Stem Rust (*Puccinia graminis* f.sp. *tritici*) Disease Indeces on Barley (*Hordeum vulgare* L.) Genotypes

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Abstract

Stem rust, caused by (Puccinia graminis f.sp. tritici) is a catastrophic disease because of its ability to cause complete annihilation of barley and wheat crops over wide areas. Previously rpg1 sr31, 24 and 36 genes for resistances were incorporated in genotypes of barley and wheat grown in Kenya. In 1999, a new race Ug99 was detected in Uganda; that has virulence to a number of genes that were initially resistant to stem rust. The new race spread rapidly and in 2001, stem rust monitoring in Kenya detected isolates of Ug99. Most of the work on wheat shows susceptibility and barley being one of the genotypes affected by stem rust limited work has been done to it. There was need to search for new materials that are resistant to the new physiological race of stem rust. In this study 20 barley cultivars locally from Kenya and imported from ICARDA were screened in controlled greenhouse environment and in the field with isolate of Puccinia graminis f.sp. tritici. The infection levels in most genotypes at seedling stage in the greenhouse, ranged from 0 to 2, except in selections CBSS99MOO349T-F-3M-IY-IM-OM and CBSW98WOOO54S-BY-2M-IY-2M-IY-OM that showed infection type 3and 3,4 respectively. At adult plant stage only selection CBSS99MOO39IT-H-IM-IY-IM-IY-OM, Nguzo and Karne were moderately resistant while the rest were susceptible or moderately susceptible. In the field, the new line 1512-5 showed the highest severity of 93% in season 1, with Sabini having the highest severity of 30% in the second season; Nguzo had the lowest disease severity of 16% and 5% in season 1 and 2 respectively. From the results in this experiment most of the Kenyan grown cultivars were susceptible to the new race of stem rust. Race PgtUg99 of stem rust has highly negative impact in limiting grain production potential of most of the commercially popular Kenyan barley cultivars. The study has demonstrated the pathogenicity of PgtUg99 to barley despite the fact that it poses a great threat to wheat production in the world. The information in this study is beneficial to both researchers, barley and wheat producers in confronting a pathogen that is devastating.

Keywords: Stem rust, Barley, Resistance, Ug99, *Puccinia graminis* f. sp. *tritici*, infection type.

Introduction

Barley (*Hordeum vulgare* L.), is one of the most important cereal crops, in Kenya. Currently about 30,000 ha of land is under the crop but potential for expansion remains (EABL, 2005). Rust fungi, responsible for diseases like stem rust, yellow rust and leaf rust is a major contributor to the sub optimal yields realized by farmers. Breeding for resistance has been used as the main method of protection against the fungi. The rust fungi can however overcome host resistance genes and spread new strains through wind dispersal of spores (Hovmøller and Justesen, 2007). Stem rust of barley and wheat, (caused by *Puccinia graminis* f.sp *tritici***,** Eriks. and E. Henn.) is historically one of the most important plant diseases. Devastating stem rust epidemics often result in major grain losses (CIMMYT, 2005). The problem of wheat stem rust was previously contained through the use of genetic resistance resulting from genes such as Sr31 (KARI, 2005b). In 1999 and 2001 a new race Ug99 or TTKS with virulence to stem rust resistant cultivars, was detected in Uganda and Kenya respectively (Wanyera *et al*., 2006; Pretorius *et al.,* 2000).

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Preliminary work indicates that race TTKS is virulent on Midwestern barley cultivars carrying the durable stem rust resistance gene *Rpg1* (Steffenson and Jin, 2006). Stem rust race Ug99 is responsible for up to 100% yield loss of wheat. The race also affects barley where control using fungicides has been unsuccessful. Much of the work including genetic manipulation of wheat to control the disease has had little success. Barley is another crop that is affected by the Ug99 race. Little work has however been done to screen the barley genotypes for resistance to the new race of stem rust. The study was carried out to find out the level of resistance in selected barley genotypes from Kenya and ICARDA to the new race of stem rust Ug99.

Materials and Methods

The experiment was carried out at the Kenya Agricultural Research Institute (KARI) –Njoro situated along the Njoro- Mau Narok road in Nakuru County, located at 0^0 20'S 35^0 56'E and at 2185 meters above sea level. Twenty barley germplasm that included three Kenyan commercial cultivars (Nguzo, Sabini and Karne), two (1512-5 and 1385-13) new introductions from the Kenya malting company and 15 selections from ICARDA were used (Table 1).

Table 1: Type and source of barley germplasm used in the experiment

Genotypes code/ Name	Selection History	Source
ICARDA- 01	CBSS99MOO39IT-H-IM-IY-IM-IY-OM	ICARDA
ICARDA- 02	CBSS99MOO317T-AH-2M-IY-IM-IY-OM	ICARDA
ICARDA- 03	CBSS00YOO113T-A-OY-OM-2Y-OM	ICARDA
ICARDA- 04	CBSS99MOO429T-L-IM-IY-OM	ICARDA
ICARDA- 05	CBSW99WMOOO95T-B-IM-IY-IM-OM	ICARDA
ICARDA- 06	CBSS00YOOO48S-OY-OM-2Y-OM	ICARDA
ICARDA -07	CBSSOOYOO475T-O-OY-OM-2Y-OM	ICARDA
ICARDA- 08	CBSSOOYOO236T-E-0Y-OM-2Y-OM	ICARDA
ICARDA- 09	CBSS99MOO349T-F-3M-IY-IM-OM	ICARDA
ICARDA- 10	CBSS99MOO468T-H-IM-IY-OM	ICARDA
ICARDA -11	CBSSOOYOO479T-D-OY-OM-IM-OM	ICARDA
ICARDA -12	CBSW98WOOO54S-BY-2M-IY-2M-IY-OM	ICARDA
ICARDA- 13	CBSS99MOO315T-F-IM-F-IM-IY-IM-IY-OM	ICARDA
ICARDA- 14	CBSSOOYOO225T-C-OY-OM-2Y-IM-OM	ICARDA
ICARDA- 15	CBSSOOYOO278D-G-OY-OM-2Y-OM	ICARDA
1385-13	-	KENYA
NGUZO	-	KENYA
1512-5	-	KENYA
KARNE	-	KENYA
SABINI	-	KENYA

Stem Rust Isolate preparation

The spores used for inoculation were collected from experimental plots in KARI-Njoro. The stem rust spore (urediniospore) samples consisted of bulk isolate collected using a portable powered suction pump (Hoover Model, 2944B) ® from wheat cultivar Chozi. The inoculum was pre-tested on differential hosts in the greenhouse according to Roelfs *et al.* (1989) and confirmed to be those of PgtUg99. To generate large quantities of urediniospores, the isolate was inoculated onto seedlings of the susceptible wheat cultivar chozi having gene Sr31. The Urediniospores were suspended in distilled water where light mineral of oil- (tween 20 solution) was added and the suspension sprayed onto the plants using a small bowler- atomizer. The plants were then kept in a dark humidity chamber overnight before taking them to clear plastic chambers in the greenhouse to prevent contamination resulting from the movement of urediniospores. Urediniospores were harvested from sporulating pustules at 14 and 21 days after inoculation using a cyclone spore collector. The spores were then dried for 2 to 3 days over silica gel in the laboratory.

The infection type at Seedling stage

The greenhouse experiment was arranged in a completely randomized design (CRD) with three replicates done by sowing the 20 barley germplasm in 15 cm plastic pots filled with forest soil that had been autoclaved at 121^0C and 15 pounds pressure for one hour (Welty *et al.,* 1992). The urediniospore suspension (1mg of urediniospore per 100ml of distilled water) was mixed with 4 drops of tween 20, surfactant using a small bowler- atomizer and inoculated on the seedlings. The inoculation involved uniform spraying of the upper and lower leaf surfaces of individual plants 10 days after germination. Immediately after inoculation the seedlings were incubated for 24 hours in a dark dew chamber kept moist by frequently spraying with water to maintain humidity of 80-100%, and temperatures between 16^0C and 22^0C. The seedlings were transferred to the greenhouse after incubation. For the first two hours, the leaves were sprayed with water for 15 minutes at an interval of 30 minutes using an atomizer. Temperature of 22-30^0C was there after maintained in the green house, following the procedure by McIntosh *et al*., (1995). Fourteen days after inoculation the seedlings were assessed for disease severity according to Stakman *et al.* (1962) scale for stem rust.

The reaction type to stem rust at adult plant stage

Twenty barley germplasm were sown in 20cm diameter plastic plots filled with sterile forest soil. Diammonium Phosphate (18%N and 46% P_2o_5) at the rate of 50Kg/ha that translates to125mg per pot was thoroughly mixed with the soil where 10 seeds of each cultivar were sown in one pot. Two weeks after emergence plants were thinned to eight. The pots were placed on trays with gravel and watering done every other day for 30 minutes. At flag leaf stage plants were inoculated with urediniospores suspension (1mg urediniospores/ 100 ml of distilled water plus 5 drops of tween 20 surfactant) using a syringe and a hypodermic needle. The urediniospore suspension was injected into elongating stems, where each plant received 0.5ml of the suspension (Welty *et al*., 1992), and then in a dark dew chamber over night. The plants were then moved to the greenhouse and placed on a bench where a spray of water was applied on the leaves every 15 minutes for 2 hours to maintain temperature between 22-30^oC. The experiment was laid out as a completely randomized design (CRD) with three replicates. The disease reaction was rated based on the size of the pustules and associated necrosis or chlorosis 14 days after inoculation and weekly thereafter as described by Roelfs *et al*. (1992).

Stem rust severity and infection type in field

Twenty barley cultivars were screened for their level of resistance to stem rust disease in the field. The experiments were planted for two seasons at KARI Njoro during the 2008/09 crop seasons. Each cultivar was sown in plots of 3m length at spacing of 20 cm, at seed rate of 125 kg/ha. The experimental design was randomized complete block design (RCBD) replicated three times. Susceptible wheat cultivars, chozi was planted perpendicular to test plots in the middle of the 1-m pathways on both sides of experimental plots to serve as spreader. The furrows were cut by machine and planting done by hand. A basal dose of DAP fertilizer (18%N and 46% P_2o_5) at a rate of 50Kg/ha was applied at planting and followed with a top dress of CAN (23%N) at 25Kg/ha a month later. Weeds were controlled using Stomp 500E, a pre-emergency herbicide at a rate of 3.0L/ha. Buctril MC (bromoxyil and iso-octyl esters) a post emergent herbicide was applied at the rate of 1.25L/ha to control broad-leaved weeds (KARI, 2005a). Metasystox insecticide at the rate of 0.75 L/ha was used to control Russian wheat aphid (RWA) and other insect pests. The spreader rows were inoculated with *P. g. tritici* pathotype TTKSK at the early jointing stage (Zadoks *et al.,* 1974) by injecting an aqueous suspension of urediniospores into the hollow culm of plants at 0.75-m intervals along each spreader. The infection type responses as described by Roelfs *et al.* (1992), McIntosh *et al.* (1995) and disease severities 0-100%, Peterson *et al.* (1948) were recorded in both trials starting from when 50% of the plants reached heading stage at an interval of 14 days until hardening stage.

Results and Discussion

Infection Levels of Stem Rust in the Greenhouse

The germplasms showed varying levels of resistance to stem rust (Table 2). At seedling stage, the infection levels ranged from 0 to 2, except in selections CBSS99MOO349T-F-3M-IY-IM-OM and CBSW98WOOO54S-BY-2M-IY-2M-IY-OM that showed infection types 3and 3,4 respectively. The resistance at seedling stage may probably be because the germplasm had resistance conferred by one single major gene that was broken down at adult plant stage.

In adult plants reaction 60% of the population were susceptible, 20% moderately susceptible, 10% moderately susceptible to moderately resistant and 10% moderately resistant. ICARDA selections CBSS99MOO39IT-H-IM-IY-IM-IY-OM and CBSSOOYOO225T-C-OY-OM-2Y-IM-OM were moderately resistant (MR) while, Nguzo was resistant to moderately resistant (R -MR). Karne was however moderately resistant to moderately susceptible reaction (MR - MS). The genotypes that showed some resistance at adult stage may contain a major single gene that remained resistant at seedling and at adult plant stage or they may have minor genes that are working together to reduce the disease (Roelfs *et al.,* 1992).

Stem rust severity under field conditions

Disease pressure was higher in season 1 compared to season 2 probably because of differences in relative humidity and temperature. Among the 20 genotypes that were evaluated for adult plant resistance, selection CBSS99MOO39IT-H-IM-IY-IM-IY-OM, Nguzo and Karne were moderately resistant while the rest were susceptible or moderately susceptible (Table 3). Genotype 1512-5 showed the highest severity of (93%) in season 1, while Sabini had the highest severity of 30% in the second season. Nguzo had the lowest disease severity of 16% and 5% in season 1 and 2 respectively. Jin *et al.* (1994) similarly noted an interaction between host genotypes and rust pathotypes which they attributed to variation in environmental temperature. Fox and Harder (1995) noted that terminal disease severities were a reliable indicator of resistance to stem rust in wheat and barley. Barley germplasm that showed the highest final disease percent were considered very susceptible.

Table 2: Seedling infection type and adult plant reaction of barley to PgtUg99 in the greenhouse

Genotypes code/ Name	Selection History	Seedling infection type[a]	Adult plant reaction[b]
ICARDA- 01	CBSS99MOO39IT-H-IM-IY-IM-IY-OM	;	MR
ICARDA- 02	CBSS99MOO317T-AH-2M-IY-IM-IY-OM	0	S
ICARDA- 03	CBSS00YOO113T-A-OY-OM-2Y-OM	0;	S
ICARDA- 04	CBSS99MOO429T-L-IM-IY-OM	0	S-MS
ICARDA- 05	CBSW99WMOOO95T-B-IM-IY-IM-OM	1	S
ICARDA- 06	CBSS00YOOO48S-OY-OM-2Y-OM	1	MS
ICARDA -07	CBSSOOYOO475T-O-OY-OM-2Y-OM	1	MS-S
ICARDA- 08	CBSSOOYOO236T-E-0Y-OM-2Y-OM	1	S
ICARDA- 09	CBSS99MOO349T-F-3M-IY-IM-OM	3	S
ICARDA- 10	CBSS99MOO468T-H-IM-IY-OM	0	S-MS
ICARDA -11	CBSSOOYOO479T-D-OY-OM-IM-OM	; 1	S
ICARDA -12	CBSW98WOOO54S-BY-2M-IY-2M-IY-OM	4, 3	S
ICARDA- 13	CBSS99MOO315T-F-IM-F-IM-IY-IM-IY-OM	; 0	S
ICARDA- 14	CBSSOOYOO225T-C-OY-OM-2Y-IM-OM	2	MR
ICARDA- 15	CBSSOOYOO278D-G-OY-OM-2Y-OM	2	S
1385-13	-	1	S
NGUZO	-	0	R-MR
1512-5	-	0, 2	S
KARNE	-	2	MS-MR
SABINI	-	1	S

[a] *Seedling infection type as described by Stakman et al. (1962). 0 – Immune, ; - Very resistant, 1- Resistant, 2- Moderately resistant, 3 - Moderately susceptible, 4 – Susceptible.*

[b] *Adult plant reaction based on modified cobb scale (Roelfs et al.,1992): R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.*

Table 3: Adult plant response to infection and disease severity of barley germplasm to PgtUg99 in the field

Genotypes code/ Name	Selection History	Season 1	Season 2	Mean%
ICARDA- 01	CBSS99MOO39IT-H-IM-IY-IM-IY-OM	23[*]MR[**]	7MR	15
ICARDA- 02	CBSS99MOO317T-AH-2M-IY-IM-IY-OM	28S	6S	17
ICARDA- 03	CBSS00YOO113T-A-OY-OM-2Y-OM	56S	18S	37
ICARDA- 04	CBSS99MOO429T-L-IM-IY-OM	46S-MS	13MS	30
ICARDA- 05	CBSW99WMOOO95T-B-IM-IY-IM-OM	41S	13S	27
ICARDA- 06	CBSS00YOOO48S-OY-OM-2Y-OM	23MS	9MS	16
ICARDA -07	CBSSOOYOO475T-O-OY-OM-2Y-OM	40MS-S	16MS	28
ICARDA- 08	CBSSOOYOO236T-E-0Y-OM-2Y-OM	45S	16MS	31
ICARDA- 09	CBSS99MOO349T-F-3M-IY-IM-OM	53S	15S	34
ICARDA- 10	CBSS99MOO468T-H-IM-IY-OM	46MS	15MS	31
ICARDA -11	CBSSOOYOO479T-D-OY-OM-IM-OM	73S	13S	43
ICARDA -12	CBSW98WOOO54S-BY-2M-IY-2M-IY-OM	50S	15S	33
ICARDA- 13	CBSS99MOO315T-F-IM-F-IM-IY-IM-IY-OM	63S	21S	42
ICARDA- 14	CBSSOOYOO225T-C-OY-OM-2Y-IM-OM	20MS	6MS	13
ICARDA- 15	CBSSOOYOO278D-G-OY-OM-2Y-OM	36S	16S	26
1385-13	-	86S	16S	51
NGUZO	-	16R-MR	5MR	11
1512-5	-	93S	21S	57
KARNE	-	46R-MR	13MR	30
SABINI	-	63S	3OS	47

[*] *Disease severity scale from 0 to 100%, as based on Peterson et al. (1948)*
[**] *Infection type responses based on Roelfs et al. (1992), McIntosh et al. (1995)*

Conclusion

The study has demonstrated the pathogenicity of PgtUg99 to barley despite the fact that it poses a great threat to wheat production in the world. Most of the genotypes were resistant at seedling stage but were susceptible at the adult plant stage.The information in this study is beneficial to both researchers, barley and wheat producers in confronting a pathogen that is devastating with feasible chemical control though expensive and unfriendly to the environment. More sources for resistance should be sought for to be incorporated in the Kenyan germplasm.

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