Asian Journal of

Plant Pathology

ISSN 1819-1541

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Plant Pathology 6 (2): 25-32, 2012
ISSN 1819-1541 / DOI: 10.3923/ajppaj.2012.25.32

Wheat Stem Rust Severity and Physiological Races in North Rift Region of Kenya

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ABSTRACT
Wheat stem rust severity was determined using stratified simple random sampling technique and physiological races of *Puccinia graminis* f.sp. *tritici* were determined using the 20 North American wheat stem rust differentials. Results revealed a significant variation in wheat stem rust severity in the region. Lower wheat stem rust severity of 12.5% was observed in high altitude areas of Kipkabus having more than 2200 m above sea level. High wheat stem rust severity of 34 and 32% was observed in Mois Bridge and Moiben, respectively. These areas had lower altitude of less than 1900 mean sea level. Four wheat stem rust races were reported in this region. The predominant race was TTKST (*Ug99+Sr24*) at 66.7% predominance, followed by race TTKSK (*Ug99*) at 21.1%, race PTKST (*Sr31+Sr24-Sr21*) at 10.5% and race TTTSK (*Ug99+Sr36*) at 1.8%. A new race PTKST was reported at Chepkoilel, Moiben and Mois Bridge. There was no significant correlation (r = 0.398) of stem rust severity and race diversity. However, there was a significant negative correlation (r = -0.461) of race diversity and altitude and a highly significant negative correlation (r = -0.713) of altitude and stem rust severity. The study concluded that wheat stem rust severity varied depending on altitude and location, and four races are present in North Rift region of Kenya, where the predominant race is TTKST (*Ug99+Sr24*).

Key words: Differentials, *Puccinia graminis* f.sp. *tritici*, race, stem rust, wheat

INTRODUCTION
Wheat is among the grain cereals which contribute significantly to food security in Kenya and ranks second after maize. Stem rust caused by *Puccinia graminis* f.sp. *tritici* is at present one of the major threats to wheat production, leading to heavy yield losses in Kenya (Njau *et al.*, 2009). East Africa is known to be hot-spot for origin of new virulent races of rust (Singh *et al.*, 2006). Occurrence of race Ug99 which is virulent to *Sr 31* (Pretorius *et al.*, 2000) and the new mutants which are virulent to *Sr 24* (Jin *et al.*, 2008) and *Sr 36* genes (Jin *et al.*, 2009) in Kenya has made all local commercial wheat varieties susceptible. Studies on wheat stem rust race distribution in East Africa are limited. With the current mutants of race Ug99 being reported, breeders were not sure how this races are distributed in East Africa. There is continued fear that race Ug99 is still mutating as previously resistant materials are increasingly losing their resistance (Pretorius *et al.*, 2010), thus the need for continued race surveys and screening the previously resistant materials against all the races reported. The use of effective resistance has for long been the most economical

way for control of wheat stem rust. In order to effectively utilize stem rust resistance in the improvement of wheat, it is necessary to monitor the virulence composition and dynamics in the stem rust pathogen population. The dynamic nature of stem rust pathogen provides a continuous threat to released wheat varieties where some are rescinded soon after (Park, 2008). Some new races are known to cause severe losses within a short time as it happened with race TTKST which was detected in 2006 and caused major losses in 2007 in Kenya. Others take some time to build enough inoculum to cause an epidemic like race Ug99 which is thought to have been present in Kenya since 1993, but it was not until 2004-2005 that farmers experienced epidemics. Although race Ug99 is known to be present in East Africa (Singh *et al.,* 2006), the extent of its distribution and its frequency of occurrence is not well understood, more so with the recent detection of race Ug99 mutants. Timely identification of a race can assist the breeders to develop a resistant variety in time before an epidemic. The information on pathogenicity will enable pathologists and breeders to utilize the most effective resistance genes in their breeding programmes. A study was therefore carried out to determine wheat stem rust severity and physiological races in the North Rift region of Kenya.

MATERIALS AND METHODS

Determination of wheat stem rust severity and distribution: Survey was carried out during May-Nov 2010 when the wheat crop was at flowering-soft dough stage (Zadoks *et al.,* 1974). Stratified simple random sampling technique (Deng *et al.,* 2009) was used, samples were collected from five zones in North Rift region where wheat is grown most and were considered to be homogenous sampling blocks i.e., Northern strata (Moiben), Southern strata (Mosoriot),Western strata (Kipkabus), Central strata (Chepkoilel/Sergoit) and Eastern strata (Mois Bridge). A total of 360 km was covered and the procedure described by Park (2008) for wheat rust sampling was followed. Wheat stem rust severity was determined by walking into the field beyond the borders and then examining approximately 33 m across the field as described by Roelfs *et al.* (1992). Stem rust severity was scored using the modified cobs scale of 0-100%, where 0 = no rust infection and 100% = complete cover by rust uredia both on leaves and stem (Peterson *et al.,* 1948). A sample was taken after every 20 km (Jin, 2005), and the number of sampling sites within a strata depended on the area covered by wheat within that strata. Once a sampling site was identified the geographic data viz., latitude, longitude and altitude were recorded using a GPS (e Trex Legend GPS System, Garmin).

Determination of wheat stem rust races: During the survey a total of fifty samples (5 cm stems of wheat that had susceptible pustules) were collected by cutting them using a sterilized pair of scissors. A cooling box was used to preserve the samples while in transit to the laboratory. Later the samples were air dried for 12 hours before storing them in a refrigerator at 3-5°C (Greens, 1981) awaiting inoculation. Twenty Northern America wheat differential hosts (Sr5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17(+13), 9a, 9d, 10, Tmp, 24, 31, 38 and McN.) were used to characterize the isolates of *Puccinia graminis* f.sp. *tritici* (Jin *et al.,* 2008).

Cultivar *Morocco* (a universal susceptible genotype to stem rust) was planted for spore isolation and subsequent spore multiplication. Three seeds of cultivar Morocco were planted in a six inch diameter pot in the greenhouse and maintained at 26-30°C (McIntosh *et al.,* 1995). Seven days after planting, the seedlings were inoculated with spores collected from each site. Inoculum was prepared by mixing distilled water and oil tween-20 at one drop/500 mL of distilled water and then suspending 2 mg of urediniospores/1 mL of the mixture (Welty and Barker, 1992). The suspension was sprayed using a hand operated sprayer onto 7-day-old seedlings of the Morocco lines. Each set of three Morocco plants received 100 mL of the inoculum which was sprayed to cover the whole plant ensuring both top and bottom of the leaves are well covered. Seedlings were incubated in a dew chamber for 14 h at 18°C in the dark and then in a greenhouse maintained at 18±2°C for 14 days. Fourteen days after inoculation pustules were separated according to their sizes/virulence pattern of large, small, round and ragged. Three pustules were derived from each site/field (Jin, 2005). This was repeated for the four sites/fields from each zone. From the five zones a total of 60 pustules were bulked separately on cultivar Morocco which were later tested on the set of twenty wheat stem rust differentials for race identification. The twenty differential hosts were seeded in plastic pots filled with a mixture of sterile silt clay, sand and forest soil. Each pot contained four differential genotypes in the following order (i) *Sr5, 21, 9e, 7b*; (ii) *Sr11, 6, 8a, 9g*; (iii) Sr*36, 9b, 30, 17*; (iv) *Sr 9a, 9d, 10, Tmp;* (v) *Sr24, 31, 38* and *McN* (Jin *et al.*, 2008). The bulked single-pustule isolate derived from each collection was inoculated on the differential seedlings (Welty and Barker, 1992). The seedlings were incubated in a dew chamber for 14 h at 18°C in the dark, and then in a greenhouse maintained at 18±2°C for 14 days. Infection types were scored (Stakman *et al.*, 1962) on designated *Sr* genes, 14 days after inoculation using 0-4 Infection Type (IT) scale (McIntosh *et al.*, 1995) when the uredia on the susceptible variety Morocco, which acted as a control appeared fully developed. A susceptible host response characterized by large uredia without chlorosis or necrosis was denoted by 4, whereas no visible uredia, flecking and small uredia with necrosis was denoted by 0 and 1, respectively. Small to medium sized uredia with green islands and surrounded by necrosis or chlorosis and medium sized uredia with or without chlorosis was indicated by 2 and 3, respectively. Each isolate was assigned a five-letter race name based on its reaction on the differential lines. The experiment was repeated twice and only differential hosts that produced similar infection types in the two experiments were considered. For infection type 0 (immune reaction), the test was repeated to exclude the possibility of disease escape.

Statistical analysis: Wheat stem rust severity from the survey was analyzed using descriptive statistics (frequencies and percentages) and direct correlation using statistical package for social scientists (SPSS 17) computer software. Correlation analysis between wheat stem rust races, severity and altitude was performed using GenStat 12th edition statistical package.

RESULTS AND DISCUSSION

Wheat stem rust severity and distribution: The general visual disease severity scores on locally grown wheat crop, made from the five zones are given in Table 1. Stem rust disease severity of 33 and 34% on locally grown wheat was observed to be significantly ($p<0.05$) higher in Moiben and Mois' Bridge area, respectively. Mois' Bridge reported the highest stem rust severity of 34%, with an altitude range of between 1714-1946 m above sea level (m.a.s.l.). The highly significant negative correlation ($r = -0.461$) of stem rust severity and altitude, indicates that the high stem rust severity recorded at this zone could be attributed to the zone having the lowest altitudes. The low altitude offers high temperatures and during the cropping season there is high humidity which provides more conducive conditions for infection, spread and growth of wheat stem rust (Roelfs *et al.*, 1992).

Moiben area recorded a mean stem rust severity of 33% which was found to be not significantly different from that of Mois' Bridge. Moiben is located at slightly higher altitude range of between 1944-2150 m.a.s.l. The region is associated with very large fields of monocropping (wheat and

Table 1: Wheat stem rust severity in Uasin Gishu region at different sites during cropping season (May-Nov. 2010)

Zone	Mean stem rust severity (%)	Standard error
Chepkoilel	23.33	3.333
Mois Bridge	34.00	2.449
Moiben	32.50	2.500
Kipkabus	12.50	2.500
Mosoriot	13.33	3.333
Total	24.21	2.456

Means whose SE overlaps are not significantly different at 0.05 level of significance

barley) and the proximity of this zone to Mois' Bridge, which had the highest stem rust severity, could explain why this region too had a high stem rust severity. Chepkoilel/Sergoit recorded a mean stem rust severity of 23% which was found to be significantly lower than that recorded at both Mois' Bridge and Moiben zones but significantly higher than that recorded at both Kipkabus and Mosoriot. With an altitude range of between 2144-2147 m.a.s.l, high stem rust severity would have been expected as that reported at Moiben. However a lower severity of about 23% was recorded, which was mainly attributed to chemical control (Tebuconazole 250 g L^{-1} at 1 l ha^{-1} applied twice during the crops cycle) which had been used at two of the three farms surveyed (Rop, Pers comm.). Both Kipkabus and Mosoriot area recorded significantly low stem rust severity than all the other zones. Kipkabus area which is located at altitudes of between 2595-2718 m.a.s.l recorded the least mean stem rust severity of 13%, with a highly significant negative correlation between altitude and stem rust severity. The high altitude of this area can perhaps explain why low stem rust severity was recorded. More so most farmers in this area are small scale practicing mixed cropping and this could also contribute to the low stem rust severity reported. Although Mosoriot area is located at altitudes favorable for rust epidemics i.e., 1996-2134 m.a.s.l, a low severity of 13% was recorded. The area is geographically isolated to the other four wheat growing zones, more so most farmers in this area were small scale and mixed cropping was common. This could perhaps explain why the area recorded a low stem rust severity.

Wheat stem rust races: Stem rust races identified from the 57 isolates derived from the samples collected from all the five zones of North Rift region are given in Table 2. Chepkoilel recorded the highest race diversity, with the nine pustules isolated yielding four races; of which two yielded race TTKSK; four were of race TTKST; one of TTTSK and two of PTKST. The high race diversity including the new race PTKST is usual, since two of the three fields where samples were taken are mostly used for research purposes and thus different wheat materials/varieties are planted and this could be the reason why there was high race diversity. Similarly, previous studies have shown that the type of race identified in a place greatly depends on the cultivars planted (Admassu *et al.*, 2009). The gene for gene theory dictates that for a virulent race to be present, there must be a resistant gene against that pathogen (Flor, 1971). At Mois' Bridge, out of the 12 pustules tested, three stem rust races were identified. These were one pustule of TTKSK; nine of TTKST and two of PTKST. Race TTTSK was not identified in this zone.

The predominance of race TTKST in this zone i.e. nine pustules out of 12 tested, can perhaps be attributed to the commonly grown wheat variety in this zone, the variety KS Mwamba which is known to carry *Sr 24*, which this race is virulent to Njau *et al.* (2009). The new race PTKST was also detected in this zone and since this race is virulent to *Sr 24* gene too, perhaps explaining its presence. At Moiben, out of the 15 pustules tested, three races were identified. These were two of

Table 2: Wheat stem rust races in Uasin Gishu region at different sites during cropping season (May-Nov. 2010)

				Number of isolates of *Pgt* races			
Site	Source	Collection	Isolate	TTKSK	TTKST	TTTSK	PTKST
Chepkoilel/Sergoit	Nursery and Field	3	9	2	4	1	2
Mois bridge	Field	4	12	1	9	0	2
Moiben	Field	5	15	2	11	0	2
Kipkabus	Field	4	12	4	8	0	0
Mosoriot	Field	3	9	3	6	0	0
Total		19	57	12	33	1	6
Total (%)				21.1	66.7	1.8	10.5

Table 3: Correlation relatiouship between race diversity, stem rust severity and altitude

	Race diversity	Stem rust severity	Altitude
Race diversity	1	0.398	-0.461*
Stem rust severity		1	-0.713**
Altitude			1

*,**Correlation is significant at the 0.05 and 0.01 level, respectively

TTKSK; 11 of TTKST and two of PTKST. Race TTTSK was not identified in this zone and race TTKST was the most predominant in this zone i.e., 11 of the 15 pustules tested. Similarly the new race PTKST was also reported in this zone. At Kipkabus, of the 12 pustules tested only two races were identified. These were four of TTKSK and eight of TTKST. In this zone the new race PTKST as well as race TTTSK was not identified. Table 3 shows that there was a significant negative correlation (r = -0.713) on number of races identified with altitude, thus the two races identified at this site could be attributed to the area being located at high altitudes. At Mosoriot, of the nine pustules tested only two races were identified. These were three of TTKSK and six of TTKST. Just like Kipkabus, this zone did not report the new race PTKST and also race TTTSK. Although the zone is located at slightly lower altitude where stem rust races diversity is expected, the low stem rust severity of 13.33% reported could be the reason why there were fewer races. Similarly the zone is geographically isolated from the other four, perhaps explaining the low race diversity. It should be noted that race TTTKS was only identified at Chepkoilel site whereas the new race PTKST, was reported at three sites i.e., Chepkoilel, Mois' Bridge and Moiben. Perhaps the proximity of these areas to each other, with no host barrier can explain this. Of the four races identified in Uasin Gishu region race TTKST had 70% predominance. The widespread cultivation of variety *KS Mwamba* which is known to carry *Sr* 24 (Njau *et al.*, 2009) can explain perhaps why race TTKST is predominant in this region. This was followed by race TTKSK (15%), PTKST (11.5%) and TTTKS (3.3%).

This study confirmed the widespread distribution of wheat stem rust in Uasin Gishu region. Wanyera *et al.* (2006) reported race Ug99 all over Kenya. However from the present study, there appear to be change in virulence dynamics within *Pgt* populations. A mutant of race Ug99 (TTKST) which was detected at Njoro in 2008 (Jin *et al.*, 2008) appears to be the most predominant race in this region. The change in virulence dynamics over time is not new as this has been reported in other places like USA (Jin, 2005) and Ethiopia (Admassu *et al.*, 2009). From the survey, it was confirmed that most farmers in this area had planted variety KS Mwamba and since this variety is known to carry *Sr 24* gene, the predominance of race TTKST (*Ug99+Sr 24*) is possible. Although it took many years for race Ug99 to cause epidemics on farmers' fields from when it was reported in Uganda (Pretorius *et al.*, 2000) up to 2005 when epidemics were reported Wanyera *et al.* (2006), this race appear to be even more virulent as it was only reported in 2007 at KARI Njoro Kenya (Jin *et al.*, 2008) and is currently causing epidemics in Uasin Gishu region.

Identification of a new race PTKST with a predominance percentage of 10.5% is significant. Considering this race is also virulent to *Sr 24* gene which is present in KS Mwamba and its widespread cultivation in this area, perhaps it is only a matter of time before this race becomes widespread in this region. Whether this race is a mutant from the existing races or an introduction currently remains unknown. However, its detection at Moiben, Mois Bridge and Chepkoilel, where high stem rust severity was reported and the three areas being in close proximity to one another and its absence in higher altitudes of Kipkabus and isolated areas of Mosoriot, could be an indication that it could be an introduction either through Chepkoilel research fields or a mutation caused by high inoculum pressure as suggested by Singh *et al.* (2006) and Jin *et al.* (2008). Latest studies have also confirmed race PTKST in South Africa (Pretorius *et al.*, 2010) where it is claimed to have been introduced from unknown sources. Though the original Ug99 race is virulent, recent studies by Pretorius *et al.* (2010) have pointed out the importance of the mutants of this race, which are being predicted to even be more virulent on the previously resistant materials.

Correlation of race diversity, stem rust severity and altitude: Correlation was performed using GenStat statistical package (version 4) and the data (Table 3) revealed that, there was no significant correlation between race diversity and stem rust severity, which implies that the number of wheat stem rust races identified in a place is not related to wheat stem rust severity. Therefore, it is possible to have a few races with a very high rust severity and vice versa. Similar studies as reported by Wanyera *et al.* (2006) showed race Ug99 (TTKS) alone in farmers' fields caused up to 70% severity in Kenya. Similarly Admassu *et al.* (2009) in Ethiopia reported up to 12 races with a severity of 27% only at Shewa area, while Jin (2005) reported five races from fields with trace amounts (<10%) of wheat stem rust severity in U.S.A. There was a significant negative correlation of $r = -0.461$ on race diversity with altitude. This implies that the number of races is likely to increase as altitude reduces. However it is important to note that altitude alone might not be the cause as previous work has shown that race diversity is also dependent on varieties planted and cultural practices employed as well as the environmental conditions (Singh *et al.*, 1991; Roelfs *et al.*, 1992). Perhaps the low altitude favors the environmental conditions necessary for evolution and survival of wheat stem rust races. There was a highly significant negative correlation of $r = -0.713$ on wheat stem rust severity and altitude. This implies that stem rust is more likely to be severe in low altitude areas than in higher altitude areas. Low altitude favors high temperatures and this conforms to report by Roelfs *et al.* (1992) who showed that an optimal temperature of 30°C and high light are necessary for growth and sporulation of wheat stem rust. The study therefore concluded that wheat stem rust is severe and widespread in North Rift region of Kenya. The predominant race in this region is race TTKST and a new race PTKST was also reported. These two races are virulent to *Sr 24* gene, which is present in the commonly grown wheat variety in this region.

The study therefore recommends that, similar studies be carried out in other major wheat growing areas of East Africa.

ACKNOWLEDGMENTS

We wish to acknowledge the International Atomic Energy Agency (IAEA) project INT/5/150 for funding. Staffs of IAEA project INT/5/150 for regular visits to trial sites. We also thank the staffs of Chepkoilel University College and Egerton University for providing trial sites and materials.

REFERENCES

Admassu, B., V. Lind, W. Friedt and F. Ordon, 2009. Virulence analysis of *Puccinia graminis* f.sp. tritici populations in Ethiopia with special consideration of Ug99. Plant Pathol., 58: 362-369.

Deng, A.L., J.O. Ogendo, G. Owuor, P.K. Bett, O.E. Omolo, M. Mugisha-Kamatenesi and J.M. Mihale, 2009. Factors determining the use of botanical insect pest control methods by small holder farmers in the lake Victoria basin, Kenya. Afr. J. Environ. Sci. Technol., 30: 231-235.

Flor, H.H., 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol., 9: 275-296.

Greens, G.J., 1981. Identification of physiological races of *Puccinia graminis* f. sp. *tritici* in Canada. Canadian J. Plant Pathol., 3: 33-39.

Jin, Y., 2005. Races of *Puccinia graminis* identified in the United States during 2003. Plant Dis., 89: 1121-1124.

Jin, Y., L.J. Szabo, Z.A. Pretorius, R.P. Singh, R.W. Ward and T.J. Fetch, 2008. Detection of virulence to resistance gene *Sr24* within race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Dis., 92: 923-926.

Jin, Y., L.J. Szabo, M.N. Rouse, T. Fetch Jr., Z.A. Pretorius, R. Wanyera and P. Njau, 2009. Detection of Virulence to Resistance Gene Sr36 Within the TTKS Race Lineage of *Puccinia graminis* f. sp. tritici. Plant Dis., 93: 367-370.

McIntosh, R.A., C.R. Wellings and R.F. Park, 1995. Wheat Rusts: An Atlas of Resistance Genes. CSIRO Publications, Australia.

Njau, P.N., R. Wanyera, G.K. Macharia, J. Macharia, R. Singh and B. Keller, 2009. Resistance in Kenyan bread wheat to recent eastern African isolate of stem rust, *Puccinia graminis* f. sp. *tritici*, Ug99. Plant Breed. Crop Sci., 1: 22-27.

Park, R.F., 2008. Breeding cereals for rust resistance in Australia. Plant Pathol., 57: 591-602.

Peterson, R.F., A.B. Campbell and A.E. Hannah, 1948. A diagrammatic scale for estimating rust intensity of leaves and stems of cereals. Can. J. Res., 26c: 496-500.

Pretorius, Z.A., R.P. Singh, W. Wagoire and T. Payne, 2000. Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Dis., 84: 203.2-203.2.

Pretorius, Z.A., C.M. Bender, B. Visser and T. Terefe, 2010. First report of a *Puccinia graminis* f. sp. *tritici* race virulent to the *Sr24* and *Sr31* wheat stem rust resistance genes in South Africa. Plant Dis., 94: 784-785.

Roelfs, A.P., R.P. Singh and E.E. Sari, 1992. Rust Diseases of Wheat: Concepts and Methods of Disease Management. CIMMIYT, Mexico, DF., ISBN: 968-6127-70-4, pp: 81.

Singh, R.P., T.S. Payne, P. Figueroa and S. Valenzuela, 1991. Comparison of the effect of leaf rust on the grain yield of resistant, partially resistant and susceptible spring wheat cultivars. Am. J. Alternat. Agric., 6: 115-121.

Singh, R.P., D.P. Hodson, Y. Jin, J. Huerta-Espino and M. Kinyua *et al*., 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Rev. Perspect. Agric. Vet. Sci. Natural Resour., 54: 1-13.

Stakman, E.C., D.M. Stewart and W. Loegering, 1962. Identification of Physiologic Races of *Puccinia graminis* var *tritici*. US Department of Agric. Agricultural Research Service, USA., Pages: 53.

Wanyera, R., M.G. Kinyua, Y. Jin and R.P. Singh, 2006. The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in Eastern Africa. Plant Dis., 90: 113-113.

Welty, R.E. and R.E. Barker, 1992. Latent period responses to stem rust in tall sescue incubated at 4 temperatures. Crop Sci., 32: 589-592.

Zadoks, J.C., T.T. Chang and C.F. Konzak, 1974. A decimal code for growth stages of cereals. Weed Res., 14: 415-421.