INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 7, No. 4, p. 1-13, 2015

RESEARCH PAPER OPEN ACCESS

Inheritance of stem rust (*Puccinia graminis* Pers. F. Sp. *Tritici* ericks and *E. Hen*) resistance in bread wheat (*Triticum aestivum* L.) lines to TTKST race

Zennah Kosgey[1*], James O. Owuoche[1], Michael A. Okiror[1], Peter N. Njau[2]

[1]*Egerton University (Njoro Campus), P.O. Box 536, Egerton, Kenya*

[3]*Kenya Agricultural and Livestock Research Organization (KALRO), P.O. Private Bag -20107, Njoro, Kenya*

Article published on October 12, 2015

Key words: Duplicate recessive epistasis, Inheritance, Stem rust, TTKST isolate Wheat.

Abstract

Stem rust disease caused by *Puccinia graminis* f.sp. *tritici* (*Pgt*) is currently one of the major biotic constraints in wheat (*Triticum aestivum*) production worldwide. Therefore, objectives of this study were (i) to identify resistant wheat lines with both adult plant resistance (APR) and seedling plant resistance (SPR), and (ii) to determine the kind of resistance to stem rust in KSL18, PCB52, PCB62 and PCB76 wheat lines. A collection of 100 wheat lines was evaluated in the field and greenhouse for stem rust resistance. The following four lines- KSL18, PCB52, PCB62 and PCB76 were identified as resistant and were crossed with known susceptible cultivars *Kwale* and *Duma*. The resulting F_1 hybrids and F_2 populations alongside the parents were then tested in the greenhouse for response to the stem rust race TTKST. The selected wheat lines exhibited infection types ';' to '2' depicting resistance while *Kwale* and *Duma* depicted infection type '3^+' to TTKST. In the F_2 populations evaluations that derived from *Kwale* × PCB52 indicated that the resistance is conferred by a single dominant gene. However, all other F_2 populations showed that the resistance was conferred by two genes complementing each other (duplicate recessive epistasis) thus the ratios 9R: 7S. These identified resistant lines could be evaluated for other qualities and passed as potential varieties or used as sources of valuable stem rust resistance.

* **Corresponding Author:** Zennah Kosgey

Introduction

The sources and inheritance of resistance genes to stem rust (*Puccinia graminis* f.sp *tritici*) (*Pgt*) in wheat (*Triticum aestivum* L.) is important to all wheat breeders in their endeavor to develop resistant varieties. Increased and sustainable productivity of this crop both locally and globally will be achieved largely if resistance to the rusts (yellow rust caused by *Puccinia striformis*; leaf rust caused by *Puccinia tauschii;* and stem rust caused by *Puccinia graminis* f.sp *tritici*) that have often reduced yields in Kenya and other wheat growing regions worldwide by as much as 100% in susceptible varieties are adopted (Leonard, 2001; Njau *et al*., 2010). The resurgence of stem rust has received high attention from breeders and pathologists in wheat breeding programs due to the yield losses incurred worldwide (Singh *et al*., 2011). Spraying with fungicides against stem rust is expensive, hazardous and a short term solution to the damage caused by this foliar disease. The foreseeable and feasible best option is bioresistance (Singh *et al*., 2006; Singh *et al*., 2008). A number of novel sources of resistance to stem rust have been reported. However, little is reported on the kind and mode of the resistances they carry. Such information is very important to all breeders wishing to design breeding strategies for its use. The mode of action of such genes is either dominant or recessive; single (monogenic) or multigenic (polygenic); autosomal or sex-linked (Bahadur *et al*., 2002; Odeny *et al*., 2009).

Inheritance studies on barley (*Hordeum vulgare*), oat (*Avena sativa*), beans (*Phaseolus vulgaris*), rye (*Secale cereal*) and pigeon pea (*Cajanus cajan*) have shown that major genes and/or modifying genes are vital in conferring resistance to various pathogens at the various growth stage i.e seedling and adult plant stages. For example, resistance to *Puccinia coronata avenae* in spring oats is mainly conferred by major genes and wheat lines possessing *Lr28* gene showed dominant genes governing resistance to leaf rust races 77-1, 77-2 and 77-5 (Bansal *et al*., 2008; Staletic *et al*., 2009) . However, the resistance to stem rust in wheat with *Tr129* background to race MCCF is conditioned by two dominant genes (Ghazvini *et al*., 2012). In barley, resistance to isolate 76-32-1335 of *Puccinia graminis* f. sp. *secalis* is conferred by one recessive gene (Steffenson *et al*., 1985). With such an array of resistances to stem rust, it is proposed that pyramiding these genes may offer even a more lasting, durable and broad based resistance. To achieve this, it is essential that the breeder is equipped with knowledge on the kind of gene action in such genes. Thus, the objectives of this study were one, to select resistant lines at adult plant and seedling stages, and two, to determine the nature and number of genes conferring this resistance in selected wheat lines

Materials and methods

Screening for resistance in recombinant inbred wheat lines

Field screening

(a) *Experimental site*

This study was conducted at Kenya Agricultural and Livestock Research Organization (KALRO) (- 0° 20' 29˝ N 35° 56' 40" E) Njoro centre-Kenya. The area experiences an average annual rainfall of 939.3 mm (average of 60 years) (Kenya Metereological Station Identification Number 9031021) and average temperatures of 9 °C (minimum) and 24 °C (maximum). The soils are predominantly mollic phaeozems with a pH of 7.0.

(b) Genotypes

A hundred semi dwarf recombinant inbred wheat lines that originated from CIMMYT; *Parcela Chica* (small plots) Bread Wheat Rainfed Lines (PCBWR) and Kenyan Selected Lines (KSL) were evaluated for stem rust resistance under field conditions over two seasons (2012 and 2013) against stem rust *Pgt* race *Ug 99* and its variants.

(c) *Experimental procedure*

Evaluation was done in the field that was previously under soyabean (*Glycine max*) crop. The field was prepared by first spraying it with a non selective herbicide, round up (glyphosate) at the rate of 360g ha^{-1} and three weeks later, disc ploughed and harrowed to a fine tilth suitable for wheat planting.

Each entry was planted to two 1.5m rows spaced 0.5m apart. The susceptible cultivar *Caccuke* was planted after every 20 entries for disease build up monitoring. Several other cultivars susceptible to TTKST and TTKSK races were used as spreaders and planted perpendicular to all entries to supply adequate rust inoculum. Di-ammonium phosphate fertilizer was applied at planting at the rate of 125 kg ha^{-1} to supply an equivalent rate of 22.5 kg ha^{-1} of N and 25 kg ha^{-1} of P. Calcium ammonium nitrate (CAN) fertilizer was applied at growth stage (GS) 20-29 at the rate of 100kg ha^{-1} to provide 33 kg N ha^{-1}. Pre-emergence herbicide, Buctril MC (Bromoxynil octanoate, 225 g ha^{-1} and MCPA Ethyl Hexyl Ester, 225 g ha^{-1}) was applied at GS 20-29 (Zadoks *et al*., 1974) to further control annual broad leaved weeds. Also, Bulldock (beta-cyfluthrin), a systemic insecticide was sprayed at the rate of 31 g ha^{-1} to control both sucking and chewing pests.

(d) *TTKST and TTKSK pathogen build up.*

Epidemics were induced through artificial inoculation with inoculants prepared from spreader row plants. Rusted stems of these plants were harvested and chopped into small pieces and soaked in a few drops of tween 20 and water to provide concentration of 4×10^6 spores ml^{-1}. The spreaders were inoculated using a syringe at the stage GS 30-49 (Zadoks *et al*., 1974) late in the evening. To enhance humidity, these plants were repeatedly irrigated thereby enhance stem rust infection and spread.

(e) *Data collection*

Evaluation was based on pustule size and the associated necrosis. Disease severities were observed as R= resistant (small uredinia surrounded by necrosis), MR= moderately resistant (medium-sized uredinia surrounded by necrosis), MS= moderately susceptible (medium-sized uredinia without necrosis), S= susceptible (large uredinia without necrosis), MSS= moderately susceptible to susceptible (medium to large-sized uredinia without necrosis) and MRMS= infection response that overlap the MR and MS categories (Roelfs *et al*., 1992). Stem rust severity scoring begun when the spreader rows had attained 50% infection based on modified Cobbs scale where 0%= immune (no uredinia or any other sign of infection) and 100%= completely susceptible (large uredinia without necrosis) (Peterson *et al*., 1948). Disease was evaluated three times at an interval of 10 days between heading (GS 50-69) and plant maturity (GS 70-89) (Zadoks *et al*., 1974).

Seedling screening in the greenhouse

The hundred inbred wheat lines were also screened under glasshouse conditions. Ten seeds of each line were planted in square 6 × 6 cm plastic pots in two batches. Infected wheat plant stems were collected from screening nurseries, chopped into small pieces and suspended in light mineral oil (Soltrol 170). The spore suspension was adjusted with more oil to obtain a spore concentration of 4×10^6 spores ml^{-1}. At growth stage 12, the seedlings were inoculated by atomizing spores solution using a hand sprayer. The plants were then air dried for 30 minutes in an inoculation chamber, then placed in a dew chamber set at 16 -18 ^{o}C and about 100 RH for 48 hours and finally transferred to a greenhouse bench maintained at 20 ^{o}C. After 14 days, seedlings were evaluated for infection types based on a 0 to 4 scale (Stakman *et al*., 1962). Evaluation was based on uredinia size and presence or absence of necrotic regions. In this scale, '0'= no uredinia or any other sign of infections, '; -fleck'= presence of hypersensitive necrotic flecks but no uredinia, '1'= small uredinia surrounded by necrotic regions, '2'= small to medium size uredinia surrounded by necrosis, '3'= medium sized uredinia without necrosis and '4'= large uredinia without necrosis. Infection types '0', ';', '1' and '2' were categorized as resistant whereas '3' and '4' as susceptible. Infection types were confirmed by re-evaluating another set of the lines for the second time.

Genetics of resistance to *TTKST* race

(a) Parental stock and development of F_1 and F_2 populations

From the screening above, four resistant lines; KSL18, PCB52, PCB62 and PCB76 were identified. These and two adapted susceptible cultivars *Kwale* and *Duma* were used in this study (Table 2). KSL18, PCB52,

PCB62 and *Kwale* are hard white spring wheat while *Duma* and PCB76 are hard red spring wheats. Also, KSL18, PCB62 and PCB76 are early maturing while PCB52, *Kwale* and *Duma* are medium in maturing. F_1 and F_2 populations were derived from straight crosses between the selected resistant recombinant inbred lines and the susceptible cultivars *Kwale* and *Duma*.

(b) Preparation of TTKST pure race of stem rust.

Spores of the race TTKST were purified on a universal susceptible wheat cultivar *Kenya Mwamba*. Five seeds of cultivar K. *Mwamba* were each sown in ten 6 cm-diameter plastic pots filled with approximately 60g of vermiculite. At growth stage GS 12, seedlings thereof were inoculated with spores collected from *Sr24* spreader wheat plants. Inoculum was prepared and seedlings of *Kenya Mwamba* inoculated as in the glasshouse experiment above. After 14 days, a single pustule was collected into a capsule using automizer machine. This pustule was then dissolved in soltrol oil and atomized on seedlings of *Kenya Mwamba* for the spore multiplication. The purified spores were then bulk-collected, packed in capsules and stored at -20 °C to ensure their viabilities.

(c) Glasshouse screening of F_1 and F_2 populations

Five seeds of each purified inbred parents and their F_1s and a hundred and fifty seeds of each F_2 population of the eight crosses were planted in 6 × 6 cm plastic pots set up in the greenhouse. At the GS 12, inoculations were made by suspending the purified *TTKST* spores in light mineral soltrol oil and spraying the seedlings. These seedlings were handled and evaluated after 14 days as above mentioned in the greenhouse experiment.

(c) Data analysis of F_2 populations

The infection types observed on parents, F_1 and F_2 genotypes were categorized into resistant ('0', ';', '1', '2', '2$^-$' '2$^+$') and susceptible ('3', '3$^-$', '3$^+$', '4'). The data of the F_2 populations were analyzed using SAS version 9.4 (SAS, 2012) for a fit into the 3:1 ratio or other ratios as follows:

$$\chi 2 = \sum \frac{(O-E)^2}{E}$$

Where χ^2 = *Chi* Square value, $\sum$ = Summation, O = Observed numbers in each category and E = Expected numbers in the corresponding category according to hypothesis

Phenotypic correlation was performed between seedling infection types observed on F_2 populations that conformed to 9:7 and 3:1 resistant: susceptible ratio using SAS version 9.4 (SAS, 2012) basing on the following formula:

$$r = \frac{n(\sum XY) - (\sum X)(\sum Y)}{\sqrt{[n\,(\sum X^2) - (\sum X)^2]\,[n\,(\sum Y^2) - (\sum Y)^2]}}$$

Where r = Pearson correlation coefficient, x = values in set of infection type data from one population, y = values in the set of infection type data from the next population and n = Total number of values (Ott and Longnecker, 2001).

Results

Screening for resistance in recombinant inbred wheat lines

Among the wheat lines evaluated for *Pgt*, severity in the field and greenhouse showed that there was genotypic variability for both adult and seedling resistance (Table 2). In the field, seasonal variations affected the severity of stem rust in the wheat lines as most lines were heavily infested in the 2013 season compared to the 2012 season (Table 2). The hard white spring wheat lines KSL 18, PCB52, PCB 62 and PCB 76 had low disease severities (10 MR to 30 MR) in 2013 than in 2012 and exhibited resistant seedling infection types '2' to '2$^+$' in the greenhouse. Thus, based on their adult and seedling reactions to *Pgt* infections, the four above mentioned lines were selected as a valuable source of resistance to stem rust. They were therefore used in crossings with known susceptible cultivars *Kwale* (40 MSS, adult plant resistance (APR); '3', seedling infections) and *Duma* (40 MSS, APR); '3', seedling infections) (Table 2). The reactions of the other lines pointed to susceptibility for instance, lines PCB 34 and PCB 35 were rated 15 MSS and 20MSS, respectively in the field (adult) and '3+' and '4', respectively in the greenhouse (seedling stage) (Table 2). Also lines PCB

65 and PCB 66, that are sister lines reacted differently. PCB 65 was susceptible to stem rust at seedling stage with infection type '3+' but responded to field infection with susceptibility severity of 60 S. However, PCB 66 was resistant at seedling stage with infection types ';', '1+' (Table 2). There was differential resistance observed between PCB 76 and PCB 77 that are sister lines. The virulence of TTKST on lines KSL 3, PCB 29, PCB 37, PCB 65, PCB 69, PCB 71, PCB 77 and PCB 79 at seedling was low (';1' to '2+') despite the fact that the severity of 20M to 40MSS were observed in the field (Table 2). This suggests that these lines have seedling resistance genes to *Pgt* but are devoid of adult resistance genes.

Table 1. Mean temperatures and rainfall experienced during the evaluation period of 2012 and 2013 at KALRO-Njoro.

Season	January	February	March	April	May
2012					
Min. Temp. °C (Mean)	10.0	16.0	18.0	14.0	12.0
Max. Temp. °C (Mean)	23.0	18.0	22.0	24.0	22.0
Mean rainfall (mm)	0.0	13.6	11.0	295.0	183.7
2013	August	September	October	November	December
Min. Temp. °C (Mean)	8.0	9.0	10.0	10.0	10.0
Max. Temp. °C (Mean)	22.0	23.0	21.0	22.0	23.0
Mean rainfall (mm)	110.6	173.3	73.9	60.6	137.5

Min. = Minimum, Max. = Maximum, Temp. = Temperature, mm= Millimeters.

Table 2. Field and greenhouse evaluation of 28% Recombinant inbred wheat lines from KALRO-Njoro stem rust screening nursery for resistance to predominant *Ug99* races.

Genotype	Pedigree	Stem rust severity 20122013		Seedling infection type
KSL1	SSERI1/CHIBIA/4/BAV92//IRENA/KAUZ/3/HUITES	30MSS	50MSS	3+
KSL2	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/PFAU/WEAVER//BRAMBLING/6/BAV92	20MSS	30MSS	3+
KSL18	WBLL1*2/KURUKU/4/BABAX/LR42//BABAX*2/3/KURUKU	20MR	10MR	2+
PCB5	WBLL1*2/BRAMBLING/3/KIRITATI//PBW65/2*SERI.1B	20M	30MSS	2-
PCB27	WBLL1*2/KUKUNA/4/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1	40M	50MSS	3+
KSL3	BAV92//IRENA/KAUZ/3/HUITES*2/4/CROC_1/AE.SQUARROSA (224)//KULIN/3/WESTONIA	20 MSS	40MSS	2+
PCB4	WAXWING/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/KIRITATI//ATTILA*2/PASTOR	30M	40MSS	3+
PCB7	FRANCOLIN #1/MESIA//MUNAL #1	25 M	25MSS	4
PCB9	ALTAR84/AE.SQUARROSA(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/KACHU/6/KIRITATI//PBW65/2*SERI.1B	30M	5MSS	3+
PCB29	KISKADEE #1//KIRITATI/2*TRCH	20M	40MSS	2+
PCB30	MUNAL/3/HUW234+LR34/PRINIA//PFAU/WEAVER	40M	30MSS	4
PCB31	PBW65/2*PASTOR/3/KIRITATI//ATTILA*2/PASTOR/4/DANPHE #1	30M	60MSS	3+
PCB34	ATTILA/3*BCN//BAV92/3/PASTOR/4/TACUPETO	10M	15MSS	3+

Genotype	Pedigree	Stem rust severity 20122013		Seedling infection type
PCB35	F2001*2/BRAMBLING/5/PAURAQ ATTILA/3*BCN//BAV92/3/PASTOR/4/TACUPETO F2001*2/BRAMBLING/5/PAURAQ	15M	20MSS	4
PCB36	KACHU/BECARD//WBLL1*2/BRAMBLING	20M	30MSS	3+
PCB37	PFAU/SERI.1B//AMAD/3/WAXWING*2/4/TECUE #1	40 M	20M	2+
PCB40	PCAFLR/KINGBIRD #1//KIRITATI/2*TRCH	30 M	30M	3+
PCB41	MUNAL*2//WBLL1*2/BRAMBLING	25M	30MSS	3-
PCB52	MUU/KBIRD	5RMR	20MR	2+
PCB62	CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92*2/4/QUAIU	30MR	30MR	2
PCB44	ND643/2*TRCH//BECARD/3/BECARD	40M	60MSS	3+
PCB65	ND643//2*ATTILA*2/PASTOR/3/WBLL1*2/KURUKU/4/WBL L1*2/BRAMBLING	40 M	50M	;1
PCB66	ND643//2*ATTILA*2/PASTOR/3/WBLL1*2/KURUKU/4/WBL L1*2/BRAMBLING	40M	60MSS	3+
PCB69	KIRITATI//ATTILA*2/PASTOR/3/AKURI	30 M	20M	;1+
PCB71	HUIRIVIS #1/MUU//WBLL1*2/BRAMBLING	10MR	20M	;1
PCB76	BABAX/LR42//BABAX*2/3/TUKURU*2/4/HEILO	50MR	30MR	2+
PCB77	BABAX/LR42//BABAX*2/3/TUKURU*2/4/HEILO	20MR	40M	2+
PCB79	PICUS/3/KAUZ*2/BOW//KAUZ/4/KKTS/5/T.SPELTA PI348530/6/2*FRANCOLIN #1	20M	40MSS	2+
KWALE	KAVKAZ/ TANORI-71/3/MAYA-74(SIB)//BLUEBIRD/INIA-66	30MS	40MSS	3
DUMA	AURORA/UP301//GALLO/SUPER X/3/PEWEE/4/MAIPO/MAYA 74//PEWEE	30MSS	40MSS	3

IT (Infecton types) are based on 0 to 4 scale as described by Stakman *et al.* (1962). Field disease response and severities are based on Roelfs *et al.* (1992) and Peterson *et al.* (1948).

Genetics of resistance to TTKST race

In the determination of the genetics of resistance in the identified four resistant lines, it was found that all the F_1s from the eight crosses were resistant (Table 3). Hybrids from *Kwale* × PCB52, *Kwale* × KSL 18, *Kwale* × PCB 76 and *Kwale* × PCB62 crosses with resistant genes placed in *Kwale* background showed infection types ';1+', '2' from PCB 52, '2', '2+' from KSL18, '; 1+' from PCB 76 and '; 1+','2' from PCB 62. F_1 genotypes derived from a cross that *Duma* was used as recipient parent of the gene exhibited '2' to '2+' infection types compared to '; 1+', '2' infection types observed on the F_1 genotypes derived from crosses involving susceptible cultivar *Kwale*. The F_2 population derived from *Kwale* × PCB 52 fitted segregating ratio of 3:1 (R: S) (χ^2= 0.6881). In this cross, 72% of the progenies exhibited '0' to '2' infection types. Among the resistant genotypes, 39% of the progenies showed resistant reactions of '0' and ';' infection types, whereas 28% showed susceptible reaction of '3' to '4' infection types (Fig. 1).

The F_2 genotypes derived from *Kwale* × KSL18, *Kwale* × PCB 76 and *Kwale* × PCB 62 crosses showed infection types that conformed to 9:7 (χ^2- 0.9900, 0.6796, 0.3608, respectively) ratio of resistance to susceptible. From these three crosses, 61, 56 and 59% of the genotypes fall in resistant class of '0' to '2' infection types, respectively (Fig. 1). Considering '0' and ';' infection types, 30, 27 and 18% of the individuals, respectively, were considered highly resistant. All the F_2 progenies that involved *Duma* as the female parent (*Duma* × PCB 52, *Duma* × KSL 18, *Duma* × PCB 76 and *Duma* × PCB 62) conformed to

9:7 ratio of resistance to susceptible, (χ^2 - 2.3179, 0.1668, 2.6840 and 1.3593), respectively (Fig. 1). The crosses showed the proportions of 63, 58, 64 and 55%, respectively, of resistant genotypes with the infection types ranging from '0' to '2' (Fig. 1). The progenies of the cross *Duma* × PCB 52 had 16% of the genotypes exhibiting '0' and ';' infection types, while 37% of the genotypes were susceptible (Fig. 1). Progenies of the cross *Duma* × KSL 18 had the least proportion (9%) of the genotypes exhibiting '0' and ';' infection types while 42% were susceptible (Fig. 1). F_2 progenies developed from *Duma* × PCB 76 progenies had 16% showing '0' and ';' infection types and 30% of the genotypes were susceptible exhibiting '3' to '4' infection types (Fig. 1). The progenies of the cross *Duma* × PCB62 had 11% of the genotypes exhibiting '0' and ';' infection types and the highest proportion of 36% showed a '3' infection type (Fig. 1).

Table 3. Infection types (IT) of parents, F_1 plants and segregation in F_2 populations to pathotype *Pgt*-TTKST of *Puccinia graminis* f.sp. *tritici* at seedling stage.

Parents	Observed Infection types	Resistant	Susceptible	Observed ratio (R:S)	*Chi*-square (χ^2)	p- value
Kwale	3^+					
Duma	3^+					
PCB52	2^+					
KSL18	2^+					
PCB76	;1					
PCB62	2+					
Crosses with *Kwale* as susceptible parent						
F_1 (*Kwale* × PCB52)	;1^+, 2					
F_2 (*Kwale* × PCB52)		78	31	3:1	0.6881	0.4068
F_1 (*Kwale* × KSL18)	2, 2^+					
F_2 (*Kwale* × KSL18)		77	50	9:7	0.9900	0.3197
F_1 (*Kwale* × PCB76)	;1^+					
F_2 (*Kwale* × PCB76)		61	47	9:7	0.6796	0.4097
F_1 (*Kwale* × PCB62)	;1^+, 2					
F_2 (*Kwale* × PCB62)		62	48	9:7	0.3608	0.5481
Crosses with *Duma* as susceptible parent						
F_1 (*Duma* × PCB52)	2^+					
F_2 (*Duma* × PCB52)		68	39	9:7	2.3179	0.1279
F_1 (*Duma* × KSL18)	1^+, 2, 2^+					
F_2 (*Duma* × KSL18)		64	46	9:7	0.1668	0.6830
F_1 (*Duma* × PCB76)	2, 2^+					
F_2 (*Duma* × PCB76)		62	49	9:7	2.6840	0.1014
F_1 (*Duma* × PCB62)	2					
F_2 (*Duma* × PCB62)		61	40	9:7	1.3593	0.2437

Infection type (IT) was based on the scale described by Stakman *et al.* (1962) with ITs ; 1, 2 considered resistant and 3 considered susceptible. Positive (+) = larger uredinia than the normal size and negative (-) = smaller uredinia than the normal size.

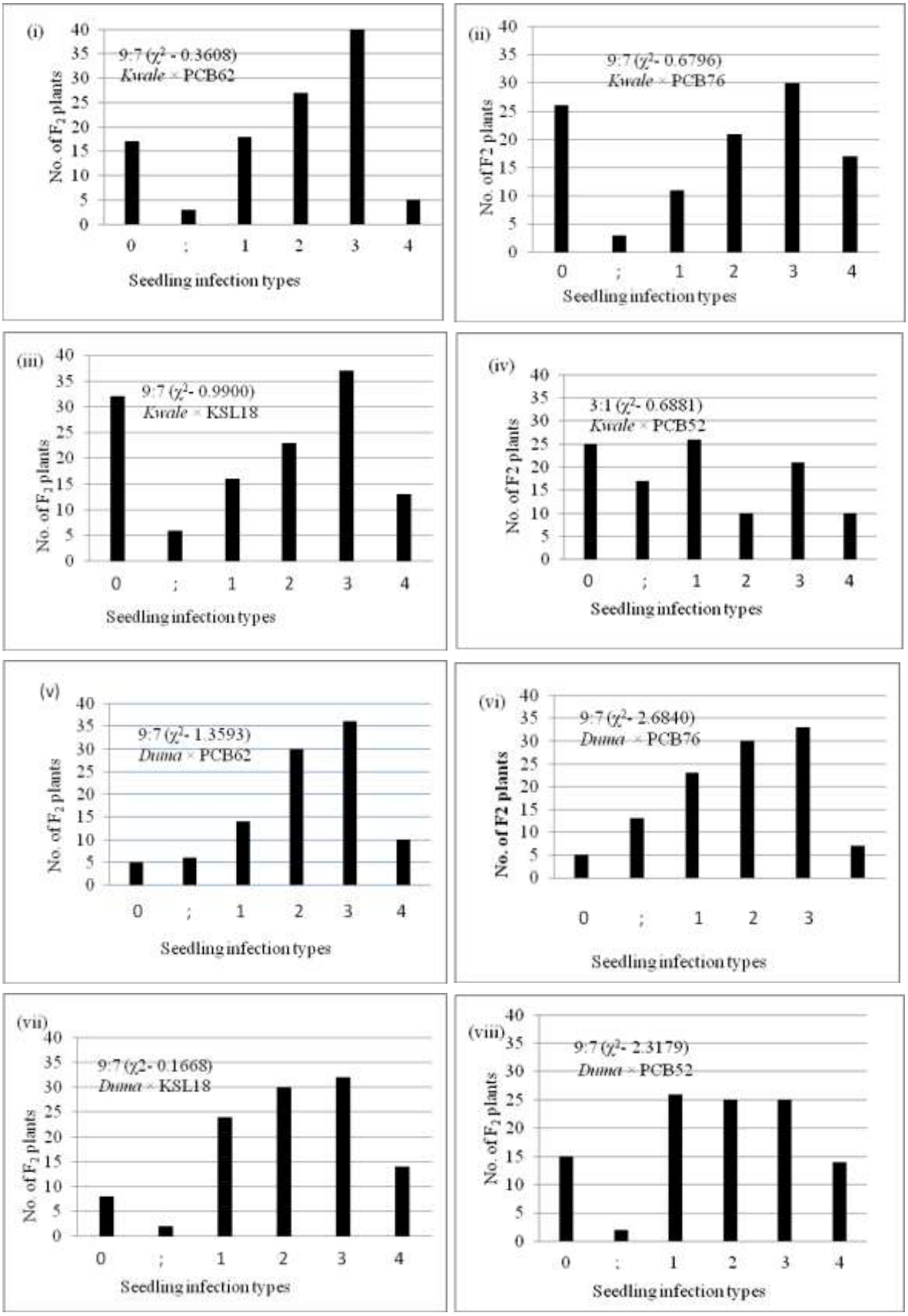

Fig. 1. Proportion of seedling infection types of F_2 wheat (*Triticum aestivum*) populations developed from eight crosses and evaluated in the greenhouse for *Puccinia graminis* f. sp. *tritici* race TTKST (category: resistant= 0, ;, 1, 2; susceptible= 3 and 4.

Table 4. Correlation among observed seedling infection types on F_2 populations evaluated for resistance to race TTKST.

	Kwale × PCB62	*Duma* × PCB62	*Duma* × PCB76	*Kwale* × PCB76	*Kwale* × KSL18	*Duma* × KSL18	*Duma* × PCB52	*Kwale* × PCB52
Kwale × PCB62		0.888 *	0.811 *	0.753	0.842*	0.847*	0.786	0.215
Duma × PCB62			0.920**	0.481	0.564	0.912*	0.716	-0.190
Duma × PCB76				0.239	0.369	0.876*	0.701	-0.023
Kwale × PCB76					0.979 ***	0.453	0.531	0.246
Kwale × KSL18						0.527	0.586	0.354
Duma × KSL18							0.925 **	-0.065
Duma × PCB52								0.169
Kwale ×PCB52								

*, **, *** = significant at 0.05, 0.01 and 0.001, respectively.

Discussion

Screening for resistance in recombinant inbred wheat lines

Stem rust severity on evaluated wheat lines in the field varied between the seasons having most of the lines severely infected in 2013 compared to the 2012 season. The genotypic variation for *Pgt* severity between the two seasons was due to weather related factors that favored infection. The ideal temperatures for the germination of the urediniospores (minimum, 2 ˚C; optimum, 15 - 24 ˚C and maximum is 30 ˚C) whereas 5 ˚C, 30 ˚C and 40 °C are the minimum, optimum and maximum, respectively, for the sporulation of the spores (Hogg *et al*., 1969, Roelfs *et al*., 1992). The temperatures in this study were within the favorable range for the establishment and development of *Pgt* pathogen in both years (Table 1). The effects of varying temperature levels on expression of genes influence either compatibility or incompatibility of the host genotypes and pathogens (Luig and Rajaram, 1972; Steffenson *et al*., 1985). Effects of temperatures on expression of these genes under controlled environments were not within the scope of this study. In addition to the ideal temperatures, rust develops in the presence of heavy dews and high humidity (Todorovska *et al*., 2009; Murray *et al*., 2010) hence the high precipitation experienced in the second year seems to have contributed and favored *Pgt* severity (Table 1). Infection of diseases such as net blotch (*Pyrenophora teres Drechs*.) in barley (*Hordeum vulgare*), is often favored by high rainfall experienced during the growing seasons (Steffenson and Webster, 1992). Because of high severity observed in the second season, the data from this season formed the basis of selection of lines showing field resistance to *Pgt*.

The genotypic variability observed in the field evaluation indicated that lines KSL 18, PCB 52, PCB 62 and PCB 76 were resistant to *Pgt*. The presence of adult plant and seedling resistance genes against race *Ug99* in wheat are often beneficial to farmers. In this study, low severity at adult plant and seedling stages suggested that these four lines possess both seedling and adult resistance genes that could be used for improvement of adapted varieties. Unfortunately, the identification and location of these *Pgt* genes in wheat genomes have not yet been identified. The occurrence of adult and seedling resistance for tanspot (*Pyrenophora tritici-repentis Drechs*), leaf rust and stripe rust (*Puccinia striiformis*) on the same background has benefited wheat breeders in most parts of the world (Park and Pathan, 2006; Park *et al*., 2008;Tadesse *et al*., 2011). The virulence of *Pgt* race TTKST on related lines PCB 34 and PCB 35 clearly indicated that these genotypes do not posses adult and seedling resistance genes (Table 2). Neither cultivar *Kwale* nor *Duma* possessed adult or seedling resistance genes to *Pgt* hence used for inheritance studies. The variation between sister lines PCB 65 and PCB 66 suggested that PCB 65 has a gene for seedling resistance but none of them has adult resistance genes (Table 2). This difference may be attributed to the recombination of genes during development of the recombinant inbred lines. Avirulence of TTKST on lines KSL 3, PCB 29, PCB 37, PCB 65, PCB 69, PCB 71, PCB 77 and PCB 79 at seedling stage indicate that these lines posses major genes for resistance.

Unfortunately, these lines did not have genes for adult resistance and this may not be useful for field resistance (Table 2).

Genetics of resistance to TTKST race

All the male parental genotypes and hybrids were resistant to *Pgt* race TTKST. Resistance depicted by low infection types on F_1 and segregation of F_2 genotypes is an indication that the resistance at F_1 is conferred by major gene(s). The resistant infection types observed on PCB 52, KSL 18, PCB 76 and PCB 62 at seedling stage suggested that these lines posses resistant genes that could be useful for improvement of wheat varieties. The segregation ratios for resistance to *Pgt* suggested that major genes that are modified due to epistatic effect are involved in conferring resistance to race TTKST. Nevertheless, apart from the *Kwale* × PCB 52 cross whose segregation ratio conformed to 3:1 (R:S), the 9:7 ratio observed on the other crosses demonstrated that modifying genes that influenced the resistance genes to *Pgt* TTKST may be due to duplicate recessive epistasis (Staletic *et al*., 2009). The observed 9R:7S ratio in this study was also depicted by barley F_2 progenies derived from the Steptoe × Q 21861 cross when they were screened against stem rust *Pgt* MCC and QCC races (Jin *et al*., 1994). However, double recessive genes (9S:7R) conferred resistance to head bug in populations developed from different sorghum genotypes (Aladele and Ezeaku, 2003).

Other than the resistance to *Pgt* in wheat lines noted in this study, a single dominant gene also conferred resistance to leaf rust and powdery mildew (*Blumeria graminis* f.sp *hordei*) in barley, chocolate spot (*Botrytis fabae*) in faba bean (*Vicia faba* L.), leaf rust in wheat cultivars and Asian soybean rust (*Phakopsora pachyrhizi*) in soybean (*Glycine max*) (Abbassi *et al*., 2004; Charan *et al*., 2006; Ghazvini *et al*., 2012; Naghavi *et al*., 2002). In addition, two dominant independent (15R:1S) and three dominant genes (63S:1S) conferred resistance to leaf rust races 0R9 (106), 29 R23 (104B), 109 R31-1 (77-2), HD 2285, Vaishali and HD 2189 in wheat (Bahadur *et al*., 2002; Charan *et al*., 2006). Crosses that involved *Kwale* cultivar (*Kwale* × PCB 62, *Kwale* × PCB 76 and *Kwale* × KSL 18) have more or less the same frequencies of '0' infection type in the resistant categories, an indication that a major/dominant gene was expressed in these crosses (Fig. 1). When infection types of *Kwale* × PCB 76 and *Kwale* × KSL18 populations were correlated, positive associations were noted between the infection types in all categories (Fig. 1, Table 4). This showed that the effect of resistant and modifying genes were the same in the *Kwale* background because of the expression of 9:7 ratios in F_2 population. The infection types in *Kwale* × PCB 62 and *Kwale* × KSL18 crosses were also positively correlated (Table 4) and this association indicated that mode of gene action was the same because the proportion of infection types in the resistant (';', '1', '2') and susceptible ('3', '4') categories were more or less the same.

Crosses that involved cultivar *Duma* (*Duma* × KSL 18, *Duma* × PCB 76 and *Duma* × PCB 62) had less number of resistant categories ('0', ';', '1', '2') compared to susceptible categories ('3', '4') (Fig.ure 1) and this suggests probably some recessive genes were involved in the modification of the resistance genes. However, single recessive genes (3S:1R) conferred resistance to *Pgt* races (ND8702 and ND89-3) in barley, resistance to fusarium wilt in pigeon pea and head bug resistance in sorghum (Aladele and Ezeaku, 2003; Karimi *et al*., 2010). Among the crosses, only *Kwale* × PCB 52 exhibited 3:1 ratio without modifying effects. In addition, there were more infection types in the resistant category ('0',';','1','2') compared to those in the susceptible category ('3', '4') in *Kwale* × PCB 52 cross. This was an indication that PCB 52 donated a major gene for resistance to *Pgt* at seedling stage. A single dominant gene also conferred resistance to *Pgt* f.sp. hordei in barley and wheat populations derived from four resistant and one susceptible bread wheat cultivars against three different leaf rust races; 0R9 (106), 29R23 (104B) and 109R31-1 (77-2) (Charan *et al*., 2006).

Conclusion

The inheritance of resistance in the wheat lines to *Puccinia graminis* f.sp *tritici* was conditioned by a single major gene due to the observed 3R:1S ratio in *Kwale* × PCB 52 cross and epistatic effect of two interacting major genes (9R:7S) in crosses derived from PCB62, PCB76 and KSL 18 lines. *Duma* cultivar could be having recessive modifying genes for *Pgt* resistance. It would be advisable to locate these genes in the wheat genome and furthermore lines that showed both adult and seedling resistance could be used for the improvement of *Pgt* resistance in adapted wheat varieties in the breeding programs.

Acknowledgment

The financial and technical support from Kenya Agricultural and Livestock Research Organization (KALRO) through Durable Rust Resistance in Wheat (DRRW) project is highly acknowledged.

References

Abbassi MA, Bahadur P, Prabhu KV, Charan R. 2004. Genetics of resistance in bread wheat cultivars HD 2687, HD2501, HW 2004, UP, 2425 and RAJ 3765 to leaf rust. Indian Phytopathology **57**, 422 - 426.

Aladele SE, Ezeaku IE. 2003. Inheritance of resistance to head bug (Eurystylus oldi) in grain sorghum (Sorgum bicolor). African Journal of Biotechnology **2**, 202 - 205.

Bahadur P, Charan R, Sharma JB. 2002. Inheritance of resistance to leaf rust in four bread wheats. Indian Phytopathology **55**, 163 -168.

Bansal UK, Saini RG, Khanna R. 2008. Inheritance of leaf rust resistance in wheat lines carrying Aegilops speltoides Tausch. Translocation in Chinese spring background. Journal of Applied Genetics **49**, 141-145.

Charan R, Bharathidasan S, Bahadur P. 2006. Inheritance of resistance to leaf rust in bread wheat cultivars HUW 510, HW 2044, K 9644 and PBW 443. Indian Phytopathology **59**, 482-485.

Ghazvini H, Hiebert CW, Zegeye T, Liu S, Dilawari M, Tsilo T, Anderson JA, Rouse MN, Jin Y, Fetch T. 2012. Inheritance of resistance to Ug99 stem rust in wheat cultivar Norin 40 and genetic mapping of Sr42. Theoretical Applied Genetics **125**, 817- 824.

Hogg WH, Hounam CE, Mallik AK, Zadoks JC. 1969. Meteorological factors affecting the epidemiology of wheat rusts. WMO Technical note no. 99 p. 143.

Jin Y, Steffenson BJ, Miller JD. 1994. Inheritance of resistance to pathotypes QCC and MCC of Puccinia graminis f. sp. tritici in barley line Q21861 and temperature effects on the expression of resistance. Phytopathology **84**, 452- 455.

Karimi R, Owuoche JO, Silim SN. 2010. Inheritance of fusarium wilt resistance in pigeonpea (Cajanus cajan (L.) Millspaugh. Indianjjournal of Genetics **70,** 1- 6.

Leonard KJ. 2001. Stem rust- Future enemy? In "Stem Rust of Wheat: From Ancient Enemy to Modern Foe" (P. D. Peterson, ed.), APS Press, St. Paul, MN. p. 119-146.

Luig NH, Rajaram S. 1972. The effect of temperature and genetic background on host gene expression and interaction to puccinia graminis tritici. Phytopathology **62**, 1171-1174.

Murray T, Milus G, McMullen M, Carson M, Chen X, Jin Y, Marshall D. 2010. Recovery Plan for Stem Rust of Wheat caused by *Puccinia graminis* f. sp. *tritici* Ug99 (race TTKSK) and its derivatives. The American Phytopathological Society, p. 2- 27.

Naghavi MR, Ghanadha MR, Yazdi-Samadi B. 2002. Inheritance of Resistance to Powdery Mildew

(*Erysiphe graminis* f. sp. *hordei*) in Barley. Journal of Agriculture Science and Technology **4**, 135-139.

Njau PN, Jin Y, Huerta-Espino J, Keller B, Singh RP. 2010. Identification and evaluation of sources of resistance to stem rust race *Ug99* in wheat. Plant Disease **94**, 413-419.

Odeny DA, Kimani PM, Githiri SM. 2009. Inheritance of resistance to fusarium wilt in pigeonpea (Cajanus cajan (L.) Millsp.). Journal of Animal and Plant Sciences **2**, 89- 95.

Ott RL, Longnecker M. 2001. An introduction to statistical methods and data analysis. Fifth edition. Texas A$M University p. 590-600.

Park RF, Pathan AK. 2006. Evaluation of seedling and adult plant resistance to leaf rust in European wheat cultivars. Euphytica **149**, 327- 342.

Park RF, Bariana HS, Wellings CR, Pathan AK. 2008. Evaluation of seedling and adult plant resistance in European wheat cultivars to Australian isolates of *Puccinia striiformis* f.sp. *tritici.* Euphytica **163**, 283-301.

Peterson RF, Campell AB, Hannah AE. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Canadian Journal Research **26**, 496 -500.

Roelfs AP, Singh RP, Saari EE. 1992. Rust Diseases of Wheat: Concepts and methods of disease management. Mexico, CIMMYT.

SAS Institute. 2012. SAS procedure for personal computers. Version 9.4 SAS Institute Inc., Cary, NC, USA.

Singh RP, Hodson DP, Jin Y, Huerta-Espino J, Kinyua MG, Wanyera R, Njau P, Ward RW. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources p. 1-13.

Singh R, Hodson P, Huerta- Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Ward RW. 2008. Will stem rust destroy the world's wheat crop? Agronomy **98**, 271-309.

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel SA, Singh PK, Singh S, Govindan V. 2011. The emergence of *Ug99* races of the stem rust fungus is a threat to world wheat production. Annual Review Phyto-pathology. **49**, 465-481.

Stakman EC, Levine MN, Loegering WQ. 1962. Identification of physiologic races of *puccinia graminis* f. sp. *Tritici*. United States Department of Agriculture, Agriculture Research service p. 1053.

Staletic MD, Milovanovic MS, Markovic AI, Delic GT. 2009. Inheritance of spring oat resistance to *Puccinia coronata avenae*. Kragujevac Journal of Science **31**, 75-83.

Steffenson BJ, Wilcoxson RD, Roelfs AP. 1984. Inheritance of resistance to *Puccinia graminis* f. sp. *secalis* in barley. Plant Disease **68**, 762-763.

Steffenson BJ, Wilcoxson RD, Roelfs AP. 1985. Resistance of barley to Puccinia graminis f.sp tritici and Puccina graminis f.sp secalis. Phytopathology **75**, 1108-1111.

Steffenson BJ, Webster RK. 1992. Quantitative resistance to *Pyrenophora teres* f. *teres* in barley. Phytopathology **82**, 407-411.

Tadesse W, Reents HJ, Hsam SLK, Zeller FJ. 2011. Relationship of seedling and adult plant resistance and evaluation of wheat germplasm against tan spot (Pyrenophora tritici repentis). Genetic Resources and Crop Evolution **58**, 339-346.

Todorovska E, Christov N, Slavov S, Christova P, Vassilev D. 2009. Biotic stress resistance in wheat-breeding and genomic selection implications. Biotechnology and Biotechnological equipment **23**, 1417-1426.

Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. Weed Research **14**, 415-421.