
Assessment of Adult Plant Resistance to Stem Rust (*Puccinia graminis* f.sp *tritici*) in Wheat (*Triticum aestivum* L.) Mutant Lines

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Abstract: Stem rust (*Puccinia graminis* f. sp *tritici*) is a destructive disease of wheat (*Triticum aestivum* L.) making it a major challenge to wheat production in Kenya as well as other wheat growing countries. Due to this, mutation breeding has been as a source of increasing variability and confers specific improvement to the Kenyan varieties without significantly altering its phenotype. The objective of this study was to determine adult plant resistance of wheat mutant lines to stem rust across three different locations. The study area was in three locations, Nakuru County (Njoro and Mau Narok) and Meru County (Timau) during 2015-2016 cropping season. Sixty three mutant lines and six checks (NJBWII, Duma and Kwale, Kingbird, Robin and Cacuke) were evaluated under field conditions with three replications in an alpha lattice (23 rows by 3 columns) design. Mean for area under disease progress curve and coefficient of infection revealed that Duma200gry (1026), Duma200gry (1124) were best disease performers. The calculated variance (*Si*) distinguished stable genotypes in terms of disease and yield which included Duma100gry (995) and Kwale100gry (1483), respectively. There was positive effect of dosage 400gry on the mutant lines in terms of disease, yield and 1000 kernel weight, mostly with the Duma mutant lines. The mean grain yield for the genotypes ranged from 5.5 to 14.1 t ha⁻¹. Genotype, location and genotype by location interaction for the area under disease progress curve, coefficient of infection and yield were significant at P< 0.01 and P<0.001. There was a negative correlation displayed between yield and disease components. R-Square values revealed 0.1508 and 0.3911 of the variation in yield was contributed by the disease severity and area under disease progress curve, respectively. Considering the best lines both in disease and yield can be taken for further screening in breeding programmes.

Keywords: Adult Plant Resistance, Multi-locations, Stem Rust, Wheat

1. Introduction

Ug99 (TTKSK) race and its variants have been resulting to huge wheat yield losses which can go up to 100% in most of Kenya wheat growing areas and worldwide [1, 13, 26, 30, 31]. Though in Kenya, wheat breeding programmes have attempted to develop resistant varieties, virulence of the pathogen has been reported on most of the varieties at both seedling stage and adult plant resistance stages [13]. Due to this, mutation induction is one of the techniques that have been employed to create variation within wheat varieties [11,

17]. Some of these variations have been achieved using physical mutagens, like X-rays, gamma-rays, neutrons and chemical mutagens.

Wheat (*Triticum aestivum* L.) is one of the major food crops in the world. [6] estimated that the world wheat production had risen to 700 million tonnes in the year 2011 from 553.92 million tonnes in 2003/2004, 607 million in 2007 and 655.7 million tonnes in 2010. In Kenya, wheat is second important crop after maize with an annual

production of 0.2 million tonnes in 2009, 0.25 million tonnes in 2010, 0.2 million tonnes in 2011 and 0.25 million tonnes in 2012. This production cannot meet the demand which has been growing at 5% per annum in the recent years to 0.9 million tonnes in 2012 [6]. To fill the gap, Kenya imports about 0.65 million tonnes of wheat annually. However, wheat production can be increased by addressing the current constraints facing the small scale farmers, especially small-scale who have limited resources and lack access to production technologies.

Biotic factors like diseases, weed and pests are the major constraints to wheat production in Kenya. They may destroy between 31% and 42% of all crops annually [16]. Important wheat diseases include; rust, smut, bunts, leaf blight, powdery mildew and head scab [20]. The most important of all the diseases are those caused by the fungal pathogens and a few caused by viruses and bacteria [12]. Rust diseases cause huge losses [20] to wheat crop in the world. Leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*) can cause up to 60% loss of yield while stem rust (*Puccinia graminis* f. sp. *tritici*) can cause up to 100% loss in case of an epidemic or when a susceptible cultivar is grown [16]. Stem rust is the most limiting factor to wheat production [19, 25] because of its wide distribution, its capacity to mutate through migration, mutation and recombination to new races that attack previously resistant cultivars. It can move long distances by wind and develop rapidly under optimal environmental conditions [20]. This study was aimed at identifying possible elite wheat mutant lines containing resistance to stem rust that can be advanced to further levels in breeding programmes.

2. Materials and Method

2.1. Development of Mutants

Two high yielding wheat varieties, Kwale and NJBWII, and one drought tolerant wheat line, Duma were irradiated at 100gy, 200gy and 400gy, to produce the M0 seeds in 2009. The mutant seeds were planted in KALRO Njoro field to develop M1 to M6 generation under rain fed and irrigation systems. Duma showed high level of variation and selection began at M2. However, for NJBWII and Kwale, variations were first detected at M4. In the year 2011, M4 seeds from individual plants were selected, cleaned and well-formed grains were treated and drilled in plots of 2rows X 5metre for Duma mutants and 2 rows x 1metre for mutants of NJBWII and Kwale. Variation was observed between the levels of mutation. Selection was based on the general deviation from the original parents and resistance to disease (stem rust). The 100gy level of mutation Duma population showed late maturity as compared to the 200gy and 400gy levels of mutation.

Mutants obtained from the variety Kwale lodged easily, a character that was not in the parental line. Most of the mutants obtained were late maturing, have larger heads but

shriveled grains. Deleterious effects were observed for NJBWII and Kwale mutants irradiated at 400gy. This was unlike what was observed for the Duma mutants that had deleterious effects at 100gy irradiation level. In the main season of 2012 the mutants of Duma were planted in non-replicated observation trials, while the selected ears of NJBWII and Kwale were planted in single rows.

In 2013/2014 80 mutant lines (M5) were selected and harvested, these included 21, 4 and 11 samples NJBWII Irradiated at 100,200 and 400 Grys respectively: 1,9 and 8 samples of Duma Irradiated at 100,200 and 400 Grys respectively and 5,10 and 11 samples of Kwale Irradiated at 100,200 and 400 Grys respectively. Later 100seeds from each line was dispensed and planted on 4th February 2014 to generate the M6 population. They were space planted in plots made up squares of 7x7 cm; two seeds were planted in each hole; where both seeds germinated one was uprooted. Out of the possible 3600 samples, 1969 survived and were tagged each as an individual plant. In the 1969 samples obtained, 69 samples were selected for the main experiment. The mutants were planted in early 2015 for seed multiplication which obtained M7 mutant generation.

2.2. Genotypes

Sixty three mutant lines used in this experiment were developed from three selected Kenyan wheat varieties including 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 NJBWII, Kwale, and Duma, the resistant wheat cultivar Kingbird [14], and the two susceptible cultivars Robin and Cacuke were also included in the experiments.

2.3. Description of Study Area

The study was conducted during the 2015 cropping season across three locations in Kenya namely KALRO, Njoro and Mau Narok in Nakuru county and Timau in Meru county. Njoro station is located at 0° 20'S, 35°56'E and 2185 m elevation, with daily average temperature ranging from 9.7°C (night) and 23.5°C (day). Variations of daily temperatures in this area is approximately + 2°C occurring mostly during the day hours and receives on average 939 mm of precipitation annually. The soil type is predominantly Mollic Andosols. Mau-Narok is situated 0° 39'S, 35°57' E at 2,900 MASL and annual rainfall of 1,200 to 1,400 mm, minimum and maximum temperatures ranging 6 to 14°C and 22 to 26°C, respectively. Timau is situated 0°5'S, 37° 20'E at 2640 MASL and annual rainfall of 896 mm, minimum and maximum temperatures of 5°C and 23°C respectively. These sites fall within agro ecological zone III [8]. These areas have been

described as ‘hot-spot’ for stem rust epidemics [23]. These microclimates have strong positive effect on the frequency of stem rust epidemics in the highlands of Kenya where most of the wheat is produced.

2.4. Land Preparation and Experimental Design

Land preparation was done to a fine tilth to allow uniform crop establishment. The first plough was done two months prior to planting using mould board plough. Second land preparation was carried out using disc harrow one week to planting. Planting was done by hand and each line was planted in plot of two rows of 1m length by 0.2m width at spacing of 0.4m and at a seed rate of 125kg ha⁻¹. Each entry was separated by 0.3m and 0.5m wide alleyways within and between the blocks, respectively. Mixtures of susceptible wheat cultivars was planted around the trial plot and in the middle of the 0.5m alleyway on both sides of plots to facilitate uniform inoculum build up and serve as spreader. The experimental design used in the three locations was alpha-lattice (23 rows × 3 columns) design with three replications. DAP fertilizer was used during planting where nitrogen and phosphorus was applied at the rate of 22.5kg N ha⁻¹ and 25.3kg P ha⁻¹. Buctril MC (225g L⁻¹ Bromoxynil octanoate and 225g L⁻¹MCPA Ethylhexylester), a post emergence herbicide was sprayed at tillering stage at the rate of 7ml L⁻¹ of water to control broad-leaved weeds. Insect pest was controlled using Bulldock Duo (225 g L⁻¹ Beta-Cyfluthrin) where it was sprayed at the rate of 10 ml L⁻¹ of water. The trial was top dressed using urea fertilizer at a rate of 30kg N ha⁻¹ at jointing stage. Hand weeding was done twice at stem elongation and booting stages to eradicate grasses.

2.5. Screening for Adult Plant Resistance and Agronomic Traits Under Field Conditions

Adult plant stage assessment was done in the field under natural inoculation in which the spreader rows and field trials across sites acted as natural infection. Assessment was done from milk to early dough stage (Zadok’s growth stage 75 to 85) [33] of grain development. The adult plant response to infection was classified into five categories according to [22] R = resistant, RMR = resistant moderate resistant, MR = moderately resistant, MS = moderately susceptible, MSS = moderately susceptible to susceptible and S = susceptible and overlapping responses between two categories were denoted using a dash (—) between the categories for example MR/MS. The stem rust severity was determined by use of the modified Cobb’s scale where the severities were ranging from 5%-100% [18].

The number of effective tillers at harvest was determined by taking five samples of wheat per plot randomly and the number of tillers was counted. The height of the plant was taken during harvesting; five samples of the crop were taken from each plot and measured from the base of the plant to the base of a spike using a one meter ruler. The length of the

spike was taken at the end of maturity where five samples were taken from each plot. One meter ruler was used in measuring the spike length. To determine the number of seeds per spike, five plants per plot were randomly selected. One spike per plant was randomly picked and the number of seeds determined by counting manually. 1000 kernel weight from each plot was counted and weighed using an electronic weighing balance.

3. Data Analysis

The integral model for area under the disease progress curve (AUDPC) function was used to calculate the mean disease severity. AUDPC values were calculated for each plot using the [32] formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} 0.5(X_i + 1 + X_{i+1})(t_{i+1} - t_i)$$

Where, X_i is the cumulative disease severity expressed as a proportion at the i^{th} observation; t_i is the time (days after planting) at the i^{th} observation and n is total number of observations. Coefficient of infection (CI) was calculated by taking into account the disease severity and the host response to infection of the final disease observation where; 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 represented immune, resistant (R), moderately resistant (MR), moderately resistant to moderately susceptible (M), moderately susceptible (MS) and susceptible (S), respectively [22].

3.1. Statistical Analysis

Analysis of variance for area under disease progress curve, coefficient of infection, 1000 kernel weight and yield across the sites was performed using the Statistical Analysis Software (SAS) version 9.1 procedural PROC GLM (SAS, 2002) with genotypes as the fixed effects while location, replicates and blocks within replicates considered random. The following statistical model was used;

$$Y_{ijkl} = \mu + G_i + L_j + R_k + B_{l(k)} + GL_{ij} + \sum_{ijkl}$$

Where; Y_{ijkl} =observations; μ =mean of the experiment; G_i = effect of the i^{th} genotype; L_j = effect of j^{th} location; R_k = effect of the k^{th} replicate (superblock); $B_{l(k)}$ =effect of the l^{th} incomplete block within the k^{th} replicate; GL_{ij} = effect of i^{th} genotype in j^{th} location; ϵ_{ijk} = experimental error. The disease severity scores were transformed using square root(x+1) and means transformed back to original score. The least significant difference was determined at ($P < 0.05$).

3.2. Correlation and Regression Analysis

Correlation analysis was conducted to quantify the degree to which the disease level was related to traits associated with wheat yield. Linear regression analysis was also conducted to find the best equation and equation line that had predicted the best traits from disease level. These analyses were performed using SAS programme.

3.3. Genotype by Environment Interaction (GEI), Disease Resistance and Stability Analysis

A combined analysis of variance procedure was used to identify the existence of disease resistance, genotype*environment interaction (GEI) and stability from replicated multi-location trials. According to [9] analysis of static stability, genotypic variance (S_i^2) was computed to determine disease resistance and yield stability. The analysis followed the equation;

$$S_i^2 = \frac{\sum_{j=1}^q X_{ij}(X_{ij}-\bar{X}_i)^2}{q-1}$$

Where, X_{ij} = disease value (AUDPC) of genotype i in location j ; \bar{X}_i = mean of genotype i across all locations; $X_{ij}-\bar{X}_i$ = deviation from the average disease value, and q = number of locations.

Table 1. Summary for means squares of disease parameters, 1000 kernel weight and grain yield of 69 wheat (*Triticum aestivum* L.) genotypes evaluated across three locations in Kenya during 2015-2016 cropping season.

Source of variance	d.f	CI	AUDPC	1000 kernel weight	Yield t/ha
Site	2	276.0396779***	4514.357504***	0.02600441***	9086.71453***
Rep	2	1.7254750	15.000306	0.00001517	17.87741***
Block (Rep)	6	0.8025722	11.497370	0.00003440**	7.02101**
Genotype	68	6.2734997***	63.690222***	0.00004561***	25.26314***
Genotype×Site	136	3.0883381***	25.578582***	0.00004848***	20.56680***
Error	406	0.994303	9.84922	0.00001213	1.84834
Total		1820.652174	21056.48699	0.06685043	23604.45741
CV%		24.60087	26.71610	10.40681	14.00653
R ²		0.778360	0.810093	0.926344	0.968208

CI; coefficient of infection, AUDPC; area under disease progress curve ** represents significance at (P<0.01), *** represents significance at (P< 0.001).

4.2. Mean Performance of Site, Genotypes and Genotype by Location (GL) Interaction Regarding to Area Under Disease Progress Curve (AUDPC) and Coefficient of Infection (CI)

Variation in disease was noted on the test sites during the cropping season. Timau had the least disease severity as evidenced by its mean values of CI (3.04) and AUDPC (6.36), followed by Mau narok with CI and AUDPC values of 3.79 and 14.15, respectively (Figure 1). Njoro had the highest disease severity with AUDPC and CI values of 14.72 and 5.32, respectively (Figure 1). There was a significant difference among the sixty nine genotypes in regard to area under disease progress curve (AUDPC) and coefficient of infection (CI) values (Table 2) on the three sites (Mau narok, Njoro and Timau). Among the genotypes, the highest mean

4. Results

4.1. Analysis of variance and Genotype by Location (GL) Interaction Effects

Of the total variance of coefficient of infection (CI), the site main effect accounted for 30.60%, genotype 23.45% and the site by genotype interaction 22.63%. In regard to area under disease progress curve (AUDPC), site was 42.88%, genotype 20.57% and the interaction 16.52% (Table 1). For yield and 1000 kernel weight, site main effect was 76.99% and 77.79%, genotype was 7.78% and 4.64% and the interaction accounted for 11.85% and 9.86% of the total variation, respectively (Table 1). These results showed that CI, AUDPC, 1000 kernel weight and yield were significantly affected by changes in environment.

values for CI were scored on Robin, *Cacuke*, Duma parent, Kwale100gry (1483), NJBWII 200gry (602) and NJBWII 100gry (50) of 7.6, 7.4 and 6.7, 5.0, 5.0, 4.8 respectively across the sites (Table 2). The least mean values for CI among the genotypes were Duma200gry (1124) and Kingbird (resistant check) in the three sites with 2.6 and 2.8 respectively. The highest AUDPC mean values were recorded on Robin, *Cacuke*, Duma parent, Kwale100gry (1483), Kwale100gry (1556) and NJBWII 100gry (50) and NJBWII parent of 25.5, 22.6, 19.5, 14.8, 14, 13.5, 13.5 respectively (Table 2). The least mean values for AUDPC among the genotypes in the three sites were recorded on Duma200gry (1026), Duma200gry (1124), Duma400gry (1304), NJBWII100gry (404) and Duma200gry (1030) of 8.8, 8.8, 9.1, 9.3 and 9.3 respectively (Table 2).

Table 2. Means of area under disease progress curve and coefficient of infection for the evaluated genotypes in Kenya.

	AUDPC				CI			
	Njoro	MauNarok	Timau	Mean	Njoro	MauNarok	Timau	Mean
NJBWIII100GRY(50)	15.90	18.27	6.27	13.5	6.40	4.87	3.10	4.8
NJBWIII100GRY(57)	11.80	11.70	7.03	10.2	4.10	3.00	3.37	3.5
NJBWIII100GRY(140)	11.87	12.63	9.83	11.4	3.90	3.47	4.27	3.8
NJBWIII100GRY(288)	14.03	13.53	3.67	10.4	5.40	3.67	2.13	3.7
NJBWIII100GRY(382)	12.93	11.57	5.47	10.0	4.40	3.00	3.10	3.5
NJBWIII100GRY(404)	9.93	9.40	8.63	9.3	4.23	2.83	3.47	3.5
NJBWIII100GRY(415)	12.67	14.47	7.43	11.5	4.53	3.87	3.30	3.9
NJBWII200GRY(602)	16.13	18.70	4.03	13.0	6.87	5.53	2.47	5.0

	AUDPC				C I			
	Njoro	MauNarok	Timau	Mean	Njoro	MauNarok	Timau	Mean
NJBWII200GRY(608)	14.17	12.77	5.47	10.8	6.17	3.60	2.73	4.2
NJBWII200GRY(612)	16.13	15.40	3.07	11.5	6.17	3.87	2.07	4.0
NJBWII200GRY(634)	15.30	15.70	8.03	13.0	6.13	3.90	3.23	4.4
NJBWII200GRY(641)	14.27	14.63	7.10	12.0	4.20	3.67	3.47	3.8
NJBWII200GRY(660)	14.73	13.50	5.90	11.4	6.37	3.90	2.60	4.3
NJBWII200GRY(662)	16.60	14.30	4.80	11.9	6.60	4.07	2.47	4.4
NJBWII400GRY(675)	16.00	17.60	3.67	12.4	6.17	4.87	2.40	4.5
NJBWII400GRY(776)	13.20	9.67	7.87	10.2	4.70	1.57	3.63	3.3
NJBWII400GRY(798)	11.53	13.70	5.10	10.1	4.57	3.60	2.47	3.5
NJBWII400GRY(849)	13.90	10.17	7.60	10.6	5.00	2.67	3.10	3.6
NJBWII400GRY(908)	13.40	11.60	3.67	9.6	4.33	2.93	2.00	3.1
NJBWII400GRY(915)	12.63	11.53	9.83	11.3	4.03	2.53	4.10	3.6
NJBWII400GRY(930)	14.07	14.83	4.10	11.0	5.00	3.93	2.40	3.8
DUMA100GRY(987)	10.53	10.03	9.47	10.0	3.27	3.00	4.47	3.6
DUMA100GRY(992)	12.40	9.90	10.13	10.8	4.27	2.47	4.23	3.7
DUMA100GRY(993)	11.53	10.03	7.27	9.6	3.93	3.37	3.37	3.6
DUMA100GRY(995)	10.40	10.63	10.10	10.4	3.20	2.80	4.30	3.4
DUMA100GRY(996)	11.93	12.17	6.73	10.3	3.80	3.37	3.77	3.6
DUMA100GRY(997)	11.77	12.83	8.37	11.0	3.73	3.60	3.80	3.7
DUMA100GRY(1010)	11.00	11.20	6.83	9.7	4.23	2.87	3.27	3.5
DUMA200GRY(1026)	11.57	10.70	4.10	8.8	3.63	2.90	2.60	3.0
DUMA200GRY(1030)	11.53	13.37	3.07	9.3	5.23	2.93	1.80	3.3
DUMA200GRY(1033)	10.50	11.83	7.83	10.1	3.27	3.00	3.57	3.3
DUMA200GRY(1099)	13.33	10.90	9.23	11.2	4.23	2.97	3.73	3.6
DUMA200GRY(1103)	12.67	12.17	8.57	11.1	4.13	2.80	3.63	3.5
DUMA200GRY(1124)	11.93	10.40	4.10	8.8	3.47	2.33	2.10	2.6
DUMA200GRY(1145)	12.17	13.67	6.23	10.7	4.87	3.73	2.77	3.8
DUMA400GRY(1299)	14.90	11.87	6.27	11.0	4.40	3.13	3.37	3.6
DUMA400GRY(1295)	14.27	15.30	3.27	10.9	5.63	4.07	2.20	4.0
DUMA400GRY(1304)	13.00	7.17	7.10	9.1	5.70	2.33	3.40	3.8
DUMA400GRY(1437)	10.03	9.67	8.47	9.4	3.47	2.80	4.00	3.4
DUMA400GRY(1349)	15.33	13.30	5.90	11.5	5.70	3.53	3.27	4.2
DUMA400GRY(1368)	13.63	13.63	6.23	11.2	5.23	4.23	2.53	4.0
DUMA400GRY(1403)	14.37	11.23	10.30	12.0	4.90	3.77	4.77	4.5
KWALE100GRY(1468)	14.07	13.87	5.47	11.1	5.47	4.00	2.83	4.1
KWALE100GRY(1470)	17.80	16.27	4.87	13.0	6.13	4.23	2.67	4.3
KWALE100GRY(1483)	19.63	16.80	7.87	14.8	6.93	4.43	3.70	5.0
KWALE100GRY(1492)	15.90	13.67	7.70	12.4	5.00	3.90	3.30	4.1
KWALE100GRY(1499)	18.00	12.97	5.47	12.1	6.13	3.83	2.73	4.2
KWALE100GRY(1502)	13.23	16.00	4.27	11.2	5.23	4.30	2.20	3.9
KWALE100GRY(1556)	16.73	17.43	7.70	14.0	6.07	4.77	3.37	4.7
KWALE200GRY(1621)	12.67	14.97	3.67	10.4	5.13	3.90	2.00	3.7
KWALE200GRY(1715)	14.43	18.50	4.70	12.5	5.17	4.87	2.47	4.2
KWALE200GRY(1731)	14.43	20.00	4.87	13.1	5.10	5.57	2.00	4.2
KWALE200GRY(1750)	19.77	13.00	6.23	13.0	6.87	3.63	2.83	4.4
KWALE200GRY(1768)	16.13	16.87	4.43	12.5	5.70	4.23	2.00	4.0
KWALE200GRY(1777)	14.07	15.83	5.90	11.9	6.27	4.63	2.37	4.4
KWALE200GRY(1818)	17.40	16.63	3.87	12.6	6.77	4.67	2.47	4.6
KWALE400GRY(1875)	14.33	18.27	3.07	11.9	5.33	3.87	1.97	4.1
KWALE400GRY(1877)	13.57	16.70	6.67	12.3	4.87	4.33	3.53	4.2
KWALE400GRY(1895)	15.87	13.03	3.23	10.7	6.17	3.43	2.07	3.9
KWALE400GRY(1907)	15.57	12.90	5.47	11.3	5.93	3.27	2.67	4.0
KWALE400GRY(1949)	14.07	12.00	8.47	11.5	5.33	3.40	3.57	4.1
KWALE400GRY(1961)	13.83	10.63	5.20	9.9	5.13	2.60	3.13	3.6
KWALE400GRY(1964)	16.57	10.90	9.57	12.3	4.63	2.93	4.40	4.0
NJBWII PARENT	16.70	16.27	7.43	13.5	7.43	4.13	3.40	5.0
DUMA PARENT	25.60	22.93	9.83	19.5	8.97	7.07	4.10	6.7
KWALE PARENT	15.67	16.23	5.47	12.5	6.83	4.27	2.73	4.6
CHECKS								
KINGBIRD	16.47	12.20	2.63	10.4	5.07	2.07	1.37	2.8
CACUKE	28.83	28.53	10.47	22.6	9.67	8.17	4.37	7.4
ROBIN	34.17	35.93	6.50	25.5	10.00	10.00	2.73	7.6

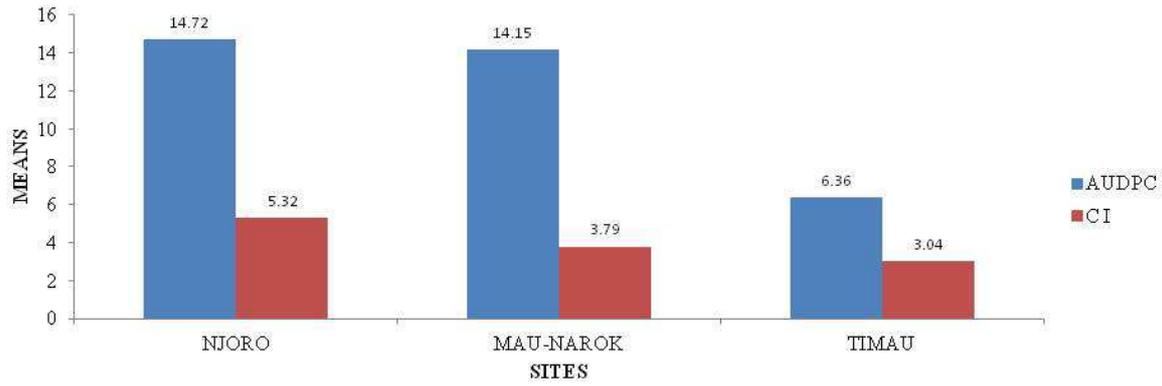


Figure 1. Mean performance of the genotypes for AUDPC and CI in the three evaluated sites.

4.3. Genotypic Performance Stability Across the Sites

Genotype stability was measured using area under disease progress curve and yield. In this case, Duma100gry (995) and Kwale100gry (1483) were the most stable genotypes among the evaluated mutant lines in

AUDPC disease and yield respectively (Tables 3 and 4). In general, Duma100gry (992), Duma100gry (995) and Duma100gry (1033) appeared the outstanding most stable genotypes on both AUDPC and yield evaluation across the sites (Tables 3 and 4).

Table 3. The stem rust (Puccini graminis f. sp. tritici) Area Under Disease Progress Curve (AUDPC), and stability values for the wheat (Triticum aestivum L.) genotypes that proved better than the resistant and susceptible check as evaluated across three locations in Kenya.

No	Genotypes	AUDPC		
		Mean	S ²	Stability rank
1	DUMA100GRY(995)	10.4	0.1	1
2	DUMA100GRY(987)	10.0	0.3	2
3	NJBWII100GRY(404)	9.3	0.4	3
4	DUMA400GRY(1437)	9.4	0.7	4
5	DUMA100GRY(992)	10.8	1.9	5
6	NJBWII400GRY(915)	11.3	2.0	6
7	NJBWII100GRY(140)	11.4	2.1	7
8	DUMA200GRY(1033)	10.1	4.1	8
9	DUMA200GRY(1099)	11.2	4.3	9
10	DUMA400GRY(1403)	12.0	4.5	10
Means	11.1			
CV%	26.7			
LSD _{0.05}	2.91			

CV; coefficient of variation, LSD; Least significant difference

Table 4. The stem rust (Puccini graminis f. sp. tritici) Yield and stability values for the wheat (Triticum aestivum L.) genotypes that proved better than the resistant and susceptible check as evaluated across three locations in Kenya.

Yield				
No	Genotypes	Mean	S ²	Stability rank
1	KWALE100GRY(1483)	6.1	5.3	1
2	DUMA200GRY(1033)	9.6	5.8	2
3	KWALE200GRY(1621)	5.5	9.6	3
4	DUMA100GRY(996)	10.2	10.1	4
5	DUMA200GRY(1124)	8.1	15.2	5
6	DUMA100GRY(995)	9.7	15.3	6
7	DUMA100GRY(992)	8.0	16.9	7
8	NJBWII100GRY(140)	10.9	18.6	8
9	KWALE400GRY(1964)	11.5	20.3	9
10	KWALE400GRY(1895)	7.6	22.5	10
Means	9.71			
CV%	14.00			
LSD _{0.05}	1.26			

CV; coefficient of variation, LSD; Least significant difference

4.4. Correlation and Regression Analysis

The Pearson correlation analysis showed a negative correlation between yield, 1000 kernel weight and disease parameters. Yield and 1000 kernel weight displayed negative correlation to area under disease progress curve (AUDPC) and coefficient of infection (CI) respectively (Table 5).

The regression equation revealed that for every percent increase in disease severity and area under disease progress curve there was yield loss of -1.1803 and -0.59, respectively. R- Square values computed for the genotypes revealed that 0.1508 and 0.3911 of the variation in yield was contributed by the disease severity and area under disease progress curve, respectively (Figures 2 and 3).

Table 5. Correlation between yield, kernel weight and the disease parameters of the wheat genotypes evaluated for stem rust during 2015-2016 main season.

Variables	Kernel weight	Yield	AUDPC	CI
Kernel weight	-	0.85768***	-0.63528***	-0.43051***
Yield		-	-0.62588	-0.44027***
AUDPC			-	0.8445***
CI				-

*** Negative relationship between the variables at (P<0.001) significant difference, AUDPC; area under disease progress curve, CI; coefficient of infection.

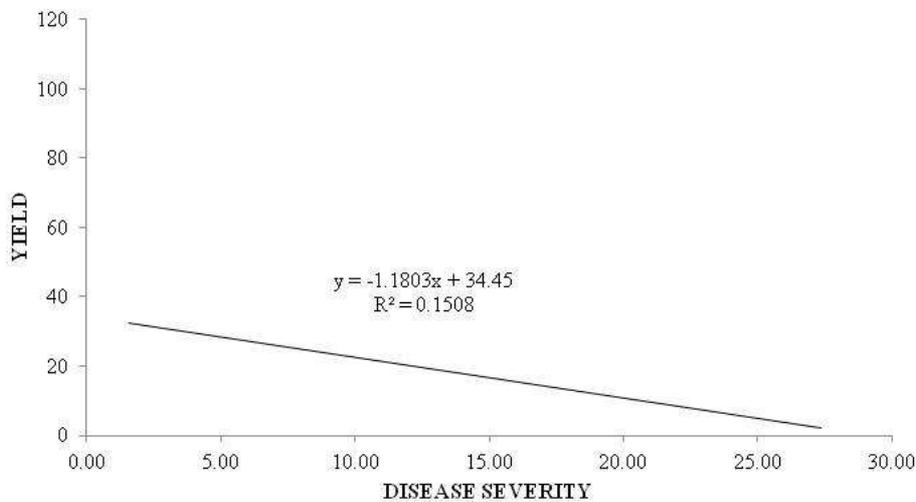


Figure 2. Association of yield with disease severity of the evaluated wheat genotypes across three sites.

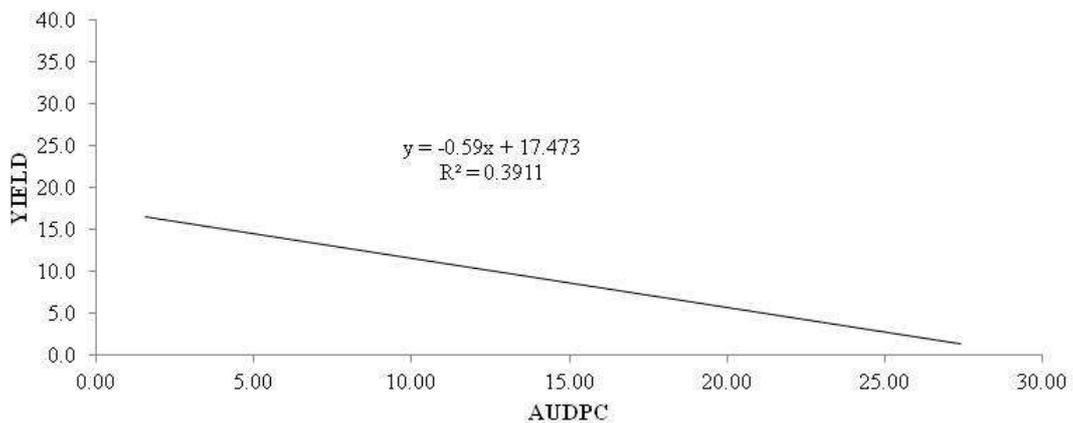


Figure 3. Association of yield with AUDPC of the evaluated wheat genotypes across three sites.

5. Discussion

There was a wide variation on disease responses among the evaluated wheat genotypes on the three study areas as shown by the area under progress curve and coefficient of infection mean values. Present results showed that some mutants exhibited high disease mean values hence highly susceptible; for instance Kwale 100gry (1483) and NJBWII

100gry (50). Some were noted with low disease mean values hence moderately resistant like Duma 200gry (1124), Duma 200gry (1026) and Duma 400gry (1304). These genotypes which were noted having moderate resistance response could be probably carrying minor genes. Similar variations in stem rust response among wheat genotypes had previously been reported [10, 29]. [28] reported significant genetic variability in disease response among various genotypes in different

environments.

In regard to sites, Njoro and Mau Narok were noted having high mean values on area under progress curve and coefficient of infection as compared to Timau. This was evident by the high infection response noted on the susceptible spreader/border rows and severity scores on genotypes showing that maximum disease pressure was reached across the three sites during the cropping season. This might be due to difference in geographical location/environmental factors, occurrence of high spore load in the atmosphere, crop management practices and type of cultivars grown.

Yield variation was observed on the evaluated genotypes and across the three sites. This could be attributed to diverse genetic backgrounds of the genotypes, climatic conditions of the sites, disease pressure in that particular area and genotype*location (GL) interaction effects. For instance, effect of improved yield was noted mainly on wheat mutants with dosage of 400 gry including Duma400gry (1295) and Duma400gry (1299) which had the highest mean values (14.14 and 13.24) as compared to the Duma parent (9.88) mean values across the sites. On the other hand, Kwale200gry (1621), Kwale100gry (1483) and Kwale100gry (1492) had the least yield means (5.54, 6.13 and 6.93) which did not deviate much from the Kwale parent (8.9) (data not shown). This shows that after irradiation chromosomes carrying genes for those traits may have been altered in the process. Besides, the parents (Duma, Kwale and NJBWII) were among the old varieties bred in the late 90s and were part of the first varieties noted to be susceptible to the new *Ug99* stem rust race [13] and consequently resulting low yield.

Nonetheless, Timau had the highest yield mean values while Njoro had the least, thus the difference in yield among the sites could be attributed to both environmental factors and stem rust infection pressure during the season. Previous studies showed that the high disease scores obtained led to low yield in Njoro since stem rust has a high prevalence in this region. Environmental conditions such as temperature and moisture considerably affect disease expressions and consequently of yield. The existence of such variation enables breeders to select both high yielding and disease resistant genotypes across different sites. Moreover, gamma rays in particular are important physical mutagen which is well known with their effects on the plant growth and development by inducing physiological, morphological and cytological changes in cells and tissues. [27] reported that stem rust reduces grain yield of wheat cultivars, which is due to the injury on the photosynthetic surface of the plant [3]. This leads to more energy expenditure impacted on plant defense mechanisms rather than for growth and grain formation [4].

As regards to [21] findings, the fungus also reduces the food and water supply within the plants. For spore formation and production, the fungus needs food and water that would otherwise be used in the formation of well developed kernels. Additionally, the fungal pustules could cause loss of water by

evaporation through numerous ruptures of the plant epidermis. Reduction in kernel weight of the genotypes could be attributed to heavy stem rust disease pressure experienced in that cropping season. Study by [15], stem rust significantly reduce kernel weight in wheat when there is severe attack in the field. During grain filling the fungus (rust) usually competes for photosynthesis resulting to reduced number and size of seeds on the plants [2]. In association to that, the heavily rusted plants had low yield, poor grain quality with shriveled seeds resulting to reduced kernel weight like "Robin. This led to a conclusion that the significant effect of stem rust on 1000 kernel weight of the genotypes was brought about by its effect on photosynthesis and grain filling.

According to [7] stability analysis for type one concept of stability indicated that genotypes are considered stable if their environment variance is small. In this case the genotypes like Duma100gry (992), Duma100gry (995) and Duma100gry (1033) were considered stable. These genotypes are useful to farmers because they would give consistent varieties that can withstand unpredictable and transient environmental fluctuations. In addition, yield stability and adaptability is an important concept in wheat breeding thus breeders and pathologists opts to develop stable, high yield and low disease varieties that can be adapted by farmers in different environments.

A strong negative correlation between the disease parameters and yield was noted in that an increase in disease resulted to decrease in yield and vice versa. Nevertheless, weak relationship between the disease and yield was observed thus the variation in yield was assumed not only due to disease response but also due to location and genotype*location interaction variability.

6. Conclusion

The study concluded that mutation breeding is necessary to enhance genetic variability in Kenyan wheat varieties so that to achieve durable and broad spectrum resistance in disease and best yield. Results of this study revealed existence of variation in stem rust resistance and grain yield among the evaluated the mutant lines. Moreover isolation of mutants with multiple traits would be ideal to grow under different environmental conditions. With respect to disease and yield performance, mutant lines Duma 200gry (1124), Duma200gry (1026) and Duma400gry (1304) portrayed moderate resistance while Duma400gry (1295) and Duma400gry (1299) performed best (disease and yield) in the locations thus they are recommended for further screening in the breeding programs. Therefore, the national wheat breeding stations should carry out more mutagenesis work to develop new varieties that could be readily adapted on varying agro-climatic conditions.

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Competing Interest

There is no competing interest among the authors.

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