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Evaluation of CIMMYT wheat (*Triticum aestivum* L.) lines for seedling and adult plant resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) race *UG99* and its variants

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Abstract

Stem rust races *Ug99* and its variants are virulent to a large number of resistant genes present in the widely grown wheat (*Triticum aestivum* L.) cultivars. This study was conducted to evaluate seedling and adult plant reaction to four stem rust races *TTKSK, TTKST, TTKTK* and *TTTSK* in CIMMYT wheat lines. The evaluation was conducted in the greenhouse with the adult plant resistance experiment conducted in a randomized complete block design (RCBD). Out of the 39 lines evaluated, only *SRG21*, *SRG34* and *SRG39* showed a reaction of 3 to race *TTKST*, *SRG22* exhibited a reaction of 3 to race *TTKTK*, *SRG25*, *SRG32*, *SRG36* and *SRG37* displayed a reaction of 3 to race *TTKSK* and *SRG27* and *SRG39* showed a reaction of 3 to race *TTTSK* the rest revealed infection types of between 0 and 2. In the evaluation of lines for adult plant reaction to stem rust race *TTKST*, only 0.13% of the lines exhibited disease severity of ≤5% while 99.87% of the lines exhibited a severity of ≥10%. In contrast, 43.59% lines showed a severity of ≤5% while 56.41% showed a severity of ≥10% to races *TTKTK* and *TTKSK*. 46.15% of the lines demonstrated a severity of ≤5% while 53.85% of the lines demonstrated a severity of ≥10% to race *TTTSK*. Lines *SRG7*, *SRG13*, *SRG24* and *SRG35* showed low final disease severity, low infection types and low AUDPC with all the four races.

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Introduction

Ug99 and its variants are major constraint to wheat (*Triticum aestivum* L.) production across wheat growing regions in Africa as they are capable of causing massive yield losses of 80 to 100% in susceptible cultivars (Singh *et al.*, 2011).

The wheat growing regions of Eastern Africa have been proven to be one of the major origins of new stem rust races of wheat (Singh *et al.*, 2006). For the last five decades, the devastating effects of stem rust races was managed by deploying resistant genes derived from bread wheat (AABBDD 2n=6x=42) and secondary gene pool of wild relatives (Haile *et al.*, 2013). However, widely deployed resistance genes *Sr24*, *Sr31*, and *Sr36* have been rendered ineffective to the stem rust by the emergence of the highly virulent race *TTKSK* (*Ug99*) and its variants (Jin *et al.*, 2009). Currently, at least eleven variants of *TTKSK* have been described that are virulent to *Sr9h, Sr13, Sr1RS^Amigo^* and *SrTmp* (Patpour *et al.*, 2016).

In most wheat breeding programs, the best and preferred strategy in the control of stem rust is to develop cultivars with durable resistance genes (Singh *et al.*, 2005). Two categories of resistance genes are widely recognized in wheat breeding for rust resistance; seedling resistance which is conferred monogenically and adult-plant resistance (APR) which is conferred polygenically (Chen, 2005). Several qualitative *Sr* genes are race specific and have been mapped on specific chromosomes (Singh *et al.*, 2011). Qualitative genes are phenotyped as present or absent by observing the characteristic low or high infection types displayed by them (Jin *et al.*, 2007).

They are usually characterized by a hypersensitive reaction upon stem rust infection, and usually confers a high level of resistance that is effective in all stages of plant development (Park *et al.*, 2011). A major limitation associated with these genes is their vulnerability when deployed singly in wheat cultivars. This has been demonstrated by *Sr24*, *Sr36*, and resistance in cultivar (cv.) 'Matlabas' becomes virulent to race *TTKSK* (Kolmer and Acevedo, 2016).

The repeated appearance of novel and virulent races of this pathogen population through a single-step mutation and/or sexual recombination events brought on by single gene deployment in wheat led to the alternative strategy of combining or "pyramiding" multiple major resistance genes into cultivars (Pink, 2002). This strategy has been effective in controlling stem rust since the 1950s in the northern Great Plains of North America and Australia (Park, 2007).

On the other hand, the polygenically inherited resistance conferred by multiple minor genes slows down pathogen infection and colonization in adult wheat plants (Collins *et al.*, 2007). APR is typically identified by phenotyping wheat plants at the seedling stage in the greenhouse, then subsequently evaluating adult plants in the field (Ellis *et al.*, 2014).

However, the accuracy of phenotyping in the field can be compromised by the effects of environmental factors such as weather patterns, inoculum pressure, sequential infection, differences in plant maturity and the presence of other diseases that influence the expression of APR genes upon infection (Hickey *et al.*, 2012). APR type of resistance can be more durable than single gene resistance due to race non-specificity of the resistance genes involved (Singh *et al.*, 2005).

Sr2, 9h, 13, 14, 15, 21, 22, 24, 25, 26, 27, 28, 29, 32, 33, 35, 36, 37, 39, 40, 42, 43, 44, 45, 46, 47, 50, 51, 52, 53, 55, 57, 58, Huw234, ND643, Yae, SrTA10171, SrTA1662, SrTA10187, SrTmp, and Sr1RS^{Amigo} are effective to at least one pathotype within the *Ug99* race group (Kielsmeier-Cook *et al.*, 2015; Singh *et al.*, 2015). However, *Sr9h, Sr21, Sr24, Sr36* and *SrTmp* have failed to confer resistance to individual *Ug99* races, while effective to others (Patpour *et al.*, 2016). Moreover, not all genes aforementioned can be introgressed into plants due to inadequate protection levels in adult plants, occurrence of virulence in other *Puccinia graminis f.sp. tritici* races, or undesirable linkage drag (Singh *et al.*, 2015).

The objectives of this study were therefore to evaluate seedling and adult plant reactions to four stem rust races *TTKSK, TTKST, TTKTK* and *TTTSK* of CIMMYT wheat lines.

Materials and methods

Preparation of pure spores of races TTKST, TTKSK, TTTSK and TTKTK (According to the North American system of nomenclature)

Single spores used for race purification were collected from *cvs. Cacuke*, *Kwale*, *Kingbird* and *Robin* planted at KALRO, Njoro trap nursery. These *cvs.* were used because they are highly susceptible to the stem rust races *TTKST*, *TTKSK*, *TTTSK* and *TTKTK*. The fresh single spores were collected into gelatine capsules from the leaves and stems of the infected plants. The process of purification was carried out in the greenhouse where the rust races *TTKST*, *TTKSK*, *TTTSK* and *TTKTK* were purified on *cvs. Cacuke (Sr24)*, *Kwale (Sr31)*, *Kingbird (Sr36)* and *Robin (SrTmp)*, respectively. The *cvs.* were planted in plastic pots of 6 cm-diameter and height of 6 cm. The pots were filled with approximately 127 cm^3 of vermiculite, levelled and 10 seeds of each *cv.* planted in 15 pots at a depth of 1 cm. The pots were then watered to field capacity and placed in greenhouse growth chamber. Separate inocula of each race were then prepared when the plants had reached GS 12 by suspending single spores collected earlier from each *cv.* in distilled water (0.01 g of spores per 100 ml of distilled water). Seedlings were sprayed with the inoculum using a hand sprayer as a fine mist at a distance of 30 cm from the sprayer to the plants. They were then placed in an incubation chamber with the incubator maintained at 16-18 ^{o} C temperature for 24 hours for sporulation to take place. Thereafter, the pots were moved to greenhouse bench where conditions were regulated at 12 hours (h) photoperiod, at temperature of 18 to 25 ^{o} C and relative humidity of 60 to 70%. Light was provided by fluorescent tube at a distance of 1.1 m above the plants and disease infection was monitored. A second set of cvs. *Cacuke*, *Kwale*, *Kingbird* and *Robin* was planted in plastic pots mentioned earlier for the increase of the spores. Single pustules were collected from the first set 14 days post inoculation, inoculum prepared from them and inoculated onto the second set. Pure fresh spores were then collected from the second set, suspended in distilled water and the solution mixed well.

Evaluation of wheat lines for seedling resistance

A total of 39 lines (Coded as stem rust gain (*SRG*)) (35 CIMMYT lines (*SRG1-SRG35*) and 4 checks (*SRG36, SRG37, SRG38* and *SRG39*)) were planted in 6 cm-diameter plastic pots and each pot was filled with approximately 127 cm^3 of vermiculite, levelled and 5 seeds from each line planted at an approximate depth of 1 cm per pot. The seeds were then watered to field capacity and then placed in the growth chamber in the greenhouse. Inoculation with races *TTKST*, *TTKSK*, *TTTSK* and *TTKTK* was done by hand spraying when plants were at GS 12 in the inoculation chamber using the inoculum prepared following the procedure explained above. The inoculated plants were then placed in an incubation chamber under natural light at 16-18 ^{o} C for 24 h after which they were moved to greenhouse bench with 12 h photoperiod, at temperature of 18 to 25 ^{o} C and relative humidity of 60 to 70% for disease evaluation after 14 days post inoculation. Seedlings were evaluated basing on 0-4 scale of infection types adopted from Stakman *et al.* (1962) where 0-2 is low infection type (IT) and 3-4 is high IT.

Evaluation of wheat lines for adult plant resistance

The same lines used in the seedling experiment were planted in 13 cm-diameter plastic pots. Each pot was filled with about 1145 cm^3 of soil levelled and 5 seeds from each line planted per pot at an approximate depth of 1 cm in a randomized complete block design (RCBD) with two replicates. Diammonium phosphate (DAP) was applied at the rate of 57 mg pot^{-1} at sowing to provide an equivalent of 10 mg N pot $^{-1}$ and 11 mg P pot $^{-1}$. Calcium Ammonium Nitrate (CAN) was applied at stem elongation stage (GS 30) (Zadok's *et al.*, 1974) at the rate of 38 mg pot^{-1} to supply a booster of 9.8 mg N pot $^{-1}$. The plants were then inoculated with races *TTKST*, *TTKSK*, *TTTSK* and *TTKTK* at booting stage GS 41-47 with inoculum prepared using the procedure explained above. The inoculation was done by injecting each and every plant using a syringe in all the pots in the evening when conditions were favourable for spore germination to create an artificial disease epidemic and ensure uniform inoculum dissemination.

Data collection for adult plant resistance in the greenhouse

Wheat lines were evaluated for severity on a scale of 0% (immune) to 100% (completely susceptible) depending on the area affected by stem rust, following a modified Cobb scale (Peterson *et al.*, 1948). Infection responses were based on the size of stem rust pustules and amount of associated chlorosis and necrosis. Infection response categories: Resistant (R), moderately resistant (MR), intermediate (M), moderately susceptible (MS) and susceptible (S) according to Roelfs *et al.*, 1992.

Overlapping infection response categories were noted when two different infection responses occurred on a single stem (MR-MS ratings indicating MR pustules on the same stem as MS pustules). The predominant category was listed first such that MR-MS differs from MS-MR. Evaluation was done at 7 days intervals beginning at the time when disease was first observed up to plant maturity (GS 70-89) (Zadoks *et al.*, 1974).

Biomass, thousand kernel weight and yield were also measured. Harvest index was then computed by dividing the yield by total biomass.

Data analyses for adult plant reactions in the greenhouse

Area under the disease progress curve was computed as follows: using the formula by and AUDPC CIMMYT programme.

$$AUDPC = \sum_{i}^{n-1}\left[\left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)\right] \quad (1)$$

(Wilcoxson *et al.*, 1975; CIMMYT, 2008)

Where; t_i is the time in days of each reading, y_i is the percentage of affected part of the plant at each reading, n is the number of readings, $t\ (i+1)$ is the second assessment date of two consecutive assessment, $y\ (i+1)$ is the disease severity on assessment date $t\ (i+1)$.

Analysis of variance was done using SAS (SAS, 2012) by applying the statistical equation below:

$$Y_{ijk} = \mu + R_i + G_j + B_k + GB_{jk} + \varepsilon_{ijkl} \quad (2)$$

Where Y_{ijkl} is the observation of experimental units, R_i is the effect due to replicate, G_j is the effect due to line in the i^{th} replicate, B_k is the effect due to race in the j^{th} line, GB_{jk} effect due to interaction between line and race and ε_{ijkl} is the random error effect.

Mean comparisons was done based on LSD procedure at 5% probability to separate the different wheat genotypes using the formula:

$$LSD = t_{\alpha/2}\sqrt{\frac{2MSE}{r}} \quad (3)$$

Where t is the error degree of freedom, r is the number of replicates, MSE is the mean square error (Gomez and Gomez, 1984).

Results

Analysis of variance for AUDPC, yield and yield components

There were significant (P≤0.001) effects due to line, race and line × race for area under disease progress curve, yield, kernel weight and harvest index. In addition, line and race effects were significant (P≤0.01 and P≤0.001, respectively) for biomass (Table 1).

Table 1. Mean squares from combined analysis of variance of thirty five wheat (*Triticum aestivum*) lines from five CIMMYT stem rust resistance screening nurseries and four checks for Area Under Disease Progress Curve (AUDPC) for stem rust, yield, thousand kernel weight, biomass and harvest index.

Source of variation	df	Area Under Disease Progress Curve	Yield	Kernel Weight	Biomass	Harvest index
Replicate	1	26972.79	3.64	256.37	27.40	9.11
Line	38	40648.04***	1.28***	147.82***	10.62**	0.72***
Race	3	16633.54***	95.23***	9416.07***	445.49***	9.11***
Line×Race	114	6796.19***	1.00***	118.68***	5.99	0.42***
Residual	155	1218.49	0.23	22.29	5.14	0.15
CV		24.59	20.98	19.47	21.49	23.24
R^2		0.93	0.92	0.93	0.75	0.84

*, **, *** represents significance at P≤0.05, P≤0.01 and P≤0.001, respectively.

Effects of stem rust races TTTSK, TTKSK, TTKTK and TTKST

Among the races evaluated, *TTTSK* exhibited the highest mean AUDPC of 153.64 while *TTKSK* exhibited the lowest mean AUDPC of 121.31. Because *TTTSK* is a variant of *TTKSK*, there was an increase of 26% AUDPC. In an attempt to determine the effect of the four races on yield, kernel weight and biomass, Lines which were inoculated with race *TTKSK* showed the highest mean yield of 1.13 g/plant while those that were inoculated with race *TTKTK* displayed the lowest mean yield of 0.46 g/plant.

Highest mean kernel weight and biomass of 11.25 g/plant and 4.52 g/plant, respectively were observed on plants which were inoculated with race *TTTSK* while lowest mean kernel weight and biomass of 4.47 g/plant and 2.85 g/plant, respectively were observed on plants which were inoculated with race *TTTSK*. Highest harvest index of 0.29 was from plants inoculated with race *TTKSK* (Table 2). *SRG39* which was used as a control revealed the highest mean AUDPC of 518.33 which was significantly higher than that for line *SRG33* of 195.77.

This revealed a difference of 61.28% between the worst performed check and the worst performed line. Line *SRG3* displayed the lowest mean AUDPC of 65.58 which was 67.31% lower than that for *SRG33* (Table 3).

Table 2. Mean comparisons for four stem rust races for Area Under Disease Progress Curve for stem rust, yield, thousand kernel weight, biomass and harvest index on CIMMYT wheat lines.

Races	Area Under Disease Progress Curve	Yield (g/plant)	Kernel Weight (g/plant)	Biomass (g/plant)	Harvest index
TTTSK	153.64 a	1.12 a	11.25 a	4.52 a	0.25 b
TTKSK	121.31 c	1.13 a	11.23 a	3.79 b	0.29 a
TTKTK	142.14 b	0.52 c	5.39 b	2.89 c	0.18 c
TTKST	150.73 ab	0.46 b	4.47 c	2.85 c	0.16 a
$LSD_{0.05}$	11.04	0.05	0.49	0.24	0.04

Means bearing same letters within the same column are not significantly different.

LSD: Least significant difference.

Table 3. Mean comparisons for CIMMYT wheat lines for Area Under Disease Progress Curve for stem rust, yield, thousand kernel weight, biomass and harvest index.

Code	Pedigree	Area under disease progress curve	Biomass (g/plant)	Kernel weight (g/plant)	Yield (g/plant)	Harvest index
SRG1	KRICHAUFF/2*PASTOR	108.8 k-p	3.84 a-f	10.69 a	1.09 a	0.28 c-g
SRG2	KRICHAUFF/2*PASTOR	136.83 f-j	4.08 a-c	4.19 n	0.95 a-e	0.23 j-k
SRG3	SERI*3//RL6010/4*YR/3/PASTOR	65.58 q	3.38 a-c	8.37 d-h	0.74 h-l	0.22 j-l
SRG4	SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92	126.02h-i	3.18 a-d	7.72 f-g	0.74 h-l	0.23 d-h
SRG5	PGO//CROC_1/AE.SQUARROSA (224)/3/2*BORL95/4/CIRCUS	149.08 e-i	3.09 g-i	6.19 j-m	0.71 h-l	0.23 e-h
SRG6	PGO//CROC_1/AE.SQUARROSA (224)/3/2*BORL95/4/CIRCUS	149.77 d-i	3.11 f-i	8.09 d-i	0.77 f-j	0.25 c-g
SRG7	THB/KEA//PF85487/3/MILAN	93.39 l-q	3.39 c-i	7.83 e-i	0.72 h-l	0.21 c-g
SRG8	THB/KEA//PF85487/3/RIVADENEIRA 4	117.27 i-m	3.58 c-i	8.06 d-i	0.83 c-l	0.23 j-k
SRG9	WHEAR/VIVITSI//WHEAR	89.02 l-q	3.11 f-i	10.39 a-c	0.97 a-d	0.31 a-b
SRG10	WHEAR/TUKURU//WHEAR	77.95 o-q	3.51 c-i	9.49 b-c	0.86 b-h	0.25 a-c
SRG11	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/CIRCUS	128.95 g-h	3.44 c-i	8.43 d-h	0.76 f-k	0.22 h-j
SRG12	PFAU/SERI.1B//AMAD/3/VARIS	138.02 f-j	3.38 c-i	7.11 h-l	0.76 f-k	0.22 h-j
SRG13	PBW343*2/KUKUNA//KIRITATI	118.39 i-m	3.99 a-d	11.12 a	0.99 a-c	0.25 a-c
SRG14	INQALAB 91*2/KUKUNA//KIRITATI	112.95 j-n	3.62 c-i	8.59 d-h	0.92 b-g	0.25 a-c
SRG15	ND643/2*WBLL1	152.52 d-h	3.48 c-i	8.58 d-h	0.86 b-g	0.25 a-c
SRG16	PFAU/SERI.1B//AMAD/3/VARIS	138.08 f-j	3.46 c-i	7.78 f-i	0.76 f-k	0.22 j-l
SRG17	TRCH/SRTU/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUI	142.70 e-j	3.15 f-i	7.90 e-i	0.79 e-j	0.25 a-c

Code	Pedigree	Area under disease progress curve	Biomass	Kernel weight	Yield	Harvest index
			(g/plant)			
	TES					
SRG18	TRCH/SRTU/5/KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES	184.08 b-d	3.32 d-i	9.06 c-g	0.93 a-f	0.28 a-c
SRG19	PBW343*2/KUKUNA//SRTU/3/PBW343*2/KHVAKI	136.14 f-j	3.57 c-i	9.20 b-f	0.95 a-e	0.27 a-c
SRG20	PBW343*2/KUKUNA//SRTU/3/PBW343*2/KHVAKI	161.95 c-g	3.67 c-h	8.02 e-i	0.81 d-j	0.22 j-l
SRG21	FRET2/KUKUNA//FRET2/3/WHEAR/4/FRET2*2/KUKUNA	79.95 n-q	3.34 c-i	8.40 d-h	0.84 c-i	0.25 c-g
SRG22	FRET2/KUKUNA//FRET2/3/WHEAR/4/FRET2*2/KUKUNA	150.02 d-i	3.41 c-i	8.60 d-h	0.83 c-i	0.24 j-k
SRG23	KACHU/KIRITATI	162.58 c-f	2.90 i	5.76 l-n	0.58 l-m	0.20 j-k
SRG24	KACHU/KIRITATI	122.95 i-m	3.04 h-i	7.49 h-j	0.71 h-l	0.23 j-l
SRG25	KIRITATI//ATTILA*2/PASTOR/3/AKURI	170.20 b-f	3.71 c-h	8.07 d-i	0.79 e-i	0.21 c-g
SRG26	KIRITATI//ATTILA*2/PASTOR/3/AKURI	129.64 g-k	3.63 c-i	9.19 b-f	0.92 b-f	0.25 a-c
SRG27	ALTAR 84/AE.SQUARROSA (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/KACHU/6/KIRITATI//PBW65/2*SERI.1B	88.77 m-q	3.66 c-h	8.63 d-h	0.87 b-h	0.24 j-k
SRG28	ALTAR 84/AE.SQUARROSA (221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/KACHU/6/KIRITATI//PBW65/2*SERI.1B	152.33 d-h	3.15 f-i	9.50 b-c	0.93 b-f	0.29 a
SRG29	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/BECARD	95.52 k-q	3.95 a-e	9.38 b-e	0.94 a-e	0.24 j-k
SRG30	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/NELOKI	95.33 k-q	3.74 b-h	7.41 h-k	0.81 d-l	0.22 j-l
SRG31	MUNAL #1*2/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07	110.02 j-o	2.98 i	4.27 m	0.59 l-m	0.19 i-l
SRG32	MUNAL #1*2/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07	75.02 p-q	3.21 e-i	7.51 h-k	0.69 i-l	0.21 c-g
SRG33	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/PBW343*2/KHVAKI/5/PBW343*2/KUKUNA/6/TRCH/SRTU//KACHU	195.77 b-c	3.15 f-i	8.07 d-i	0.72 h-l	0.23 j-l
SRG34	SOKOLL/3/PASTOR//HXL7573/2*BAU*2/4/EGA BONNIE ROCK	108.89 k-p	4.02 a-d	9.19 b-c	1.02 a	0.25 a-c
	Cont.					
Code	Pedigree	AUDPC	Biomass	Kernel weight	Yield	Harvest index
SRG35	SOKOLL/3/PASTOR//HXL7573/2*BAU*2/4/GLADIUS	125.64 h-i	3.52 c-i	7.60 g-j	0.79 e-j	0.22 j-l
SRG36	BABAX/LR42//BABAX*2/3/TUKURU	202.58 b	4.47 a-b	6.74 i-l	0.65 j-l	0.15 l-m
SRG37	TAM200/TUI/76//CAR422/ANA/5/BOBWHITE/CROW/BUC/PAVON76/3/YR/4/TAP	180.77 b-e	4.53 a	6.58 i-m	0.60 k-m	0.13 m
SRG38	R1122(KSRRVI)ND643//2*WBLL1	152.52 d-h	3.98 a-d	5.99 k-m	0.76 g-k	0.19 j-l
SRG39		518.33 a	3.31 d-i	5.07 m-n	0.44 m	0.13 m
$LSD_{0.05}$		34.48	0.75	1.55	0.10	0.12

Means bearing same letters are not significantly different. LSD: Least significant difference.

The lines showed varying levels of resistance to the four stem rust races with most lines showing more than one infection types to the same race and same line exhibiting different infection types with different races at seedling stage. The seedling infection types (IT) ranged from 0 to 2 except for a few lines which showed susceptible response (IT 3).

Out of the 39 lines evaluated, only *SRG21, SRG34* and *SRG39* showed a susceptible reaction (IT 3) to race *TTKST*, *SRG22* exhibited a susceptible reaction (IT 3) to race *TTKTK*, *SRG25, SRG32, SRG36* and *SRG37* displayed a susceptible reaction (IT 3) to race *TTKSK* and *SRG27* and *SRG39* revealed a susceptible reaction (IT 3) to race *TTTSK* the rest showed infection types of between 0 and 2.

In evaluation of lines for adult plant reaction to stem rust race *TTKST*, only four lines exhibited disease severity of ≤5% while 35 lines exhibited a severity of ≥10%. In contrary, 17 lines showed a severity of ≤5% while 22 lines showed a severity of ≥10% to races *TTKTK* and *TTKSK*. 18 lines displayed a severity of ≤5% while 21 lines displayed a severity of ≥10% to race *TTTSK*. All lines revealed a MSS response to race *TTKST* and *TTKTK* except for lines SRG13 which was moderately susceptible (MS) to race *TTKST* and SRG27 which was MS to race *TTKTK*. For race *TTKSK*, 82.05% of the lines exhibited a MSS response, 5% exhibited a MS response, 7% exhibited a moderately resistant/ moderately susceptible (M) response and 2.56% exhibited a susceptible (S) response while for race *TTTSK*, 84.61% displayed a MSS response, 10.25% showed moderately resistant (MR) and 5.12% showed MS responses (Table 4).

Table 4. Infection types and responses of CIMMYT wheat lines to four stem rust races evaluated in the greenhouse at seedling and adult plant stages, respectively.

Code	TTKSK			TTKST			TTTSK			TTKTK		
	Final rust severity (%)	AUDPC	Seedling IT	Final rust severity (%)	AUDPC	Seedling IT	Final rust severity (%)	AUDPC	Seedling IT	Final rust severity (%)	AUDPC	Seedling IT
SRG1	25 MSS	270	0 1	10 MSS	128	; 1	30 MSS	163	1 ;	5 MSS	110	2
SRG2	20 MSS	128	1 ;	20 MSS	238	0 1	15 MSS	178	2 1	15 MSS	128	1 ;
SRG3	10 MSS	36	1 0	15 MSS	80	; 1	5 MS	22	0 ;	15 MSS	110	; 0
SRG4	5 MSS	22	0 ;	20 MSS	115	0 ;	5MS	22	0 ;	10 MSS	220	; 0
SRG5	5 MSS	110	0 ;	25 MSS	180	0 ;	10 MSS	128	; 1	15 MSS	248	;
SRG6	10MSS	142	2 1	25 MSS	180	0 1	20 MSS	160	; 0	5 MSS	110	;
SRG7	5 MS	22	0 1	5 MSS	92	0 ;	5 MSS	110	; 0	5 MSS	110	0
SRG8	10 MSS	62	; 1	10 MSS	128	0 ;	10 MSS	110	; 0	10 MSS	110	0
SRG9	5 MSS	110	0 ;	15 MSS	54	0 ;	10 MSS	110	; 0	10 MSS	54	;
SRG10	5 MSS	110	0 ;	20 MSS	54	0 ;	5 MSS	22	0 ;	10 MSS	62	0
SRG11	10 MSS	110	1 ;	30 MSS	288	0 ;	5 MSS	110	2	5 MSS	36	; 1
SRG12	10 MSS	143	1 ; 2	10 MSS	128	0 ; 1	5 MSS	110	1	15 MSS	178	2 3
SRG13	5 MSS	110	0 ;	5 MS	36	0 ;	15 MSS	112	0 ;	5 MSS	110	1 ;
SRG14	15 MSS	110	2 2+	15 MSS	128	2 1	5 MSS	110	2	10 MSS	110	1 ;
SRG15	5 MR	110	1 ;	20 MSS	160	1 ;	5 MR	110	0 ;	10 MSS	160	; 0
SRG16	20 MSS	160	0 ;	25 MSS	195	0 ;	5 MSS	133	1	5 MSS	110	; 1
SRG17	5 S	110	2 1	10 MSS	128	1 0	5 MSS	128	1	10 MSS	110	2 1
SRG18	20 MSS	270	1 ;	15 MSS	160	2 1	15 MSS	128	2	10 MSS	128	2 1
SRG19	10 MSS	92	; 1	15 MSS	210	1 ;	15 MSS	183	;	5 MSS	110	1 ;
SRG20	15 MSS	160	; 1	15 MSS	145	; 1	10 MSS	128	1 0	5 MSS	92	1 ;
SRG21	5 MSS	36	0 1	15 MSS	80	2 3	5 MS	22	1 ;	5 MSS	36	2 ;
SRG22	10 MSS	110	0 1	30 MSS	195	2 1	5 MSS	110	1	20 MSS	248	2 3
SRG23	15 MSS	160	; 1	10 MSS	80	2 1	15 MSS	270	;	5 MSS	110	2 1
SRG24	15 MSS	178	2 1	5 MSS	110	1 ;	5 MR	110	; 0	10 MSS	110	2 1
SRG25	5 MSS	110	3 2	20 MSS	265	; 1	15 MSS	198	1 ;	10 MSS	110	2 1
SRG26	5 MSS	110	; 1	30 MSS	283	; 1	20 MSS	178	2 ;	10 MSS	62	; 0
SRG27	10 MSS	62	1 ;	15 MSS	160	0	5 MSS	110	3	5 MS	36	; 0
SRG28	10 MSS	110	2	10 MSS	160	0 ;	10 MSS	128	0 ;	15 MSS	178	1 ;
SRG29	5 M	22	0 ;	10 MSS	110	2 1	5 MSS	22	2	5 MSS	110	1 ;
SRG30	5 M	110	2 1	10 MSS	80	2 1	5 MSS	92	0	10 MSS	110	0 ;
SRG31	10 M	62	0 1	10 MSS	160	; 0	10 MSS	92	0 ;	5 MSS	110	; 0
SRG32	5 MS	22	3	10 MSS	92	0 ;	10 MSS	62	0;	5 MSS	62	; 1
SRG33	5 MSS	110	1 0	20 MSS	178	1 0	5 MSS	110	1;	10 MSS	198	1 2
SRG34	5 MSS	110	0	10 MSS	62	3 2+	5 MSS	92	2 1	5 MSS	110	; 1
SRG35	5 MSS	110	0 ;	5 MSS	92	2 1	10 MSS	128	1 0	10 MSS	110	1 ;
SRG36	15 MSS	160	3	15 MSS	128	1 0	30 MSS	315	1 ;	15 MSS	215	2 1
SRG37	10 MSS	110	3 2	10 MSS	220	1 ;	10 MSS	220	1 ;	5 MSS	92	2 2+
SRG38	10 MSS	110	0	10 MSS	62	1 ;	20 MSS	220	0	5 MSS	110	0 1
SRG39	30 MSS	253	0	30 MSS	518	2 3	50 MSS	713	3	20 MR	270	1 2

MR= moderately resistant, M=moderately resistant/Moderately susceptible; MS= moderately susceptible; S= Susceptible. IT infection type AUDPC Area under disease progress curve N/B For seedling infection type, the predominant infection types were listed first.

Agronomic traits of wheat

Highest mean biomass of 4.08 g/plant was displayed by line *SRG2*, this was 9.93% lower than that for *SRG36* (check) which showed a mean biomass of 4.58 g/plant while line *SRG23* exhibited the least mean biomass of 2.90 g/plant. Line *SRG13* showed the highest mean kernel weight of 11.12 g/plant while the least mean kernel weight of 4.19 g/plant was observed on line *SRG2*. Highest mean yield of 1.09 g/plant was demonstrated by line *SRG1*, this was significantly higher than that of *SRG38* (check) of 0.76 g/plant. Lowest mean yield of 0.59 g/plant was observed on line *SRG31* but this was still higher than the mean yield of the least performed check (*SRG39*) of 0.44

g/plant. Line *SRG28* showed the highest harvest index of 0.29 while least harvest index of 0.19 was exhibited by line *SRG31* (Table 3).

Discussion

Significant effect due to line suggest that the lines had genetic variations for the traits measured. Significant effect due to race suggests that the four races differed in their virulence as shown by their different effects on the parameters measured. Significant effects due to line×race indicates that there were variations in response of the lines to the races used in this experiment. Since the new races identified after race *TTKSK* showed higher mean AUDPC and lower yields than race *TTKSK*, this indicates that the new races are more virulent than the original race and are capable of reducing yield significantly as they exhibited lower mean yields than the mean yield for race *TTKSK*. This further demonstrated that, as much as the *Sr*31 gene was broken down, the varieties having it still bare some level of resistance to stem rust, thus preventing reduction in yields.

Lines which showed higher AUDPC displayed lower mean yield, this demonstrated that AUDPC is negatively correlated with yield because AUDPC indicate the level of infection. The results showed that line *SRG39* which showed the highest AUDPC had the lowest mean yield, therefore, AUDPC is directly related with the yield loss (Subba Rao *et al.*, 2008). Varieties which revealed low AUDPC may have good level of adult plant resistance (Wang *et al.*, 2005), in this study, lines showed good level of APR to race *TTKSK* since the mean AUDPC was low.

Results of the reaction of wheat lines to the four races showed that the tested lines differ in their resistance to stem rust races, the reason could probably be due to the difference in virulence among the stem rust races used in the greenhouse, differences in the number of resistance genes present and mode of gene action. Low infection types at seedling stage could have been due to the lines having resistance conferred by one single major gene that was broken down at adult plant stage (Mwando *et al.*, 2011). Those that revealed high infection types do not have effective seedling resistance genes against the races, these lines may possess race non-specific resistance (Sawhney, 1995) and may provide durable resistance when their field assessment results confirm their slow rusting character. Some of the lines showed moderately resistance reactions at adult plant stage. The lines which showed some resistance at adult stage may contain a major gene that remained resistant at seedling and at adult stage or they may have minor genes that are working together to reduce the disease (Roelfs, 1992). Also, those that exhibited low values of slow rusting at adult plant stage could have durable resistance (Sing *et al.*, 2005). Performance among sister lines suggested that they all had effective APR genes since they all showed a severity of ≤30% with the four races. The variation between sister lines *SRG31* and *SRG* 32 suggested that they both have a gene for seedling resistance to all the four races except for SRG 32 which lacks seedling resistance gene to race *TTTSK*. This difference may be attributed to the recombination of genes during development of the recombinant inbred lines.

In this study, most of the lines showed a final severity of ≤30%, Safavi and Afshari (2012) proposed that wheat lines with final rust severity values of 1-30%, 31-50% and 51-70% were regarded as possessing high, moderate, and low levels of slow rusting resistance, respectively. Therefore the lines in this study had high levels of slow rusting. Lines with a low final disease severity under high disease pressure may possess more additive genes (Singh *et al.*, 2005). Safavi and Afshari (2012) also used final severity as a parameter to assess slow rusting behaviour of wheat lines. The steady increase in spore sizes in some of the lines tested could have been due to lack of effective resistant genes in those lines. While those that their spore sizes remained constant had some resistant genes which lead to necrosis and chlorosis of the infected areas, consequently the death of the spores, therefore preventing the progress or development of the spore. Significant correlation between race *TTKTK* and season one and *TTKSK* and season one suggests that the two races were present in the field when the lines were being evaluated in the field in season one.

The difference in biomass among the lines tested might be as a result of genetic makeup of parental material of these lines, because all the lines were provided with the same environmental and management conditions. Similar results were observed by Dahleen *et al.* (1991) who found varied quantities of total biomass for varieties developed in the diversified regions. Moreover, Yagbasanlar and Ozkan (1995) also found similar results regarding the biomass in different wheat varieties. High grain yield and kernel weight observed in some lines might also be associated with parental genetic makeup of these lines, since under similar environmental and crop management conditions, the grain yield differed significantly. Grain yield of wheat varieties is mostly associated with the environmental conditions (Porfiri *et al.,* 2001) and the difference in grain yield of pigeon pea varieties developed in different ecologies were observed by Wamatu and Thomas, (2002).

The low yield and low kernel weight observed in some lines could be because the plants respond to inoculation with energy demanding physiological processes, probably defense reactions, using stored host energy that otherwise would go to growth and seed production. In addition, a reduction in photosynthetic leaf area due to hypersensitive flecking also can cause yield reductions (Khanna *et al*., 2005). In a study by Sayre *et al.* (1998), grain yield losses due to leaf rust in bread wheat were associated with reductions in kernel weight, kernels per square meter, spikes per square meter, and grain-filling rate. The relationship between reduction in kernel weight and yield losses caused by rust has been found by other authors (Singh and Huerta, 1994). In addition, the kernel weight, which is a function of size and weight/ density of individual kernels, has been shown to be affected by rust in several studies (Griffey *et al*., 1994).

Conclusion

Evaluation of wheat varieties for their resistances is very important in integrated stem rust management. Most of the tested bread wheat varieties do not have adequate resistance for dominant races (*TTKSK*, *TTKSK*, *TTKTK* and *TTTSK*) at seedling and adult plant stages. However, lines *SRG7, SRG13, SRG24* and *SRG35* showed low final disease severity (both in the field and greenhouse), low infection types and low AUDPC with the four races. These lines can be used as sources of stem rust resistance genes for the study area. Therefore, regular assessment and evaluation of wheat varieties against stem rust races is compulsory for virulence and/or avirulence information in Kenya. Moreover, there should be an urgent need for developing new wheat varieties resistant to the dominant stem rust races (*TTKSK*, *TTKSK*, *TTKTK* and *TTTSK*).

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