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## Sources of resistance to stem rust among selected wheat germplasm

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### ABSTRACT

Wheat (*Triticum aestivum*) is an important staple food crop contributing to food security and income generation among resource poor farmers. However, the crop is threatened by stem rust which pose a major constraint to wheat production in East Africa. This is because the Ug99 (TTKS) a virulent strain of the *Puccinia graminis fsp tritici* Eriks and Henns, has overcome major resistance genes; *Sr31*, *Sr36* and *Sr24* previously deployed against the stem rust. This has led to significant reduction in the wheat yields or sometimes to total crop failure under heavy epidemics. Thus, host resistance remains vital in combating the *ug99* spread. A study carried out at Kenya Agricultural Research Institute (Njoro) in the field aimed at identifying sources of resistance to stem rust. This study revealed some promising wheat lines; R07F4-21258 and THELIN#2/TUKURU CGSS02Y00118S-099M-099Y-099M-16Y-OB which should constitute appropriate material for breeding programs. These promising lines have already been used in intercrosses and populations are being advanced into further generations for genetic studies and mapping of the resistance genes. The recurrent selection will be used to accumulate these resistance genes into high yielding wheat background in further breeding work to help avert further wheat yield losses in East Africa which is faced with acute malnutrition, famine and drought.

**Keywords:** Host resistance, *Puccinia graminis*, *Triticum aestivum*, virulent strain, *ug99*

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### Introduction

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 at different agro ecological zones provides a green bridge for rust inoculum throughout the year (Singh *et al.*, 2008). This has led to unacceptable 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 the high investment crop.

The fungus, *Puccinia graminis fsp tritici*, exhibits high genetic diversity (Groth *et al.*, 1995) that has resulted in breakdown of major resistance genes namely *sr31*, *sr24* and *sr36*, which were deployed in stem rust resistant varieties. This has been associated with mutation, sexual and para-sexual genetic recombination and the current climate change.

Over 45 stem rust resistance (*Sr*) genes have been deployed worldwide against the different races of stem rust (Roelfs, 1988; McIntosh *et al.*, 2003 and Steffenson *et al.*, 2007). However, most of these resistance genes show dominant inheritance and are race specific (Spielmeyer *et al.*, 1998). Thus, the use of single resistance genes has been considered a threat to wheat production and is highly discouraged.

The erosion of the few resistance genes against the *ug99* race has been attributed to the arms race between the pathogen and the host. The host has to continuously evolve to produce new forms of resistance genes while the pathogen is forced to alter effector genes to avoid its recognition. Hence, this calls for use of durable sources of resistance to stem rust which can remain effective for long duration over generations under environments with disease pressure (Johnson, 1984). This has involved the use of wheat lines containing *Sr2* gene which is non race specific. This would help to stabilize the existing populations of the pathogen preventing further evolution. These resistance genes provide resistance in mature plants close to immune when used in combination. Thus, this study set out to identify new sources of resistance effective against *Ug99* strain of the *Puccinia graminis fsp tritici*.

## Materials and Methods

Twenty five wheat lines plus susceptible checks containing *Sr24* gene were planted in the field at Kenya Agricultural Research Institute (KARI) Njoro (0° 20'S; 35° 56' E, and 2166 m above sea level) during the off season, 2009 (November 2009 to April, 2010) and main season, 2010 (June to October, 2010). The site is a hotspot for stem rust disease with all the major pathotypes including the *ug99*. It also has the facilities and fits well with the shuttle breeding program adopted by CIMMYT. The shuttle breeding has been involved in the screening of segregating populations developed by wheat breeding programs at CIMMYT-Mexico nurseries and other international wheat breeding programs (Singh *et al.*, 2009).

Each wheat line was grown on two rows each of one metre in length in an alpha lattice design. Disease spreaders involving a mixture of the seven susceptible *Sr24*-gene containing wheat lines were grown perpendicular to all the plots as infector rows and inoculated with the stem rust spores using a syringe (Liu and Kolmer, 1998).

The field assessment for disease resistance was achieved by making a score of the disease severity which includes estimating the proportion of the stem affected by the disease from time of disease appearance until physiological maturity. The stem rust disease severity and reaction was scored based on modified Cobb's scale (0-100%) and their field responses. The field responses involved resistant (R) to moderately resistant (MR) (small-size uredinia); moderately

resistant (MR) to moderately susceptible (MS) (medium-size uredinia) while the moderately susceptible (MS) to susceptible (S) showed medium to large-size uredinia (Peterson *et al.*, 1948). Other agronomic data collected were plant height, stem lodging, yellow rust and maturity. All these data was subjected to ProcGLM of the SAS program (SAS institute Inc., 1996).

## Results and Discussion

All the traits measured among the wheat genotypes showed variation at  $p < 0.001$ . It was also noted that the disease severity did not show variation across the two seasons (Table 1). There were favourable environmental conditions for rust development in both seasons.

The genotypes R07 F4-21258 and THELIN#2/ TUKURU CGSS02Y00118S-099M-099Y-099M-16Y-OB exhibited the best agronomic traits. These lines had statistically low disease severities, early maturity, low plant height, low yellow rust severity and resistance to stem lodging (Table 2). The early maturing lines exhibited disease resistance whereas the short plants produced thick stems which withstood stem lodging while supporting larger seed heads. From this work, wheat lines with trace responses (TR) were noted. These lines were characterized by chlorotic flecks implying they may contain major genes attributed to hypersensitive responses. The use of wheat lines containing only major genes has been prohibited due to the erosion of the resistance through mutations and sexual recombination (Ayliffe *et al.*, 2008). Further improvement of these lines could be achieved through pedigree selection to select for major gene effects. The involvement of these lines in intercrosses and recurrent selection could enhance the value of these lines.

**Table 1 ANOVA for agronomic traits among selected wheat germplasm**

Source	df	% stem lodging		a%DS		maturity		plant height		yellow rust	
		mean deviance	chi pr	MS	P>F	MS	P>F	MS	P>F	MS	P>F
Pedigree	24	31.075	<.001	370.5	<.001	50.26	0.101	147.29	<.001	396.51	<.001
season	1			28.4	0.609	4746.33	<.001	12168.85	<.001	4872.04	<.001
Pedigree. Season	24			97.4	0.587	18.39	0.935	40.45	0.41	208.58	<.001
Residual	48										
Total	98										

<sup>a</sup>%DS = percentage disease severity; MS= mean squares

**Table 2 Means of the stem rust disease and other agronomic traits among the wheat germplasm**

Entry	Pedigree	%DS (0-100)	$\alpha$ Field Response	yellow rust (0-100)	<sup>b</sup> PBC	maturity (days)	plant height (cm)	stem lodging (0-100)
1	1168.6	1	TR	16.5	+	84.5	66.31	70
19	SUNCO//TNMU/TUI	1	TR	7.5	-	78.5	74	35
7	SERI.1B*2/3/KAUZ*2/BOW// KAUZ/4/PBW343*2/TUKURU/5/ C80.1/3*BATAVIA//2*WBLL1	1.8	TR	18.75	-	85.5	67.06	65
21	R07 F4-21258	4.5	TR	19	-	82	70.31	0
3	MON'S'/ALD'S'//TOWPE'S'	12.5	RMR	3.75	+	87.75	85.12	0
5	THELIN#2/ TUKURU CGSS02Y00118S- 099M-099Y-099M-16Y-OB	12.7	MR	8.75	-	74.25	71.19	0
4	87	15	RMR	10.25	-	83	78.56	0
24	CHEN/AEGILOPS SQUARROSA (TAUS)// BCN/3/VEE#7/BOW/4/PASTOR/5/ VERDIN CMSS02M00361S-030M-16Y- 0M-040Y-16ZTB-0Y-03B-0Y	16.2	MR	20	-	79	79.94	0
20	CHEN/AEGILOPS SQUARROSA (TAUS)// BCN/3/VEE#7/BOW/4/PASTOR/5/ VERDIN CMSS02M00361S-030M-15Y- 0M-040Y-6ZTB-0Y-03B-0Y	17.5	RMR	23.75	+	81.75	76.56	0
2	CWANA 1st SR RESIS. ON - ETH - OS71	20	RMR	5.25	+	87	86.16	0
9	WHEAR/KUKUNA//WHEAR	21.2	MR	32.5	+	78.5	85.56	0
14	WHEAR/VIVITSI//WHEAR	21.2	RMR	33.75	-	80.25	83.12	0
15	WHEAR/SOKOLL	21.2	RMR	13.75	-	75.5	84.88	0
22	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	21.2	M	12.5	-	80.5	82.97	0
13	SUPER SERI#1	23.7	MR	22.5	+	81.25	86.88	0
25	(yield trial 2007)	23.7	MR	7.75	-	87	85.38	0
11	PBW343*2/KUKUNA//PBW343*2/ KUKUNA/3/PBW343	25	MR	21.25	-	82.5	79	0
12	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	25	M	37.5	-	83	78.44	0
10	WHEAR/JARU//WHEAR	26.2	M	35	-	79	77.62	0
17	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	27.5	RMR	15	-	81.27	84.44	0
16	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	28.7	M	14	+	75	81.84	30
23	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	28.7	MR	33.75	-	83.5	84.94	0
18	WHEAR/VIVITSI/3/ C80.1/3*BATAVIA//2*WBLL1	30	M	17.5	-	81	83.94	0
8	WHEAR/VIVITSI//WHEAR	32.5	M	18.75	-	81.25	81.06	0
6	IGW3207	33.8	MS	6.5	-	79.5	84.56	0
	Grand mean	19.7		18.22		81.29	79.99	
	Standard error of differences (s.e.d)	7.31		4.914		4.041	4.35	

<sup>a</sup> Field responses involved trace response (TR), resistant (R), moderately resistant (MR), resistant to moderately resistant (RMR), moderately resistant to moderately susceptible (M), moderately susceptible (MS), and susceptible (S).

<sup>b</sup> 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 (-).

%DS implies percentage disease severity

Among the 25 wheat lines screened for resistance to stem rust, *ug99*, 72% of them expressed resistance responses (R to MR). The wheat lines used in this experiment had been selected during the 2008 (main season) international screening nursery grown at KARI, Njoro based on their level of resistance. Some of these lines exhibited the pseudo black chaff phenotype (Table 2). The pseudo black chaff phenotype has been associated with the *Sr2* gene. The *Sr2* gene in combination with other minor genes forms the '*Sr2*' complex forming the basis of durable resistance in wheat against the stem rust. The *Sr2* has been effective against *Puccinia graminis fsp tritici* since 1920 (Ayliffe *et al.*, 2008; Liu and Kolmer, 1998). With durable sources of resistance, the *Sr* genes remain effective over a vast area and longer period of time (McIntosh and Brown, 1997). This will make it difficult for new races of *Puccinia graminis fsp tritici* to overcome the prevailing resistance. 24% of these lines expressed moderately resistant to moderately susceptible responses. It is crucial to test these lines for partial resistance to stem rust for further screening in combination with the resistant wheat lines. These lines could also be involved in gene pyramiding experiments to make use of any available resistance.

The results of this experiment suggest the presence of good sources of resistance to the stem rust *ug99* among the wheat lines involved.

## Conclusion

More work is ongoing and intercrosses have been made. The population is also been advanced through the single descent method to study the genetics of resistance and map the stem rust resistance genes. It is crucial to accumulate the resistance into high yielding wheat background while testing the advanced generations for adaptability in areas that are vulnerable to the *ug99* strain especially in East Africa which is faced with acute malnutrition, drought and famine. The genotypes could also be used to replace the susceptible wheat varieties to ensure food security within the region.

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## References

- Ayliffe, M., Singh, R., Lagudah, E. 2008. Durable resistance to wheat stem rust needed. *Curr. Opin. Plant Biol.* 11:187–192
- Dixon, J., Braun, H.J., Kosina, P. and Crouch, J. (Eds.). 2009. *Wheat facts and futures*. CIMMYT. Mexico, D.F.:
- Groth, J. V., McCain, J. W., and Roelfs, A. P. 1995. Virulence and isozyme diversity of sexual versus asexual collections of *Uromyces appendiculatus* bean rust fungus. *Heredity*. 75: 234–242.
- Johnson, R. 1984. A critical analysis of durable resistance. *Annual Review of Phytopathology*. 22:309-30.
- Liu, J. Q. and Kolmer, J. A. 1998. Genetics of stem rust resistance in wheat cultivars Pasqua and AC Taber. *Phytopathology*. 88: 171-176
- McIntosh, R. A., Wellings, C.R. and Park, R.F. 1995. *Wheat Rusts: An Atlas of Resistance Genes*. CSIRO, Australia. ISBN. 0 643 05428 6.
- McIntosh, R. A., Yamazaki, Y., Devos, K. M., Dubcuisky, J., Rogers, J., Appels, R. 2003. Catalogue of gene symbols for wheat. In: *Proceedings of the Tenth International Wheat Genetics Symposium*. Paestum, Italy, 2003. 5: 56-60
- Roelfs, A. P. 1988. Genetic Control of Phenotypes in Wheat Stem Rust. *Annual Review. Phytopathology*. 26:351-67
- SAS institute Inc. 1996. *The SAS system for windows*. Version 6.12. SAS. Inst., Cary N.S
- Singh, D., Girma, B., Badebo A., Woldeab, G., Njau, P., Wanyera, R., Singh, R.P., Bhavani S., Huerta-Espino, J. and Ward, R. 2009. Screening for stem rust resistance in East Africa. <http://www.globalrust.org/>.
- Singh, D., Park, R.F., McIntosh, R.A. and Bariana, H.S. 2008. Characterisation of stem rust and stripe rust Seedling resistance genes in selected wheat cultivars from the United Kingdom. *Journal of Plant Pathology*. 90: 553-562
- Spielmeyer, W., Robertson, M., Collins, N., Leister, D., Schulze-Lefert, P., Seah, S., Moullet, O. and Lagudah, E.S. 1998. A super family of disease resistance gene analogs is located on all homoeologous chromosome groups of wheat (*Triticum aestivum*). *Genome*. 41: 782–788
- Steffenson, Brian J., Olivera, P., Roy, Joy K., Jin, Y., Smith, Kevin P. and Muehlbauer, Gary J. 2007. A walk on the wild side: mining wild wheat and barley collections for rust resistance genes. *Australian Journal of Agricultural Research*. 58:532–544.
- Wanyera, R. 2008. Status and Impact of TTKS (Ug99) in Kenya. In: Singh, G.P., Prabhu, K. V. and Singh, Anju M. (Eds.) *Proceedings of International Conference on Wheat Stem Rust Ug99- A Threat to Food Security*. Indian Agricultural Research Institute, New Delhi. 12-14
- Wanyera, R. Kinyua, M.G., Njau, P., Kamundia, J.W. and Kilonzo, S. 2004. Current Status of Stem Rust in Wheat Production in Kenya. 12th Regional Wheat Workshop for Eastern, Central and Southern Africa. Nakuru, Kenya, 22-26 November 2004. 1-243