Determination of Stem Rust Ug99 Disease Severity in Selected Wheat Backcrosses in Kenya

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Abstract: *Stem rust (Puccinia graminis tritici) is a disease of economic importance in wheat (Triticum aestivum L.) worldwide. The objective of this study was to determine the disease severity of the BC_1F_1 plants in the nursery at Kenya Agricultural and Livestock Research Organization, Njoro. Kingbird (resistant) was crossed with Njoro Bw_2 (susceptible) to obtain F_1 plants. The F_1 plants were backcrossed with Njoro Bw_2 to obtain a BC_1F_1 progeny. Two hundred and forty seeds of the BC_1F_1 plants, twenty eight resistant and susceptible parents were planted in two replicates and the Modified Cobb's Scale was used to record their disease severity scores. An excel work sheet programmed with the AUDPC formula was used to compute the Area Under Disease Progress Curve (AUDPC) values for BC_1F_1 plants. Variation of the disease severity scores and mean AUDPC among the wheat plants was analysed using Analysis of variance (ANOVA). Results showed 47.9% of resistant plants that portrayed the pseudo black chaff (PBC) trait had low disease severity mean scores ranging from 0 to 1.0414 with mean AUDPC values below 99.8. The resistant plants without PBC were 43.3 % and had disease severity mean score ranging from 0.9098 to 1.3686 with mean AUDPC values ranging from 99.8 to 260.The BC_1F_1 plants that showed susceptibility to stem rust disease were 21 (8.8 %) and had the highest disease severity mean score ranging from 1.3223 to 1.4914 with the highest mean AUDPC values ranging from 282.5 to 431.3. The ANOVA computation showed that, variation in the disease severity and in the mean AUDPC values among the individual plants (P < 0.001) was significant, but variation in mean AUDPC values from one replicate to another (P > 0.001) was not significant. The BC_1F_1 wheat plants showed a low disease severity score in the field indicating that these plants received the APR genes for stem rust and this could be useful for wheat improvement through breeding.*
Key words: *Stem rust, Ug99, resistance, backcross, improvement, disease severity*

I. Introduction

Wheat (*Triticum* species) is one of the cereal grains known to have been domesticated first from the Middle East region and Ethiopian Highlands, but currently cultivated globally [1];[2]. The wild einkorn's wheat genetic study, suggests that it was first grown in the Karacadag Mountains in Southeastern Turkey [3]. Compared to other main cereals, wheat is the most significant source of carbohydrate in a majority of countries, and is the primary source of protein in human food as it has a higher protein content [4]. Flour for most baked products, beer and other alcoholic drinks are from the wheat grain [5];[6].

1.1 Wheat Production Constraints

Farmers and agricultural researchers face a major challenge of trying to develop and apply technology that can increase the global cereal yields enough for consumption, and use ways that are affordable, with minimum level of pollution to the environment [7]. Other than constraining factors like low soil fertility and drought, there are also biotic constraints comprising of weeds, insect attacks and diseases such as rusts, smuts, bunts, leaf blight, powdery mildew and head scab [8]. The most significant production challenges in most of the wheat growing areas are disease and particularly those triggered by the fungal pathogens and a few caused by viruses and bacteria [9]. Stem rust can cause up to 100% loss [10] in the presence of susceptible host and favorable environmental conditions while leaf and stripe rust cause 60% loss of yield. Stem rust is the most limiting factor to wheat production [11];[12] due to its extensive distribution, its ability to mutate to new races and attack the previously resistant cultivars.

In 1998 stem rust caused by the fungus *Puccinia graminis tritici* emerged in Uganda and was found on wheat lines well-known to have the stem rust resistance gene *Sr31* (no virulence anywhere in the world had been reported on this gene) [13]. In 2005, a similar virulence was detected in Kenya and in Ethiopia [14], raising

a major concern over the spread of race Ug99 across Africa, Asia and the Middle East [11]. As such, it is inevitable to produce varieties of wheat that can withstand Ug99 [15];[11].

The TTKSK (Ug99) is predicted to spread to the world's most important wheat growing regions in the near future [16]. Plant breeders and pathologists however, still have time to identify resistant genotypes, introgress the genes and increase the seed for farmers before the Ug99 causes a major problem in the Saudi Arabian Peninsula, South Asia and East Asia. This can reduce the devastating effect of the pathogen on susceptible cereals. Introgressing stem rust resistance genes into Njoro Bw_2 variety, using the Kingbird variety derived from CIMMYT germplasm as the donor parent, might produce good results. The Kingbird variety has some adult plant resistance genes (non- race specific, polygenic genes) to Ug99 and matures early [17]. Njoro Bw_2 (recipient) is high yielding, drought resistant, has late maturity and is susceptible to Ug99 [18]. In order to cope with the rising rate of consumption, it is essential to breed for new high yielding cultivars, tolerant against biotic and abiotic stresses to ensure food security and to meet the challenges of the 21st century [16].

The main objective of this work was to determine the disease severity of the BC_1F_1 plants in the nursery at Kenya Agricultural and Livestock Research Organization, Njoro.

II. Materials and Methods

2.1 Experimental Site
The field trials were carried out in a nursery, at Kenya Agricultural and Livestock Research Organization (KALRO) Njoro Centre, in Nakuru, Kenya from June 2012 to December, 2013. The experimental site is located at 0.3 ^{0}S and 35.9 ^{0}E at 2185 m above sea level. The area has an average annual rainfall of 939 mm (average of 61 years) (Kenya Meteorological Station Identification Number 9031021) with an average daily minimum and maximum temperature of 9.7 and 23.5 ^{0}C respectively.

2.2 Plant Materials
The seeds of the 'Kingbird' variety were obtained from CIMMYT and those of Njoro Bw_2 from KALRO, Njoro Centre. Njoro Bw_2 wheat variety grown in Kenya is susceptible to Ug99 though it contains the *Sr2* gene. Kingbird variety is resistant to stem rust and known to have the *Sr2* gene with other slow rust resistant genes that form the "*Sr2* complex" [19];[20].

2.3 Experimental Design
There were two blocks measuring 2 m × 4.5 m. The two blocks were each divided into two plots of 2 m × 2 m with 0.5 m wide alley-ways between the plots. These plots were used for planting the parents and the first filial generation (F_1). There were five rows of each variety per plot, which were 0.4 m apart. There were two other plots of 6 m × 6 m in the same nursery which were used for planting the BC_1F_1 seeds in two replicates. The plots were made of twelve rows, 0.5 m apart. The experimental design was a randomized complete block design (RCBD).

2.4 Field Practices
The planting was carried out in June 2012, in long narrow furrows dug by hand a few inches (1-1.5 inches) deep to the ground, in the two plots of 2 m × 2 m with 0.5 m wide alley-way in between. Seeds of Njoro Bw_2 were planted in furrows made in plot one which had five rows that were 0.4 m apart. Two weeks later in plot two, the same was carried out for the seeds of Kingbird variety which is early maturing to synchronize flowering. During planting, the advised rate of Di-ammonium phosphate (DAP) was applied at 150 kg/ha, as the source of nitrogen and phosphorus (18:24). Two times between stem elongation and booting stages, weeding was carried out by hand. At flowering stage, hand emasculation was carried out on Njoro Bw_2 (female parent) two days before anthesis, to prepare for cross pollination with pollen from 'Kingbird' (donor parent) which was carrying the gene of interest. The highest and lowest florets of NjoroBw_2 were clipped off leaving 4 florets per spikelet. Scissors were used to chop the top part and forceps were used to remove the anthers. This was immediately followed by bagging to prevent random pollination. Pollination bags were used for bagging where they were tied at the base of the inflorescence with a thread. After bagging, the emasculated flowers were tagged to avoid confusion. The tags were labelled to show date of emasculation, name of female and male plant. The date of pollination was indicated on the tag after pollination. Three days after emasculation, pollen from dehisced anthers of 'Kingbird' were collected and dusted onto the stigmas of emasculated flowers of 'Njoro Bw_2'. Bagging was carried out after pollination to prevent random pollination. After pollination, the seeds bagged with the pollination bags were allowed to ripen (develop, fill up, and become harder and drier) for six weeks, then the mature seeds were harvested to obtain the F_1 progeny (first filial generation). Harvesting was carried out by hand using a knife by snipping off the head from the stem. Each head was threshed separately and kernels stored in different medical envelopes.

2.5 Determination of Disease Severity in BC_1F_1

To obtain BC_1F_1 seeds (backcross F_1 seeds), a single backcross was made with Njoro Bw_2. At maturity, harvesting was carried out by cutting off the head from the stem. Each head was threshed separately and the kernels were stored in different envelopes. Based primarily on pustule size and the associated necrosis and chlorosis, the infection responses to rust infection were classified into four discrete categories: R = resistant, MR = moderately resistant, MS = moderately susceptible and S = susceptible (large uredia) (Plate 1.) [21]. Infection responses that overlapped between MS and S categories were denoted as MSS (medium to large sized uredia without chlorosis or necrosis) and those that overlapped between MR and MS were denoted as M. The infection responses that overlapped between R and MR were denoted as RMR. The Pseudo black chaff (PBC) trait was also scored on the two hundred and forty plants and the parents. The plants which showed PBC were scored as (+) while those without PBC were scored as (-).The evaluations for infection responses and stem rust severity were carried out on 17th, 24th and 31st Dec. 2013, respectively. The three scores that were obtained for each plant were used to determine the area under disease progress curve values (AUDPC) for each plant (see formula 1).
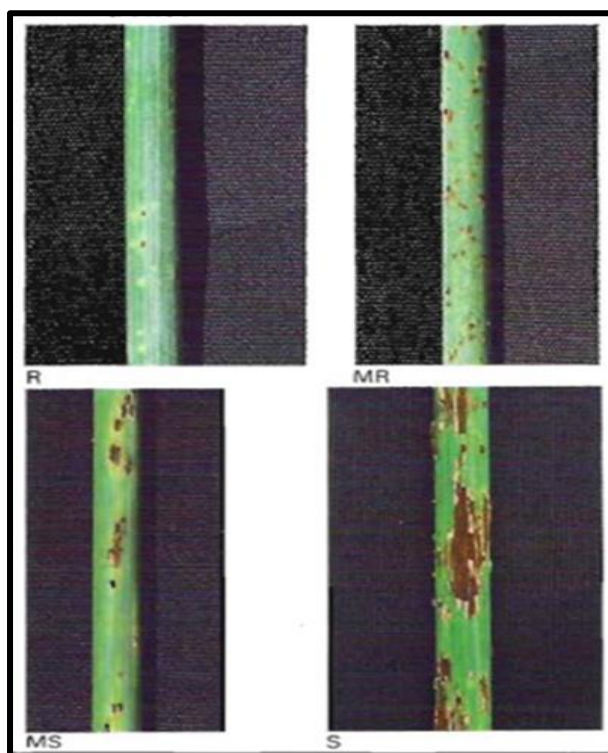
2.6 DATA ANALYSIS
The area under disease progress curve (AUDPC) was computed using the following formula:

(1) AUDPC= ∑[i(t+1) –i)y[]it +1)iy +] /2 (http://www.apsnet.org.)

Where
it is the first assessment date of two consecutive assessment
iy is the disease severity on assessment date it
t(i+1) is the second assessment date of two consecutive assessment
The disease severity scores of individual plants were transformed using $Log_{10}(X+1)$ for homogeneity. One way-ANOVA was used to analyse data on disease severity and AUDPC among the wheat plants. Using the computer software SAS, the means were separated by use of the Honestly Significant Difference test (HSD at $P < 0.05$) (SAS Version 9.2, SAS Inc.) [22].

III. Results and Discussion
3.1 Disease Severity of Adult Plants to Stem Rust Disease

Assessment of adult plants on disease severity to stem rust categorized the BC_1F_1 into three groups based on the final disease severity (FDS). There were 115 plants resistant to stem rust with pseudo black chaff (PBC) trait representing 47.9 % of the total population, 104 wheat plants representing 43.3 % of the total

population, showed resistance to stem rust but did not express the PBC trait and 21 (8.8 %) plants that showed susceptibility to stem rust disease (Table 1.). The 115 resistant BC_1F_1 plants and the resistant parent (Kingbird) which expressed the PBC trait, had low disease severity score with means ranging from 0 to 1.0441 in the final disease score (3rd score). There were four plants (76, 81, 134 and 200) with PBC that had the lowest disease severity with mean of 0.1505 while plant 138 had disease severity mean of 0.00 (Table 1).

Table 1: Disease severity scores to *Puccinia graminis sp tritici* race Ug99 among selected crosses

	Mean of Disease severity Score					
Plant	**1st score (Trans)**	**2nd score (Trans)**	**3rd score (Trans)**	**IF type**	**PBC**	**AUDPC Means**
138	0.00 (0.0000) ^{a}	0.00 (0.0000) ^{a}	0.00 (0.0000) ^{a}	RMR	+	0.0 (0.0)^a
76	0.00 (0.0000) ^{a}	0.50 (0.1505) ^{a}	0.50 (0.1505) ^{b}	R	+	7.0 (2.1)^b
134	0.00 (0.0000) ^{a}	0.50 (0.1505) ^{a}	0.50 (0.1505) ^{b}	RMR	+	7.0 (2.1)^b
200	0.00 (0.0000) ^{a}	0.50 (0.1505) ^{a}	0.50 (0.1505) ^{b}	RMR	+	7.0 (2.1)^b
81	0.50 (0.0000) ^{a}	0.50 (0.1505) ^{a}	0.50 (0.1505) ^{b}	RMR	+	7.0 (2.1)^b
68	7.50 (0.9098) ^{b}	12.50 (1.1228) ^{b}	20.00 (1.3096) ^{c}	M	-	257.5 (21.0)^c
14	7.50 (0.9098) ^{b}	12.50 (1.1228) ^{b}	20.00 (1.3222) ^{c}	M	-	257.5 (21.1)^c
27	7.50 (0.9098)^b	15.00 (1.2041)^c	17.50 (1.2632) ^{c}	M	-	260.0 (21.3)^d
74	10.00 (1.0414)^c	10.00(1.0414)^d	15.00 (1.2041) ^{d}	MS	-	217.5 (20.3)^e
215	5.00 (0.7782) ^{b}	10.00 (1.0414) ^{d}	15.00 (1.2041)^c	MS	-	195.0 (19.1)^f
221	5.00 (0.7782) ^{b}	10.00 (1.0414) ^{d}	15.00 (1.2041) ^{c}	MS	-	95.0 (19.1)^f
***17	12.50 (1.1228) ^{d}	22.50 (1.3686)^e	27.50 (1.4532) ^{c}	MS	-	03.8 (24.8)^g
***9	15.00 (1.2041)^d	22.50 (1.3686) ^{e}	30.00 (1.4914) ^{c}	MSS	-	431.3 (25.4)^h
***25	12.50 (1.1228) ^{d}	20.00 (1.3222) ^{f}	27.50 (1.4532) ^{c}	MS	-	385.0 (24.4)^g
**255	10.00 (1.0414) ^{d}	10.00 (1.0414) ^{d}	10.00 (1.0414) ^{d}	MR	-	185.0 (19.3)^f
**266	5.00 (0.7782) ^{b}	5.00 (0.7782) ^{g}	10.00 (1.0414) ^{d}	MR	-	125.0 (16.1)^h
**267	10.00 (1.0414) ^{c}	10.00 (1.0414) ^{d}	10.00 (1.0414) ^{b}	MR	-	185.0 (19.3)^f
P values MSD	P< 0.0001 0.7698					

*Resistant parent (Kingbird); **Susceptible parent (NJBW$_2$); ***Susceptible BC_1F_1 plants; Trans, Log$_{10}$(X+1) transformed; (+), pseudo black chaff present (PBC); (-), pseudo black chaff absent; IF, Infection response; R, resistant; RMR, resistant to moderately resistant; MR, moderately resistant; MS, moderately susceptible; M, moderately resistant to moderately susceptible; MSS, moderately susceptible to susceptible; AUDPC, area under disease progress curve values are means of two replications and means with same letters within the column are not significantly different at P < 0.05 according to Tukey HSD test; means in parenthesis are transformed means of disease severity and AUDPC (Log$_{10}$(X+1)); 1st, 2nd and 3rd score values are means of two replications; MSD, mean significance difference.

The final disease severity (FDS) mean scores of the 104 resistant plants without PBC ranged from 0. 9098 to 1.3686 which was slightly higher compared to the disease severity mean scores of the plants with PBC (Table 1.). Although all the resistant plants with and without PBC showed infection responses type R, MR and RMR, plants (27, 68 and 14) with disease severity mean scores of 1.2632, 1.3096 and 1.3222 respectively, showed an intermediate (M) type of response to infection. Plants 74, 215 and 221 with a disease severity mean score of 1.2041 and plant 216 with a disease severity mean score of 1.1228 showed an MS response to infection though the disease severity was low (Table 1.).

The final mean disease severity scores for the 21 susceptible BC_1F_1 plants was higher ranging from 1.3222 to 1.4914. Plants 9, 17 and 25 were the most susceptible with the highest disease severity means of 1.4914, 1.4532 and 1.4532 respectively (Table 1.). Although all the 21 BC_1F_1 plants that showed susceptibility to stem rust had infection responses type MS and MSS , there were susceptible parent plants (NJBW$_2$) (255, 266 and 267) all with a disease severity mean score of 1.0414, which showed infection response type MR instead of the MS and MSS reaction expected in the susceptible plants (Table 1.). There was a significant difference in the disease severity mean scores between the plants (P < 0.001).

3.2 Stem Rust AUDPC Values of The BC_1F_1 Wheat Plants and the Parents
The area under disease progress curve (AUDPC) calculated, indicated that differences in the AUDPC means between the 240 BC_1F_1 plants with the parents was significant (Table 2.). All the 115 resistant BC_1F_1 plants with the resistant parents which showed expression of PBC (+), had mean AUDPC values ranging from 0 to 151, with plants 76, 81, 134, and 200 showing the lowest mean AUDPC value of 7 (Table 2.). Plants that had no expression of PBC (-) had higher mean AUDPC values compared to plants that had the PBC. The lowest mean AUDPC values for the plants with no PBC was registered in plants 32 and 75 which was 99.8 followed by plants 88 and 206 which had a mean AUDPC value of 116. Plants without the PBC trait but with mean AUDPC values ranging from 99.8 to 195 showed higher resistance to stem rust as compared to plants with mean AUDPC values ranging from 217.5 to 260 (Table 2).

Table 2: Pseudo black Chaff (PBC) response and area under disease progress curve AUDPC values

Plant number	PBC	AUDPC values
76	+	7.0^a
81	+	7.0^a
134	+	7.0^a
200	+	7.0^a
32	-	88^b
75	-	206^c
9	-	431.3^d
17	-	403.8^d
25	-	385^d
266	-	125^e
255	-	185^c
267	-	185^c
262	-	195^c

(+), pseudo black chaff present (PBC); (-), pseudo black chaff absent; AUDPC, area under disease progress curve values are means of two replications and means with same letters within the column are not significantly different at P < 0.05 according to Tukey HSD test.

The 21 BC_1F_1 plants that showed susceptibility to stem rust disease had mean AUDPC values ranging from 282.5 to 431.3 and plants 9, 17 and 25 had the highest AUDPC means of 431.3, 403.8 and 385 respectively. All the susceptible parent plants had AUDPC means ranging from 285 to 357.5 except for parent plants 266, 255, 267 and 262 which had low AUDPC means of 125, 185, 185 and 195 respectively (Table 2.).The mean AUDPC values among the plants was significantly different (P < 0.001), but from one replicate to another there was no significant difference (P > 0.001).

4.1 Stem Rust AUDPC Values of the BC_1F_1 Plants and Parents

The low disease severity on the 115 plants that had the PBC trait, showed the true linkage of the expression to the *Sr2* gene that slows the rate of infection of plants to stem rust disease [23]. The low mean AUDPC values and low disease severities indicated a high level of resistance to stem rust in the 115 plants with PBC, which is in agreement with the outcomes of [24], where the resistant cultivars had low disease severity and lowest AUDPC values throughout the disease assessment period. There were 104 BC_1F_1 plants (Table 1) that lacked the PBC expression but showed resistance to stem rust. These plants had higher mean AUDPC values compared to the plants which expressed the pseudo black chaff. This is an indication that the *Sr2* gene might be present in a plant interacting with other polygenic genes to bring about resistance but fail to express the PBC trait phenotypically. Plants with mean AUDPC values below 151 showed low disease severity, those with mean AUDPC values above 151 but less than 255 showed moderate severity to stem rust while plants with mean AUDPC values above 255 (Susceptible plants) showed high disease severity which was in agreement with the findings of [25], who found out that there was correspondence between genotypes susceptibility and AUDPC showing that the most susceptible wheat cultivars recorded high AUDPC values.

IV. Conclusion

This study demonstrated that BC_1F_1 wheat plants received the Adult Plant Resistance (APR) genes for stem rust evidenced by the low disease severity mean scores and this could be useful for wheat improvement as far as resistance to stem rust is concerned.

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