Race Analysis of Stem Rust Inoculum and its Virulence on Commercial Wheat Varieties at Seedling in Kari-Njoro, Kenya

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ABSTRACT— Stem rust (Puccinia graminis pers. f. sp. tritici) is one of important foliar diseases of an economic importance in wheat (Triticum aestivum L.) worldwide. Commercial varieties are valuable genetic resource for wheat improvement. The objective of this study was to determine stem rust race in Narok and the seedling resistance of commercial wheat varieties to stem rust race from Narok. In this experiment, five sets of stem rust differential from CIMMYT were inoculated with stem rust inoculums from Narok North District, Kenya. There were different infection types as far as stem rust virulence is concerned on the commercial varieties and the stem rust differentials. From the five sets of differentials, the inoculums from Narok were found to be TTKST (Ug99+Sr24). Twenty commercial wheat varieties were evaluated for resistance to stem rust race Ug99 in three replicates in the green house. The infection type ranged between "0" to "4" with 55% of the genotypes showing resistance which could be considered for improvement of wheat varieties. About 45% of the genotype was susceptible to Ug99 suggesting elusiveness of resistant gene for stem rust. Kingbird and robin were among the resistant varieties indication of having resistant genes. Farmers need to adopt the new varieties such as kingbird and robin for better yield.

Keywords--- wheat, Stem rust, Puccinia graminis pers. f. sp. tritici, Triticum aestivum L., Ug99 stem race

1. INTRODUCTION

Stem rust race TTKSK (Ug99) was discovered in Uganda in 1998/99(Pretorius et al., 2000) since then it has caused havoc to global wheat crop. Seedling resistance protects the plant against virulent pathogen isolate during their entire growing period and it is race specific controlled by major genes. However, when used extensively over along period of time, new races of Ug99 usually overcomes it leading to susceptibility of the released germplasm (Rajaram et al.,1988). The virulence shown by races in the Ug99 lineage is exceptional in the range of resistance genes conquered and in the area sown globally to cultivars protected previously by these defeated genes. These factors make the Ug99 lineage unique and a clear threat to a large proportion of existing commercial wheat cultivars (Singh et al., 2008). Rust pathogens are capable to change and evolve through mutation or sexual recombination (Park, 2007). Ug99 is no exception, and is mutating and migrating rapidly. Seven variants are now recognized within the Ug99 lineage and confirmed occurrence is known in 10 countries (Singh et al., 2011).

Within East and Southern Africa, available data indicates that members of the Ug99 lineage are now the predominant stem rust pathotypes throughout the entire region. The screening of barley and wheat germplasm against a new variant TTKSK is a major threat in the highlands, particularly in Eastern Africa (Bonman et al., 2007). In any breeding program, the aims of germplasm screening are to evaluate the scope of virulence of the new stem rust races and secondly, to identify the source of resistance to stem rust over a large number of germplasm (Jin et al., 2006). The global production of wheat and barley is at risk due to the emergence of devastating race Ug99 which has caused yield losses ranging from 30 to 70% in the case of susceptible varieties (Rodriguez et al., 2008). Unlike other races of rusts, this only partially affects crop yields, the destruction of Ug99 results in yield loss ranging 80-100 (CIMMYT, 2005b). The Sr2, Sr30 and Sr31 are among the 45 stem rust genes that have been evaluated for virulence in different backgrounds (Bansal et al., 2008).

2. MATERIALS AND METHODS

The study was conducted in the greenhouse at Kenya Agricultural Research Institute (KARI) at Njoro (0° 20'S and 35° 56'E) situated at an elevation of 2185 m above sea level. Twenty wheat commercial varieties from Kenya Agricultural Research Institute (KARI) NJBWII, King bird, Chozi, Duma, Mbega, Chiriku, Kwale, Yombi, Pasa, Nyangumi, Tembo, Fahari, Kulungu, Swara, Kongoni, Mbuni, Ngamia, K. Ibis, Robin and Eagle 10 were screened for seedling resistance in the green house. A susceptible check wheat variety Sr24 and 50 CIMMYT stem rust (SR) differentials were planted

The samples were taken to Kenya Agricultural Research Institute-Njoro together with test genotypes. Wheat samples with stem rust urediniospores were collected in Narok during the 2012 main season of crop cycles.

2.1 Experimental Procedures

The samples were taken to Kenya Agricultural Research Institute-Njoro (KARI-Njoro) for their conservation at -20 °C and increase. The rust samples were increased by inoculating the wheat variety with Sr24 gene due to the fact that this genotype is susceptible to stem rust. The experiment was performed under greenhouse conditions on perforated aluminum trays measuring $20\text{cm} \times 20\text{cm}$ filled with soil and ten seeds were planted per tray. Uredinospores of the stem rust isolate were removed from -20°C and heat shocked in water bath at 40°C for 3 minutes.

Uredinospores were suspended in soltrol 130 light mineral oil and atomized on seedling about 7 days after planting (Feekes stage 1) in isolation room. The test genotypes were placed in dark mist with a relative humidity of 100% in incubation chamber, which was regulated at 13° C for 24 - 36 hrs. Seedlings were then transferred to the growth chamber that was maintained at about 22° C day and $20 - 21^{\circ}$ C night temperature

At 2 leaf stage, (Feekes stage 1), seedlings were assessed for disease severity using 0-4 infection type (IT) scale according to Roelfs (1992). To designate physiological races of wheat stem rust, the response (avirulence / virulence) exhibited by the genes used as differentials were reported. In this paper, the nomenclature proposed by Singh (2008) was used.

3. RESULTS AND DISCUSSION

Among the tested twenty commercial genotypes for seedling resistant, 55% were resistant to stem rust pathotype *Ug99*. This constituted of NJBWII, Kingbird, Chozi, Yombi, Nyangumi, Swara, Kongoni, Ngamia, Robin and Eagle 10. On the other hand, 45% of the tested genotypes were susceptible at seedling and this included Duma, Mbega, Chiriku, Kwale, Pasa, Tembo, Fahari, Mbuni and K. Ibis. These findings are not surprising as resistance against pathogens may be classified as specific or non-specific. Specific resistance genes determine a great protection against the disease and are expressed usually at all stages of plant development. They are also assumed to follow the gene-for-gene relationship⁴. The genetics of non-specific resistance was postulated to be mainly quantitative. However, the detection of highly comparable resistance behaviors in descendants of varieties showing high levels of non-specific resistance gave some indication that also major genes may exist (Meinel et al., 1980).

Moreover, 65% of the genotypes conditioned "0" as the most common infection type and this constituted NJBWII, Kingbird, Chozi, Duma, Yombi, Fahari, Kulungu, Swara, Ngamia, K. Ibis, Robin and Eagle 10. However, genotype Nyangumi conditioned ';" as the most common infection type while Chiriku, Pasa and Kwale were susceptible with common infection types of "3", "3", and "4" respectively which can be compared to the infection type of *Sr 24*. Mbega was the only genotype with "2⁺" as the most common infection type (Table 1).

Amongst the test genotypes 35% exhibited "4" as the highest range which included Duma, Mbega, Chiriku, Kwale, Pasa, Tembo and Fahari. Genotypes NJBWII, Eagle 10, Ngamia, Kongoni, Chozi and Yombi exhibited "2" as the highest range which was 30% of the genotypes. Moreover Kingbird, Nyangumi and Kulungu were virulent with "1" as the highest range. Mbuni and K. Ibis were susceptible with "3" as the highest range. In addition, Swara and Robin were resistant with infection type of "0" as the highest range (Table 1).

Table 1. Seedling Reaction of twenty wheat (*Triticum aestivum L.*) Commercial Lines to stem rust (*Puccinia graminis* sp. *tritici*) race *Ug99* in the green house at KARI- Njoro

CULTIVAR	Infection types (IT) (0-4 Scale) ¹		RESISTANT/ SUSCEPTIBLE
	Most Common	Range	
NJBWII	0	0 -2+	Resistant
King bird	0	; - 1+	Resistant
Chozi	0	; -2	Resistant
Duma	0	0 - 4	Susceptible
Mbega	2+	0 - 4	Susceptible
Chiriku	3	; - 4	Susceptible
Kwale	4	0 - 4	Susceptible
Yombi	0	0 - 2	Resistant
Pasa	3	0 - 4	Susceptible
Nyangumi	;	; - 1	Resistant
Tembo	None	2 - 4	Susceptible
Fahari	0	; - 4	Susceptible
Kulungu	0	; - 1	Resistant
Swara	0	; - 0	Resistant
Kongoni	0	; - 2	Resistant
Mbuni	None	0 - 3	Susceptible
Ngamia	0	0 - 2 +	Resistant
K.Ibis	0	0 - 3	Susceptible
Robin	0	; - 0	Resistant
Eagle 10	0	0 - 2	Resistant

Infection type scale: 0= no uredinia or flecks visible 0; = very Faint hypersentive flecks; = hypersentive flecks 1= small uredinia surrounded by necrosis 2= small uredinia surrounded by chlorosis 3= moderate size uredinia without chlorosis 4= large uredinia without chlorosis += slightly larger uredinia than expected for the infection type -= slightly smaller uredinia than expected for the infection type

Table 2: Infection types of Twenty CIMMYT differential lines inoculated with stem rust inoculum from Narok, stem rust genes and race nomenclature.

Set 1 1 Sr5 3 H 2 Sr21 3 H T 3 Sr9e 4 H H 4 Sr7b 3 H T set 2 1 Sr11 3 H T 2 Sr6 3 H T 3 Sr8a 4 H T 4 Sr9g 4 H K Set 3 I Sr36 0 L L 2 Sr9b 3 H K 3 Sr30 4 H K 3 Sr30 4 H K Set 4 I Sr9a 4 H S 2 Sr9d 4 H S 3 Sr10 4 H H 4 SrTMP 2+ L		Sr genes	Infection Type		
2	Set 1				_
3 Sr9e 4 H 4 Sr7b 3 H set 2 T T T 1 Sr11 3 H T 2 Sr6 3 H T 3 Sr8a 4 H H 4 Sr9g 4 H K Set 3 Sr9b 3 H K 3 Sr30 4 H K 3 Sr30 4 H H 4 Sr17 4 H S 2 Sr9d 4 H S 2 Sr9d 4 H H 3 Sr10 4 H H 4 SrTMP 2+ L	1	Sr5	3	Н	
4 Sr7b 3 H set 2 1 Sr11 3 H 2 Sr6 3 H T 3 Sr8a 4 H H 4 Sr9g 4 H K Set 3 Sr36 0 L K 2 Sr9b 3 H K 3 Sr30 4 H K 4 Sr17 4 H S Set 4 1 Sr9a 4 H S 2 Sr9d 4 H S 3 Sr10 4 H H 4 SrTMP 2+ L		Sr21	3	Н	T
set 2 1 Sr11 3 H 2 Sr6 3 H T 3 Sr8a 4 H 4 Sr9g 4 H Set 3 1 Sr36 0 L 2 Sr9b 3 H K 3 Sr30 4 H K 4 Sr17 4 H S Set 4 Sr9d 4 H S 2 Sr9d 4 H S 3 Sr10 4 H H 4 SrTMP 2+ L	3	Sr9e	4	Н	
1 Sr11 3 H 2 Sr6 3 H T 3 Sr8a 4 H H 4 Sr9g 4 H H Set 3 I Sr9g 4 H K 2 Sr9b 3 H K 3 Sr30 4 H K 4 Sr17 4 H S Set 4 H S 2 Sr9d 4 H S 2 Sr9d 4 H H 3 Sr10 4 H H 4 SrTMP 2+ L	4	Sr7b	3	Н	
2 Sr6 3 H T 3 Sr8a 4 H 4 Sr9g 4 H Set 3 I Sr36 0 L 2 Sr9b 3 H K 3 Sr30 4 H K 4 Sr17 4 H S Set 4 Sr9a 4 H S 2 Sr9d 4 H S 3 Sr10 4 H H 4 SrTMP 2+ L	set 2				
3 Sr8a 4 H 4 Sr9g 4 H Set 3 I Sr36 0 L 2 Sr9b 3 H K 3 Sr30 4 H H 4 Sr17 4 H S Set 4 I Sr9a 4 H S 2 Sr9d 4 H S 3 Sr10 4 H H 4 SrTMP 2+ L	1	Sr11	3	Н	
4 Sr9g 4 H Set 3 1 Sr36 0 L 2 Sr9b 3 H K 3 Sr30 4 H H 4 Sr17 4 H S Set 4 1 Sr9a 4 H S 2 Sr9d 4 H S 3 Sr10 4 H H 4 SrTMP 2+ L	2	Sr6	3	Н	T
Set 3 1 Sr36 0 L 2 Sr9b 3 H K 3 Sr30 4 H 4 Sr17 4 H Set 4 1 Sr9a 4 H 2 Sr9d 4 H 3 Sr10 4 H 4 SrTMP 2+ L	3	Sr8a	4	Н	
1 Sr36 0 L 2 Sr9b 3 H K 3 Sr30 4 H 4 Sr17 4 H Set 4 1 Sr9a 4 H 2 Sr9d 4 H 3 Sr10 4 H 4 SrTMP 2+ L		Sr9g	4	Н	
2 Sr9b 3 H K 3 Sr30 4 H 4 Sr17 4 H Set 4 1 Sr9a 4 H 2 Sr9d 4 H 3 Sr10 4 H 4 SrTMP 2+ L	Set 3				
3 Sr30 4 H 4 Sr17 4 H Set 4 1 Sr9a 4 H 2 Sr9d 4 H 3 Sr10 4 H 4 SrTMP 2+ L				L	
4 Sr17 4 H Set 4 1 Sr9a 4 H 2 Sr9d 4 H 3 Sr10 4 H 4 SrTMP 2+ L		Sr9b	3	Н	K
Set 4 1 Sr9a 4 H S 2 Sr9d 4 H 3 Sr10 4 H 4 SrTMP 2+ L	3	Sr30	4	Н	
1 Sr9a 4 H S 2 Sr9d 4 H 3 Sr10 4 H 4 SrTMP 2+ L	4	Sr17	4	Н	
1 Sr9a 4 H S 2 Sr9d 4 H 3 Sr10 4 H 4 SrTMP 2+ L					
2 Sr9d 4 H 3 Sr10 4 H 4 SrTMP 2+ L	Set 4				
3 Sr10 4 H 4 SrTMP 2+ L			4		\mathbf{S}
4 SrTMP 2+ L			4	Н	
	3		4		
Set 5 T	4	SrTMP	2+	L	
Set 5					
	Set 5				T

1	Sr24	3	Н	
2	Sr31	4	Н	
3	Sr38	4	Н	
4	SrMcN	3	Н	

Five sets of differentials were inoculated with inoculum obtained from Narok District. The infection type ranged from '0' to '4' for these differentials. For set one, two and five exhibited High infection type '3' and '4' (H, H, H, H) hence was designated as "T" (Table 2). Set three exhibited both low and High infection type "0", "3", and "4"(L,H,H,H) hence designated as K. Set four exhibited both low and high infection type "2+" and "4"(H,H,H,L) and this set was designated as "S" (Table 2). The stem rust inoculums from Narok was virulent on the following genes (Sr5, Sr21, Sr9e, Sr7b, Sr11, Sr6, Sr8a, Sr9g, Sr9b, Sr30, Sr17, Sr9a, Sr9d, Sr10, Sr24, SrMcN, Sr31 and Sr38) but avirulent on Sr36 and, SrTMP. The stem inocula from Narok District were therefore identified as Ug99 variant TTKST (*Ug99+Sr24*) (Table 2).

4. CONCLUSION

The study established that wheat rust infection type ranged between "0" to "4" with 55% of the genotypes showing resistance which may be considered for improvement of wheat varieties. About 45% of the genotypes was susceptible to stem rust. Kingbird and Robin were among the resistant varieties which properly indicate the presence of resistant genes.

Analysis of the five sets of differentials using the inocula from Narok District identified the race of stem rust as TTKST (Ug99+Sr24).

5. ACKNOWLEDGMENTS

We acknowledge National Council of Science and Technology (NCST) for funding the study, Kari-Njoro for providing facilities and technical services and Maasai Mara University for granting authority to conduct research.

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