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Evaluation of advanced wheat (*Triticum aestivum* L.) lines for stem rust (*Puccinia graminis* f. sp. *tritici*) resistance and yield

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Abstract

Stem rust disease caused by *Puccinia graminis* f. sp *tritici* is a major challenge to wheat (*Triticum aestivum*) production in Kenya and other wheat growing countries of Africa and Asia. The current study aimed at evaluating sixty four wheat genotypes for stem rust resistance at seedling stage in a greenhouse; as well assessing the genotypes for stability in adult plant resistance to stem rust and yield across three sites in Kenya in an alpha lattice design with three replications. Seedling disease Infection Type (IT) ranged from "0" (immune) to "4" (susceptible), while adult plant infection assessed by disease Coefficient of Infection (CI) and Area Under Disease Progress Stairs (AUDPS) ranged from means of 0.2 to 1.7 and 30.2 to 1174.2, respectively. Mean grain yield ranged from 2.0 to 7.8 t ha^{-1}. Genotype, location and genotype × location interaction for the AUDPS, CI, and yield were significant ($P \leq 0.01$). There was a significant ($P \leq 0.01$) linear and inverse relation of grain yield to AUDPS and CI. Considering the disease response, yield potential, and yield stability, genotypes KSL 42 and KSL 3 were consistently well ranked. These genotypes are suitable candidates for utilization in yield and stem rust resistance improvement programs in Kenya and potentially in other major wheat growing areas in Kenya and potentially in other major wheat growing areas globally, where stem rust is significant.

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Introduction

Stem rust caused by *Puccinia graminis* f.sp *tritici* is a devastating disease of Wheat (*Triticum aestivum* L.) and has historically constrained wheat production in Kenya as well as other wheat growing countries of Africa and Asia (Admassu *et al.*, 2008; Njau *et al.*, 2009; Singh *et al.*, 2006; 2008; Wanyera *et al.*, 2006). Currently, yield losses associated with stem rust under on farm conditions in Kenya is about 70% but up to 100% yield loss can be recorded under severe disease condition (Wanyera *et al.*, 2008). Although wheat breeding programme in Kenya has attempted to develop resistant varieties, virulence has been reported on most the varieties at both seedling stage and adult plant resistance stages (Njau *et al.*, 2009). Seedling resistance to stem rust is regulated by major genes which can be completely effective against some races of the pathogen, but can also be vulnerable to at least one other race of the same pathogen hence not reliable (Lowe *et al.*, 2011). On the other hand adult plant resistance (APR) is based on minor genes (Ginkel and Rajaram, 1993) and can be used over a large area, for a long time especially when the host is exposed to a wide range of the pathogen but still remains resistant (Johnson, 1983). This kind of resistance is often undetectable at seedling stage as opposed to adult plant stage, and is known to be limited to specific physiologic races of the pathogen (Lowe *et al.*, 2011).

For several decades in recent years, the stem rust had been contained to some extent especially through utilization of more than fifty resistance genes in wheat (McIntosh *et al.*, 1998). This resulted in a decline in research activity over the last three decades (Singh *et al.*, 2006) until the emergence of a stem rust pathotype Ug99 in Uganda in the year 1999 (Pretorius *et al.*, 2000). This pathotype is virulent to Sr31 which was introgressed into wheat from rye, Secale cereale (Todorovska *et al.*, 2009) and is known to be present in many wheat cultivars through the world (Singh *et al.*, 2006). In 2007, a mutant race of Ug99, TTKST overcame Sr24 (Jin *et al.*, 2008a; Njau *et al.*, 2010) which was originally transferred from the tall wheat grass (*Elitrigia elongata*) to bread wheat (Smith *et al.*, 1968). Another Ug99 mutant race (TTTSK) has overcome Sr36 (Jin *et al.*, 2008b). Sr36 is derived from Sanduri wheat (*Triticum timopheevii*) (Allard and Shands, 1954). Nonetheless, major genes when combined with APR genes offer a primary means of durable resistance and can also confer desired levels of resistance near immunity (Singh *et al.*, 2005). Efforts to tackle the stem rust problem were initiated in 2006 through a Mexico - Kenya shuttle breeding program with the objective of transferring the APR identified in CIMMYT wheat to a range of important wheat germplasm (Singh *et al.*, 2008). Through this program, CIMMYT germplasm are used in wheat breeding activities for durable resistance in Kenya have been developed (Njau *et al.*, 2010).

Quantitative traits such as yield are usually influenced by genotype, environment and genotype × environment (GE) interaction (Breese, 1969; Yan and Hunt, 2002). The cross over GE interaction type results in inconsistent performance of genotypes across environments (Yan and Hunt, 2002). It complicates the plant breeders' aim of developing varieties which are best performing and most stable, hence reducing the progress from selection in any one environment. This can be managed by selecting genotypes that are broadly adapted to a range of environments (Yau, 1995). For stem rust disease, the pathogen as well as seasonal variation is part of the environment, which strongly affects resistance and its durability (Parveliet, 1993). Therefore, to increase the level of durable resistance, the breeder should select genotypes with lower levels of disease severity continuously over seasons or target locations where they will be exposed to a wide spectrum of the pathogen races (Johnson, 1983; Parveliet and Van Ommeren, 1988).

Durably resistant wheat varieties to stem rust will be of less importance to the farmer unless it has other important traits such as yield. Measurement of GE interactions for disease resistance and yield enables the plant breeder to identify broadly adapted genotypes that offer stable performance across a wide range of sites, as well as under specific conditions

such as high disease pressure (Yan and Tinker, 2005). This could aid in the development of an optimum breeding strategy for releasing varieties adapted to a target environment (Ahmad *et al.*, 1996). Consequently, it is important to crosscheck the disease resistance as well as yield potential of the breeding lines at every breeding stage to ensure that novel sources of resistance to the emerging strains of the pathogen as well as good yield potential are identified, gathered and utilized. Development of durably resistant varieties will reduce the cost of production and frequency of serious epidemics; this will clearly enhance wheat production in Kenya and other wheat dependent countries. Introgression of resistance genes in the material used for the current study had been conducted by CIMMYT and selections carried out at a 'hot spot' in Kenya. The objectives of this study were to evaluate the wheat genotypes for stem rust resistance at seedling stage in a greenhouse; as well assessing the genotypes for stability in adult plant resistance to stem rust and yield across three sites in Kenya.

Materials and methods

Plant materials and site description

Sixty-four advanced wheat breeding lines together with two checks, susceptible cultivar Cacuke and a resistant cultivar Robin were evaluated in the current study. The lines showing high resistance to stem rust were selected from CIMMYT nurseries as part of a larger international stem rust screening nursery in Njoro, Kenya in 2011. Robin is known to be resistant, while Cacuke is completely susceptible to TTKST (Ug99+Sr24 virulence) race of Ug99 which was the predominant race in Kenyan fields by the year 2011 (Singh *et al.*, 2011).

Seedling experiment was conducted in a greenhouse at Kenya Agricultural Research Institute (KARI), Njoro research center. Njoro is located 0°20'S, 35°56'E at an altitude of 2185 m above the sea level (a.s.l). The field experiments were carried out at three sites namely, Njoro, Timau and Mau Narok during the 2012-2013 cropping season. Njoro site falls within agro-ecological zone III. It receives an annual rainfall of 939 mm (15 yrs) distributed over one cropping season and an average daily minimum and maximum temperatures of 9 and 24°C, respectively. Timau is located 0°5'S, 37°20'E at 2640 m a.s.l with an average minimum temperature of l5°C and a maximum temperature of 23°C, the average annual rainfall is 896 mm (15 yrs). This site falls within agro-ecological zone II. Mau Narok is located 0°38' S, 35°47′ E at 2863 m a.s.l. The site falls within agro-ecological zone II. It receives annual rainfall of 1200 mm (15 yrs) with a minimum temperature of 11°C and a maximum temperature of 24.5°C. Planting in Njoro was done during the optimal rainy season in mid-June 2012. Planting in Timau and Mau Narok was done in early-November 2012. The sites were selected due to their significance in wheat production and a high frequency of natural population of stem rust hence 'hot spots' and suitable sites for screening wheat genotypes for the disease reaction (Wanyera *et al.*, 2006).

Seedling resistance experiment

Sixty four advanced wheat breeding lines mainly obtained from CIMMYT and selected from the Njoro stem rust resistance screening nursery (SRRSN) and two checks (Cacuke as a susceptible cultivar and Robin as a moderately resistant cultivar) were evaluated in a greenhouse at KARI, Njoro. Ten seeds of each entry were planted in 5 cm diameter pots filled with a potting mix (six parts peat moss, four parts vermiculite, two parts perlite, three parts Roxana silt loam soil and three parts sand) and placed in a plastic tray with each pot in a fixed position.

Race identification was done based on a set of 20 differentials, each with a different single stem rust (Sr) resistance gene proposed by Fetch (2009). Four seeds of each differential line were planted in each corner of the square pot (5 cm span) filled with the potting mix. The pots were also placed in plastic trays in fixed positions. For both the differentials and experimental genotypes, the 9 day old seedlings were inoculated with urediniospores of the common race obtained from the stem rust trap nursery of KARI, Njoro SRRSN in 2012. The spores were suspended in

light mineral oil (Soltrol 170) at a concentration of 6×106 spores/ml of oil. Inoculated plants were then air-dried for 1 hr and incubated for 24 hrs in dark dew chamber kept moist by frequently spraying with distilled water to maintain humidity of 80% to 100%. The temperatures were maintained between 18°C and 20°C. The seedlings were then transferred to the greenhouse at 23°C after incubation. For the first 2 hrs after the transfer, misty condition was created by spraying on the leaves indirectly with distilled water at an interval of 30 min in order to maintain high relative humidity. Misting was done three times a day for the rest of the time until the first sign of infection appeared. The disease infection type was scored through a procedure proposed by Stakman *et al.* (1962) based on "0" to "4" scale, 15 days after inoculation, where "0" is no disease and the genotype is resistant while "4" shows the highly susceptible genotype(s). ITs "0", ";", "1", "2", or combinations indicated low infection type. Infection type "3" to "4" was considered high infection types. The symbols; = hypersensitive flecks, 1 = small uredinia surrounded by necrosis, 2 = small uredinia surrounded by chlorosis, 3 = moderate size uredinia without chlorosis, 4 = large uredinia without chlorosis, + = slightly larger uredinia than expected for the infection type, and x = mixed infection types. The experiment was repeated once.

Field experiments

The plant materials (64 genotypes) used in seedling test was planted during 2012-2013 cropping season across three sites (KARI Njoro, Timau, and Mau Narok) in Kenya. The experimental design used at all the three locations was alpha-lattice (22 rows × 3 columns) with three replications. Each entry was planted in plots of 2 rows × 1.2 m long × 0.2 m apart at a seed rate of 125 kg ha-1. The entries were separated by 0.3 m and 0.5 m wide alleyways within and between the blocks, respectively. Susceptible wheat cultivar Cacuke was planted around the trial plot and in the middle of the 0.5 m alleyways on both sides of plots as a spreader to facilitate uniform inoculum build up. Nitrogen and Phosphorus were applied at the rate of 22.5 kg N ha^{-1} and 25.3 kg P_2O_5 ha^{-1}. Buctril MC (bromoxynil + MCPA) a post emerging herbicide was sprayed at tillering stage at the rate of 225 g Bromoxynil octanoate ha-1 to control broad-leaved weeds. The trial was top dressed with 30 kg N ha^{-1} at jointing. Manual weeding was done by hand two times between stem elongation and booting stages to eradicate grasses. Field trials across sites were under natural infection of the disease.

Assessment of plants for APR extended from milk to early dough stage (Zadok's growth stage 75 to 85) (Zadoks *et al.*, 1974) of grain development and when the spreader reached 50% severity. The adult plant response to infection was classified according to Roelfs *et al.* (1992) into four categories; R resistant, MR moderately resistant, MS moderately susceptible, S susceptible and overlapping responses between two categories was denoted using a dash between the categories for example MR/MS. The stem rust severity was determined by use of the modified Cobb scale, where the severities ranged from 5 to 100% (Peterson *et al.*, 1948). Disease observations were made 3 times on an 8 day interval. Whole plots for each entry were harvested for grain yield assessment. Grain yield for each entry was adjusted to 12.5% grain moisture before conversion to t ha^{-1} for statistical analysis.

Data analysis

For comparison of lines based on stem rust resistance, the mean disease severity was used to calculate the area under disease progress stairs (AUDPS) and the (Coefficient of infection (CI) was calculated by taking into account the disease severity and their infection response 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 (Roelfs *et al.*, 1992).

Since disease observations were made on a regular interval, the following formula as described by Simko and Piepho (2012) was applied;

$$\text{AUDPS} = \bar{y} \times \frac{Dn}{n-1}$$

Where, is the arithmetic mean of all assessments, is time duration (in days) between the first and the last observation and, is the number of observations.

A combined analysis of variance for AUDPS, CI and yield across sites was performed using a linear mixed model following restricted maximum likelihood (REML) procedure in GenStat 14th edition statistical software (VSN international Ltd, 2010). Genotypes, locations, replicates, and Genotype × location were considered as fixed effects while incomplete blocks nested in replicates (Replicate × Block) were considered as random. The following statistical model was used;

$$Y_{ijk} = \mu + G_i + L_l + R_j + B_{(kj)} + GL_{il} + \varepsilon_{ijk}$$

Where; Y_{ijk} = observations; μ = mean of the experiment; G_i = effect of the i^{th} genotype; L_l = effect of l^{th} location; R_j = effect of the j^{th} replicate (superblock); $B_{(jk)}$ = effect of the k^{th} incomplete block within the j^{th} replicate; GL_{il} = effect of i^{th} genotype in l^{th} location and ε_{ijk} = experimental error. The least significant difference was determined at $P \leq 0.05$.

Relation between the two disease parameters and yield was determined using the simple linear regression using GenStat 14th edition statistical software (VSN international, Ltd 2010). For analysis of stability in disease resistance, genotypic variance ($\mathcal{S}i^2$) (Lin *et al.*, 1986) was computed using IBWorkFlowSystem. Finlay and Wilkinson's analysis (Finlay and Wilkinson, 1963), Wricke's ecovalence (Wi) (Wricke, 1962), and GGE biplot (Yan et al., 2000) models in IBWorkbench were used to analyze genotype yield sensitivity, stability and for graphic exploration of relationships between genotypes and/or sites, respectively.

Results

Seedling reaction to stem rust

Based on a set of differentials, the inoculum used in seedling experiment was identified as TTKST (Ug99 with virulence on Sr24). Twenty nine percent of the screened genotypes exhibited resistance (IT's of ";", "1", "2" or combinations), one line, KSL 31 showed a heterogeneous reaction "x" while the rest 60% lines showed susceptible reactions. Results for seedling reactions of the genotypes which were considered to be resistant and checks are presented in Table 1. Robin and Cacuke showed resistance (1+) and susceptibility (4), respectively.

Table 1. Seeding infection type to TTKST race of stem rust (*Puccinia graminis* f. sp *graminis*) for wheat (*Triticum aestivum)* genotypes that were considered resistant and checks as evaluated in a greenhouse.

Genotype	Pedigree	IT[1]	Genotype	Pedigree	IT[1]
KSL 1	MERCATO//JNRB.5/PIFED	2	KSL 33	KIRITATI//ATTILA*2/PASTOR/3/AKURI	2+
KSL 5	PREMIO/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/PIFED	; 1+	KSL 40	MILAN/S87230//BAV92/3/KINGBIRD #1	2+
KSL 9	MERCATO/VORB	; 1	KSL 42	KIRITATI//PRL/2*PASTOR/5/OASIS/SKAUZ//4*BCN/3/PASTOR/4/KAUZ*2/YACO//KAUZ/6/KIRITATI//PRL/2*PASTOR	;1
KSL 10	MERCATO/5/CHEN/AE.SQ//2*OPATA/3/BAV92/4/JARU	0	KSL 44	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/WHEAR	;1
KSL 11	MERCATO//JNRB.5/PIFED	2+	KSL 46	KINGBIRD #1/INQALAB 91//INQALAB 91*2/KUKUNA	;1
KSL 12	MERCATO//JNRB.5/PIFED	0	KSL 52	SHORTENED SR26 TRANSLOCATION//WBLL1*2/BRAMBLING	;1
KSL 13	MERCATO//JNRB.5/PIFED	1+	KSL 53	SITE/MO//PASTOR/3/TILHI/4/MUNAL #1	2
KSL 14	MERCATO//JNRB.5/PIFED	2	KSL 55	TUKURU//BAV92/RAYON/3/WBLL1*2/BRAMBLING/4/WBLL1	;1
KSL 16	PREMIO/VORB	;1+	KSL 56	TUKURU//BAV92/RAYON/3/WBLL1*2/BRAMBLING/4/WBLL1	;1
KSL 21	EGA BONNIE ROCK/6/CP18/GEDI/3/GOO//ALB/CRA/4/AE.QQUARROSA (208)	1	KSL 62	Svevo	1+
KSL 24	SW89-5124*2/FASAN/3/ALTAR 84/AE.SQ//2*OPATA/4/ARREHANE	;1	KSL 63	BR 35/CEP 9291/4/BR 32/3/CNO 79/PF 70354/MUS"S"	2+
KSL 25	SOKOLL*2/ROLFO7	1+	KSL 64	PF 70100/J15157-69	2+
KSL 30	WAXWING/KIRITATI*2//YANAC	;1	ROBIN	BABAX/LR42//BABAX*2/3/TUKURU	1+
			CACUKE	CANADIAN/CUNNINGHAM//KENNEDY	4

[1]IT: Infection type, KSL: Kenyan Selection.

Adult plant reaction to stem rust

Significant (P ≤ 0.01) main effects of genotypes and locations were obtained for both CI and AUDPS (Data not shown). Similarly, two-way interaction between genotype (G) and location (L) (GL) were significant (P ≤ 0.01) for the parameters measured. Because of the significant interaction, stability values were also computed for the two disease parameters. The CI, AUDPS, and stability values for genotypes that proved better than the resistant check based on AUDPS values are presented in Table 2. The genotypes were ranked according to their AUDPS means across sites. Based on AUDPS means, genotypes KSL 42, KSL 51, and KSL 3 ranked top with means of 30.2, 42.7 and 74.5, respectively.

Table 2. Stem rust (*Puccinia graminis*) coefficient of infection (CI), the area under disease progress stairs (AUDPS) and stability values wheat (*Triticum aestivum*) genotypes that proved better than the resistant check as evaluated across three location of Kenya. Ranking was based on AUDPS means.

			Coefficient of infection (CI)					Area under disease progress stairs (AUDPS)				
	Genotype	Pedigree	Mau Narok	Njoro	Timau	Mean	Stability (1000)	Mau Narok	Njoro	Timau	Mean	Stability (1000)
1	KSL 42	KIRITATI//PRL/2*PASTOR/5/OASIS/SKAUZ//4*BCN/3/PASTOR/4/KAUZ*2/YACO//KAUZ/6/KIRITATI//PRL	0.6	0.1	0.0	0.2	0.01	72.0	18.7	0.0	30.2	1.40
2	KSL 51	SHORTENED SR26 TRANSLOCATION//WBLL1*2/BRAMBLING	0.8	0.2	0.1	0.4	0.15	98.7	24.0	5.3	42.7	2.44
3	KSL 3	MERCATO//JNRB.5/PIFED	0.9	0.3	0.2	0.5	0.16	178.7	32.0	13.3	74.7	8.20
4	KSL 57	R09 RC1F5-5292	1.1	0.1	0.7	0.6	0.26	173.3	2.7.0	82.7	86.2	7.29
5	KSL 13	MERCATO//JNRB.5/PIFED	0.7	0.4	0.0	0.4	0.04	141.3	120.0	0.0	87.1	5.81
6	KSL 7	SW89-3218/VORONA//SUNCO/2*PASTOR	1.0	0.4	0.2	0.5	0.16	216.0	34.7	18.7	89.8	12.01
7	KSL 34	KIRITATI//ATTILA*2/PASTOR/3/AKURI	1.0	0.3	0.1	0.5	0.26	245.3	34.7	8.0	96.0	16.90
8	KSL 59	PAMUKOVA-97*3/3/88 ZHONG 257//CNO79/PRL	0.9	0.3	0.6	0.6	0.09	152.0	64.0	85.3	100.4	2.11
9	KSL 5	PREMIO/4/CROC_1/AE.SQUARROSA (205)//KAUZ/3/PIFED	0.9	0.4	0.1	0.5	0.18	202.7	101.3	8.0	104.0	9.48
10	KSL 62	Svevo	0.9	0.4	0.1	0.5	0.18	216.0	98.7	5.3	106.7	11.14
11	KSL 29	KFA/5/2*KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	1.0	0.9	0.5	0.8	0.07	152.0	162.7	45.3	120.0	4.21
12	KSL 8	SW89-3218/VORONA//SUNCO/2*PASTOR	1.0	0.3	0.6	0.6	0.13	256.0	34.7	80.0	123.6	13.67
13	KSL 9	MERCATO/VORB	1.0	0.5	0.3	0.6	0.11	269.3	98.7	10.7	126.2	17.30
14	KSL 61	Carisma	1.1	0.8	0.3	0.7	0.14	232.0	160.0	45.3	145.8	8.86
15	KSL 39	MILAN/S87230//BAV92/3/KINGBIRD #1	1.1	0.7	0.3	0.7	0.19	306.7	125.3	32.0	154.7	19.51
16	KSL 58	PARULA	0.9	0.6	0.7	0.7	0.03	229.3	125.3	114.7	156.4	4.01
17	KSL 63	BR 35/CEP 9291/4/BR 32/3/CNO 79/PF 70354/MUS"S"	1.2	0.3	0.6	0.7	0.23	349.3	45.3	85.3	160.0	27.29
18	KSL 43	ALTAR 84/AE.SQUARROSA(221)//3*BORL95/3/URES/JUN	1.1	0.7	0.4	0.7	0.13	298.7	138.7	48.0	161.8	16.11
19	KSL 30	WAXWING/KIRITATI*2//YANAC	1.1	0.3	0.3	0.6	0.2	413.3	29.3	48.0	163.5	46.88
20	Robin	BABAX/LR42//BABAX*2/3/TUKURU	1.0	0.6	0.3	0.6	0.12	333.3	162.7	26.7	174.2	23.61
21	Cacuke	CANADIAN/CUNNINGHAM//KENNEDY	2.0	2.0	1.1	1.7	0.24	1693.3	1693.3	229.3	1174.2	67.14
Means			1.2	0.8	0.5			441.6	273.3	69.3		
CV%				20.81					37.49			
LSD(0.05)[a]				0.16					90.81			
LSD (0.05)[b]				0.03					19.36			

[1]IT: Infection type, [2]AUDPS: Area under disease progress stairs; KSL: Kenyan Selection [a]: LSD for comparing means within locations and [b]: LSD for comparing means between locations.

Similarly, these genotypes significantly ranked better compared to the check variety Robin, with mean CI values of 0.2, 0.4 and 0.5, respectively. Cacuke had the highest means for AUDPS (1174.2) and CI (1.7). Among the three sites, for all the 66 evaluated wheat genotypes, Timau had the lowest disease pressure depicted by lowest mean values of CI (0.5) and AUDPS (69.3), followed by Njoro with CI and AUDPS values of 0.8 and 273.3, respectively. Mau Narok had the highest disease pressure with AUDPS and CI values of 1.2 and 441.6 respectively. Low stability values indicate high stability of genotype. With regard to AUDPS stability values, the most stable genotypes include; KSL 42 (1400), KSL 59 (2110), and KSL 51 (2440). In spite of its stability, KSL 59 had strikingly higher AUDPS means across all sites.

A simple regression analysis revealed a significant linear and inverse relationship ($p \leq 0.01$) between grain yield and CI (Y= -1.198x + 6.224, s.e = 0.12, R^2=14.3) (Fig. 1), and grain yield and AUDPS (Y= -0.0025 + 5.878, s.e = 0.00019, R^2= 21.4%) (Fig. 2).

Genotype sensitivity and yield stability across sites

There was a significant ($p \leq 0.01$) genotype (G) and location (L) main effects and ($p \leq 0.05$) GL interaction across sites for grain yield (Data not shown). The mean yields for the best 20, the least yielder, and the two checks of wheat genotypes evaluated are presented in Table 3. Looking at how the locations differentiated performance of genotypes, Njoro (5.4 t ha^{-1}) and Timau (5.4 t ha^{-1}) recorded equal mean yields, while Mau Narok (4.8 t ha^{-1}) recorded lower yield. Genotypes KSL 42, KSL 3 and KSL 9 ranked the highest with; 7.8 t ha^{-1}, 6.6 t ha^{-1} and 6.6 t ha^{-1} mean yields across sites, respectively. The commercial variety Robin (Check) had 4.9 t ha^{-1}, while the susceptible check Cacuke had 3.6 t ha^{-1}.

Table 3. The mean yield for the best 20, the least yielder, and the two checks of wheat (*Triticum aestivum)* genotypes evaluated across the three sites in Kenya during 2012-2013 cropping season.

			Yield t ha^{-1}			
	Genotype	**Pedigree**	**Mean**	**Mau Narok**	**Njoro**	**Timau**
1	KSL 42	KIRITATI//PRL/2*PASTOR/5/OASIS/SKAUZ//4*BCN/3/PASTOR/4/KAUZ*2/YACO//KAUZ/6/KIRITATI//PRL/2*PASTOR	7.8	7.6	8.2	7.6
2	KSL 3	MERCATO//JNRB.5/PIFED	6.6	6.5	6.7	6.6
3	KSL 9	MERCATO/VORB	6.6	5.1	8.0	6.8
4	KSL 4	MERCATO//JNRB.5/PIFED	6.5	7.4	6.7	5.5
5	KSL 61	Carisma	6.5	4.9	7.3	7.4
6	KSL 7	SW89-3218/VORONA//SUNCO/2*PASTOR	6.3	7.1	7.3	4.6
7	KSL 29	KFA/5/2*KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	6.3	7.2	6.4	5.3
8	KSL 11	MERCATO//JNRB.5/PIFED	6.2	7.2	4.6	6.7
9	KSL 13	MERCATO//JNRB.5/PIFED	6.2	7.7	5.7	5.2
10	KSL 14	MERCATO//JNRB.5/PIFED	6.2	6.5	6.0	6.3
11	KSL 28	KFA/5/2*KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	6.2	5.1	7.1	6.3
12	KSL 58	PARULA	6.1	5.3	6.0	6.9
13	KSL 34	KIRITATI//ATTILA*2/PASTOR/3/AKURI	6.0	7.7	5.9	4.5
14	KSL 10	MERCATO/5/CHEN/AE.SQ//2*OPATA/3/BAV92/4/JARU	5.9	5.1	5.9	6.5
15	KSL 60	Pamukova-97/Arostor	5.9	3.5	7.8	6.6
16	KSL 63	BR 35/CEP 9291/4/BR 32/3/CNO 79/PF 70354/MUS"S"	5.9	2.9	7.0	7.8
17	KSL 26	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/CROC_1/AE.SQUARROSA (224)//OPATA	5.8	7.8	5.1	4.7
18	KSL 32	PBW343*2/KUKUNA/3/PGO/SERI//BAV92	5.8	5.2	6.8	5.3
19	KSL 55	QUAIU/5/2*FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	5.8	4.8	5.9	6.6
20	KSL 12	MERCATO//JNRB.5/PIFED	5.7	7.2	5.6	4.3
21	KSL 57	R09 RC1F5-5292	2.0	1.4	2.2	2.3
22	ROBIN	BABAX/LR42//BABAX*2/3/TUKURU	4.9	3.8	5.3	5.6
23	CACUKE	CANADIAN/CUNNINGHAM//KENNEDY	3.6	1.4	1.7	7.5
		Means		4.8	5.4	5.4
		CV%			12.87	
		LSD (0.05)[a]			0.62	
		LSD (0.05)[b]			0.13	

KSL: Kenyan Selection, [a]: LSD for comparing means within locations, [b]: LSD for comparing means between locations.

Further analysis of GL interaction according Finlay and Wilkinson (1963) revealed significant ($p \leq 0.01$) G, L effects and sensitivities (Table 4).

Table 4. Analysis of variance in Finlay and Wilkinson modified joint regression analysis for yield of advanced wheat (*Triticum aestivum*) genotypes evaluated across three sites in Kenya during 2012-2013 cropping season.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Genotypes	65	493.9228	7.5988	8.44	<0.001
Locations	<0.001	54.2388	27.1194	30.12	<0.001
Sensitivities	65	363.7472	5.5961	6.22	<0.001
Residual	461	415.0384	0.9003		
Total	593	1326.947	2.2377		

The adaptability and average yield performance for each genotype based on sensitivities across the locations are presented in a two dimension scatter plot (Fig. 3). From the scatter plot, the three locations favoured generally adapted genotypes, although with a little bias towards varieties specifically adapted to low yielding locations. The most adapted genotypes to unfavorable locations are located towards the bottom of the plot. Such genotypes are; KSL 13 (13), KSL 26 (26), KSL 34 (34), KSL 8 (8), and KSL 24 (24). The most adapted genotypes to favorable location are located towards the top of the plot, such genotypes are KSL 20 (20), KSL 63 (63), and KSL 2 (2). The generally adapted genotypes to all locations are located on or close to b=1.0 regression coefficient line. The further a genotype is to the right, the higher the yield. With regard to general adaptability, KSL 42 (42) is the best since it is close to the b=1.0 regression line and the furthest to the right (Fig. 3).

Table 5. The results for Wricke's ecovalence values for the best 20 high, the least yielder, and the two checks of wheat (*Triticum aestivum)* genotypes evaluated across three sites in Kenya during 2012-2013 cropping season.

	Genotype	Pedigree	Mean yield t ha^{-1}	Ecovalence
1	KSL 42	KIRITATI//PRL/2*PASTOR/5/OASIS/SKAUZ//4*BCN/3/PASTOR/4/KAUZ*2/YACO//KAUZ/6/KIRITATI//PRL/2*PASTOR	7.8	0.17
2	KSL 3	MERCATO//JNRB.5/PIFED	6.6	0.21
3	KSL 9	MERCATO/VORB	6.6	2.54
4	KSL 4	MERCATO//JNRB.5/PIFED	6.5	3.21
5	KSL 61	Carisma	6.5	2.07
6	KSL 7	SW89-3218/VORONA//SUNCO/2*PASTOR	6.3	5.73
7	KSL 29	KFA/5/2*KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	6.3	3.22
8	KSL 11	MERCATO//JNRB.5/PIFED	6.2	5.44
9	KSL 13	MERCATO//JNRB.5/PIFED	6.2	5.91
10	KSL 14	MERCATO//JNRB.5/PIFED	6.2	0.74
11	KSL 28	KFA/5/2*KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	6.2	0.91
12	KSL 58	PARULA	6.1	0.60
13	KSL 34	KIRITATI//ATTILA*2/PASTOR/3/AKURI	6.0	7.53
14	KSL 10	MERCATO/5/CHEN/AE.SQ//2*OPATA/3/BAV92/4/JARU	5.9	0.43
15	KSL 60	Pamukova-97/Arostor	5.9	6.15
16	KSL 63	BR 35/CEP 9291/4/BR 32/3/CNO 79/PF 70354/MUS"S"	5.9	10.27
17	KSL 26	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/CROC_1/AE.SQUARROSA	5.8	8.71
18	KSL 32	PBW343*2/KUKUNA/3/PGO/SERI//BAV92	5.8	1.13
19	KSL 55	QUAIU/5/2*FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	5.8	0.70
20	KSL 12	MERCATO//JNRB.5/PIFED	5.7	6.39
21	KSL 57	R09 RC1F5-5292	2.0	0.04
22	ROBIN	BABAX/LR42//BABAX*2/3/TUKURU	4.9	0.73
23	CACUKE	CANADIAN/CUNNINGHAM//KENNEDY	3.6	22.06

KSL: Kenyan Selection.

According to Wricke's stability analysis (Table 5), KSL 42 and KSL 3 were the best two genotypes after KSL 57. Since Wricke's stability test only measure stability, average yield performance of the genotype/s across sites should also be considered when selecting genotypes for breeding purpose. In spite of high stability, KSL 57 had the least mean yield and therefore cannot be recommended for use in breeding (table 5).

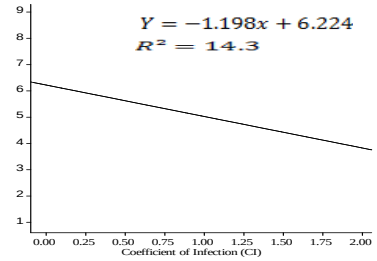

Fig.1. Association of grain yield with CI for the all the tested wheat genotypes.

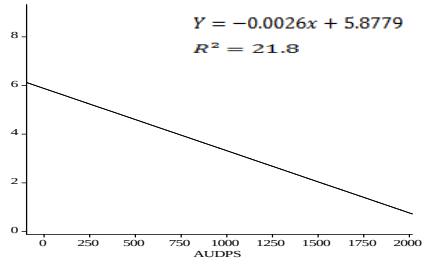

Fig. 2. Association of grain yield with stem rust AUDPS for all the tested wheat genotypes.

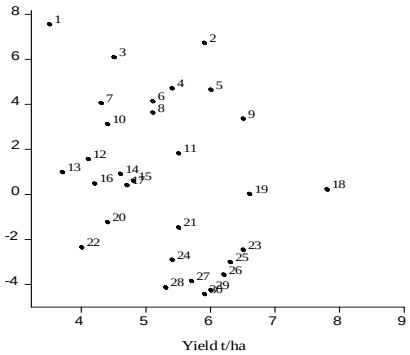

Fig. 3. Relationship of genotype adaptation and genotypic mean yield for the genotypes representing specific adaptability to favorable environments, general adaptability, and specific adaptability for unfavorable environments across the locations.

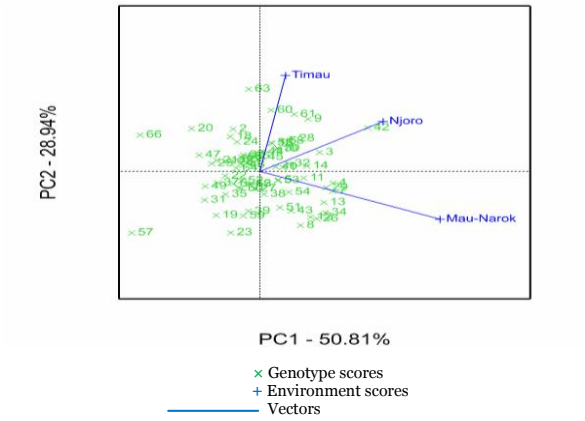

Fig. 4. A GGE biplot for the average grain yield (t/ha) (environmental scaling) across the sites.

There was a negative relationship between disease and yield (Fig. 1 and 2). The GGE biplot presentation of the average grain yield under stem rust disease pressure across sites is presented in Fig. 4. The first and the second principal components (PC1 and PC2) explained more than 79% of the total variation across sites. There was an acute angle between the environmental axes of Timau and Njoro and indicating similarity among the sites with regard to genotype yield performance. However, with regard to disease, the two sites had slightly different means (Table 2). Although the variation among the sites was not large an obtuse angle between Timau and Mau Narok indicated that these sites were negatively correlated and therefore ranked the genotypes differently. Mau Narok made the largest contribution of the GE interaction as it had the largest projection from the biplot origin. Also, the site was the most discriminative being the farthest from the biplot origin (Fig. 4) and had the highest disease pressure (Table 2); making it an ideal site for evaluating grain yield under the disease pressure. The high yielding genotypes are concentrated on the right side of the biplot while the low yielding ones are located on the left side of the biplot. This includes KSL 42 (42) on the extreme right and the two genotypes KSL 57 (57) on the extreme left (and below the origin). Genotype KSL 60 (60) has a good yield as it is situated on the right side of the biplot and projected on Timau axis above origin indicating a positive interaction with that site. Genotype KSL 42 interacts positively with Njoro as it projected above that site's vector. On the other hand, KSL 7 (7) interacts positively with Mau Narok as its vector projected above that site's vector. These three genotypes were best performers in their respective sites in spite of the stem rust disease pressure.

Discussion

Stem rust is among the most destructive of wheat diseases and can cause heavy yield loss if uncontrolled. It is however possible to mitigate this yield loss through the use of fungicides, but this has a serious cost implication to a resource poor farmer. Consequently, the plant breeders have emphasized the use of genetic sources of resistance. Major gene resistance/seedling resistance can offer complete protection and significant economic benefits to farmers. Nevertheless, this kind of resistance is known to lack durability (Johnson, 1983). Adult plant

resistance (APR) is not complete and not limited to specific physiological races of the pathogen but unlike major gene resistance, it can be durable, hence a major concentration for wheat breeders and valuable to farmers. However, the APR genes can render the plant completely susceptible to the pathogen at seedling stage.

From the field experiments, the high stem rust infection of Cacuke which was used as a susceptible check and disease spreader implies that the disease response recorded in all the three sites was predominantly due to TTKST (Ug99 + Sr24 virulent). This postulation is also supported by previous reports of Singh *et al.* (2011) which revealed TTKST as the predominant race of Ug99 in Kenyan fields by 2011. Nonetheless, there was evidence of significant genetic variability in disease response and severity among the genotypes across locations. However, most lines exhibited some resistance. Similar variations among wheat genotypes have previously been reported (Tabassum, 2011; Macharia and Wanyera, 2012). Some lines exhibited high susceptibility at seedling stage but low severity in the field e.g. KSL 3. Similar trends of genotypes showing susceptible reactions at the seedling stage and maintaining low severity in the field across a range of environments have been reported; this phenomenon is common with genotypes based on the *Sr2* (Njau *et al.*, 2010; Singh *et al.*, 2011). The stem rust resistant gene Sr2 is so far the only characterized minor gene and it can offer moderate levels of resistance when alone (Mago *et al.*, 2005; Singh *et al.*, 2005) but in case of a high disease pressure, the resistance offered may not be effective unless it is in combination with other unknown APR genes (Singh *et al.*, 2005). This might explain the high values of AUDPS and CI in Mau Narok. Effective major gene/s in combination with APR gene/s may explain the kind of resistance in KSL 42 which showed resistant reactions both at seedling stage and in the adult plants across sites. The pedigree information also shows that KSL 42 has Kiritati in the parentage, this CIMMYT genotype is known to possess the Sr2; this postulates the source of APR gene/s in KSL 42. According to Johnson (1983), there is a need to employ adult plant resistance since it is durable and can be used for a long time especially when the host is exposed to a wide range of the pathogen. In our stability analysis, KSL 42 and KSL 3 were the most stable genotypes across the three locations. The two genotypes also displayed high mean yields. Besides disease resistance, farmers' preference is also for high yielding and stable varieties, hence yield stability and adaptability is an important concept in wheat breeding.

Yield variability existed across locations; this is due to diverse genetic backgrounds for the genotypes (as depicted by pedigree information), location, and GL interaction. There was a negative relationship between disease and yield (Fig. 1 and Fig. 2). For instance, Mau Narok had the lowest means for yield (4.8 t/ha) and the highest means for the two disease parameters (Table 1). Similar relationship have been reported by Bathal *et al.* (2003); Ali *et al.* (2007; 2009); Macharia and Wanyera, (2012). Most genotypes that showed high values of CI and AUDPS had lower yields, however some inconsistencies were observed, KSL 4 which did not rank among the best 20 genotypes as per the field disease score ranking (Table 2), ranked among top five high yielders (Table 4). This may be attributed to some capacity of tolerance of the line and therefore detailed studies should be performed to explore this phenomenon. The weak relationship between the disease and yield (Fig. 1 and Fig. 2) is because variation in yield is not only due to disease response but also other sources of variability mentioned above. The existence of such variation enables the breeder to select both high yielding and disease resistant genotypes across sites and in presence of disease pressure. Genotypes KSL 42 and KSL 3 combined both field disease resistance and high yield and such genotypes are potential candidate lines for variety release.

According to Wricke's ecovalence values (Wi) (Wricke, 1962), the lower the values, the more stable the genotype is; and hence KSL 57 and KSL 42 proved to be the most stable genotypes compared to Robin (a check variety) with regard to grain yield. These two

genotypes were the top phenotypic stable genotypes with consistent performance in the test locations. Such genotypes may possibly be useful to farmers because they would give consistent varieties that can withstand unpredictable and transient environmental fluctuations.

Selection for specific adaptability is useful because farmers are able to utilize high yielders for their respective environments. According to Finlay and Wilkinson (1963) regression coefficients, genotypes characterized by b=1.0 were considered to have average phenotypic stability and considered to be adapted to all environments, b>1 are highly adapted to high yielding environment while b<1 are highly adapted to poor environment. Most genotypes exhibited average stability as they were clustered around the center of the scatter plot (Fig. 3), a few example include, KSL 5 (5), KSL 40 (40), and KSL 27 (27). The genotypes that had b<1 such as; KSL 13 (13), KSL 26 (26), KSL 34 (34), KSL 8 (8), and KSL 24 (24) were able to resist environmental change (above average stability), and therefore had specific adaptability to the low yielding environment (Mau Narok). However, plant breeders ignore the results obtained in low yielding environments for reason that such environments provide very low yields and are therefore not able to discriminate the selections. The genotypes with b>1 were sensitive to environmental changes and are suitable to be cultivated under favorable environments (Timau and Njoro). In general, specifically adapted genotypes have high yields and are able to be differentiated from the selections (Mevlüt *et al.*, 2009).

The GGE biplots afford a platform for breeders to graphically explore the relationship/s between genotypes and/or environments; the closer the genotypes and/or environments the higher the similarity (Malosetti *et al.*, 2013). This is determined by the angle between the vectors for each factor projected to the biplot origin. It shows those sites which are ideal and representative environment for experimentation and the effect of specific traits of interest e.g. stem rust resistance for each wheat genotype on yield performance, adaptability, and stability across environments. In this study, Njoro and Mau Narok were similar in genotypic yield performance under the disease pressure due to an acute angle between the environmental axes of the two sites. The same relationship was detected for Timau and Njoro. However, there was a negative correlation in genotypic yield performance between Timau and Mau Narok. Mau Narok proved to be a good site for wheat selection for yield under stem rust pressure since it had a larger projection from the biplot origin, indicating that it made the largest contribution of the GL interaction. According to Malosetti *et al.* (2013), the projection of a genotype onto a site vector reflects the performance of that genotype in that environment. Therefore, KSL 63 has a good yield and is positively associated with Timau; KSL 42 interacted positively with Njoro while KSL 13, KSL 4, KSL 26 and KSL 34 interacted positively with Mau Narok. This positive association of genotypes with sites was in spite of the stem rust disease in these sites. The GGE biplots have been previously used to identify superior wheat genotypes with regard to yield and other agronomic traits (Mohammadi *et al.*, 2011).

Conclusion

The screening of stem rust disease in a greenhouse and the three field locations allowed evaluation of stem rust at both levels. The field experiments also allowed assessments of yield potential of the genotypes in presence of the disease. The results of this study revealed a number of superior advanced lines which combined both good yield and disease resistance. With regard to disease resistance and yield performance, the genotypes KSL 42 and KSL 3 consistently ranked among the top performers and have good potential for variety release in Kenya. These outstanding lines can be included in the regional and national trials and used as parental lines for obtaining a segregating population for stem rust disease resistance and yield related traits. Therefore, it is imperative to carry out genetic analysis to identify the kind of gene action to guide in effective introgression of these important traits into the

Kenyan adapted but stem rust susceptible commercial cultivars.

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