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Full Length Research Paper

Assessment of advanced Kenyan selected wheat lines for resistance to the prevailing stem rust races (*Puccinia graminis* f.sp.*tritici*) in Kenya

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Stem rust (*Puccinia graminis* f.sp.*tritici*) of wheat (*Triticum aestivum*) has caused wheat yield losses in Kenya for years and the trend shows the situation has worsened. The objective of the research was to identify elite genotypes for adult plant and seedling stage resistance. Adult plant resistance study was done under natural conditions in three locations. Scoring was done following the modified Cobbs scale. Seedling stage resistance was done in the greenhouse and scored following the Stakmans scale. Genotype KSL 144, 71, 50, 31 44, 115 were identified as having seedling stage resistance. Area Under Disease Progress Curve (AUDPC) and Final Disease Severity (FDS) when used for adult plant revealed KSL 142, 71, 144, 50, 31, 44, 115, 146, 69 and 76 as having resistance. The variance (S_i) and Coefficient of Variation (CV_i) was calculated from the FDS and yield values, which distinguished stable genotypes. The stable genotypes for disease severity were KSL 69 (8.8%), 161 (14.9%), 54 (12.4%), 156 (18.24%). The relationship between yield and AUDPC was strong and negative, r=-0. 943 same as yield and FDS relationship r= -0.84. Variation for yield performance was recorded KSL 137 (2.63t/ha), KSL 31 (2.52 t/ha) showing high performance. The thousand kernel weight values were not significant for the three location at (P<0.05). The advanced genotypes that consistently performed better should be released as varieties or used in improving local varieties in the Kenyan wheat stem rust breeding programme or potentially in the Eastern Africa region.

Key words: Ug99, disease severity, Area Under Disease Progress Curve (AUDPC), resistance.

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the worlds' most productive and important crop in the 21st century. There is increased consumption and demand for grain, for fuel as well as food (Curtis and Halford, 2014). Wheat yields must be increased which is seen as an important strategy to prevent food shortages (Curtis and Halford, 2014). It is one of the key staple crops for global food security, providing more than 35% of the cereal calorie intake in

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the developing world, 74 % in the developed world and 41 % globally from direct consumption (Shiferaw et al., 2013). Wheat is the second most important cereal staple food after maize in Kenya (USAID, 2010). In Kenya it is mostly grown in the Rift Valley, some areas of upper Central province (Nyandarua, Nyeri) and parts of Meru (Timau) (USAID, 2010).The crop is susceptible to three types of rust; stem (black) rust (*Puccinia graminis* f.sp.*tritici*), leaf (brown) rust *Puccinia triticina*, and stripe (yellow) rust *Puccinia striiformis* f.sp. *tritici* (Dubin and Brennan, 2009).

In most wheat-growing regions of the world, existing environmental conditions would favour stem rust infection, which could lead to epidemic buildup (Singh et al., 2011).The stem rust is the most devastating of the rust diseases and can cause losses of 50% in one month when conditions for its development are favourable. Losses of 100% can occur with susceptible cultivars (FAO, 2002). An estimated 80-90% of all global wheat cultivars growing in farmer's fields are now susceptible to *Ug99* or variants (*Ug99* factsheet, 2010). *Ug99* is the only known race of wheat stem rust that has virulence for an extremely important resistance gene - *Sr31*. In addition, *Ug99* has virulence against most of the resistance genes of wheat origin and other resistance genes from related species (*Ug99* factsheet, 2010).The stem rust resistance gene *Sr31* derived from rye has been used as an important source of stem rust resistance in many wheat cultivars worldwide. However, isolates of stem rust with virulence to *Sr31* were identified from Uganda in 1999. Similarly stem rust susceptibility in wheat lines with *Sr31* was observed in Kenya in 2003 and 2004 (Jin and Singh, 2006).

Ug99 possesses broad virulence, especially virulence to genes commonly used in combinations for stem rust resistance in wheat cultivars (Jin and Singh, 2006; Njau et al., 2009). Detection in Kenya of a new variant *TTK*ST in 2006 with virulence to gene *Sr24*, which caused severe epidemics in 2007 in some regions of Kenya and rendered about half of the previously known *Ug99*-resistant global wheat materials susceptible, has further increased the vulnerability globally (Singh et al., 2008). The emergence of virulence on *Sr24* within the *TTKST* race cluster has probably increased the vulnerability of wheat to stem rust worldwide because of the widespread use of this gene in breeding (Jin et al., 2008). Nearly all Kenyan germplasm are known to be susceptible or partially susceptible to *Ug99* (Njau et al., 2009). The stem rust resistance gene *Sr36* confers a near-immune resistance reaction to many races of Stem rust and is highly effective against race *TTKSK*, which possesses unusually broad virulence combinations. Because this gene is widely used in United States soft winter wheat germplasm and cultivars, it has been considered to be an important source of resistance to *TTKSK* (Jin et al., 2009).

The spread of *Ug99* race group of stem rust in Eastern and Southern Africa and beyond has brought back stem rust research and development activities back onto the international wheat improvement agenda under the BGRI (Singh et al., 2015). Currently, the research of stem rust in wheat is focusing on identifying further resistance genes to control *Ug99* and its derivatives (Haile and Roder, 2013). Despite the identification and deployment of a number of rust resistance genes to protect wheat crops, the emergence of virulent pathogen pathotypes can restrict their durability and use (Pathan and Park, 2006). Therefore resistance in wheat varieties has to be constantly improved to avoid having susceptible genotypes in production. Genetic improvement to minimize yield loss under disease is an attractive goal, as it exerts little or no selection pressure on pathogen populations, and could form a useful component of durable disease management programmes (Bingham et al., 2009). Because of this, there is a constant need to identify, characterize and deploy new sources of resistance (Pathan and Park, 2006). With world population increasing, food security is projected to become more critical; therefore increasing wheat yield potential in the developing world remains a high priority (Duveiller et al., 2007). Breeding resistant wheat varieties that have superior yields than currently grown popular varieties is the best (Singh et al., 2011).

MATERIALS AND METHODS

Seedling stage experiment

Experimental genotypes

The genotypes were made up of forty five advanced wheat lines and five local checks of the commonly grown varieties (Table 1). The advanced lines are mainly selection from the CIMMYT durable resistance rust nursery. The CIMMYT germplasms are used in Kenya for breeding to develop varieties that are resistant. The genotypes are selected continuously over seasons and tested both in Kenya and Mexico. The advanced lines were selected from CIMMYT lines that showed promising traits for both yield and stem rust resistance.

Inoculum preparation for seedling stage resistance

The inoculum used was collected from the trap nurseries of KALRO Njoro usually in the evening when it was cold. The trap nurseries were planted using the highly susceptible variety *Cacuke* for high amounts of Urediniospores used for inoculation. The trap nurseries were planted early before the main crop. It contained a bulk of Urediniospores of the common two races of *TTKST* and *TTKSK.* The inoculum was made up of a mixture of pathotypes for both *TTKST* and *TTKSK* stem rust races occurring in Kenya. The inoculum measured was based on the amount of spore number per unit dilute spores in a 1:1 mixture (Table 1).

Seedling stage experiment

The experiment conducted in the greenhouse was at the Kenya

Table 1. Description of bread wheat (*Triticum aestivum* L.) genotypes used in the experiment.

Genotype	Source	Pedigree/selection history
KSL1	CIMMYT	SERI1/CHIBIA/4/BAV92//IRENA/KAUZ/3/HUITES
KSL15	CIMMYT	WBLL1*2/BRAMBLING/5/BABAX LR42//BABAX*2/4/ SNI/TRAP31/3KAUZ*2/TRAP//KAUZ
KSL16	CIMMYT	WBLL1*2/BRAMBLING/5/BABAX/ LR42// BABAX*2/4 /SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
KSL17	CIMMYT	BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP KAUZ/5/WBLL1*2/TUKURU
KSL19	CIMMYT	WBLL1*2/TUKURU/7/CNDO/ R143/ENTE/MEXI_2/3// AEGILOPSSQUARROSATAUS)/4/WEAVER/5/2* KAUZ/6/FRET2
KSL21	CIMMYT	BW343*2/KUKUNA/3/ SERI//BAV92
KSL22	CIMMYT	PBW343*2/KUKUNA/3/ PGO/SERI//BAV92
KSL13	CIMMYT	QUAIU/5/FRET2*2/4/SNI/TRAP#1/3 /KAUZ*2/TRAP//KAUZ
KSL14	CIMMYT	QUAIU/5/FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
KSL28	CIMMYT	KFA/5/2*KAUZ//ALTAR84/ AOS/3
KSL29	CIMMYT	TUKURU//BAV92/RAYON*2/3/JUCHI
KSL31	CIMMYT	UP2338*2/KKTS*2//YANAC
KSL32	CIMMYT	UP2338*2/KKTS*2//YANAC
KSL33	CIMMYT	UP2338*2/KKTS*2//YANAC
KSL37	CIMMYT	CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2*KAUZ/6/ PASTOR/7/YANAC/8/CAL/ NH//H567.71/3/SERI/4/CAL/NH / /H567.71/5/2*KAUZ/6/PASTOR CAL/NH//H567.71/3/SERI/4/CAL/H567.71/5/2*KAUZ//PASTOR
KSL40	CIMMYT	/7/YANAC/8/CAL/NH//H567.71/3/SERI/4/CALNH//H567.71/5/2* KAUZ/PASTOR
KSL46	CIMMYT	TACUPETOF2001/6/CNDO/R143/ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/PASTOR/7/ROLF07
KSL47	CIMMYT	TACUPETO01/6/CNDO/R/R143//ENTE/MEXI2/3/AEGILOPS SQUARROSA(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07
KSL48	CIMMYT	TACUPETO01/6/CNDO/R/R143//ENTE/MEXI2/3/AEGILOPS SQUARROSA(TAUS)/4/WEAVER/5/PASTOR/7/ROLF07
KSL50	CIMMYT	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42// BABAX
KSL51	CIMMYT	KSW/7/CAL/NH//H567.71/3/SERI/4/CAL/NH//H567.71/5/2 KAUZ/6/PASTOR/8/CAL/NH//H567.71/3 SERI/4/CAL/NH //H567.71/5/2*KAUZ/6/PASTOR
KSL53	CIMMYT	TILILA/JUCHI/4/SERI.1B// KAUZ/HEVO/3/AMAD
KSL54	CIMMYT	28th SAWSN /09
KSL57	CIMMYT	C 30 SAWSN 2010
KSL59	CIMMYT	C 30 SAWSN 2010
KSL42	CIMMYT	FRANCOLIN #1/KIRITATI
KSL44	CIMMYT	BABAX/LR42//BABAX*2/3/ KUKUNA/4/TAM200/ PASTOR//TOBA97
KSL52	CIMMYT	KENYANYANGUMI/3/2*KAUZ/PASTOR//PBW343
KSL58	CIMMYT	C 30 SAWSN 2010
KSL63	CIMMYT	4th SRRSN 2010
KSL69	CIMMYT	Ethiopia 2010
KSL71	CIMMYT	SOUTHAFRICAN BETHLEHEM2010
KSL72	CIMMYT	4th SRRSN 2010
KSL73	CIMMYT	Bangladesh 2010

Table 1. Contd.

KSL76	CIMMYT	K.YOMBI/R1066
KSL81	CIMMYT	NJBW/CHIRIKU
KSL115	CIMMYT	R1071/MBUNI
KSL118	CIMMYT	R1075/KWALE
KSL126	CIMMYT	R1089/R1069
KSL137	CIMMYT	K8676/NJBWII
KSL142	CIMMYT	KONGONI/1083
KSL144	CIMMYT	KWALE/ZABADI
KSL146	CIMMYT	PAKA/R8665
KSL156	CIMMYT	RWAPT60/MBUNI
KSL161	CIMMYT	R960/R1088
Checks	KALRO	
Korongo[a]	KALRO	
Kingbird[a]	KALRO	
Eagle10[a]	KALRO	
Robin[a]	KALRO	
Wren[a]	KALRO	

KSL: Kenyan Selection; CIMMYT, Center for Maize and Wheat Improvement; a: commonly grown varieties.

Agricultural Livestock and Research Organization (KALRO) Njoro. Fifty pots of 5 cm diameter each filled with a potting media (Hygromix) were used for planting ten seeds of the genotypes. The pots were placed in a plastic tray of ten pots each. The inoculated plants were air dried for half an hour. The pots were then placed in the growth chamber and removed after ten days for inoculation. The inoculum prepared before containing a bulk of the stem rust races mainly *TTKS*T and *TTKS*K was sprayed on the genotypes and local checks using a hand sprayer. The pots were then kept in a dark humidity chamber for 48 h before taking them to the incubation chamber.In the incubation chamber the pots were left until spores started forming for data collection. Data collection was done fourteen days after inoculation when most of the leaves showed infection. To test for resistance the experiment was repeated five times in the greenhouse and data collected was used to determine which genotypes had resistance.

Data collection

Assessment was done to show which genotypes were consistent for low levels of infection types. The genotypes were scored following a scale of 0-4 according to Stakman et al. (1962) as described below. The numbers indicate the infection type while the host response is described as immune to very susceptible as follows; 0=immune, ; = nearly immune, 1=very resistant, 2=moderately resistant, X, Y, Z= heterogenous types,3=moderately susceptible and 4=susceptible. All data was collected and compared for consistency for the seedling stage resistance.

Field experiment

Experimental locations

The experimental locations used were established at three Locations: namely Mau-Narok, Njoro and Lanet.Kenya Agricultural livestock and Research organization (KALRO), situated at Njoro location with an altitude of 2185 meters above sea level (masl), average annual rainfall of 935 mm and minimum and maximum temperatures of 9.7°C and maximum of 23.5°C, respectively. Agricultural Development Corporation (ADC) Enchili farm Mau-Narokis situated at Mau-Narok location with an average annual rainfall of 752 mm, an altitude of 2900 masl and an annual rainfall range of 1,200 to 1,400 mm, minimum and maximum temperatures ranges of 6 to 14°C and 22 to 26°C, respectively. Kenya Plant Health Inspectorate Service (KEPHIS) Lanet is situated at Dundori location, 1920 masl with a minimum temperature of 10°C and maximum temperature of 26°C and annual rainfall of 800 mm.

Experimental procedure

Land preparation was done with one plough and two harrows for all the three locations to obtain fine seedbed. The trial design at all the three locations was an alpha lattice of 5 blocks with 10 plots within blocks and replicated three times and plot sizes were 1m by 2m. Spacing was 20 cm between rows by drill. Planting was done by hand in all the three locations. The genotypes were mainly fifty wheat advanced genotypes selected from CIMMYT nursery including five checks of the commonly grown commercial varieties. The genotypes were tested for resistance to stem rust under natural infection. Genotypes possessing *Sr24* genes with susceptibility to *TTKST* were used as a spreader. Four rows of the *Sr24* susceptible genotypes used as spreader were planted around the experimental plot and between replicates. A seed rate of 125 kg ha^{-1} which amounts to 25 g^{-1}plot and 5 g^{-1} for 5 rows in a plot was used. During planting fertilizer was applied at the rate of 22 kg of N ha^{-1} and 25 kg of P ha^{-1}. At five weeks after planting nitrogen was top dressed at the rate of 32 kg of Nha^{-1}. Weed control was done using Hussar evolution herbicide at the rate of 0.15 ml $1m^{-2}$. Scoring of stem rust was done when 50% of the susceptible spreader genotypes had been affected. Scoring was done three times across all the locations after twelve days and ten days from the first reading and second reading respectively.

Data collection

Data on diseases severity were scored following the modified Cobb

scale as described by Peterson et al*.* (1948). Cobbs scale key of 0.37 representing 1% of the actual affected tissue by disease to 37.0 represented 100% leaves covered by pustules. The percentages indicated the infection type used to determine the disease severity of 0-100%. The host response was assessed as described in Roelfs et al*.* (1992). The adult plant response to infection in the field was scored using 'R' indicating resistance, 'MR' indicating moderate resistance, 'MS' indicating moderately susceptible, 'S' indicating full susceptibility. The overlapping responses between two categories scored as 'M' were indicatedusing a slash between the two which was MR/MS.

Yield and thousand kernel weight

Grain yield^{-1} plot of the entire experimental plots was weighed in grams and converted to tones ha^{-1}for all the plots in the three locations having a total of 450 data entries. The weight of thousand kernels of grains harvested from each experimental plot was also measured. The thousand kernel weight was a yield component.

Data analyses

The Area under the Disease Progress Curve (AUDPC) was calculated for all the forty five elite genotypes and five local checks according to the formula of Shaner and Finney (1977):

$AUDPC=\sum_{i=1}^{n} [(Y_{i+ n1} + Y_i)/2] [X_{i+1}- X_i]$
Y_i=the disease severity at the i^{th} observation, X_i=time in days at the i^{th} observation, n= total number of observations. Analysis of variance was used to find the mean values of AUDPC using *SAS* version 8.02 (SAS/STAT software 1999). The experimental model is shown below;

$Y_{ijkl} = \mu + G_i + R_k + L_j + B_{l(k)} + GL_{ij} + \varepsilon_{ijkl}$
j= 1…3 k=1..3 i=1…50 l=1… 5,Y_{ijk}-overall response of the genotypes
μ- the overall mean, G_i-effect due to the i^{th} genotype in the k^{th} replicate and l^{th} block
$B_{l(k)}$- effect of the l^{th} block in the k^{th} replicate, R_k- effect due to k^{th} replicate, L_j- effect due to j^{th} location, GL_{ij}- interaction between the i^{th} genotype, j^{th} location and ε_{ijkl}- random error component.

Analysis for stability of the genotypes done using the variance for a genotype across environments (S_i^2) was used to determine the most stable genotype on disease across the three locations using the formula described by Francis and Kannenberg (1978),

$$S_i^2 = \sum_{i=j}^{q} (x_{ij} - \bar{x}_i)^2 / q-1,$$

Where S_i^2 is the variance for a genotype across environments, q= number of locations, x_{ij}= is the observed mean of the genotype,$\bar{x}_i$=the mean of the genotype in the three locations. The Coefficient of Variation of each genotype (CV_i) was used to determine the most stable line on disease and yield across the three locations using formula described by Francis and Kannenberg (1978),

$CV_{i=} S_i/ \bar{x}_i \times 100$

Where CV_i is the Coefficient of variation of each genotype in percentage, S_i is the standard deviation for each genotype, $\bar{x}_i$ is the mean of the genotype i across locations.

The correlation coefficient *r* between yield and AUDPC and between yield and final disease severity was calculated following the formula of Mead et al*. (*1993).

RESULTS

Seedling stage resistance experiment

Variation was observed among the genotypes for seedling stage infection after a repeated score of five times (Table 2). From the results considering top 25 genotypes (Table 2), the genotypes with small sized Uredinia surrounded by necrosis were very resistant and these were genotypes KSL50, 31, 44, 54, 51, 156, 81 and KSL33. The Uredinia that were medium often being surrounded by chlorosis or necrosis were moderately resistant; they are genotype KSL144, 115, 146, 69, 76, 161, 53, 137, 37, 52, 17 and KSL 57.On the other hand genotype KSL 142, 71, 72 and KSL73 Medium Uredinia and chlorosis were moderately susceptible. Large Uredinia without chlorosis were susceptible. The best performing genotypes at seedling stage resistance were entry KSL 144 (2+), 50 (1+), 31 (1+), 44 (1+), 115 (2+), 146 (2+), 69 (2+) and 76 (2+) (Table 3).The percentage of the very resistant genotypes at seedling stage of the best performing twenty four genotypes was 32% compared to the rest at 44% of moderately resistant and 24% for moderately susceptible.

Field experiment

Performance of genotypes across location

The area under the disease progress curve values ranged from KSL 142 (28.9) for the best performing genotypes to KSL 42 (1085) which was the worst (Table 4). The lowest values were for the most resistant varieties and highest values for the most resistant. The final disease severity values showed the best genotype having the lowest and worst having the highest at KSL 142 (2.8%) to KSL 42 (80%). The diseases severity progressed as the growth of plant increased the first had low disease levels by the third reading the levels increased. Under natural infestation Mau-Narok crop had most of the stems and leaves with a lot of Urediniospores at 80% for the worst genotype KSL 42 compared to Njoro at 73% and Lanet 56.7% for the three locations. The genotypes had the lowest at 10% in Mau-Narok and 0% Njoro and Lanet. For the AUDPC values Mau-Narok had 1080 for KSL 42, with Njoro at 1040 and Lanet at 916.1 as the worst performing genotype.

The analysis of variance for Area Under Disease Progress Curve (AUDPC), Final Disease Severity (FDS) , yield and 1000-kernal weight was performed using *SAS* version 8.02 (SAS/STAT software 1999). The ANOVA for AUDPC revealed variation among the genotypes and locations (Table 5). The locations were significantly different in performance at P<0.05; the genotypes were

Table 2. Seedling stage resistance for the top twenty four selected Kenyan wheat genotypes based on the AUDPC values from the three locations of Mau-Narok, Njoro and Lanet.

Genotypes	Seedling infection types	Host response
KSL142	3+	Moderately susceptible
KSL71	3+	Moderately susceptible
KSL144	2+	Moderately resistant
KSL50	1;	Very resistant
KSL31	1;	Very resistant
KSL44	1+	Very resistant
KSL115	2+	Moderately resistant
KSL146	2+	Moderately resistant
KSL69	2+	Moderately resistant
KSL76	2+	Moderately resistant
KSL161	2+	Moderately resistant
KSL53	2+	Moderately resistant
KSL73	3+	Moderately susceptible
KSL54	1+	Very resistant
KSL51	1+	Very resistant
KSL156	1+	Very resistant
KSL81	1+	Very resistant
KSL137	2+	Moderately resistant
KSL 37	2+	Moderately resistant
KSL72	3+	Moderately susceptible
KSL52	2+	Moderately resistant
KSL33	1+	Very resistant
KSL17	2+	Moderately resistant
KSL57	2+	Moderately resistant
Checks		
Kingbird[a]	2+	Moderately resistant
Eagle 10[a]	1+	Very resistant
Korongo[a]	3+	Moderately susceptible
Kenya Wren[a]	3+	Moderately susceptible
Robin[a]	3+	Moderately susceptible

KSL: Kenyan Selection KEY: 1=very resistant, 2=moderately resistant 3=moderately susceptible and 4=susceptible[a:] Local checks.

also significant. The Analysis of Variance (ANOVA) detected significant relationships between location and FDS (Final Disease Severity) at P<0.05, P<0.01 and P<0.001 being highly significant. Mau-Narok had the highest mean at 35.7%, Lanet 23.9% and Njoro at 23.3%. There was also a highly significant relationship between genotype and FDS with KSL 142, 71 and 144 having high resistance levels to disease as compared to the other genotypes. The genotype and location interaction for FDS was highly significant with the genotypes KSL 142, 71, 115, 146 and 69 having performed well overall across the three locations.

The same case applied to the AUDPC across the location which was highly significant at P<0.05, P<0.01 and P<0.001 with Mau-Narok having the highest mean at 363.18 followed by Njoro at 326.87 and Lanet at 231.95. The genotype and AUDPC relationship was highly significant with less consistency in performance among most of the genotypes. The genotype and location interaction was highly significant with the genotypes with low values in one location having low values across all the three locations with Mau-Narok having consistently higher AUDPC values compared to Njoro and Lanet.

Stem rust disease effect on the genotype yield and thousand kernel weight (TKW)

The grain yield relationship between location and yield

Table 3. Mean squares derived from analysis of variance for stem rust disease resistance and yield components of wheat genotypes.

Source of variation	d.f	FDS	AUDPC	YIELD	TKW
Rep	2	5068.95**	41719.79**	6832.39***	0.000169***
Location	2	7275.17***	688756.54***	2436705.46***	0.001141***
Block (rep)	12	15044.1***	241179.9***	8313.02***	0.0002228*
Genotype	49	3046.92***	510471.46***	14311.70***	0 .0001528
Genotype*Location	98	27821.88***	33016.39***	12184.86*	0.000098**
Error	10.0	9.17	111.33	64.692	0.0085737
CV		33.2	36.22	54.57	34.2
R^2		0.89954	0.90194	0.852	0.51773

*, **, *** represent significance at P < 0.05,P < 0.01 and P < 0.001respectively, d. f, degrees of freedom; FDS, Final Disease Severity; AUDPC, Area Under Disease Progress Curve, TKW, Thousand Kernel Weight.

was highly significant at P<0.05, P<0.01 and P<0.001, the highest mean yield for the genotypes was at Mau-Narok with 2.82 t ha^{-1} followed by Njoro 1.27 t $ha^{-1}$and then Lanet 0.51482 t ha^{-1}.Genotype and grain relationship was highly significant with variations from one location to the other. In Mau-Narok the highest grain yield was obtained by KSL 137 (2.63 t ha^{-1}), 31 (2.52 t ha^{-1}), 50 (2.46 t ha^{-1}) and KSL 33 (1.98 t ha^{-1}). The same genotypes performed well in Njoro and Lanet. The interaction between genotype and location for yield was highly significant with Mau-Narok reporting the highest grain yield per genotype. Njoro had better grain yield per genotype with Lanet having less grain yield per genotype.

Genotypic performance for TKW showed no significant genotypic variation under stem rust infection. For example genotype KSL 50, 31, 44, 115, 146 and 69 had high TKW in terms of overall genotype performance but not significant at P<0.05, P<0.01 or P<0.001.The interaction between genotype and location for TKW was only significant at P<.0.05 and P<0.01 not significant at P<0.001. Njoro appeared to positively interact with genotypes giving high values. On the other hand Mau-Narok some genotypes with high and others low but slightly lower general mean of 0.2555 than Njoro of 0.0274. The thousand kernel weight was not significant at P<0.05 for genotype, there were no variations from one genotype to the other. There were highly significant differences for location and thousand kernel weight at P<0.05, P<0.01 and P<0.001.

There was a negative relationship between disease severity, progress and yield while using the AUDPC and Final Disease Severity values. The more the disease pressure the lower the yields across the study locations of Mau-Narok, Njoro and Lanet.

Adult plant response to infection for the genotypes in the three locations

In Lanet the genotypes that had a resistant (R) reaction to stem rust were KSL 142, 71 and 144. The ones possessing a moderately resistant (MR) reaction were genotypes KSL 161, 69, 50, 156, 81, 137 and 57. The genotypes with moderately resistant to moderately susceptible (M) were KSL 44, 115, 146, 76, 53, 73, 54, 51, 72, 33 and 17 (Table 6). The genotype with moderately susceptible reaction (MS) was 52. In Njoro the genotypes that had a resistant reaction were KSL 71, 50, 31, 115 and 137. Genotypes possessing moderately resistant reaction were KSL 142, 144, 81, 44, 37 and 57. The genotypes with moderately resistant to moderately susceptible were KSL 146, 69, 76, 53, 54, 51, 156, 72, 33 and 17. The genotypes KSL 52 were moderately susceptible. In Mau-Narok most of the genotypes showed a moderately susceptible reaction which were genotypes KSL 69, 76, 161, 53, 53, 73, 54, 37, 72, 52, 33, 17 and 57. The genotypes with moderately resistant to moderately susceptible were KSL 142, 71, 144, 31, 115, 146, 156 and 137. The genotypes with moderately resistant reaction were KSL 50, 44 and 51.

Genotypic stability

The Coefficient of Variation (CV_i) and Variance (S^2_i) identified stable genotypes across the three locations. Generally stable genotypes had lower values of CV_i and S^2_i compared to those that were less stable (Table 7). Amongst the genotypes the most stable were KSL 69, 161, 54 and 156 with less than 20% coefficient of variation values. While the most unstable had higher values which were KSL 137, 44 and 76 among the top twenty four. Genotype KSL 21, 58, 42 and 16 were the least stable. The values were directly proportional to each other; when the variance increased the coefficient of variation also increased. The yield data show that the genotypes were very unstable, the CV_i percentage ranged from 42.93 to 98.8% which are far from the acceptable 20%. Although, lines KSL 142, 71, 144, 50, 31 44, 115 and 146 had relatively low stability.

Table 4. Area Under Disease Progress Curve (AUDPC) and Final Disease Severity means for the best twenty four genotypes in the three locations.

AUDPC					Final disease severity			
Genotype	**Lanet**	**Njoro**	**Mau- Narok**	**Means**	**Lanet**	**Njoro**	**Mau-Narok**	**Means**
KSL142	0.000	25.80	60.80	28.90	0.00	0.00	8.30	2.80
KSL71	75.00	5.800	27.50	36.10	5.00	1.70	3.30	3.30
KSL144	0.000	25.80	82.50	36.10	0.00	0.00	10.0	3.30
KSL50	11.70	0.000	110.0	40.60	5.00	0.00	15.0	6.70
KSL31	60.80	5.800	137.5	68.10	8.30	1.70	16.7	8.90
KSL44	33.30	31.70	141.7	68.90	5.00	1.70	13.0	6.50
KSL115	33.30	170.0	72.50	91.90	5.00	0.00	11.7	8.90
KSL146	76.70	63.30	165.8	101.9	8.30	3.30	11.7	7.80
KSL69	98.30	112.5	140.0	116.9	11.6	10.0	11.7	11.1
KSL76	45.00	96.70	211.7	117.8	8.30	8.30	33.3	16.7
KSL161	88.30	213.3	66.70	122.8	11.7	13.3	10.0	11.7
KSL53	94.30	152.5	165.0	137.2	13.3	5.00	21.7	13.3
KSL73	17.50	258.3	151.7	142.5	5.00	20.0	13.3	12.8
KSL54	110.0	217.5	131.7	153.1	13.3	11.7	15.0	13.3
KSL51	215.0	69.20	180.8	155.0	16.7	5.00	23.3	12.8
KSL156	167.0	130.0	169.0	155.6	16.7	11.7	16.7	15.0
KSL81	141.0	76.70	267.5	161.9	15.0	10.0	30.0	18.3
KSL137	66.70	5.800	438.3	170.3	10.0	1.70	50.0	20.1
KSL 37	88.30	245.0	204.2	179.2	11.7	15.0	28.3	18.3
KSL72	120.0	221.7	296.5	212.8	13.3	11.7	40.0	21.7
KSL52	157.5	154.2	351.7	221.2	16.7	10.0	43.3	23.3
KSL33	82.50	167.5	416.7	222.2	10.0	16.7	50.0	25.6
KSL17	120.0	171.7	385.0	225.6	13.3	23.3	46.7	27.8
KSL57	100.0	290.8	328.3	239.7	15.0	11.7	40.0	22.2
Checks								
Kingbird	280.0	295.0	177.5	250.8	23.3	8.30	13.0	14.9
Eagle 10	480.0	398.0	225.0	367.8	33.3	32.3	16.0	27.2
Korongo	698.3	686.7	395.0	593.3	53.3	28.3	53.3	45.0
Kenya Wren	530.0	745.0	623.3	632.8	50.0	53.3	70.0	57.8
Robin	875.8	970.0	1093	979.7	45.0	80.0	80.0	68.3
Means	231.95	326.87	363.18	307.33	23.9	23.3	35.7	27.6
CV%	36.22				CV%	36.212		

LSD 0.05 between locations 25.3; LSD 0.05 between locations 2.09; LSD 0.05 within locations 103.3; LSD 0.05 within locations8.513; KSL: Kenyan Selection, [a:] Local checks.

Correlation between yield, AUDPC and final disease severity

The correlation coefficient (r) for AUDPC and grain yield was found to be - 0.943, while coefficient of determination (r^2) was 0.890 (Figure 1). Similarly Final Disease Severity and yield r was -0.84 and r^2 was 0.0705 (Figure 2). The r value revealed a strong negative relationship between yield and AUDPC and also for yield and FDS within the linear model explaining 84% of the variation relationship. For the yield and FDS relationship 70.5% was explained.

DISCUSSION

Seedling stage resistance

In the seedling stage resistance 84% of the top twenty four genotypes had adequate resistance levels of 1+ and 2+ for infection types and being very resistant and moderately susceptible. Seedling resistance according to Pathan and Park (2006) by comparison, is effective at all growth stages. As suggested by GRDC, (2012) protection at the seedling stage is provided by ‘major’ or seedling

Table 5. Grain yield per plot in t/ha for the three locations and thousand kernel weights of the best performing twenty four genotypes.

Grain yield in t/ha					Thousand Kernel Weight in grams			
Genotype	**Lanet**	**Njoro**	**Mau-Narok**	**Means**	**Lanet**	**Njoro**	**Mau-Narok**	**Means**
KSL 142	0.642	2.19	0.992	1.28	0.0260	0.0263	0.0357	0.0270
KSL 71	0.537	1.01	2.46	1.33	0.0270	0.0330	0.0340	0.0269
KSL 144	0.569	1.65	1.70	1.31	0.0220	0.0287	0.0363	0.0290
KSL 50	0.570	2.19	4.63	2.46	0.0273	0.0340	0.0297	0.0303
KSL 31	0.774	2.18	4.63	2.52	0.0343	0.0320	0.0330	0.0331
KSL 44	0.625	1.93	2.96	1.84	0.0227	0.0250	0.0240	0.0290
KSL115	0.255	1.43	2.10	1.26	0.0247	0.0270	0.0343	0.0287
KSL 146	0.700	1.70	2.03	1.48	0.0290	0.0273	0.0283	0.0282
KSL 69	0.352	1.20	2.12	1.22	0.0223	0.0337	0.0180	0.0276
KSL 76	0.434	1.96	2.48	1.63	0.0230	0.0297	0.0297	0.0274
KSL 161	0.607	1.82	2.84	1.76	0.0263	0.0356	0.0193	0.0271
KSL 53	0.600	1.38	4.79	2.26	0.0223	0.0310	0.0280	0.0271
KSL 73	0.375	1.57	3.19	1.71	0.0253	0.0300	0.0260	0.0210
KSL 54	0.834	2.01	2.10	1.65	0.0247	0.0290	0.0270	0.0269
KSL 51	0.550	1.86	1.98	1.46	0.0193	0.0267	0.0330	0.0263
KSL 156	0.424	1.59	3.32	1.78	0.0243	0.0293	0.0250	0.0262
KSL 81	0.227	1.37	1.63	1.08	0.0217	0.0287	0.0270	0.0258
KSL 137	0.844	2.01	5.03	2.63	0.0260	0.0263	0.0350	0.0210
KSL 37	0.255	1.04	2.75	1.36	0.0190	0.0263	0.0313	0.0200
KSL 72	0.312	1.29	3.44	1.68	0.0200	0.0270	0.0287	0.0252
KSL 52	0.514	1.61	1.62	1.26	0.0223	0.0260	0.0267	0.0250
KSL17	0.600	1.02	3.69	1.77	0.0213	0.0277	0.0214	0.0241
KSL33	1.161	1.29	3.82	1.98	0.0207	0.0257	0.0283	0.0249
KSL57	0.290	1.39	1.74	1.14	0.0190	0.0250	0.0277	0.0239
Checks								
Korongo[a]	0.480	2.18	3.20	1.96	0.0257	0.0220	0.0183	0.0220
Kingbird[a]	0.480	0.87	3.16	1.51	0.0187	0.0247	0.0327	0.0253
Kenya wren[a]	0.485	0.25	2.79	1.45	0.0223	0.0267	0.0267	0.0240
Eagle 10[a]	1.200	1.09	2.40	1.28	0.0130	0.0313	0.0160	0.0228
Robin[a]	1.140	1.09	1.24	1.16	0.0203	0.0290	0.0230	0.0218
Means	0.514	1.27	2 .82	1.53	0.0220	0.0274	0.0255	0.0250

LSD 0.05 between locations 0.188; LSD 0.05 between locations 0.0019; LSD 0.05 within locations 0.769; LSD 0.05 within locations 0.079; KSL: Kenyan Selection, [a:] Local checks.

Table 6. Adult Host response for the genotypes across the three locations.

Genotype	Lanet	Njoro	Mau-Narok
KSL 142	R	MR	MR/MS
KSL 71	R	R	MR/MS
KSL 144	R	MR	MR/MS
KSL 50	MR	R	MR
KSL 31	MR/MS	R	MR/MS
KSL 44	MR/MS	MR	MR
KSL 115	MR/MS	R	MR/MS
KSL 146	MR/MS	MR/MS	MR/MS
KSL 69	MR	MR/MS	MS
KSL 76	MR/MS	MR/MS	MS

Table 6. Contd.

KSL 161	MR	MR	MS
KSL 53	MR/MS	MR/MS	MS
KSL 73	MR/MS	MR	MS
KSL 54	MR/MS	MR/MS	MS
KSL 51	MR/MS	MR/MS	MR
KSL 156	MR	MR/MS	MR/MS
KSL 81	MR	MR	MS
KSL 137	MR	R	MR/MS
KSL 37	MR/MS	MR	MS
KSL 72	MR/MS	MR/MS	MS
KSL 52	MS	MS	MS
KSL 33	MR/MS	MR/MS	MS
KSL 17	MR/MS	MR/MS	MS
KSL 57	MR	MR	MS
Checks			
Kingbird	MR	MR	MR/MS
Korongo	MR/MS	MS	MSS
Eagle 10	MR/MS	MR/MS	MS
Kenya Wren	MS	MR/MS	MS
Robin	MSS	MSS	S

R-Resistant, MR- Moderately Resistant, MR/MS- Moderately Resistant to Moderately Susceptible, MS- Moderately Susceptible, MSS- Moderately susceptible to Susceptible, S-Susceptible.

resistance genes, which have much larger effect and often provide complete resistance at all growth stages.

ANOVA for the four parameters AUDPC, FDS, TKW and yield

There was a highly significant genotype and location interaction for FDS and AUDPC ($P<0.001$), for yield it was only significant at $P<0.05$. As illustrated by Finlay and Wilkinson, (1963) that adaptability has proved to be of particular importance, because edaphic variation between localities and the seasonal variation in any one locality are very great. Thus the mean values for Mau-Narok were slightly high for AUDPC at 363.18 much higher than Lanet but comparable to Njoro at 231.97 and 326.57 respectively. Genotype KSL 142, 71, 144, 50, 31 and 44 showed resistance to stem rust disease across the three locations. At Mau-Narok all the genotypes had high disease severity levels.

Grain yield mean for the three locations also had variations with Mau-Narok at 2.82 t/ha, Njoro 1.27 t/ha and Lanet 0.514 t/ha (Table 5). Mohammadi, et al. (2012) established that grain yield in wheat is frequently the sink limited, and for this reason, the 1000 kernel weight has been reported as a promising trait for increasing grain yield in wheat under different conditions. The TKW showed less variation among the genotypes except for location. The grain yield values showed consistency with the genotypes performance across the locations. From the ANOVA the grain yield data identified KSL 137 at 2.63 t ha^{-1}, KSL 31 2.52 t ha^{-1}, KSL 50 2.46 t ha^{-1} and KSL 53 2.63 t ha^{-1} as the best performing across the three locations. The AUDPC was expressed in %-days (accumulation of daily percent infection values) and interpreted directly without transformation. The higher the AUDPC, the more susceptible was the genotype as verified by Ali et al. (2012). There was also a correspondence between genotype susceptibility and AUDPC showing that the most susceptible recorded higher AUDPC values.

Genotype by environment (location) interaction for the three locations

There were variations among the three locations which revealed the genotypes KSL 137, 54, 31, 146, 44, 161, 17 and KSL 53 having good grain yield performance in Lanet. Genotypes KSL 142, 50, 31, 54, 137, 76, 44, 51, 161 and 146 performed well in Njoro. Genotype KSL 137, 50, 31, 44, 53, 33, 17, 156, 72 and 161 were the best performing in Mau-Narok. As stated by Yan (2002) that the measured yield of each cultivar in each test

Table 7. Coefficient of Variation (CV_i) and variance (S_i^2) for the top twenty four genotypes based on the FDS values and yield.

Genotype	FDS S_i^2	FDSCV_i	YieldS_i^2	YieldCV_i
KSL142	3.630	31.10	0.664	63.86
KSL71	2.730	49.50	0.998	74.90
KSL 144	8.300	43.50	0.409	48.90
KSL50	39.96	81.10	4.160	82.80
KSL31	56.52	84.20	3.750	77.20
KSL44	35.70	89.60	1.370	63.60
KSL115	12.13	38.20	0.873	74.10
KSL146	17.32	55.00	0.478	46.80
KSL69	0.963	8.800	0.778	72.20
KSL76	108.3	86.57	1.130	65.50
KSL161	2.860	14.90	1.250	63.78
KSL53	69.70	62.60	4.990	98.85
KSL73	56.50	58.74	2.008	82.76
KSL54	2.730	12.38	0.052	42.93
KSL51	85.89	62 .00	0.626	54.17
KSL156	7.330	18.24	2.120	82.08
KSL81	108.3	56.70	0.556	69.37
KSL137	433.3	96.70	4.658	82.16
KSL37	166.5	70.36	0.873	74.10
KSL72	252.7	73.38	2.550	95.20
KSL52	310.2	75.42	0.404	50.94
KSL33	458.9	83.70	2.550	80.80
KSL17	371.9	78.99	2.821	98.80
KSL57	239.5	69.71	0.573	66.37
checks				
Kingbird[a]	58.30	50.00	2.696	96.20
Eagle10[a]	91.85	34.50	1.160	84.30
Korongo[a]	203.3	32.00	1.887	70.34
Kenya Wren[a]	114.9	18.50	1.420	82.30
Robin[a]	408.3	29.56	1.420	6.730

KSL; Kenyan selection, FDS: Final Disease Severity, $S_i^{2:}$: Variance, CV_i: Coefficient of Variation, KSL: Kenyan Selection, [a:] Local checks.

environment is a mixture of environment main effect (E) genotype main effect (G) and genotype and environment (GE).

The TKW values were related to yield as the same genotypes tended to have a slightly higher weight than the ones with low yields for example genotypes KSL 50, 31, 44, 115, 144, 142, 146, 69 and 76 although not applicable to a few of the high yielders such as KSL 137. According to Yan (2002) that typically E explains most (up to 80% or higher) of the total yield variation and G and GE are usually small. The environments showed that wheat grain yield was significantly affected by environment as in the case of Mau-Narok reporting greater grain yields. Mohamed (2013) added that the large yield variation explained by environments indicated that the environments were diverse, with large differences between environmental means contributing most of the variation in grain yield.

Seedling and adult stage resistance of the genotypes

Seedling and adult stage resistance genes as explained by Morgounov et al. (2010) in wheat fall under two broad categories and are referred to as seedling and adult plant resistance (APR) genes. Seedling resistance genes are detected during both the seedling and adult plant stages and as such constitute an all stage resistance phenotype. APR is commonly detected at the post-seedling stage and often as field resistance.

Therefore the genotypes that had seedling stage reflected well with resistance in the field. The genotypes

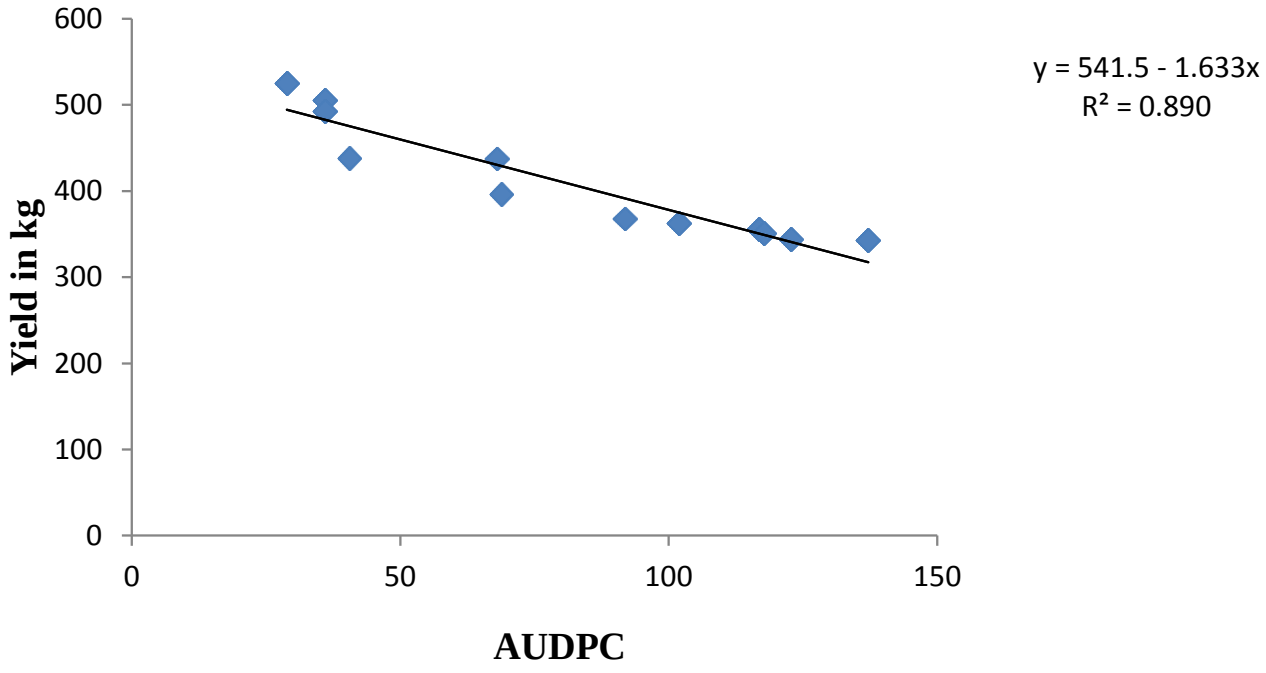

Figure 1. Relationship between AUDPC and genotype yield in the three locations of Mau-Narok, Njoro and Lanet.

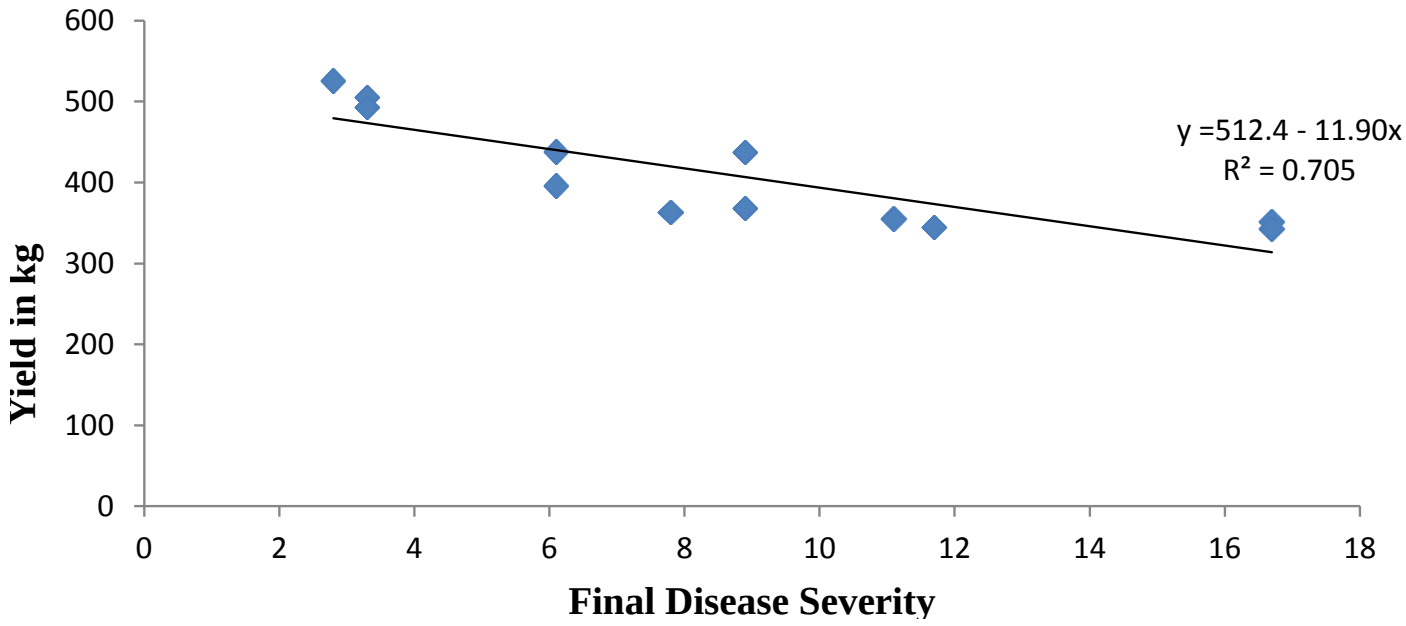

Figure 2. Relationship between Final Disease Severity and genotype yield in the three locations of Mau-Narok, Njoro and Lanet.

that posed both seedling and adult stage resistance were KSL 144 (2+), 50 (1+), 31 (1+), 44 (1+), 115 (2+), 146 (2+), 69 (2+) and 76 (2+) based on the AUDPC and Final Disease Severity values. According to Wang et al. (2005) all genotypes with APR showed lower values for AUDPC than susceptible cultivars. Apparently most of the best performing genotypes were pedigrees of already released varieties such as Kenya Nyangumi, Kongoni, Kwale, Zabadi, Mbuni, Paka and NjoroBWII. There is therefore need to improve on already released varieties

for trends have shown that the agronomic performance is superior. Wang et al. (2005) explained that the adult plant resistance (APR) is of major importance in breeding for an efficient genetic control strategy and added that it is possible to combine major resistance genes and APR genes to achieve durable resistance.

Adult plant host response of the genotypes to stem rust in the three locations

In Lanet 12.5% of the genotypes showed resistance to stem rust, 29.2% were moderately resistant, 54.2% were between being moderately resistant and moderately susceptible and 4% had a moderately susceptible reaction. In Njoro the genotypes with resistance were 20.8%, moderately resistant, 33.3%, moderately resistant to moderately susceptible 41.7% and moderately susceptible 4.2%. In Mau-Narok there were no genotypes showing resistance, 12.5% showed a moderately resistant reaction, 33.3% had moderately resistant to moderately susceptible and 54.2% had moderately susceptibility. The implication of host response across the locations is that there were less than 15% of the genotypes with resistance. There was a tendency where genotypes with resistance or moderately resistance in Lanet and Njoro having good yield performance across the locations such as KSL 137, 31, 33 and 50.

The relationship between FDS and genotype yield in the three locations

There was heavy disease pressure evidenced by 90% FDS values on the spreader rows and genotype Robin especially in Mau-Narok and proved by Singh et al. (2008) and Singh et al. (2011). The spreader rows of *Sr 24* susceptible genotypes had the highest Final Disease Severity of 90% which implies that the races were mainly *TTKST* and *TTKSK.* Mau-Narok had many Ureniospores expressed on the crop and progressed at a faster rate than the two locations of Njoro and Lanet. Mau-Narok had the genotypes KSL 137, 53, 50, 31, 33, 17, 156, 161, 72 and KSL 44 which reported good performance in grain yield. The genotypes KSL 137, 33, 17 and 72 had FDS values ranging from 40 -50% showing that despite high disease pressure the grain yield was good. The grain yield ranged from 5.03 to 3.44 t ha^{-1} which outperformed the other genotypes. The genotypes therefore may be used in breeding purposes or released as varieties with good stem rust management the grain yields may increase. The genotype interacted well with the environment. In Njoro genotypes KSL 142, 50, 31, 54, 137, 44, 51 and KSL 146 reported good grain yield ranging from 2.19 to 1.70 t ha^{-1} with FDS values ranging from 0 - 5%, there was a clear manner which showed that the genotypes with low FDS values reported high grain yields. In Lanet the same case occurred where KSL 33, 137, 54, 31, 146 and 142 had grain yield ranging from 1.161 to 0.642 t ha^{-1} and FDS value from 0 to 13.3%.

Correlation coefficient (r) and coefficient of determination (r^2) for AUDPC and yield, FDS and yield

In the study stem rust severity and yield relationship was explained by the negative and high correlation coefficient (r=-0.943) for AUDPC and yield (Figure 1). The Final disease Severity and yield was at (r=-0.839) (Figure 2) also having a strong negative relationship, Jeger (2004) explained that even where disease resistance is a major target in breeding programs, the effect on yield and productivity is an important trait, thus the additional value of the relationship between AUDPC and yield components. There is strong evidence from the study that grain yield loss and stem rust disease are highly associated. The coefficient of determination (r^2) was based on the amount of variability in one variable (yield) that was explained by the linear function of the other variable (AUDPC). The same case applied to FDS and yield by Gomez and Gomez, (1984). The correlation values for AUDPC and Final Disease Severity signify that yield losses increased under disease presence in a progressive manner.

Coefficient of variation (CV_i) and variance (S_i) for AUDPC and yield and final disease severity and yield

The coefficient of variation (CV_i) was used to determine stability for FDS and yield among the genotypes, from Yan (2002) visualization of the genotype stability is always an important issue in cultivar evaluation. For FDS KSL 69 (8.8%) 54 (12.38%), 161 (14.9%) and 156 (18.24%) were identified as the most stable with less than 20% CV_i from Lin et al. (1986) and the most unstable were KSL 137 (96.7%) 44 (89%) and KSL 76 (86.57%) among the top twenty four genotypes. While using the yield data to identify stability most of the genotypes were unstable.

Conclusion

The parameters used were adequate enough to distinguish resistant/susceptible, stable/unstable high yielding/low yielding genotypes where stem rust disease occurred. The genotypes KSL 161, 73 and KSL 156 were consistent in performance for the seedling, adult stage, yield, FDS stability and thousand kernel weight performances as the best. The genotypes KSL 137, 50, 161, 31, 44, 53, 33 and KSL 73 had overall performance for the seedling, adult stage, yield and thousand kernel

weight performances except for FDS and yield stability across the three locations. The genotypes KSL 156, 72, 52 and KSL 57 performed well in Njoro and Mau-Narok. In Mau-Narok genotypes KSL 137, 72, 17 and 33 performed well. The same genotypes expressed resistance or moderately resistance host response therefore superior on grain yield. The genotypes should be recommended for production or used for improving the already existing varieties. The results confirm that stem rust disease pressure was high and also caused grain yield loss. These suggest that wheat production in Kenya has to be done with effective management options available for stem rust, which may also be applicable in the Eastern Africa region. Management options should be maximized which may include a holistic approach such as an integrated disease management. To identify genotypes with yield stability more work needs to be done to identify the ones with wide adaptability across all major growing locations.

Conflict of Interests

The authors have not declared any conflict of interests.

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Correlation and path co-efficient analysis for grain quality traits in F_1 generation of rice *(Oryza sativa* L.)

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A study on correlation and path co-efficient analysis was conducted on eleven F_1 generation derived from crosses between aromatic and non-aromatic parental landraces genotypes. The results showed that high standard deviation was observed on head rice recovery (16.12) followed by gel consistence (13.81) and milling recovery (10.74). The results also showed that correlation between brown rice length and paddy grain width (r= 0.692); paddy grain length and brown grain length (r = 0.558); head rice recovery and milling recovery (r = 0.511) and brown rice shape and grain rice length (r = 0.404) were positive highly significant, while correlation between brown rice shape and brown rice length were negative (r = -0.497). These highly correlated characters could be used for indirect selection and improvement of grain rice quality. The path co-efficient analyses revealed a low direct and positive effect for paddy grain length on brown grain length (0.009). The direct effect of brown rice width (2.774) and brown grain shape (2.481) on brown grain length was high and positive showing that these characters have a direct effect and influence on brown grain length thus indicating their importance in grain rice quality improvement.

Key words: Rice, correlation, path co-efficient analysis, F_1 generation, quality, aroma.

INTRODUCTION

Rice *(O. sativa* L*)* is one of the most important cereal crops for human consumption. It feeds billions of people around the world, and more especially in less developed countries in Asia, Latin America and Africa. Rice is one of most important crop in Mozambique, being the fourth most consumed crop in the country after cassava, maize and wheat (Ministry of Agriculture, 2013). It is the second most consumed crop in Malawi after maize (Magreta et al., 2013). According to Abade et al. (2016), Kilombero, Faya, and Nunkile are the most aromatic landraces that are produced and have high marketability value in Malawi. However the low yield, and longer maturity time contribute to less production. Mozambique has a great diversity of local genotypes of rice, mostly concentrated in the provinces of Zambézia, Sofala and Nampula. However, there is a lack of information on grain quality characteristics for the rice germplasm in the country and most of them are non-aromatic (Ministry of Agriculture, 2013).

Rice landraces are the groups of lineages that originated and evolved in the field over millennia through selective breeding by generations of farmers, who chose

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random mutants and gene combinations in domesticated rice, for better yield, grain size and other agronomic or cultural values (Ray et al., 2013). According to this author, those landraces, carry appreciable genetic information on their genome that can be exploited for developing new varieties with desirable characteristics for grain quality. Selection of promising genotypes, in a breeding program, is based on various criteria, most importantly final crop yield and its quality (Kiani and Nematzadeh, 2012).

The persistence of rice farms growing local landraces with low yield over the improved high yielding varieties has been attributed to their adaptability to environmental conditions, resistance to pests and diseases, grain quality, test, aroma and also marketability, which are not present in many cases in the improved varieties (Dey, 2009). This phenomena presents a special challenge for plant breeders in such a way that they have to take in consideration all of those aspect in order to develop a variety that fulfills the needs of the farmers as well as the consumers. Grain quality in rice, is defined in two different criteria: the millers' basis of quality is dependent upon total recovery and the proportion of head and broken rice on milling; While the consumers base their concept of quality on the grain appearance, size and shape of the grain, the behavior upon cooking, the taste, tenderness, flavor of cooked rice and today the nutritional value (Khush and Cruz, 2000).

Grain quality is an important character to study not only because of its contribution to yield, but also because of its influence in rice marketing and trade (Prem et al., 2010). Grain weight and grain shape (length, width, length/width ratio) are positively correlated characters (Anandakumar et al., 2015). Studies on grain quality characters have shown different results concerning its genetic control. Nevertheless it's possible to study rice grain quality in first generation of crosses as were proposed by Yan and De-lin (2004).

Most of the high-quality preferred varieties in major rice growing countries are aromatic (Khush and Cruz, 2000). Thus, introgression of this character into varieties with aroma absent is a quit difficult because of that genetics of the aroma characteristic is somewhat complex has been associated with the presence of 2-acetyl-1-pyrroline (Bradbury et al., 2005). Although many other compounds are also found in the headspace of fragrant rice varieties possibly due to secondary effects related to the genetic background of the rice variety, 2-acetyl-1-pyrroline is widely known to be the main cause of the distinctive basmati and jasmine fragrance (Khush and Cruz 2000). The desirability of fragrance has resulted in strong human preference and selection for this trait. Non- fragrant rice varieties contain very low levels of 2-acetyl-1- pyrroline, while the levels in fragrant genotypes are much (Khush and Cruz, 2000; Jewel et al., 2011).

The data obtained from correlation coefficient can be augmented by path analysis. Path coefficient analysis splits the genotypic correlation coefficient into the measure of direct and indirect effects (Train *et al.,* 1999). In rice, those analyses can be performed in order to assess the inheritance of earlier generation of crosses with their parents. Because there was no report about breeding program involving these parental genotypes in Mozambique and Malawi, and the preferences of aromatic rice is high than the non-aromatic, there was a need to produce crosses and evaluate then in order to generate enough information for their use in the next breeding program for quality improvement, that is why the main objective of this study was to determine the correlation and path co-efficient analysis for grain quality character improvement on first generation of rice *(Oryza sativa* L) derived from aromatic and non-aromatic landraces genotypes from Mozambique and Malawi under irrigation.

MATERIALS AND METHODS

Three non- aromatic parental landraces genotypes brought from Mozambique namely (Marista Djissa, Chibiça, and three aromatic genotypes (Faya, Kilombero and Nunkile) from Malawi were planted and crossed with each other under field and greenhouse conditions to produce sufficient F_1 generation seeds for the trial during December, 2014 to May, 2015. The field crossing blocks were established in Lifuwo Rice Research Station and the greenhouse crossing blocks were established at Bunda College Campus (Lilongwe). Eleven (11) crosses involving aromatic versus non-aromatic landraces genotypes, namely Nunkile x Chibiça, Chibiça x Nunkile, Nunkile x Marista, Kilombero x Djissa, Nunkile x Djissa, Kilombero x Chibiça, Kilombero x Marista, Djissa x Nunkile, Faya x Marista, Djissa x Kilombero, and Chibiça x Kilombero which were selected for the field trial.

Field trial site description

The trial was conducted at Lifuwu Rice Research Station in Salima-Malawi; is situated at the foot of the Lifuwu hill and is part of the expansive and seasonally flooded Katete dambo. The station is located at an altitude of 513 meters above sea level (masl) with coordinates sited on Latitude 13°40' South and Longitude 34°35' East. Paddy fields are predominantly clay soils types characterized by low Nitrogen and Phosphorus content, with pH 7 to 8 and annual average rainfall of 1,200 mm. Mean minimum and maximum temperatures are 19°C and 29°C, respectively. Between May and August, absolute air temperatures may drop to as low as 16°C, and because of that the growth rate of dry season irrigated rice is slowed and also, prolongs the growth duration of rice varieties by 10 to 30 days depending on variety and other weather parameters.

Experimental design, field layout and crop management

In the winter season, on 24th June, 2015, the eleven (11) F_1 generation and their six (6) parent were sowed on the petri-dishes after surface-sterilization as recommended by Coffman and Herrera (1980). Soon after germination and before the roots entangled, the seedlings were transferred to the pots while the parents were directly planted in the pots as nursery. Thirty five (35) days old plants were transplanted in the field trials in a completely randomized block design with 3 replicates under irrigation.

Table 1. Descriptive statistics for grain quality in 11 F_1 rice generation.

Characters	Range	Mean	Std. deviation	Coefficient of variation (%)
GrL	2.80	9.52	0.65	6.84
Grw	1.40	3.09	0.23	7.49
BrL	2.90	6.45	0.68	10.50
BRw	1.70	2.67	0.36	13.54
BrS	1.80	2.44	0.41	16.90
MR	51.00	62.66	10.74	17.13
HRR	75.90	71.18	16.12	22.65
GTr	6.00	4.22	2.50	59.31
Ass	3.00	2.25	1.00	44.43
GCs	62.00	145.67	13.81	9.48

Grw: Paddy grain width (mm); BRw: Brown rice width; BrS: brown rice shape; GrL: paddy grain length; HRR: Head rice recovery; MR: milling recovery; GTr: Gelatinization temperature; Ass: Aroma sense and BrL: Brown grain length, GCs: gel consistence.

Single plant per hill were transplanted in 3 rows with 5 plants per row in total and only the middle plants were considered for that collection as described by Kiani and Nematzadeh (2012). The first line was planted with the female parent genotypes; the second line was planted with the F_1 derived from the cross between the two parents and the last line was planted with the male parent spaced by 25 cm x 25 cm (Shanthala et al., 2005). The plot size was 0.94 m^2 with a total of eleven (11) plots per replication. The block size was 10.34 m^2 and a total trial area of 31.02 m^2. NPK (12-24-12) compound fertilizer was applied at a rate of 100 Kg/ha at transplanting time and 60 Kg of Urea 46% as topdressing at 45 days after sowing and at 60 days after sowing respectively. The harvesting time varied from middle October to middle November of 2015 and the laboratory analyses were conducted three months later (February, 2016).

Data collection on paddy grain length GrL (mm), paddy grain width Grw (mm), brown rice length BrL (mm), brown rice width BRw (mm), brown rice shape BrS (mm), percentage of milling recovery (MR), percentage of head rice recovery (HRR), gelatinization temperature/alkali spread GTr (mm) and aroma sense (Ass) were recorded according to Yan and De-lin (2004).

Samples were dried for 4 h under the sun and stored for the sometime at room temperature, rough rice was processed to brown rice and milled rice using a conventional rice miller machine. Paddy grain length and grain width, brown rice length, brown rice width, brown rice shape, of head rice was measured using caliper with the precision of 0.02 mm as described by Khush and Cruz (2000).

For each trait of every F_1 generation, 10 grains were measured in three replicates. Percentage of milling recovery (MR) and percentage of head rice recovery (HRR) were accessed following the procedure developed by International Rice Research Institute (IRRI) and used by Khush and Cruz (2000). Gelatinization temperature (alkali spread value, ASV) were measured and graded while gel consistency was measured and classified according to the method as used by Rafii et al. (2014). The Amylose content was determined according to Hu et al. (2010).

For the aroma sense assessment, 40 milled grains for each sample were soak with 10 ml 1.7% KOH solution in a glass Petri-dishes for 1 h. The samples were scored on a scale of 1 to 4 where 1,2,3 and 4 corresponding to absence of aroma, slight aroma, moderate aroma and strong aroma as recommended by IRRI (2007). The test for aroma was assessed by a rating panel of eight people selected and trained for their ability to differentiate between the smelling of the genotypes with aroma and the ones with do not have aroma by smelling and chewing the grains, this methodology was used by Khush and Cruz (2000). Statistical package for social sciences (SPSS) 20th edition was used for statistical analyses at 95% of the probability.

Data analysis

Correlation coefficients were calculated for all the characters and the path coefficient analysis splits the genotypic correlation coefficient into direct and indirect effect according to Bhati et al. (2015). The path coefficient analysis was estimated by the formula below used by (Yakubu, 2010).

$P_{YXi} = b_i(S_{xi}/S_y)$; Where: $P_{y.xi}$ = path coefficient from Xi to Y (i= Grw, BR, BrS, GrL, HRR, GTr, Ass); b_i = regression coefficient; S_{xi}= standard deviation of Xi; S_y= standard deviation of Y.

RESULTS AND DISCUSSION

It's important to have high genetic variability in crops for particular traits in order to achieve successful plant breeding programs (Kiani and Nematzadeh, 2012). Estimates for range, mean, standard deviation and coefficient of variation (CV) for selected F_1 generation evaluated are shown in Table 1. The maximum standard deviation was observed on head rice recovery (16.12) followed by gel consistence (13.81) and milling recovery (10.74). Among the grain quality traits, gelatinization temperature measured as alkali dispersion, aroma sense, head rice recovery, milling recovery and brown rice shape with the CVs of 59.31, 44.43, 22.65, 17.13 and 16.90 percent had more phenotypic variation, respectively.

The paddy grain rice length (6.84%), paddy grain width (7.49%), gel consistence (9.48), paddy grain length (10.50%) and brown rice width (13.54%) had less variation. Plant breeder uses selection for improving of traits of interest of crop by management of available genetic variability and landraces are known to have larger range of variability (Shanthala et al., 2005; Kiani and

Table 2. Correlation coefficients recorded among various rice grain quality traits.

Variable	Grw	BRw	BrS	GrL	HRR	MR	GTr	Ass	BrL
Grw	1.000	-	-	-	-	-	-	-	-
BRw	0.692**	1.000	-	-	-	-	-	-	-
BrS	-0.497**	-0.787**	1.000	-	-	-	-	-	-
GrL	-0.100	-0.080	0.404**	1.000	-	-	-	-	-
HRR	0.040	0.122	-0.080	0.000	1.000	-	-	-	-
MR	0.046	0.238	-0.104	-0.065	0.511**	1.000	-	-	-
GTr	-0.205	-0.068	0.116	0.225	0.212	0.109	1.000	-	-
Ass	-0.278*	-0.171	0.094	0.087	-0.052	0.004	0.157	1.000	-
BrL	0.036	0.116	0.476**	0.558**	-0.009	0.095	0.241	0.001	1

**Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed). Grw: Paddy grain width; BRw: Brown rice width; BrS: brown rice shape; GrL: paddy grain length; HRR: Head rice recovery; MR: milling recovery; GTr: Gelatinization temperature; Ass: Aroma sense and BrL: Brown grain length.

Nematzadeh 2012). This study reveals the possibility of effective selection for improvement of rice grain quality in subsequent segregating populations for these landraces genotypes.

Correlation and path coefficient analysis for grain quality and aroma

The analyses of correlation for grain quality including aroma sense (Table 2), showed strong and highly significant positive correlation between brown rice width and paddy grain width (r = 0.692**). There was a highly significant and negative, but strong correlation between brown rice shape and paddy rice width (r= -0.497**) while for aroma sense the correlation was statistically significant and negative, although weak (r= -0.278*). The strong and positive correlation observed between brown rice width and paddy grain width reveals that an increase on the grain width leads to 69.2% increase on paddy grain width and vice-versa. The negative relationship between brown rice shape and paddy grain width means that an increase of rice shape leads to a decrease of 49.7% in grain width and vice-versa. The results elsewhere shows that an increase in 27.8% of width of the brown rice can decrease the aroma scent by 27.8% (Anandakumar et al., 2015).

The brown rice length was statistically high and negatively but strong correlated with brown rice shape (-0.787**). There was no correlation between brown rice length and brown rice width, paddy grain length, head rice recovery, milling recovery, gelatinization temperature and aroma sense. There was a statistically highly significant positive correlation between the brown rice length and brown rice shape (0.476**). A positive correlation between these two character were also reported by Golam et al. (2014)**.** The paddy grain length was strongly and positively correlated with brown rice length (r = 0.558**). There was no significant correlation between paddy grains lengths and 8 other character evaluated in this study. Brown rice shape statistical highly significant correlated positively with paddy grain length (r = 0.404**). There were no correlations between the brown rice shape and head rice recovery, milling rice recovery, gelatinization temperature and aroma sense.

Head rice recovery was highly and positively correlated with milling rice recovery (r = 0.511**), and there were no relationship between head rice recovery and other characters evaluated. The aroma sense correlated negatively with grain width (r = - 0.278*). Jewel et al. (2011) reported that aroma had significant and positive relationship with paddy grain length-width ratio; significant and negative association with grain width, significant and negative association with gelatinization temperature, and no significant association with grain length.

Regression and path co-efficient analysis

The regression anova (Table 3) showed a highly statistically significant regression model (p=0.000). The brown rice length was considered as resultant variable (dependent) while paddy grain width, brown rice width, brown rice shape, paddy grain length, head rice recovery, milling recovery, gelatinization temperature and aroma sense were considered as casual (independent) variables and the model mean square was equal to 2. 619. The model summary provide the values of R = 0.956; coefficient of multiple determination (R^2 = 0.915) and the standard error of the estimate was 0.215.

The high R^2 obtained on the regression model indicates that it explains all the variability of the response variable are around the mean. In other ways the variation on the characters was more likely due to genetic makeup rather than environmental influences.

The regression analysis was used to determine the direct effect of the characters that contribute to brown grain length. The B values presented in Table 4, are the

Table 3. Analysis of variance for the regression model.

Sources of variance	df	Sum squares	Mean square	F	Sig.
Regression	8	20.950	2.619	56.202	0.000[b]
Residual	42	1.957	0.047	-	-
Total	50	22.907	-	-	-

adependent variable: BrL: brown grain length.

Table 4. Regression co-efficient (B), standard error and P values for the brown rice length (BrL).

Variable	B	Std. Error	Sig.
(Constant)	-5.091	0.762	0.000
Grw	-0.563	0.197	0.007
BRw	2.774	0.198	0.000
BrS	2.481	0.152	0.000
GrL	0.009	0.060	0.885
HRR	-0.002	0.002	0.413
MR	-0.005	0.004	0.144
GTr	0.037	0.013	0.007
Ass	0.023	0.032	0.478

Grw: Paddy grain width; BRw: Brown rice width; BrS: brown rice shape; GrL: paddy grain length; HRR: Head rice recovery; MR: milling recovery; GTr: Gelatinization temperature; Ass: Aroma sense and BrL: Brown grain length.

Table 5. Direct (diagonal bolded) and indirect effect of studied grain quality traits on brown grain length in eleven F_{1s} and their six parents**.**

Variable	Grw	BRw	BrS	GrL	HRR	MR	GTr	Ass	R
Grw	**-0.563**	0.443	-0.227	0.000	-0.288	-0.061	-0.089	-0.001	0.036
BRw	-0.390	**2.774**	1.121	0.001	1.417	0.302	0.436	0.668	0.116
BrS	0.280	-2.183	**2.481**	0.001	1.268	0.270	0.390	0.235	0.476
GrL	0.056	-0.223	-0.199	**0.009**	0.004	0.001	0.001	0.000	0.558
HRR	-0.023	0.337	-0.258	0.000	**-0.002**	0.000	0.000	-0.001	-0.009
MR	-0.026	0.661	-0.258	-0.001	-0.001	**-0.005**	-0.001	-0.003	0.095
GTr	0.115	-0.188	0.287	0.002	0.000	-0.001	**0.037**	0.003	0.241
Ass	0.157	-0.474	0.233	0.001	0.000	0.000	0.006	**0.023**	0.001

Grw: Paddy grain width; BRw: Brown rice width; BrS: brown rice shape; GrL: paddy grain length; HRR: Head rice recovery; MR: milling recovery; GTr: Gelatinization temperature; Ass: Aroma sense and BrL: Brown grain length; The bold values represent the direct effect for the brown grain length.

regression co-efficient that indicate the direct effect character on the path co-efficient analysis. The results on path co-efficient analysis showed direct and indirect effect on brown grain length for 8 grain quality characters (Table 5). The results are as followed:

Paddy grain length (GrL)

There was a positive direct effect of paddy grain length on brown grain length, although very low (0.009). The indirect effect of paddy grain length was detected for paddy grain width (0.056), head rice recovery (0.004), milling recovery (0.001), and gelatinization temperature (0.001). There was no indirect effect of aroma sense (0.000) for GrL. The negative indirect effect for the GrL was reflected by brown rice width (-0.223) and brown rice shape (-0.199).

This results shows that selecting for paddy grain length could use indirect characters such as paddy grain width, head recovery, milling recovery and gelatinization temperature. The path analysis shows that aroma sense character does not have any influence on paddy grain length. Brown rice width and brown rice shape have a

negative indirect influence of selection for improvement of paddy grain length. The results were not in agreement with those of (Anandakumar *et al*. 2015) and Nandan and Singh, (2010), who found high positive direct effect on the paddy grain length.

Paddy grain width

There was a negative direct effect of paddy grain width (-0.563). The indirect effect was high and positive only for brown rice width (0.443) and no indirect effect were found for paddy grain length. The indirect and negative effect was observed on the brown rice shape, head rice recovery, milling rice recovery, gelatinization temperature and aroma sense (Table 5). These results suggest that brown grain length could be utilized for indirect selection and improvement. This results was similar to those of (Nandan and Singh, 2010), but contradicted with findings by Sedeghi, (2011) who found a positive direct effect of paddy grain width on paddy grain length.

Brown rice width

The direct effect of brown rice width on brown rice length was very high and positive (2.774), and the indirect effect was negative for paddy grain width (-0.390). There was high indirect effect of brown rice width thru head rice recovery (1.417), milling rice recovery (0.302), and gelatinization temperature (0.436) and aroma sense (0.668). The paddy grain length indirect effect was positive but negligible (0.001). These results suggest that direct selection for brown rice length could be made by directly selecting for brown rice width and also indirectly through brown rice shape, head rice recovery, milling recovery, gelatinization temperature and aroma sense. Kumar et al. (2016) reported high positive direct effect for brown rice width and other grain quality character studied and concluded that this is one of the most important contributing character toward brown rice length.

Brown rice shape

There was a high and direct positive effect of grain rice shape (2.481) on brown rice length. The indirect effect was high but negative for brown rice width, (-2.183) only. The indirect effect was high and positive for paddy rice width (0.280), head rice recovery (1.268), milling recovery (0.270), gelatinization temperature (0.390) and aroma sense (0.235), however low for paddy grain length (0.001). These results suggest that selection in earlier breeding material through the brown rice shape can have positive improvement on brown rice length directly. The same could be indirectly done through paddy grain width, paddy grain length, head rice recovery, milling rice recovery, gelatinization temperature, and also aroma sense. The results are in agreement with findings by Kumar et al., (2016).

Head rice recovery

The direct effect of head rice recovery on brown rice length was lower and negative (-0.002). The indirect effect was high and positive for brown rice width (0.337). There was no indirect effect for paddy grain length (0.000), milling recovery (0.000) and gelatinization temperature (0.000). The indirect effect was high and negative for brown rice shape (-0.258) and negative and low for paddy rice width (-0.023) and aroma sense (-0.001). These results show that selection of brown grain length through head rice recovery is not possible because the direct effect and indirect effect are negative and largely insignificant. Results do not agree with those of Kumar et al. (2016) but are supported by findings from Vanisree et al. (2013).

Milling rice recovery

The direct effect of milling rice recovery was negative and negligible (-0.005). The indirect effect was high and positive for brown rice width (0.661) and negative for brown rice shape (-0.258). Other characters had negative and very low indirect effect. These results show that selection of brown rice length trough milling recovery has no direct effect but indirectly could be done through brown rice width. The same could be achieved through selection of brown rice shape. Vanisree *et al*. (2013) evaluated for brown rice length and Brown rice width and the results were high and positive for direct effect.

Gelatinization temperature

There was a low and positive direct effect on gelatinization temperature (0.037) on brown rice length. The indirect effect was high and positive for brown rice shape (0.287) and paddy rice width (0.115). A lower positive indirect effect was recorded for brown rice length via paddy grain length (0.002) and aroma sense (0.003). Head rice recovery showed no effect (0.000). The high indirect and negative effect was recorded only for brown rice width (-0.188). Verma et al. (2014) reported differences on the grain quality characters for the study done to evaluate aromatic short grain rice cultivars and elite lines for yield and quality character.

Aroma sense

The direct effect of aroma sense over brown rice length was positive (0.023) although lower and insignificant. The

high indirect effect was observed on the paddy grain width (0.157) and brown rice shape (0.233). There was a high and negative effect of brown rice width (-0.474) for brown rice length selection. Verma et al. (2014) found similar results in evaluating grain quality character on aromatic short grain rice cultivars.

Conclusions

Estimates for range, mean, standard deviation and coefficient of variation (CV) for selected F_1 generation evaluated were good enough, revealing the possibility of effective selection for improvement of rice grain quality in subsequent segregating populations. The correlation and path-coefficient analysis for grain quality and aroma show that improvement of these two important rice quality characters could be done through selection starting from earlier generations.

There was a strong and positive correlation between brown rice width and paddy grain width; brown rice shape and paddy grain length; paddy grain length and brown grain length; brown rice shape and brown grain length; and also head rice recovery and milling recovery concluding that there is a cause and effect on those characters. There was a negative and strong correlation between brown rice shape and brown rice width; brown rice width and brown rice shape and also aroma sense and paddy grain width. These results show that the variation recorded on the characters under evaluation was due to genetic makeup of the genotypes used for crossing program rather than the environmental effect.

This conclusion is also supported by the highly statistically significant result obtained on the regression model. The path co-efficient analyses revealed a low direct positive effect for paddy grain length through brown grain length. The direct effect of brown rice width, brown grain shape through brown grain length was high and positive. Selecting for paddy grain length could be done using indirect characters such as paddy grain width, head recovery, milling recovery and gelatinization temperature. Aroma sense character does not have any influence on paddy grain length. Brown rice width and brown rice shape have a negative indirect influence of selection for improvement of paddy grain length showing that these characters have a direct effect and influence on brown grain length indicating their importance in grain rice quality improvement.

Conflict of interests

The authors have not declared any conflict of interests.

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