African Crop Science Journal, Vol. 24, Issue Supplement s1, pp. 25 - 33ISSN 1021-9730/2016 \$4.00Printed in Uganda. All rights reserved©2016, African Crop Science Society

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## DEVELOPMENT OF WHEAT GERMPLASM FOR STEM RUST RESISTANCE IN EASTERN AFRICA

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### ABSTRACT

Wheat (*Triticum aestivum*) rust outbreak is the primary production constraint in Eastern Africa. Ethiopia, Kenya and Uganda are hot spots for the epidemic of rusts, due to higher rates of evolution of new pathogen races, especially of the virulent stem rust (*Puccinia graminis*) race, Ug99. The objective of this study was to identify sources of resistance to the major pathotypes of stem rust prevalent in some countries of Eastern Africa. Three hundred and six elite breeding lines, selected and advanced at the Wheat Regional Centre of Excellence (WRCoE) in Ethiopia, were planted in stem rust hot spot areas of the country (Arsi-Robe) and Kenya (Njoro) under natural infections. Stem rust scores of 18, 25.8 and 56.2% of lines at Arsi-Robe; and 35, 49 and 16.7% of lines at Njoro were resistant to moderately resistant, intermediate and moderately susceptible to susceptible category, respectively. Overall coefficient of infection (ACI) at Arsi-Robe (24) was greater than that of Njoro (13), indicating higher disease pressure in Ethiopia than Kenya. Among the lines, 67% at Njoro and 49% at Arsi Robe showed good levels of resistance, with a severity less than 20%. However, only 32% of the lines showed a good level of resistance (<20% severity) in both locations. These lines could have combined resistance to the multiple stem rust races prevailing at both locations. Generally, most of the lines, which showed better resistance at Njoro were susceptible at Arsi-Robe.

Key Words: Hot spots, Puccinia graminis, Triticum aestivum

# RÉSUMÉ

La rouille de la tige chez le blé (*Triticum aestivum*) est l'un des plus importants problèmes rencontres dans la production du blé en Afrique de l'Est. L'Ethiopie, le Kenya et l'Ouganda sont des pays à fort taux d'épidémie de rouille de la tige. Ceci à cause du taux élevé de d'agents pathogènes, ainsi que l'apparition de nouveaux pathogènes, dont le plus redoutable (*Puccinia graminis*) race Ug99. L'objectif de cette étude était d'identifier des sources de résistance aux pathotypes majeurs de la rouille de tige qui sevit dans certains pays de l'Afrique de l'Est. Trois cent six lignées élites, sélectionnées au centre régionale d'excellence du blé (WRCoE) en Ethiopie, ont été plantées dans des zones a fort taux de prévalence de la rouille de tige en Ethiopie (Arsi-Robe) au Kenya (Njoro). Respectivement, 18, 25.8 et 56.2% des lignées a Arsi-Robe; et 35, 49 et 16.7% des lignées a Njoro se sont révélées résistantes ou modérément résistantes, intermédiaires, modérément susceptibles a susceptibles. En somme, le coefficient d'infection (ACI) à Arsi-Robe (24) était plus élevé qu'à Njoro (13), ceci indique que la pression de la rouille de tige est plus élevée en Ethiopie qu'au Kenya. Parmi les lignées évaluées, 67% à Njoro et 49% a Arsi Robe sont exhibe des bons niveaux de résistance, avec 20% de sévérité. Néanmoins, seul 32% des lignées ont exhibe une bonne résistance (<20% sévérité) dans les deux localités. Ces lignées porteraient surement des gènes

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de résistance multiple contre les différentes races de champignon présents dans les deux localités. Généralement, la plupart des lignées ayant exhibe une bonne résistance a Njoro étaient susceptible a Arsi-Robe.

Mots Clés: Zone a fort taux de prévalence, Puccinia graminis, Triticum aestivum

#### INTRODUCTION

Wheat (*Triticum aestivum*) stem rust, caused by Puccinia graminis f. sp. tritici, is the major biotic constraints for wheat production in the world, and in the Eastern part of Africa in particular. Countries such as Kenya and Ethiopia experience recurrent epidemics of stem rust due to evolution of new stem rust races (Wanyera et al., 2006; Admassu et al., 2009). This has been evidenced in the evolution of new stem rust races, with additional virulence to the deployed stem rust resistance genes in wheat cultivars. For instance, variants with virulence for Sr24 (Jin et al., 2008) and Sr36 (Jin et al., 2009) genes were detected in Kenya. Similarly, virulence to SrTmp (Olivera et al., 2015) gene was detected from stem rust samples collected during the 2013 season epidemics that wiped out Digalu in Bale zone of Ethiopia. However, the race responsible for the loss of effectiveness of SrTmp gene in Digalu was reported in Turkey (Zafer et al., 2012), prior to its discovery in Ethiopia.

Currently, race Ug99 (TTKSK) is continuously evolving and undergoing stepwise mutations, to bear additional virulence. There are eight variants of this race reported across many parts of Africa (Singh et al., 2015) and its migration and spread to other parts of Africa, Asia and other parts of the world is inevitable (Singh et al., 2011). In view of the recurrent epidemics of stem rust in eastern Africa, an internationally aggressive breeding strategy was followed and resistant cultivars were released in countries most vulnerable to this race. For instance, in Kenya two bread wheat cultivars, Eagle-10 and Robin, were released in 2009 (Njau et al., 2011); and in Ethiopia, Kakaba and Danda'a were released in 2010 as resistant cultivars to Ug99.

Although the release of resistant cultivars to Ug99 has paramount importance for those countries that were at immediate risk of the pathogen, the continuous evolution of new races and/or variants of the same race requires sustainable efforts to deal with epidemics of the kind of Ug99 or the one that wiped out mega bread wheat cultivar Digalu in Ethiopia by the race TKTTF. The objective of this study was to identify sources of resistance to the major pathotypes of stem rust prevalent in some countries of eastern Africa.

# MATERIALS AND METHODS

A total of 306 elite bread wheat lines that were either selected from international nurseries or developed from the WRCoE, including susceptible and resistant checks, were used in the study. Among the cultivars resistant to Ug99 included in the study, were Danda'a, Kakaba and Digalu. The susceptible cultivar, Hawi was also included. Millenium, which carries Sr24 gene was included in the test to evaluate its performance across the two locations (Arsi Robe in Ethiopia and Njoro in Kenya). These elite bread wheat lines were planted in two rows of 1-metre length.

At Arsi Robe station, there was no artificial inoculation of stem rust, since the disease occurred at epidemic proportions most of the time under natural conditions. However, a spreader row of PBW343 was used to encourage further development of stem rust race Ug99. In Kenya at Njoro, disease development was encouraged by making artificial inoculations at different growth stages of the crop development and spreader rows of super susceptible bread wheat Cacuke, completely susceptible to Ug99 (Kimani et al., 2015) was utilised. When the susceptible check started to show stem rust development, scoring of severity was carried out using the modified Cobb scale (Peterson et al., 1948). Average Coefficients of Infection (ACI) were calculated by taking into account the severity of stem rust of the lines and cultivars, and their field response (Stubbs et al., 1986). The scores were converted into average coefficients of infection, by multiplying severity by an assigned value for the field response, as suggested by Stubbs et al.

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(1986) (Table 1). The constant value was further modified to include infection responses of resistant to moderately resistant (RMR=0.3) and moderately susceptible to susceptible (MSS=0.9).

## RESULTS

**Reactions of elite lines.** Out of the 306 lines tested at Arsi Robe, ten were immune to the pathogen populations (Table 2). However, at

TABLE 1. Field response values according to Stubbs *et al.* (1986) with some modifications

Field response	Symbol	Constant value
Zero (Very resistant)	0	0
Resistant	R	0.2
Resistant to moderately resistant	RMR	0.3
Moderately resistant	MR	0.4
Intermediate	M (MRMS)	0.6
Moderately susceptible	MS	0.8
Moderately susceptible to susceptible	MSS	0.9
Susceptible	S	1.0

R = resistant, RMR = resistant to moderately resistant, MR = moderately resistant, M = moderately resistant to moderately susceptible, MS = moderately susceptible, MSS = moderately susceptible to susceptible and S = susceptible

TABLE 2. Number of elite bread wheat lines in resistance and susceptibility categories in Ethiopia and Kenya

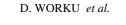
No.	Reaction category	Number o	of lines
	category	Arsi Robe	Njoro
1	0	10	0
2	R	11	4
3	RMR	5	12
4	MR	29	91
5	Μ	79	150
6	MS	31	24
7	MSS	45	16
8	S	96	9

R= resistant, RMR= resistant to moderately resistant, MR= moderately resistant, M= moderately resistant to moderately susceptible, MS= moderately susceptible MSS= moderately susceptible to susceptible and S= susceptible Njoro, none of the lines were immune to stem rust infection. Eleven lines were resistant at Arsi Robe, but only four lines were resistant at Njoro. Five and 12 lines displayed resistant to moderately resistant reactions at Arsi Robe and Njoro, respectively. Twenty nine lines at Arsi Robe and 91 lines at Njoro exhibited moderately resistant reactions; whereas 79 and 150 lines displayed intermediate reactions at Arsi Robe and Njoro, respectively. Thirty one lines at Arsi Robe showed moderately susceptible reactions, and 24 at Njoro. With respect to moderately susceptible to susceptible reactions (MSS), (45), displayed this reaction at Arsi Robe. However, only 16 lines displayed MSS reactions at Njoro.

Ninety-six lines were completely susceptible at Arsi Robe and only nine lines were susceptible at Njoro. In general, considering the disease reactions at Arsi Robe and Njoro, more lines were in the moderately susceptible and susceptible categories at Arsi Robe than at Njoro (Fig. 1 and Table 3). However, in reaction categories ranging from resistant to intermediate, the number of lines at Njoro exceeded by far the number of lines at Arsi Robe (Fig. 1 and Table 3).

**Severity of stem rust.** About half of the elite lines (151) tested at Arsi Robe (Fig. 2) showed a severity less than or equal to 20%; whereas at Njoro, almost two-third of the lines (204) showed the same severity levels (Fig. 2). Twenty one and 20% of the lines showed a severity of 25-30% at Arsi Robe and Njoro, respectively. Ninety one (30%) of the lines at Arsi Robe and 42 (14%) of the lines at Njoro displayed a severity of greater than 30%.

Terminal stem rust severity, and coefficient of infection. Considering the three parameters, the highest score was recorded for bread wheat cultivar Hawi (Table 3). The least score was recorded for elite bread wheat line ETBW6655 (Table 3). Five elite lines depicted an average coefficient of infection (ACI) value of less than two. These included lines ETBW6261, ETBW6406, ETBW6680, ETBW6681 and ETBW6690. Generally 67 (83%) of the test genotypes displayed an ACI value of less than or equals to ten. However, 11 (13%) of the



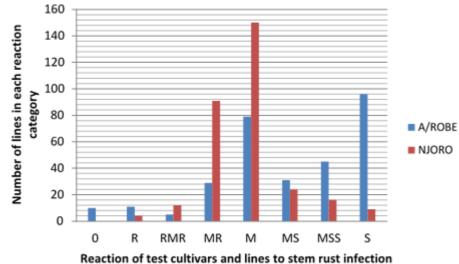
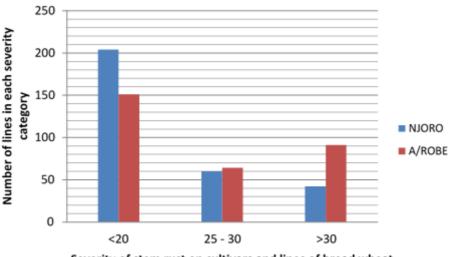


Figure 1. Number of bread wheat elite lines in different classes of stem rust reactions at Arsi Robe in Ethiopia and Njoro in Kenya in 2012.



Severity of stem rust on cultivars and lines of bread wheat

Figure 2. Elite lines of bread wheat evaluated in Ethiopia and Kenya and their severity levels.

genotypes showed ACI value of greater than or equals to ten.

# DISCUSSION

The variability in both severity and infection response of elite lines, and check cultivars at both testing locations, Arsi Robe and Njoro (Tables 2 and 3), clearly shows that the stem rust pathogen populations have differences in their virulence profiles. More lines were found to be more susceptible at Arsi Robe (Ethiopia) than at Njoro (Kenya). Cheruiyot *et al.* (2015) reported Narok as the most stem rust prone compared to Njoro in Kenya. In the present study, more lines were found resistant, which could be attributed to the low initial stem rust pressure compared with Arsi Robe in Ethiopia. During the 2012 season, stem rust race analysis reports depicted the dominance of race TTKSK across many wheat growing areas

Entry	Genotype	Terminal stem	rust severity	CI-	value	ACI value	Entry	Genotype	Terminal ste	em rust severity	CI-	value	ACI value
		Njoro	Arsi Robe	Njoro	Arsi Robe	value			Njoro	Arsi Robe	Njoro	Arsi Robe	value
1	Danda'a	tMR	10M	0.4	6	3.2	193	ETBW 6664	5RMR	5MSS	1.5	4.5	3
2	ETBW 6432	ťR	20MS	0.2	16	8.1	194	ETBW 6668	10MR	20M	4	12	8
3	ETBW 6434	10M	15M	6	9	7.5	196	ETBW 6679	5MR	20M	2	12	7
5	ETBW 6440	ťR	10M	0.2	6	3.1	197	ETBW 6681	5MR	0	2	0	1
7	ETBW 6468	5MS	5MR	4	2	3	198	ETBW 6689	10MR	5M	4	3	3.5
3	ETBW 6469	10MS	5M	8	3	5.5	200	ETBW 6219	20M	10M	12	6	9
16	ETBW 6491	10MR	10RMR	4	3	3.5	202	ETBW 6238	20MR	5M	8	3	5.5
17	ETBW 6493	10RMR	20MSS	3	18	10.5	204	ETBW 6281	15MR	5M	6	3	4.5
18	ETBW 6499	15MR	10MR	6	4	5	206	ETBW 6453	20M	tMR	12	0.4	6.2
28	ETBW 6142	20M	MS	12	0.8	6.4	207	ETBW 6474	15M	10M	9	6	7.5
17	ETBW 6133	20M	15M	12	9	10.5	210	ETBW 6506	10MR	10M	4	6	5
18	ETBW 6145	20MR	ťR	8	0.2	4.1	211	ETBW 6523	15MS	10M	12	6	9
50	ETBW 6158	10MR	20MS	4	16	10	213	ETBW 6530	10M	5M	6	3	4.5
53	ETBW 6173	5M	20MSS	3	18	10.5	215	ETBW 6534	15MR	20M	6	12	9
54	ETBW 6181	20M	ťR	12	0.2	6.1	216	ETBW 6543	20M	15M	12	9	10.5
55	ETBW 6183	20M	ťR	12	0.2	6.1	217	ETBW 6655	5RMR	0	1.5	0	0.75
60	ETBW 6300	10M	5MSS	6	4.5	5.25	218	ETBW 6682	15MR	5MR	6	2	4
61	ETBW 6317	20MS	10M	16	6	11	219	ETBW 6690	20M	0	12	0	6
2	ETBW 6320	10MS	5R	8	1	4.5	221	ETBW 6256	5MR	tMR	2	0.4	1.2
65	ETBW 6355	10RMR	20MSS	3	18	10.5	224	ETBW 6278	20M	10M	12	6	9
69	ETBW 6216	10M	10MS	6	8	7	227	ETBW 6445	20M	10M	12	6	9
86	ETBW 6488	20M	tMR	12	0.4	6.2	234	ETBW 6651	10MR	10M	4	6	5
87	ETBW 6653	20MR	tRMR	8	0.3	4.15	238	ETBW 6674	10MR	tMSS	4	0.9	2.45
88	ETBW 6654	10MR	20M	4	12	8	239	ETBW 6686	20MR	5MSS	8	4.5	6.25
90	ETBW 6197	20MR	20M	8	12	10	243	ETBW 6702	5RMR	10MS	1.5	8	4.75
8	ETBW 6261	tR,5MS	5MR	0.2	2	1.1	244	Digalu	10MR	20M	4	12	8
99	ETBW 6264	20M	10MSS	12	9	10.5	245	Kakaba	15MR	20M	6	12	9
100	ETBW 6266	5MS	5MR	4	2	3	259	ETBW 6177	20M	20M	12	12	12
103	ETBW 6457	20M	10M	12	6	9	283	ETBW 6384	15M	20M	9	12	10.5
105	ETBW 6461	20M	5MR	12	2	7	290	Hawi	30MR	50S	12	50	31

TABLE 3. Terminal stem rust severity, coefficient of infection and average coefficient of infection of selected elite wheat lines and check cultivars in Ethiopia and Kenya

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Entry	intry Genotype		Terminal stem rust severity	CI-	CI-value	ACI	Entry	Genotype	Terminal ste	Ferminal stem rust severity	CI-	CI-value	ACI
		Njoro	Arsi Robe	Njoro	Arsi Robe	value			Njoro	Arsi Robe	Njoro	Arsi Robe	value
166	ETBW 6496		10RMR	4	с	3.5	291	Millennium	20M	5S	12	5 2	8.5
170	ETBW 6529	15M	10M	7.5	9	6.75	298	ETBW 6367	5MR	20M	2	12	7
171	ETBW 6647		tMR	9	0.4	3.2	299	ETBW 6393	10MR	10MR	4	4	4
172	ETBW 6658		10M	9	9	9	300	ETBW 6377	10M	£	9	0.2	3.1
173	ETBW 6662		5M	9	ო	4.5	301	ETBW 6314	15M	£	6	0.2	4.6
174	ETBW 6680		tMR	2	0.4	12	302	ETBW 6404	20MR	20MS	ω	8	œ
175	ETBW 6683		20M	2	12	7	303	ETBW 6315	20M	tM	12	0.6	6.3
185	ETBW 6257		£	12	0.2	6.1	304	ETBW 6406	5MR	tMR	2	0.4	1.2
187	ETBW 6287		tMR	4	0.4	6.2	305	ETBW 6373	15MR	5M	9	ო	4.5
190	ETBW 6485	20M	tMR	12	0.4	6.2	306	ET13-A2	<b>5</b> RMR	20M	1.5	12	6.75

Arsi Robe (Woldeab et al., 2013). The susceptibility of major bread wheat cultivars in the Ethiopian wheat rust trap nursery (EWRTN), during the season at Arsi Robe, is additional evidence for the development of stem rust epidemics (Worku, Unpublished data) and this epidemics occurred on both bread wheat cultivars and durum wheat landraces (Worku, 2014). Ismail et al. (2012) reported race TTKST as the dominant stem rust race in major wheat growing regions of Kenya, because of the widespread production of bread wheat cultivar KS Mwamba. This particular race is still the most dominant stem rust race prevalent in major wheat growing regions of Kenya, and a recent virulence survey in Narok resulted in the identification of race TTKST which is virulent on Sr24 gene (Stella et al., 2015). The fact that none of the tested lines was immune to stem rust infection at Njoro, and ten lines were immune at Arsi Robe depicts that there could be bread wheat lines that carry effective race-specific seedling stem rust resistance genes, to the pathogen population at Arsi Robe that are not effective to the prevailing stem rust races in Njoro. This further suggests that the races prevalent in Njoro may have a wider virulence spectrum compared to the races in Arsi Robe as three of the Ug99 lineage reported in Kenya (Wanyera et al., 2006; Jin et al., 2008; Jin et al., 2009) and only two of them reported in Ethiopia (Admassu et al., 2009; Getaneh, Unpublished data).

in Ethiopia, including the current testing site, viz.

The 73 lines that displayed resistant to moderately resistant reactions to the prevalent races at both Arsi Robe and Njoro (Tables 1 and 3), could be potential sources of resistance to race Ug99 and its derivative with virulence for Sr24 gene. The Arsi zone of Ethiopia is a known hotspot for the development of stem rust epidemics and many stem rust race analysis works indicated the presence of broad races with wider virulence spectrum (Admassu and Fekadu, 2005; Admassu *et al.*, 2009); and this recurrent epidemics has resulted in the susceptibility of most tested lines in the current evaluation.

Generally, the Average Coefficient of Infection (ACI) of most elite lines was small compared to the ACI value of the susceptible check, Hawi (ACI=31). Six lines displayed an ACI value of <2.

**FABLE 3. Contd** 

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CI = coefficient of infection, ACI = average coefficient of infection

The least ACI value was recorded for elite line ETBW6655 (ACI=0.75). Hawi is postulated to have seedling stem rust resistance gene(s), Sr30+/Sr31 (Naod *et al.*, 2005), both of which are not effective to the prevalent stem rust races in the major wheat growing regions of Ethiopia (Admassu and Fekadu, 2005; Admassu *et al.*, 2009; Abebe *et al.*, 2012). Bread wheat cultivar ET13A2, was resistant to moderately resistant to the prevalent pathotypes of stem rust in Kenya and Ethiopia. It is, therefore, essential to incorporate such types of bread wheat cultivars as ET13A2 in the regional wheat breeding/ improvement programme as possible sources of stem rust resistance.

The Shuttle-Breeding Programme between Mexico and Kenya has resulted in the identification and development of high-yielding and stem rust resistant bread wheat lines, with a high level of APR to the prevailing stem rust races in Kenya and other countries of Africa (Singh et al., 2012). Taking the success of CIMMYT's shuttle breeding strategy, a shuttle breeding programme was initiated between Ethiopia and Kenya that resulted in the identification and development of stem rust resistant and high yielding bread wheat lines that fit in the agroecologies of east Africa countries, such as Tanzania and Uganda. Selection and advancement of breeding lines, with low level of severity (usually < 20%), with moderately susceptible or moderately resistant infection types (responses) to the prevailing stem rust races in the test environments is a dependable breeding option to identify resistant wheat lines which has been utilised for wheat improvement in Ethiopia (Bechere et al., 1995).

# CONCLUSION

The 73 lines that displayed low level of stem rust severities and resistant to moderately resistant reactions to the stem rust pathogen populations of both test locations, can directly be utilised as resistant parental lines in the regional wheat breeding programme, or they can be advanced to regional yield trials for the development of high yielding and stem rust resistant cultivars to combat with the ever evolving races of stem rust.

# ACKNOWLEDGEMENT

The authors thank the Wheat Regional Centre of Excellence (WRCoE) under the Eastern Africa Agricultural Productivity Project (EAAPP) for funding this research work. The collaborating institutions of the Ethiopian Institute of Agricultural Research (EIAR) and Kenya Agricultural and Livestock Research Organization (KALRO) are grateful. The Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) is acknowledged for facilitating publication of this paper.

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