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Full Length Research Paper

Resistance in Kenyan bread wheat to recent eastern African isolate of stem rust, *Puccinia graminis* f. sp. *tritici,* Ug99

P. N. Njau[1]*, R. Wanyera[1], G. K. Macharia[1], J. Macharia[2], R. Singh[3] and B. Keller[4]

[1]Kenya Agricultural Research Institute (KARI) Njoro, Kenya.
[2]Egerton University Njoro. P. O. Box 536 Njoro, Kenya.
[3]CIMMYT Mexico. Apdo - Postal 6-641, 06000 Mexico, DF.
[4]Institute of Plant Biology, University of Zurich, Switzerland

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Stem or black rust, caused by *Puccinia graminis*, has historically caused severe losses to wheat (*Triticum aestivum*) production worldwide. The causal race, commonly known as Ug99 and designated as TTKS based on the North American nomenclature, carries virulence for several genes commonly present in wheat germplasm. All Kenyan germplasm are known to be susceptible or partially susceptible to Ug99 although no proper documentation has been done. This study was aimed at evaluating the Kenyan bread wheat varieties on their response to Ug99. The varieties were screened for resistance at seedling stage and adult plant resistance stage. None of the varieties apart from Bonny were resistant at seedling stage. Some old Kenyan varieties were found to have adult plant resistance probably due to the presence of non-race specific gene Sr2 complex which among others can be exploited in breeding for resistance in Kenyan wheat.

Key words: Race specific resistance, adult plant resistance, genetic erosion.

INTRODUCTION

Stem or black rust, caused by *Puccinia graminis*, has historically caused severe losses to wheat (*Triticum aesti-vum*) production worldwide. Its control for over 30 years through the use of genetic resistance in wheat is a remar-kable success story. In 1999, high susceptibility of Inter-national Maize and Wheat Improvement Center (CIMMYT) germplasm was noted in Uganda and an increase in stem rust incidence and severity was seen in Kenya.

McIntosh (2000) describes cereal rusts as 'social' diseases because the individual farmer is subject to air-borne spores from areas beyond the farm and part of any inoculums produced on farm is dispersed to others. Control of rust diseases must therefore be addressed at the community level, preferably through the widespread use of resistant varieties. The causal race, commonly known as Ug99 and designated as TTKS based on the North American nomenclature, carries virulence for seve-ral genes commonly present in wheat germplasm. A germplasm screening process was initiated in Kenya and Ethiopia to document the scope of virulence of the new race and also to identify any source of resistance. Over 80% of all the germplasm screened were susceptible (Wanyera et al*.,* 2006; Singh et al., 2006; Jin et al., 2006).

The stem rust resistance gene Sr31, derived from Petkus rye (MacIntosh et al., 1995; Zeller et al., 1983) has been used worldwide in spring wheat through the widespread use of Russian and other East European wheat Kavkaz, Aurora and Loverin that originally carried the 1BL.1RS wheat–rye translocation (Zeller et al., 1973). This led to the release of numerous popular cultivars worldwide including several spring wheat cultivars de-rived from the spring wheat germplasm in CIMMYT. Gene Sr31 provided the main component for stem rust resistance in many wheat cultivars and continued to re-main effective until recently, when isolates of *P. graminis* f. sp. *tritici* with virulence to Sr31 were detected from Uganda in 1999 (Pretorius et al., 2000). Similar virulence was observed in Kenya in 2003

*Corresponding author. E mail: njaupnn@yahoo.com. Tel.: 073 3775229, 5161576.

and 2004 (Wanyera et al., 2006).

A few lines carrying Sr2 were found to have adult plant resistance. Gene Sr2, transferred to wheat from 'Yaroslav emmer' by McFadden (1930), is the only catalogued gene that is not race-specific. Sr2 can confer slow rusting (Sundrwirth and Roelfs, 1980) resistance of adult-plant nature. Resistance gene Sr2, in addition to other unknown minor genes derived from cultivar hope and commonly known as 'Sr2-Complex', provided the foundation for durable resistance to stem rust in germplasm from University of Minnesota in the USA, Sydney Univer-sity in Australia, and the spring wheat germplasm deve-loped by Dr N.E. Borlaug as part of a programme spon-sored by the Mexican Government and the Rockefeller Foundation (McIntosh 1988, and Rajaram et al., 1988).

This study was aimed at evaluating the Kenyan germplasm and their response to Ug99. This will help the breeder in identifying the best parents to be used in the breeding program in fight against Ug99.

MATERIALS AND METHODS

Evaluation of seedling infection types

An isolate (04KEN156) of *P. graminis f.* sp*. tritici*, collected from Kenya in 2004 and identified as race TTKS (Wanyera el al*.,* 2006) based on the 16 differentials in the *P. graminis f* . sp.*tritici* differential set of North America (Roelfs et al*.,* 1990), in addition to six Pgt isolates of races QTCS,QTHS, RCRS, RKQQ, TPMK and TTTT representing broad virulence in the North American stem rust population, were included for comparisons of infection types for the 30 Kenyan varieties at St. Paul, MN, USA. The 30 Kenyan lines were planted in tray and allowed to grow to seedling stage as explained in yue Jin (2007). Urediniospores from long-term storage in a -80°C freezer were heat shocked at 40°C for 10 min and placed in a rehydration chamber for 2 - 4 h, where approximately 80% relative humidity was maintained by a KOH solution (Rowell, 1984).

The urediniospores were then suspended in a light mineral oil (Soltrol 170) and inoculated onto the fully expanded primary leaves of 7 - 9 day old seedlings of wheat lines. The inculated seedlings were then incubated in a dew chamber for 14 h at 18°C in the dark and then for an additional period of 3 - 4 h under fluorescent light. The plants were then placed on a greenhouse bench at 18 ± 2°C with a photoperiod of 16 h. Infection types (ITs), described by Stakman et al. (1962), were assessed 14 days post inoculation. ITs 0, 1, 2 or combinations thereof were considered low ITs indicating that the corresponding resistance gene is effective. ITs 3 - 4 were considered high ITs, indicating that the corresponding resistance gene is not effective against the race tested. In each test, 6 - 10 seedlings were evaluated and the test was repeated twice.

Field stem rust evaluations in Njoro

The 30 Kenyan bread wheat varieties were tested in 2006 and 2007 as part of a larger field stem rust screening nursery in Njoro, Kenya established by the Kenyan agricultural research institute in conjunction with CIMMYT and the global rust initiative (GRI).

The nursery site was located at 0°20 S, 35°56 E and 2,185 m in elevation with an average daily minimum temperature of 9.7°C (night) and an average daily maximum temperature of 23.5°C (noon). Variations of average daily temperatures are approximately ±2°C, occurring mostly during the day hours of the field evaluation period. Dew was formed nearly daily.

Entries were planted in single 1 m row plots on 30th June of 2006 and 15th June 2007. To facilitate inoculum build-up and uniform dissemination within the nursery, a continuous row of stem rust spreader (a mixture of susceptible cvs. Chozi and Duma carrying Sr31) was planted perpendicular to all entries. The spreader rows were inoculated once by dusting them with a mixture of urediniospores and talc powder. The source of inoculum was a bulk of urediniospores collected from experimental plots of Duma variety grown within the field where the experiments were carried out in Kenya. Plant response to rust infection at the adult plant stage was termed "infection response"

According primarily to the size of pustules and associated necrosis or chlorosis, infection responses were classified into four discrete categories; R = resistant, MR = moderately resistant, MS = moderately susceptible and S = susceptible (Roelfs et al., 1992). Infection responses overlapping between any particular two categories were denoted using a dash. For instance, "MR-MS" denoted an infection response class overlapped between the MR and MS categories. Stem rust severity was assessed following the modified Cobb scale (Peterson et al., 1948). Entries were evaluated for infection responses and stem rust severity two to three times between heading and plant maturity. The infection responses and stem rust severity at the soft-dough stage of plant growth were used to represent the final disease scores in this report.

RESULTS AND DISCUSSIONS

Seedling infection type of Kenyan wheat varieties

Kenyan varieties showed high level of resistance to the stem rust races found in the USA. All the varieties apart from Duma was resistant to the QTCS race (Table 1). This trend was the same for all the other races but with race

TTTT which is a more recent race the older Kenyan varieties showed more susceptibility at seedling stage. These included, K. Kudu, K. Nyangumi, K. Tama, K. Tembo among others as shown in Table 1. Such report had been reported earlier by Singh et al. (2006) while comparing various sources for durable or slow rusting type of resistance. When it comes to race TTKS which is popularly known as Ug99, all the Kenyan varieties become susceptible with the exception of Bonny which remained resistant whereas Tama showed two types of reactions. Given that the races have been evolving over time, it is clear that most of the Kenyan varieties have remained effective against the new races until the emergence of the new race Ug99. This can be explained from the point of view that most of our Kenyan varieties, especially those derived from CIMMYT carry the Sr31 stem rust resistance gene (Singh, 2006). The alien resistance gene Sr31 has been used in agriculture on the largest scale since 1980s in spring, facultative and winter wheat breeding programs worldwide except Australia. Its use in CIMMYT wheat improvement resulted in the release of several popular cultivars worldwide. The use of 1BL.1RS

Table 1. Seeding Infection type (IT) of Kenyan wheat varieties to various stem rust races including Ug99 tested at the at St. Paul, MN, USA.

Line	Test Race						
	QFCS	QTHJ	RCRS	RKQQ	TPMK	TTTT	TTKS
K-popo	0	23-	0;	0;	;1	;1	3
K-kudu	0	2/3	;	4	;	;1+/3(2 pl)	3
K-kulungu	0;	;2/3	4	;	;	;1	3
K-fahari	0;	2+/;	;1	0;/;2	;1	;	3-
Mbega	;/2	2-	1-	;1	2	2-	3+
K-nyangumi	0	;1--	;	0;	;1	3	3+
K-paka	0	;1	0;	0;	;1/2	;	3+
Tama	0	;	2=	;3	;	3-	;13
K-tembo	;	-	0;	-	-	3	3+;
Ngamia	1	23	;	22+	3	3	3+
Kwale	0	0/1	2-	2-	2	2-	3+;
Kipapu	0;/1	2	;	2+	;	2	2/4
K-chiriku	-	;1	2-	;	2/3	;/3/2	3+
Bonny	0/;1+	0	;	3	;	3; /;	-
Pasa	;	2	;1	2-	2	1	4
Swara	0;	1-	;/2-	0;/2+	;1	3	3+
Romany	0	;/3	0;	;/3	;1	;C	4
A-mayo	;1	2	2	0;	;1	3	3-;
Catcher	0;	2-	2-	2	2	2	3
Chozi	0;	;2-	2-	2-	2-	2-	3+
R-sabanero	;1	;1+	2+	2C	2	2	3+
Duma	2-	3-	;2-	2	3-	2/3	4
Bounty	0	-	-	-	0?	1	3+
K-yombi	0/;	2-	1;	2-	2-	1;	3+
K-heroe	0;	2	;1-	;1	2;	;2-	3+
K-mbweha	;2	2-	2-	2-	2-	2-;	3+
Regent	2	2	2+3	3	3+	3+	4
K-zabadi	2;	2	2	1;	;1	3-	3+
Gem	0;	;1	;/1	23-	3	;	3-
Ngiri	0;	;	0 esc?	0;	0;	0;	3

translocation was initially associated with increased grain yields and resistance to all three rusts and powdery mildew as it carried resistance genes for all these diseases on the same translocation. Large-scale deployment of Sr31 surprisingly did not result in its breakdown until the detection of race Ug99 in Uganda. In fact this gene probably further reduced the already low stem rust survival to almost nonexistent levels in most wheat growing regions to the extent that stem rust started to become a forgotten curse. This degree of stem rust susceptibility to a single race in Kenyan bread wheat has not been observed previously. It may indicate that the virulence combination of race TTKS is very unusual. More likely, however, it may indicate a serious erosion of the resistance package in the spring wheat germplasm that has provided stable stem rust resistance in the Kenya for over 50 years Jin and Singh (2006) compared seedling reactions of US wheat cultivars and germplasm with highly virulent races present in the USA and race Ug99. Several wheat lines, especially spring wheat that were highly resistant to US races and did not carry the1BL.1RS translocation were also found to be susceptible to Ug99. This further supports the hypothesis that race Ug99 carries a unique combination of virulence to known and unknown resistance genes present in wheat germplasm. The major susceptibility is due to the specific nature of avirulence /virulence combination that Ug99 possesses which had led to the susceptibility of many wheat materials irrespective of where they were developed.

Adult plant resistance of Kenyan bread wheat toUg99

Among the 30 Kenyan wheat varieties evaluated for adult plant resistance in Kenya in 2006 and 2007, 20% were

Table 2. Adult plant response to infection and disease severity of the Kenyan bread wheat varieties.

Variety	Parentage	Disease sore		
		2006	2007	Mean (%)
K.kongoni	6410-2/6647-5=CI8154/2*Fr/2/3*ROM/3/Wis.245-II-50-17/CI8154/2/2*Fr	40S	40S	40
Mbuni	Za75/Ld357E-Tc3xGU^{-} 30520-1B-1B-3Y-0Y	40s	10MS	25
K.heroe	MBUNI/SRPC64//YRPC1	70s	40S	55
Chozi	F12.71/COC//GEN CM76689-3Y-08M-02Y-4B-2Y-OB	40S	30MS	35
Duma	AU/UP301//GLL/SX/3/PEW "S"/4/MAI "S"//PEW "S" CM67245	60S	60S	60
Mbega	Fink "S",CM41860-A-5M-2Y-3M-1Y-1M-1Y-OB-Opt2	30S	10MS	20
K.yombi	MBUNI/SRPC64//YRPC1	30S	20MS	25
Pasa	Buc "S"/Chat "S"	40S	30MS	35
K.chiriku	KTB/Carpintero "s"	30S	40MS	35
Kwale	Kinglet,CM33089-W	40MSS	10MSS	25
K.nyangumi	TZPP//SK^{E6}/LR64HDM/3/AFM/4/KSW/K4500-6	10M	5MSS	7
K.tembo	WIS.245/II-50-17//C.I 8154/2*Fr/3/2*Tob.66	30MSS	5MSS	18
K.fahari	TOBARI66/SRPC527//CI8154/3/2*FROCOR	60S	10MS	35
K.paka	(Wis245/II-50-17//CI.8154/2*Fr/3/2*Tob.66	40S	10M	25
K.popo	KL. Atl/Tob66//cfn/3/Bb	60S	10MS	35
Ngamia	SW 53=BUCKBUCK "S" CM31678	40S	10MS	25
Njbw I	KM14(PASA MUTANT)	40S	60MS	50
Njbwii	TNMU CM81812-12Y-06PZ-4Y-5M-0Y-2AL-0Y-2AL-0AL-OM	30S	40MS	35
K.kulungu	On/Tr. 207/3/cno//Son64/4/6661-53	50S	10MS	30
K.swara	(CI8254xFr2) x (T-K^{2}xY.59.2.B)	15M	1MSS	8
K.kudu	K131xK184.P.2.A.I.F K1008.K.7.K.2	40S	5MSS	23
Bonny	YF3xBza^{2} VI-116-2-4B-1T-2B-1T	5R	1MSS	3
Bounty	T-Kenya^{2}x Bonza^{2} VI-106-2t-3b-3t-1b-2t	10R	5RMR	8
Regent	H44xReward RL975.6	20M	5RMR	13
K.mamba	Africa Mayo48x/3/[(Wis.245xsup51)x(Fr.Fn/Y)^{2} A]	4OS	5MSS	23
Gem	908-Frontana x Cajeme 54	20MS	5MS	13
K.grange	360.FxGranaderoklein	40MSS	MISSING	40
catcher	Thatcher-Santa Catalina x Frocor	60S	20MS	40
R.sabanero	Single Plant Selection	60S	5MSS	33
K.ngiri	CI8154/2*Fr//5*WRT.TC/3*MIT/3/2*Tob66	30S	10M	20
Tama	Yaktana54xLerma 52	20MS	5MS	13
K.zabadi	Son64/450^{5E}//Gto/3/Inia/4/K4500/Ksw/Tob66//CIANO	20S	10MS	15
K.mbweha	CI8154/2*F/3/2*GB54/36896//II-53-526	70S	10MS	40

moderately resistance (Table 2). These were mainly the old varieties which included K. Nyangumi, K. Swara, Bonny and Bounty which contain the Sr2 resistance gene complex usually associated with the Pseudo black chaff (PBC). All these varieties showing adult plant resistance were released during the early sixties and seventies when Sr2 complex was one of the main sources for stem rust resistance. The adult plant resistance gene Sr2 confers slow rusting (Sunderwirth and Roelfs, 1980).

Combination of Sr2 with other unknown slow rusting resistance genes possibly originating from Thatcher and Chris commonly known as the ‘‘Sr2-Complex’’ provided the foundation for durable resistance to stem rust in germplasm from the University of Minnesota in the United States, Sydney University in Australia and the spring wheat germplasm developed by Dr. N. E. Borlaug (McIntosh, 1988; Rajaram et al., 1988) . Unfortunately, not much is known about the other genes in the Sr2 complex and their interactions. Knott (1988) has shown that ade-quate levels of multigenic resistance to stem rust can be achieved by accumulating approximately five minor genes. The varieties released in the eighties and nineties are the most suscepti-

ble with an average disease seve-rity of 40% and above (Table 2). These include varieties like Kwale released in 1987, Chozi released in 1999 and Duma released in 1994. Again, this may indicate a se-rious erosion of the resistance package in the spring wheat germplasm over time especially when breeding was done in the absence of stem rust. The susceptible varieties also are suspected to have only single race spe-cific type of resistance.

The decrease in incidence of stem rust to almost non significant levels by the mid-1990s throughout most of the wheat producing areas worldwide were coincident with a decline in research and breeding emphasis to such a level that in many countries including Kenya breeding was done in the absence of this disease. Most of the Kenyan wheat varieties released in the late 1980's and early 90's were selections from CIMMYT.

CIMMYT scientists continued to select for stem rust resistance in Mexico using artificial inoculation with six *P. graminis tritici* races of historical importance. New stem rust races have rarely occurred since the 'Green Revolution' in Mexico (Singh, 1991). Moreover, a majority of wheat lines selected in Mexico remained resistant at international sites either due to absence of disease, inadequate disease pressure, or presence of races that lacked necessary virulence for the resistance genes contained in CIMMYT wheat germplasm.

Almost 50 different stem resistance genes are now catalogued (McIntosh et al., 1998), several of which are incurporated in wheat from alien relatives of wheat. All but *Sr2* resistance genes are race-specific, and are expressed in both seedling and adult plants. Race specificity derives from the gene-for-gene relationship between the host plant resistance gene and corresponding virulence genes in the pathogen.

CONCLUSIONS AND RECOMMENDATIONS

Most of the Kenyan lines are susceptible to the new race of stem rust TTKS (Ug99). This could be due to their common source for resistance Sr31 from CIMMYT germplasm. Some of the old Kenyan varieties like Bonny, Bounty, K. Swara and K. Nyangumi show adult plant resistance which could be associated to the presence of the adult plant resistance gene Sr2 complex. These varieties may be used in breeding for durable resistance to stem rust especially in combination with other major genes. More sources for resistance should be sought for to be incorporated in the Kenyan germplasm. Race specific type of resistance should be avoided to minimize epidemic as has been caused by Ug99 in future.

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