean disease severity was used to calculate the area under disease progressive curve (AUDPC) using the formulae; $AUDPC = \sum^{n-i} 0.5 (Xi + 1 + Xi) (ti + 1 - ti)$. Where, Xi - is the cumulative disease severity expressed as a proportion at the ith observation; ti - is the time (days after planting) at the ith observation and *n* - is total number of observations.

3. RESULTS

3.1 Yield Performance

Mutants showed wide variation in their yields measured when compared to their parents and commercial checks. Some mutant lines showed superiority while others were inferior when compared to their parents and commercial checks. Two mutant lines (SP-26 and SP-21) gave higher yields (4.34 and 4.04 t ha^{-1} respectively) than their parents SP-K and SP-N (2.92 and 1.91 t ha^{-1} respectively) and commercial checks. For genotype x location interaction, Eldoret and Kitale recorded similar grain yields (1.99 and 2.02 t ha^{-1} respectively) while Njoro was the lowest in terms of mean grain yields per location (1.70 t ha^{-1}) (Table 3).

3.2 Genotypic Response to Stem Rust

Analysis of variance showed significant difference in disease severity scores among genotypes ($P < 0.001$) but no significant difference between one replication to another ($P > 0.1$) (Table 1). Seasonal variations were significant at $P \leq 0.05$ for AUDPC (Table 2). Significant $P \leq 0.05$ variation for coefficient of infection was observed in genotype, location and genotype x location interactions. Genotypes with ACI values of 10 and below were considered stable and resistant against stem rust. Mutant lines SP-21 and SP-26 were moderately resistant and stable genotypes against stem rust (Table 3). At 95% confidence level, the mean for Kitale (ACI=30.8) was found not to be different from that of Eldoret (ACI=31.3) but was significantly different from the mean of Njoro (ACI=34.2) (Table 3) Summary scores of ACI and FDS for 17 genotypes are presented. Genotypes with FDS values of 20% and below were considered stable against stem rust. But, FDS values in some cases did not reflect on the infection type. For example, SP-F had an FDS score of 44.6% with an IT of susceptible while SP-M had an FDS scores of 50.9% with an IT of moderately susceptible. Severity score of SP-M is an indicator of some resistance but an illustration of breakdown of genes responsible for resistance while in SP-F indicates resistance is completely broken down.

A simple regression analysis revealed a significant linear and inverse relationship ($P \leq 0.01$) between grain yield and average coefficient of infection (ACI) where; (Y = -0.00825x +4.7315, s.e = 0.13, R^2 = 2.8). Among the 17 genotypes, the AUDPC ranged from 112-523. Moderately resistant genotypes had a high level of disease control with an AUDPC value of less than 150 while susceptible genotypes had AUDPC of above 400 (Table 3). But it was observed the AUDPC values seemed not to depend on whether a genotype was resistant or susceptible. SP-M had a higher AUDPC value (492.00) than SP-20 (433.00) though SP-20 was susceptible and SP-M was moderately susceptible. The Pearson Correlation coefficient considered between grain yields and respective disease parameters were significant ($P \leq 0.05$) (Table 4) and this indicated that as the stem rust severities increases it had negative effects on grain yields.

4. DISCUSSIONS

The re-emergence of stem rust race (Ug99) brought up new challenges to breeding for stem rust resistance. None of the 17 wheat genotypes screened were completely resistant to stem rust however, some genotypes were found to have some good level of resistance. There is still lack of genotypes that combine both high yields and stem rust resistance across different environments justifying the need for more studies [7]. Mutation technique is one of

Table 1. ANOVA Table highlighting disease severity scores of the 17 wheat genotypes

Source.	d.f	Sum of squares.	Mean square	F Value	Pr> F
Rep.	2	3.3345	1.6667	0.069	0.9254
Genotypes	16	9148.8549	571.803	18.76	<0.001
Error	32	1.764	1.470		
Totals	50	9153.2855			

Analysis of variance (ANOVA) showed that there was significant variations in AUDPC values among genotypes (P < 0.001) but no significant difference was observed from one replication to another (P > 0.1) as shown in table 3.0.

Table 2. ANOVA for AUDPC values of the seventeen wheat genotypes

Source.	d.f	Sum of squares.	Mean square	F Value	Pr> F
Rep.	2	604.02	302.01	0.10	0.7291
Genotype	16	937082.672	58567.667	16.36	<0.001
Error	32	2.537	2.132		
Totals	50	937689.229			

Table 3. Results of combined mean of Yields, ACI, FDS, AUDPC and IT of 17 wheat genotypes evaluated in UOE, KARLO Njoro and Kitale in Kenya during 2012 – 2013 seasons

Exp name	Yields (t ha^{-1})	Average coefficient of infection (ACI)	Final disease severity scores (FDS)	Area under disease progressive curve (AUDPC)	Final disease infection type (IT)
SP- D	1.22	45.7	45.7	437.00	Susceptible (S)
SP- P	0.69	50.5	50.5	523.67	Susceptible (S)
SP- S	2.27	29.7	37.2	391.83	Moderately Susceptible (MS)
SP- F	1.36	44.6	44.6	418.00	Susceptible (S)
SP- R	1.97	24.3	30.3	288.00	Moderately Susceptible (MS)
SP- N	2.92	21.1	26.4	247.50	Moderately Susceptible (MS)
SP- M	1.38	40.7	50.9	492.00	Moderately Susceptible (MS)
SP- C	0.70	50.2	50.2	513.00	Susceptible (S)
SP- K	1.91	34.2	42.8	421.67	Moderately Susceptible (MS)
SP- 9	1.95	33.1	41.2	393.33	Moderately Susceptible (MS)
SP- 16	2.32	24.6	30.8	297.00	Moderately Susceptible (MS)
SP- 20	1.22	45.7	45.7	433.00	Susceptible (S)
SP- 21	4.34	5.1	8.5	112.50	Moderately Resistant (MR)
SP- 26	4.04	6.0	10.0	123.61	Moderately Resistant (MR)
SP- 29	1.67	28.3	35.3	395.50	Moderately Susceptible (MS)
SP- 31	1.75	14.1	23.4	229.67	MR – MS = (M) Intermediate
SP- 34	0.89	47.1	47.1	463.50	Susceptible (S)

Table 4. Pearson's correlation coefficient for disease parameters among 17 wheat genotypes

Source.	Yields	ACI.	AUDPC	FDS.
Yields	-			
ACI	-0.6465***	-		
AUDPC	-0.5465***	1.000***	-	
FDS	-0.5867 ***	0.899***D***	0.759***	-

**** Significant relationship between the variables at P $\leq$ 0.05, ACI = average coefficient of infection, AUDPC = area under disease progressive curve, FDS = Final disease severity*

the breeding methods used in wheat breeding for developing mutant lines with disease resistance and increased agronomic value [8]. Wheat farmer's expectation is to have a variety that is high yielding, stem rust resistant and adaptable across various locations. Mutant lines with ACI values of 10.0 and below, AUDPC values of below 150 and with an average yield above the best parent or commercial check were considered [9]. Two mutant lines SP-21 and SP-26 were found to have high yields with moderate resistance. One mutant line SP-31 showed intermediate infection but with low yields. But the three mutants had low disease infection indicating a possibility they carry stem rust resistant major and minor genes. The remaining fourteen genotypes showed susceptibility of varying degrees from moderately susceptible to susceptible (Table 3). There was a negative correlation between yield and stem rust which revealed a linear and an inverse relationship between yield and stem rust. Yield variability and disease pressure existed across the three sites due to diverse genetic background of the genotypes and genotype x environment interactions. Similar results were reported [10]. Njoro location had the lowest yields but highest mean for disease while Eldoret and Kitale recorded similar yields and disease infection scores (Table 1). Stem rust is favored by warm and moist environment which is the characteristic of Njoro location and similar results were reported [12] and [13]. There was low genetic distance between the genotypes in each sub-cluster attributed to the high genetic similarity between the mutants and their parents.

5. CONCLUSION

The two mutant lines SP-21 and SP-26 combining both high yields and stem rust resistance can be considered as candidates for variety release and will be recommended to National performance trials for further evaluation and future release as new varieties. They could also be used as donors for introgression of resistance to adapted Kenyan wheat background. The differences observed on genotypes across various locations were an indication of presence of genotype x environment interactions which is important in selection of genotypes for different environments [11]. Further studies needs to be carried out to identify the exact genes conferring resistance to stem rust among the elite mutant lines selected. Genotype SP-16 and SP-31 which showed moderate susceptibility to stem rust are potential mutant lines for gene staking which could be advanced for future release [14].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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