Research Application Summary

Field screening of bread wheat for partial sources of resistance to stem rust

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Abstract

The stem rust *Puccinia graminis tritici* race ug99 (TTKSK) and its variants results into wheat grain losses ranging from 70-100%. This study was set up at the Kenya Agricultural Research Institute (KARI), Njoro to identify sources of partial resistance to stem rust in Kenya. Twenty five wheat lines selected from 2008 international wheat screening nursery were grown in two replicates and screened for two seasons (December 2009 to March 2010 and then June to October 2010). Final rust severity (FRS), area under the disease progress curve (AUDPC) and the coefficient of infection (CI) were used to assess these lines for resistance. The wheat lines showed diversity in their responses ranging from trace to susceptibility. The line R07F4-21258 showed small chlorotic flecks and had no stem lodging. The entries 2, 3 and 20 had the least ACI, rAUDPC and rFRS values. These elite entries had the pseudo black potential sources of partial resistance to stem rust
Key words: pseudo black chaff, *Puccinia graminis tritici tritici,* resistance, *Sr2*, Ug99, yield loss

Résumé

La rouille de la tige *Puccinia graminis tritici* race ug99 (TTKSK) et ses variantes ont comme conséquence les pertes de grains de blé allant de 70-100%. Cette étude a été effectuée à l'Institut de Recherche Agronomique du Kenya (KARI), à Njoro, pour identifier les sources de résistance partielle à la rouille de la tige au Kenya. Vingt-cinq lignées de blé sélectionnées à partir de la pépinière internationale de dépistage de blé de 2008 ont été cultivées en deux répétitions et observées pour deux saisons (de Décembre 2009 à Mars 2010, puis de Juin à Octobre 2010). La gravité finale de la rouille (FRS), la zone sous la courbe de progression de la maladie (AUDPC) et le coefficient d'infection (CI) ont été utilisés pour évaluer ces lignées de résistance. Les lignées de blé ont montré la diversité dans leurs réponses allant de la trace à la sensibilité. La lignée R07F4-21258 a montré de petites taches chlorotiques et n'avait pas de logement de la tige. Les entrées 2, 3 et 20 avaient les plus petites valeurs d'ACI, rAUDPC et RFRs. Ces entrées d'élite avaient les pseudo-sources potentielles noires de résistance partielle à la rouille noire

Mots clés: Pseudo-balles noires, *Puccinia graministritici tritici*, résistance, Sr2, l'Ug99, perte de rendement

Background

Wheat (*Triticum aestivum*) production is constrained by many factors despite its economic importance in terms of calorific input of 16% in the developing countries (Dixon *et al.*, 2009). In Kenya, the growing of wheat in different agro ecological zones provides a green bridge for rust inoculum throughout the year (Singh *et al.*, 2008). This has led to high yield losses of over 70% by the small scale farmers who produce 20% of the wheat consumed in East Africa (Wanyera *et al*., 2004). In 2007, 100% yield losses were reported among farmers in Kenya (Wanyera, 2008). The stem rust disease caused by *Puccinia graminis f. sp. tritici* (Eriks and E. Henn) is currently the greatest threat to wheat production due to the emergence of the *Ug99*, a virulent strain of the *Puccinia graminis fsp tritici* which was designated TTKS based on North American pathotype nomenclature system (Wanyera *et al*., 2004). This has led to increased prices of wheat grain and its food products, increased alternative food prices due to increased demand, increased net food imports and loss of high investment in the crop. The only way to manage the disease is through the use of genetic resistance. This study aimed at identifying possible elite wheat lines containing partial resistance to stem rust.

Literature Summary

The race ug99 (TTKSK), a virulent strain of the *Puccinia graminis tritici tritici* Eriks and E. Henn first identified in Uganda in 1998 has made stem rust the greatest threat to wheat production globally. The rust fungus is easily dispersed by wind, which partly explains its rapid spread from the East African region to North Africa, Middle East and West-South Asia (Ali *et al*., 2009). The wheat monoculture, growing of wheat of narrow genetic base coupled with vertical resistance has led to high wheat yield losses ranging from 70-100% especially among highly susceptible cultivars (Mackenzie**,** 2007; Wanyera, 2008). The nature of the rust fungus (obligate biotroph, heteroceous and heterothallic), and the current climatic changes has further led to increased disease inocula and new virulences leading to the current breakdown of the resistance genes due to the *Ug99*. The race *Ug99* first overcame resistance conditioned by the locus *Sr31* (TTKSK = *Ug99*) and subsequently, *Sr*36 (TTTSK= *Ug99*+*Sr*36) and more recently *Sr*24 (TTKST = *Ug99*+*Sr*24 virulence) (Xu *et al*., 2009). Other factors like point mutations, short duplication events and indels under the tropical environment, occasioned by sexual and the para-sexual genetic recombination and the selection of sexual progeny which do not contain recognised effector genes has aggravated the problem further (Semenov and Halford, 2009). The frequency of this new mutant in natural populations of *Puccinia graminis* could have been increased by selection due to wide spread deployment of susceptible loci *Sr 31* and subsequently *Sr36* and *Sr24*. Given the gene for gene relationship, there is a possibility of the occurrence of resistance loci in cultivated wheat for example the presence of *Sr39* from wild populations with which the pathogen has co-evolved. These resistance loci could however be at very low frequency to be easily detected.

Currently, about 25% of the world's wheat crop is susceptible to *ug99* while 90% of the wheat is grown in the *ug99* spore path (Ayliffe *et al*., 2008). This pathogen therefore demonstrates a clear case of boom and burst cycle. However, due to this arms race between the fungi and the host plant, this resistance tends to be broken especially among varieties containing only race specific genes characterised by hypersensitive responses. Thus this existence is plausible given the gene for gene relationship in the *Puccinia graminis*- wheat pathosystem (Jin *et al.*, 2007). These rust fungi appear highly adaptable and their quick evolution leaves many currently grown resistant cultivars vulnerable especially where infection occurs early in crop growth (Lagudah and Griffiths, 2009). Resistance to *Ug99*, conditioned by the *Sr*39 (obtained from wild wheat relatives) is associated with linkage drag (Yu *et al*., 2010). The only way to counter the threat from *ug99* is through use of genetic resistance which adds no superfluous cost to the resource constrained small scale wheat farmers. Thus, more durable sources of resistance containing race non-specific resistance are indispensable given the high wheat yield losses being experienced globally. Thus, this study aimed at identifying possible elite wheat lines containing partial resistance to stem rust.

Study Description

The 25 wheat lines selected from the international wheat screening nursery (2008) were planted at Kenya Agricultural Research Institute (KARI) Njoro during the 2009 and 2010 season (Table 1). This site lies at 0 20'S; 35^0 56' E, and 2185 metres above sea level (Ooro *et al.*, 2009). It has minimum and maximum temperatures of 9.7 and 23.5^{0}C respectively and mean rainfall of 900mm. KARI Njoro is used for large scale field-screening nursery for stem rust, established by the Kenyan Agricultural Research Institute in collaboration with the International Maize and Wheat Improvement Centre (CIMMYT) and the Global Rust Initiative (Singh *et al*., 2009).The selection of these lines was based on their resistance to the rust diseases, desirable plant height and earliness. Seven highly susceptible wheat lines were included in the experiment as checks. The wheat lines were grown as double 1m row plots in an alpha lattice in two replicates. Disease spreaders or infector rows were grown perpendicular to all the plots and later inoculated to increase disease pressure.

Table 1. Correlation coefficients between disease severity, AUDPC and other agronomic traits.

	% stem lodging	ACI	AUDPC	Days to 50% flowering	TKW	rAUDPC	rFRS
% stem lodging	-						
ACI	-0.4	-					
AUDPC	-0.55*	0.91***	-				
Days to 50% flowering	0.1	-0.31	-0.12	-			
TKW	-0.18	0.09	0.14	-0.25	-		
rAUDPC	-0.58**	0.90***	0.99***	-0.1	0.16	-	
rFRS	-0.62**	0.89***	0.93***	-0.19	0.21	0.95***	-

AUDPC = Area Under the Disease Progress Curve for the stem rust disease progression for averaged across two seasons; rAUDPC = relative AUDPC for season 1 and 2 respectively which was calculated as a percentage of the value of the most susceptible line, CANADIANCUNNINGHAM/KENNEDY;
rFRS = relative final rust severity obtained by comparing respective rust severities values of each entry with the most susceptible check CANADIANCUNNINGHAM/KENNEDY;
ACI = average coefficient of infection obtained by multiplying the final disease severity of each season by the values 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 for infection response of trace responses (TR), resistant (R), moderately resistant (MR), moderately resistant-moderately susceptible (M), moderately susceptible (MS) and susceptible respectively;
TKW= thousand kernel weight was measured by threshing the kernels. A thousand seeds from each plot were randomly weighed and weights recorded in grams;
***, ** and *= significance at p<0.001, p<0.01 and p< 0.05 respectively.

The estimation of disease severity was done weekly beginning with peak rust infection until wheat attained physiological maturity. This was done as a percentage based on the modified Cobbs' scale (Peterson *et al*., 1948). The wheat infection responses were noted at each stage of note taking. The field infection responses included; TR= trace responses, R = resistant, MR = moderately resistant, RMR = resistant to moderately resistant, MRMS = moderately resistant to moderately susceptible, MSS= moderately susceptible to susceptible, MS = moderately susceptible, and S = susceptible.

The final rust severity (FRS), area under disease progress curve (AUDPC), coefficient of infection (CI), and correlation coefficient between the FRS and AUDPC were used as criterion to identify any possible source of partial resistance to black rust. The stem rust disease severity scores taken at different times were used to calculate AUPDC of each line following Wilcoxson *et al.* (1975) method using the relationship below:

$$AUDPC = \sum_{i=1}^{n-1} 0.5\left(x_{i+1} + x_i\right)\left(t_{i+1} - t_i\right)$$

Where; X_i is the cumulative disease severity; t_i is the time (days after planting) and n is total number of observations.

The most susceptible check CANADIANCUNNINGHAM/ KENNEDY was used as a reference to obtain the relative AUDPC and relative FRS values due to its complete susceptibility to stem rust. The coefficient of infection (CI) was obtained by multiplying the final disease severity of each season by the numerical notation for the host response; 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 for infection response of trace responses (TR), resistant (R), moderately resistant (MR), moderately resistant-moderately susceptible (M), moderately susceptible (MS) and susceptible (S), respectively (Ali *et al*, 2009). The coefficient of infection of each entry per each season was then averaged to give the average coefficient of infection, ACI (Afzal, *et al*., 2009). A correlation coefficient was used to determine the relationship between the different disease parameters; FRS, the AUDPC, rAUDPC and rFDS. Additional data collected included stem lodging, days to 50% flowering and thousand kernel weight (TKW). Days to heading were recorded when 50% of spikes completely emerged from the boot. The stem lodging was taken as a percentage of the stems which have lodged per plot. The analysis of variance was used for analysis of all the data collected from the parental lines to determine the significance of the differences among the wheat lines for the partial resistance parameters. The wheat genotypes were considered as fixed whereas seasons were random effects.

Results

During the season 2009/2010 (December 2009 to March 2010), disease scoring began on the 5th March 2010 when the most susceptible check expressed 30% stem rust severity with susceptible responses. Most of the wheat lines were at stage

5 based on Zadoks *et al*. (1974) scale. In the second season (June 2010 to October, 2010), disease scoring begun on 7th October 2010 when the susceptible check CANADIAN/ CUNNINGHAM//KENNEDY had severity of 60% with susceptible response (S). There was high disease pressure during the two seasons and this enabled uniform disease spread. The spreader rows used during the experiment had completely susceptible responses with 100% disease severity. They were characterised by total crop death in the two seasons.

The infection responses noted ranged from trace responses (TR) for the immune wheat lines to susceptible (S) where the checks included in this experiment expressed 90% disease severity. Four wheat lines had trace responses expressing chlorotic flecks (Table 2). These four lines had statistically least ACI, AUDPC, final rust severities across the season (FRS1 and FRS2) and the least relative final rust severity (rFRS). Out of these four lines expressing trace responses (TR), only R07F4-21258 showed resistance to stem lodging.Twenty one of the remaining wheat lines had compatible host-pathogen responses and showed varied final rust severities and responses. These lines had ACI values ranging from 2.63 to 19.50 compared to the seven checks used in this experiment which had ACI values of 85-90. The relative AUDPC of these 21 lines ranged from 8.41% to 24.09% with reference to the checks which had rAUDPC ranging from 91.44% to 100%. For the relative final rust severities (rFRS), these lines showed variation ranging from 12.8 % to 43.1% compared to the checks which had rFRS ranging from 94.44% to 100%. The rust infection responses of these wheat lines varied from resistant to moderately resistant (RMR) to moderately susceptible (MS). Across the two seasons, infection responses varied among the lines. Some wheat lines with moderately resistant to moderately susceptible (M) responses had statistically low rust severity while others with resistant responses had high rust severities in this experiment. In this study, seven wheat lines expressed the pseudo black chaff trait (PBC).

All the parameters used to assess partial resistance in this study showed highly significant correlations at $p<0.001$ (Table 1). The final rust severity was strongly and positively correlated with AUDPC, $R^2=0.70$; ACI, $R^2= 0.62$ and rAUDPC, $R^2= 0.87$.

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All the parameters used to assess the wheat genotypes for resistance to stem rust showed very high variation. These wheat

Table 2. Table of means showing the different parameters used to assess partial resistance among the wheat lines.

Entry	Origin	Pedigree	ACI	rAUDPC	rFRS	DTF	SL	PBC	TKW (g)
1	Argentina	1168.6	0.1	1.02	1.11	84.5	70	+	20.45
2	Syria	CWANA 1st SR RESIS. ON - ETH - OS71	4.5	10.11	16.7	87	0	+	28.39
3	CWANA	MON'S'/ALD'S'//TOWPE'S'	4.5	8.89	13.9	87.8	0	+	25.73
4	Australia	87	2.63	11.61	19.4	83	0	-	31.98
5	Mexico	THELIN#2/ TUKURU CGSS02Y00118S-099M-099Y-099M-16Y-OB	8.25	9.04	12.8	74.3	0	-	22.8
6	Australia	IGW3207	19.5	24.09	43.1	79.5	0	-	28.63
7	Mexico	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/PBW343*2/TUKURU/5/C80.1/3*BATAVIA//2*WBLL1	0.1	2.01	1.11	85.5	65	-	27.3
8	Mexico	WHEAR/VIVITSI//WHEAR	15.8	18.3	30.6	81.3	0	-	28.73
9	Mexico	WHEAR/SOKOLL	8.25	14.19	27.8	78.5	0	+	36.53
10	Mexico	WHEAR/JARU//WHEAR	11	20.18	26.4	79	0	-	19.75
11	Mexico	WHEAR/VIVITSI/3/C80.1/3*BATAVIA//2*WBLL1	9.63	15.59	27.8	82.5	0	-	26.67
12	Mexico	PBW343*2/KUKUNA//PBW343*2/KUKUNA/3/PBW343	10.3	13.57	33.3	83	0	-	23.57
13	Mexico	SUPER SERI#1	9.38	12.18	26.4	81.3	0	+	33.79
14	Mexico	WHEAR/VIVITSI//WHEAR	6.13	11.76	20.8	80.3	0	-	28.88
15	Mexico	WHEAR/KUKUNA//WHEAR	6.63	11.73	26.4	75.5	0	-	28.81
16	Mexico	WHEAR/VIVITSI/3/C80.1/3*BATAVIA//2*WBLL1	18	16.46	29.2	75	30	+	30.68
17	Mexico	WHEAR/VIVITSI/3/C80.1/3*BATAVIA//2*WBLL1	9.5	12.84	26.4	81.3	0	-	25.04
18	Mexico	WHEAR/VIVITSI/3/C80.1/3*BATAVIA//2*WBLL1	15.3	19.91	36.1	81	0	-	25.69
19	Mexico	SUNCO//TNMU/TUI	0.1	1.02	1.11	78.5	35	-	30.9
20	Mexico	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/5/VERDIN CMSS02M00361S-030M-15Y-0M-040Y-6ZTB-0Y-03B-0Y	5.75	8.41	19.4	81.8	0	+	28.71

Table 2. Contd.

Entry	Origin	Pedigree	ACI	rAUDPC	rFRS	DTF	SL	PBC	TKW (g)
21	Uruguay	R07 F4-21258	0.1	2.58	6.11	82	0	-	23.94
22	Mexico	WHEAR/VIVITSI/3/C80.1/3*BATAVIA//2* WBLL1	10.1	18.38	26.4	83	0	-	24.5
23	Mexico	WHEAR/VIVITSI/3/C80.1/3*BATAVIA//2* WBLL1	15	17.83	29.2	83.5	0	-	24.59
24	Mexico	CHEN/AEGILOPS SQUARROSA (TAUS) //BCN/3/VEE#7/BOW/4/PASTOR/5/VERDIN CMSS02M00361S-030M-16Y-0M-040Y-1 6ZTB-0Y-03B-0Y	5.75	10.36	18.1	79	0	-	27.5
25	Mexico	(yield trial 2007)	8	18.26	26.4	87	0	-	27.61
Check 1		THELIN/3/2*BABAX/LR42//BABAX	85	91.44	94.44	78.5	0	-	
Check 1		THELIN/3/BABAX/LR42//BABAX/4/ BABAX/LR42//BABAX	90	93.58	100	79.5	0	-	
Check 2		THELIN/3/BABAX/LR42//BABAX/4/ BABAX/LR42//BABAX	90	94.69	100	79.5	0	-	
Check 3		THELIN/3/2*BABAX/LR42//BABAX	90	94.69	100	73.5	0	-	
Check 4		THELIN/3/2*BABAX/LR42//BABAX	90	94.69	100	83.5	0	-	
Check 5		THELIN/3/2*BABAX/LR42//BABAX	90	96.61	100	68	0	-	
Check 6		CANADIAN/CUNNINGHAM//KENNEDY	90	100	100	66.5	0	-	
		Grand mean (GM)	8.2	12.4	21.8	81.3	0.17		27.25
		Least significant differences (l.s.d)	6.4	10.1	20	8.12	0.36		3.02
		% coefficient of variation (%CV)	39	40.5	45.5	7	107		5.4

ACI = average coefficient of infection obtained by multiplying the final disease severity of each season by the values 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 for infection response of trace responses (TR), resistant (R), moderately resistant (MR), moderately resistant-moderately susceptible (M), moderately susceptible (MS) and susceptible respectively. This was averaged out for the two seasons;
AUDPC = area under disease progress averaged across two seasons per each entry; rAUDPC= relative area under disease progress curve which was calculated as a percentage of the value of the most susceptible line, CANADIANCUNNINGHAM/KENNEDY;
FRS2, FRS1= final rust severity season for one and season two respectively; this was estimated based on modified Cobb's scale where 0%= immune and 100%= completely susceptible; rFRS = relative final rust severity scores obtained by comparing respective rust severities values of each entry with the most susceptible check CANADIANCUNNINGHAM/KENNEDY;
PBC is pseudo black chaff suggesting presence of the *Sr2* gene; its presence is indicated by a plus (+) while the lack of it is represented by a minus (-).
TKW= thousand kernel weight was measured by threshing the kernels. A thousand seeds from each plot were randomly weighed and weights recorded in grams

lines were selected during the 2008 main season from the international wheat screening nursery at KARI Njoro. This site is a hot spot for stem rust disease and has all the known stem rust races currently threatening global wheat production. There was heavy disease pressure during the seasons December 2009 to March 2010 and June 2010 to October, 2010 as shown by the high disease pressure on the most susceptible check included in this experiment, CANADIAN/ CUNNINGHAM//KENNEDY (the highest AUDPC and 90% final rust severity).

Among the four lines with the trace responses (TR), only entry 21 (pedigree R07F4-21258) which showed no stem lodging in the two seasons was promising. Trace responses could either be as a result of hypersensitive responses or due to the combination of many minor genes of small (additive) effects which provide near immunity (Afzal, *et al*., 2009). For those lines with immune responses due to hypersensitive responses, resistance often breaks down due to the arms race between the fungi and the host plant. This arises when virulent stem rust races increase in frequency. Strong selection pressure wielded upon the pathogen population ultimately lead to emergence of new dominant races which end up overcoming the available race specific resistance as is the case with the Ug99 which has led to heavy crop losses (Wanyera *et al*., 2008). A suitable breeding strategy is thus imperative in the exploitation of any of these wheat lines which may be containing race specific genes. In the case with genes with additive effects, the resistance increases as the number of genes increase and more work is needed to determine whether the resistance in these lines with trace responses could be additive.

The remaining 21 wheat lines had varied responses with regard to ACI, rAUDPC and rFRS. The degree of resistance as measured by the rAUDPC was done by recording severity scores at four different times during the epidemic. Among these lines, disease progression varied with the different genotypes and seasons. In this study, entries 2, 3, 20 and 24 (Table 2) had statistically the least ACI, rAUDPC and rFRS values among the lines which contained non-hypersensitive response. Of the 25 wheat lines evaluated in this study, 21 had below 30% rFRS relative to the most susceptible check (CANADIAN/ CUNNINGHAM//KENNEDY) despite the high disease pressure noted in the field with many pathotypes of the *Puccinia graminis fsp tritici* including the *Ug99* virulent strain.

The 21 lines supposedly have some level of partial resistance and these affected the manner of disease infection and spread at the various wheat growth stages. The available resistance in these materials could have also played a role in controlling the time of disease attack and the size of pustules. The available resistance genes supposedly overcame the stem rust virulence in the field and led to statistically low disease severities despite presence of visible and compatible interaction between host plant and the pathogen. Another trait of great significance was the presence of the pseudo black chaff among the entries 2, 3 and 20. The pseudo black chaff trait is a morphological marker for the presence of the *Sr2* gene; non-race specific gene which forms the basis for durable resistance in wheat.

All these 25 wheat lines were initially selected as promising entries in 2008 during the main season wheat screening nursery at KARI, Njoro. Further more, in two more seasons of screening (2009 to 2010), they have shown appreciable level of partial resistance. Moreover, even the wheat lines with M and MS responses were observed to possess statistically low disease severities which are attributed to a combined effect of all the resistance factors during disease progression (Ali *et al*., 2008). These lines could be good sources of partial or slow rusting resistance to stem rust conditioned by additive gene action (Kaur and Bariana, 2010). The disease progression was highly retarded among these lines. This could have occurred by the reduced selection pressure on the pathogen population (Khan and Saini, 2009). Use of these lines could highly delay evolution of new rust pathotypes because multiple point mutations will barely occur in normal circumstances (Tsilo *et al*., 2010; Ali *et al*., 2008).

It was also noted high and strong positive correlations among all disease assessment the parameters; AUDPC, FRS, rAUDPC and rFRS at $p<0.001$ (Table 1). Strong positive correlations have been reported previously (Parlevliet, 1993; Qamar *et al*., 2007). Thus, the parameters AUDPC, FRS, rAUDPC and rFRS are reliable estimators of partial or slow rusting resistance to stem rust. They give a dependable rate of disease increase and are related with components of partial resistance like low receptivity, longer latent period and smaller pustules (McNeil *et al*., 2008).

Recommendations

The further improvement of these lines would go a long way in combating the current global food crisis and ensure food security especially in Kenya which is faced with recurrent food shortage. Further knowledge of the nature of resistance in these lines will make it easier for breeders and pathologists to exploit the genetic variability revealed. Genetic studies are being carried out on some of these promising lines and these will add to the knowledge pool concerning current *Puccinia graminis fsp tritici* pathogen dynamics. These promising lines could also be used to broaden the genetic diversity of the available Kenyan wheat germplasm since these materials have shown diverse levels of resistance. This could be achieved by introgressing the resistance into adapted but susceptible wheat cultivars through backcross, pedigree selection, mass selection and/or bulk population breeding. This will help offset further wheat yield losses and lower the country's level of wheat importation.

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