The Occurrence and Pathological Characterization of Rust Causing Fungi of Brachiaria Grass in Kenya

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Abstract

Brachiaria grass is one of the most important grasses distributed throughout the tropics especially in Africa. The quality of forage yields and biomass of brachiaria is negatively affected by diseases among which leaf spot, leaf blight and leaf rust are the most common. This research was conducted to characterize the rust pathogen of brachiaria grass infected selected genotypes in Kenya based on pathological and morphological characteristics. Leaves with leaf rust pustules were collected from improved cultivars and natural population from brachiaria production and demonstration plots at the International Livestock Research Institute, Nairobi, Kenya. Pathogenicity of rust isolates was determined on the brachiaria cultivars in glasshouse. The growth conditions of rust pustules were determined by exposing into different regimes of relative humidity, temperature and time to germ tube development were established under rust ideal environmental conditions. The cultivars tested (MG4, Piata, Xareas and Marandu) were susceptible to rust diseases in the field as well as in controlled environment. In the field the percent disease incidence of 100% was recorded while disease severity was higher in MG4 (7.5 and 8.5) in 2016 and 2017, respectively in a disease severity scale of 0 to 9. The disease was least severe in Piata (2.5) in 2016 and in Marandu (3.0) in 2017. Under artificial inoculation and controlled growth conditions MG4 proved more susceptible (6.5) while, Piata (1.7) was less severe in glasshouse. However, under growth chamber Xareas was most affected with a disease score of 6.5. The isolate from MG4 was more virulent on MG4 than isolates obtained from other bracharia cultivars followed by isolates from Xareas , which caused disease severity of (3.5) on MG4. When the isolates were subjected to different environmental conditions it was established that optimum relative humidity of 75 % and temperature of between 20-25 ^0C was ideal for germ tube germination. Germ tube germination started after the 8^{th} hour but stopped extending at the 24^{th} hour. Morphologically the isolates varied in spore size from 20µm to79µm and in spore shape which were oval or globose, but all were yellow in colour. These findings indicated high occurrence of leaf rust disease in bracharia cultivars with varying tolerance levels which can provide a potential source of resistance genes. Further it is advisable to institute control measures against leaf rust whenever relative humidity is above 75% and temperature of 20-25 ^0C prevails.

Keywords: Brachiaria, Leaf rust, characterization, disease severity, growth conditions

INTRODUCTION

Brachiaria grass is one of the most important tropical grasses distributed throughout the tropics especially in Africa (Renvoize *et al*., 1996). It has high biomass production potential and produces nutritious herbage thus increase livestock productivity (Holmann *et al*., 2004). Brachiaria is adapted to drought and low fertility soils, sequesters carbon through its large

roots system, enhance nitrogen use efficiency and subsequently minimize eutrophication and greenhouse gas emissions (Subbarao *et al*., 2009; Rao *et al*., 2014). Brachiaria plays important roles in soil erosion control and ecological restoration. Some of these varieties have shown susceptibility to pests and diseases thus limiting future expansion of the brachiaria grass in Africa (Ghimire *et al*., 2015). There is therefore a need for an Africa based brachiaria improvement program to develop varieties that are tolerant to biotic and abiotic stresses for different production environments (Ondadu *et al*., 2016). The prerequisite for any crop improvement program is a germplasm with a broad genetic base. The best approach to increase genetic variations in apomictic species like Brachiaria is by tapping natural variations from the centre of diversity (Keller-Grein *et al.,* 1996). Little is known about brachiaria rust. Since identification of rusts based on morphology can be ambiguous, molecular tools should be incorporated to confirm host- and morphology-based identifications (Pizarro *et al.,* 2013). Accurate identifications of species, timely detection, and a more accurate reporting of incidences of rust can be incorporated into a broader integrated pest management program for Brachiaria grass. Resistance in certain cultivars offers an efficient approach to control of several of the more serious fungal, bacterial, and viral-caused diseases (Miles *et al.,* 2004; Rao *et al.,* 2011). Maintaining resistance in a cultivar may require continuous effort because disease resistance often loses effectiveness (Peters *et al.,* 2012). Rust pathogen can inflict substantial foliar damage on susceptible genotypes of an extremely wide range of plant species (Baker, *et al.,* 1970), including brachiaria and various tropical and subtropical crops (Yang *et al.,* 1990). The fungus can survive for a long time in the soil or in infected plant debris as sclerotic, which are first seen as white masses on infected tissues. Little is known about bracharia rust (Nzioki *et al*., 2016). These would facilitate the development of Brachiaria rust management practices that can be incorporated into a broader integrated pest management program for Brachiaria grass. Thus, the objective of this research was to study the phylogeny diversity of Bracharia rust from urediniospores collected from four different genotypes from MG4, Marandu, Piata and Xaraes in Kenya. This generates information and resources that will form part of the disease management plan that result in sustainable. Management of Brachiaria grass diseases especially in smallholder cropping systems in Kenya and also to identify pathogens associated with rust diseases of Brachiaria using molecular technique and confirm the pathogenicity of these fungi on different Brachiaria species

METHODOLOGY

Study Area

Rust infected leaves were obtained from Biosciences Eastern and Central Africa-International Livestock Research Institute hub, (BecA ILRI Hub). All the experiments were conducted in the laboratory and glass-house (*in-vitro*) of Department of Plant Pathology, Biosciences Eastern and Central Africa- International Livestock Research Institute hub, (BecA ILRI Hub) Nairobi.

Collection of rust urediniospores

Fresh urediniospores were collected using artist airbrush from Biosciences Eastern and Central Africa-International Livestock Research Institute hub, (BecA ILRI Hub) trials field, using artist airbrush. Samples were obtained from varieties of Brachiaria Piata, MG4 Marandu and Xereas, which were more affected with leaf rust from the year 2016 and 2017. The collected urediniospores were taken to the Biosciences Eastern and Central Africa-International Livestock Research Institute hub laboratory and were maintained at -20^0C for

until analysis. The samples were taken from naturally occurring rust infections. The disease severity on cultivars were visually rated on a scale of 1 to 9, where 1 represented the least disease and 9 the most disease on leaf area exhibiting signs of foliar infection, (Cobby scale ,1995).

Selection of Inocula

Four rust susceptible Brachiaria cultivars, MG4, Piata, Marandu and Xereas having mean disease severity of 4.0, 6.4, 4.5, and 5.8 respectively, were selected for use in greenhouse-based inoculation trials. The Brachiaria cultivars were seeded (3 seed per pot) in a 10-cm diameter pots filled with sterile soil and in another trial a post germination plant was also placed in 10-cm diameter pots. No fungicides or fertilizers were applied. Before inoculation experiments, urediniospores were removed from -20^0 C storage and were allowed to acclimate to room temperature for 30min to one hour. Urediniospore germination was tested and observed using microscope. Observations of the germ tube were done using Xereas isolate from the field collected in 2017, as it showed sufficient germination.

Pathogenicity test

The pathogenicity test was performed under glasshouse and growth chamber with seedlings germinated from glasshouse using split cuttings of brachiaria. The same isolates of rust spores collected from fields were used for artificial inoculation under growth chamber conditions and glasshouse for comparison.

Inoculation of Brachiaria

All brachiaria cultivars were inoculated separately with one isolate that was collected from MG4 2015, Piata 2016 and Xereas cultivar 2017. The inoculum was of approximately 0.10g of urediniospores. Three collection methods were used: spraying, brushing and taping. Tapping was done by applying adhesive tape segments (2 × 5 cm) bearing leaf rust spores to the central part of the leaves. The tape segments were previously 'inoculated' in a settling tower with 6 mg of a mixture (1:6) of spores and talc. Immediately after spore deposition, the tape segments were transported to the field and placed on the leaves. The thin air layer between the tape and the leaf surface rapidly became saturated with humidity, ensuring proper conditions for infection. The tapes were put into place after 17:00 h and covered with polythene bags for 72 hours in a glasshouse, as show in Plate 4. The plants were subsequently placed in the growth chamber maintained at 80% relative humidity $27/22^0$ C day/night temperature and 16h photoperiod. Plants previously inoculated with rust spores were kept in the metallic bench top in the greenhouse until pustules developed. All plants were watered at the base to avoid wetting the inoculated foliage. Inoculated plants were monitored until rust pustules developed.

Morphological Characterization of rust fungus

Morphological evaluation to measure urediniospore size, shape, colour, and the number of germ pores were conducted using the protocol of Cummins *et al.,* (1971) as a guide. Germ pores were stained using glycerine and visualized using the microscope (Optika microscope Italy model IM-3FL/3FL4).

Morphological variations in causal agent

Urediniospores collected from different cultivars were used to determine their size by measuring the length, width and shape under compound microscope using an ocular micrometer. The spores were measured using the digital image and adjusted using Student's

t-test. Using the statistical analysis the samples were put in different groups. Data on incidence of rust in brachiaria grass was analysed by (ANOVA) procedure using the GenStat computer Software 14th Edition, release 14.10.5943, 2013 (VSN International Ltd). Means separation was by Fishers unprotected least significant difference (LSD) at 0.05.

RESULTS

The incidence and severity of rust on brachiaria in the field during the two seasons

The incidence and severity of rust on brachiaria in the field are shown in Table 1. The findings showed that Brachiaria genotypes MG4, Piata, Xareas and Marandu were infected by rust disease in the field with a disease incidence of 100%, while disease severity ranged between 2.5 and 7.5. Results further indicated that MG4 had significant highest susceptibility with severity of 7.5 and 8.5 for leaf rust during 2016 and 2017 respectively. Xareas and Marandu were moderately infected with the disease severities of 5.6; 4.6 and 4.0; 3.0 in 2016 and 2017 respectively. However, Piata recorded significantly lowest severity (2.5 and 3.5) during both seasons.

Table 1: Incidence and Severity of rust on Bracharia grass in the field during 2016 and 2017

	Season One (2016)		Season Two (2017)	
Genotype	Severity	Incidence (%)	Severity	Incidence (%)
Mg4	7.5±0.23d	100	8.5±0.19d	100
Piata	2.5±0.41a	100	3.5±0.37a	100
Xareas	5.6±0.12c	100	4.6±0.17c	100
Marandu	4.0±0.37b	100	3.0±0.21b	100
LSD	4.9± 0.24	100	4.9± 0.24	100

Means followed by different letters within a column are significantly different at p<0.001

Severity and pathological susceptibility of brachiaria genotypes in the glasshouse

Comparison of the disease severity among genotypes under controlled environment was determined between the two seasons. Results indicated that upon inoculation of brachiaria genotypes the severity varied among the genotypes. Higher occurrence was recorded in 2016 than 2017. During the first season of the study (2016), disease severity was highest in MG4 (6.5), followed by Marandu (5.5), Xareas (4.0) and Piata (3.5) (Figure 1). Similarly, during the second season, disease severity of 5.5 was reported in MG4, followed by Marandu (5), Xareas (3.5) with Piata recording less severity of 1.5 (Figure 1). However it was noted that during inoculation in 2017 the disease severity was lower for all the brachiaria genotypes as compared with severity levels of 2016.

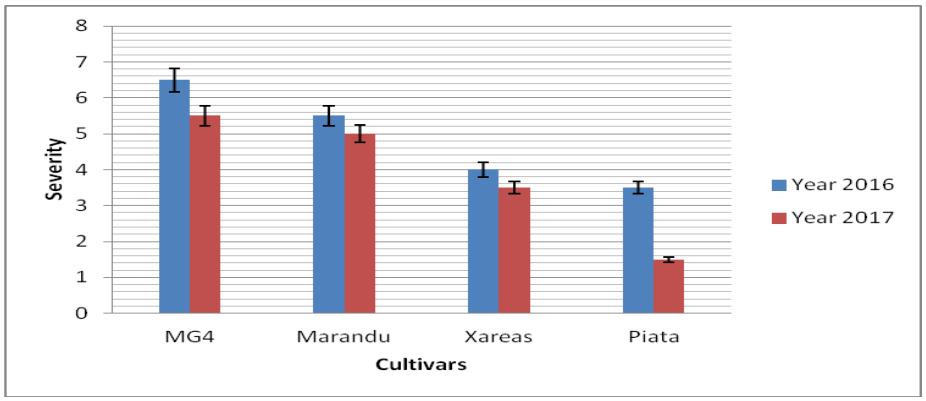

Figure 1: Severity of rust disease on brachiaria genotypes under glasshouse condition

Disease severity on brachiaria under growth chamber

Results in Figure 1 showed that genotypes inoculated under growth chamber showed significant difference in disease severity in 2017. MG4 genotype had the highest severity, with a disease score of 6.5, followed by Xareas with 6.1, Marandu with 4.2, and Piata genotype with the lowest severity of 2.7.

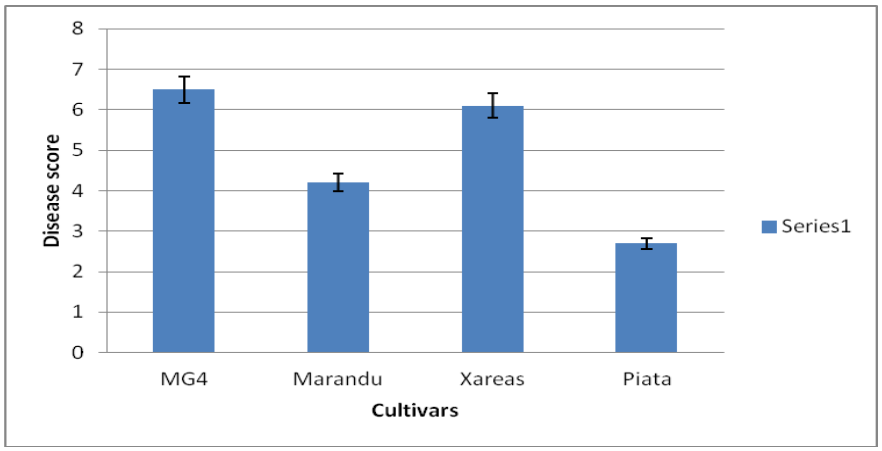

Figure 2: Reaction of Bracharia genotypes to rust fungi under growth chamber

Comparison of severity on bracharia genotypes under growth chamber and glasshouse

Effect of growth condition on rust disease severity was determined as shown in Figure 2. Higher rust disease severity was recorded in growth chamber than glasshouse. However, there was no significant difference between the 2 conditions ($p<0.001$), (fig 3). It was further noted that under growth chamber, the disease severity was significantly higher in MG4 than for the other three genotypes under both conditions (Fig 2).

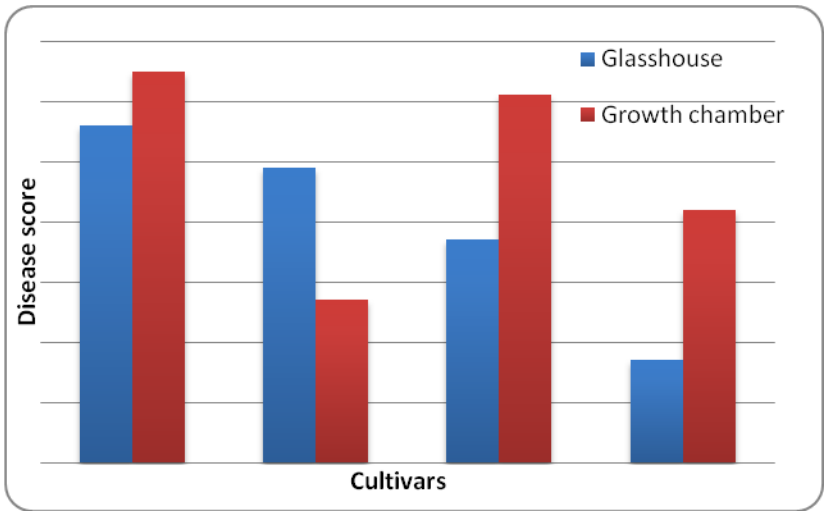

Figure 3: Disease score on brachiaria genotypes under glasshouse conditions

Pathological characterizations

The pathological characterization of rust isolates was determined by inoculation of the rust isolates collected from the different genotypes on MG4. The result showed that fungi isolates from MG4 were more severe on MG4, the original host genotype with mean severity of 6.8 followed by isolates from Marandu 5.6. Isolates collected from Piata caused the least severity disease severity of (2.5) (Figure.4).

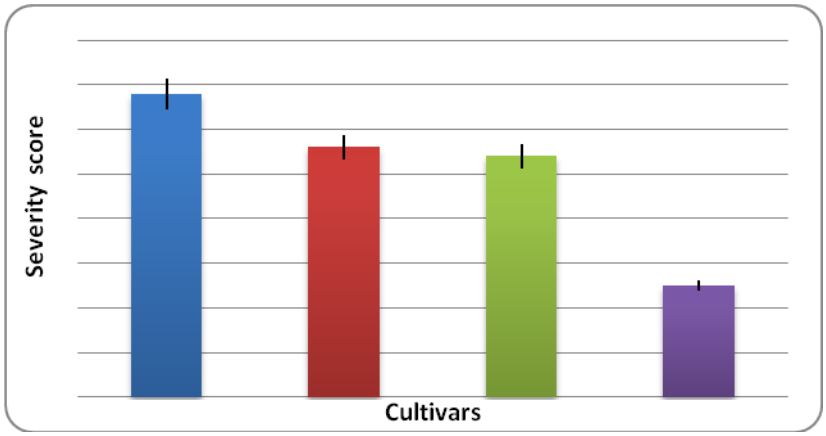

Figure 4: Pathological characterization of rust isolate on susceptible genotypes MG4

The influence of various factors on spore germination and germ tube length of Urediniospores under growth chamber

Period to spore germination and germ tube formation

The uredospore germination and growth of germ tube under 20^0C started after 8 hours of incubation, with an initial length of 17.76 µm. The length gradually increased to 54.28 µm at 22 hours after which it remained constant even after 24 hours (Table 2). This indicated that

at 20^0C, the spores of the rust fungus germinated and penetrate host cell after 8hours and reached its maximum length after 22 hours. That allowed it to reach the stomata openings of the plant leaf.

Table 2: The duration of incubation to uredospore germination and germ tube development of rust

Hours	Spore germination (%)	Germ tube length (μm)
2	0.0	0.0
4	0.0	0.0
6	0.0	0.0
8	18.3	17.8
10	21.6	21.9
12	36.7	24.6
14	51.7	32.8
16	90.0	43.7
18	90.0	49.9
20	90.0	54.3
22	90.0	54.3
24	90.0	54.3

Relative humidity (RH)

The data presented in Table 3 depicts the percentage spore germinating of brachiaria rust causing fungi, which ranged from 20.1 to 79.2 %, when subjected to various levels of relative humidity (RH). The highest spore germination of 79.2% ($p<0.05$) was recorded at 75 percent relative humidity, followed by 32% at 50 percent relative humidity. However, this was not significantly different from RH at 100% and 25%, with a percentage germination of 22.5% and 20.1% respectively. Similarly, the longest germ tube length of 47.9 μm was observed at 75 per cent RH ($p<0.05$). Germ tube length of 42.0 μm was observedat 50% RH and 41.0 μm at 100 % RH, however there was no significant difference between these 2 RH levels ($p<0.05$). The shortest germ-tube length of 34.2 μm was observed at 25 % RH.

Table 3: Effect of relative humidity on uredospore germination and germ-tube development of rust

Relative Humidity (%)	Spore germination (%)	Germ tube length (μm)
25	20.1	34.2
50	32.5	42.0
75	79.2	47.9
100	22.5	41.0
CD (5%)	8.760	2.40

Temperature

The spore germination and germ tube length varied significantly when spores were incubated at different temperatures from 5 to 50^0C. Significant highest uredospore germination of 90.3% was recorded at 20^0C, followed by 25^0C (13.3%), while significant lowest uredospore germination of 6.7% was recorded at 15^0C. Similarly, maximum germ

tube length of 51.6 µm was recorded at 20^0C, followed by 25^0C (31.4 µm) with significant shortest length of 28.7 µm reported at 15^0C. It is worth noting that no spore germination and germ-tube length were observed at 5, 10, 30, 35, 40, 45 and 50^0C (Table 4).

Table 4: Effect of temperature on spore germination and germ tube length of urediniospores of rust

Temperature (^{0}C)	Spore germination (%)	Germ tube length (µm)
5	*0.0 (0.6)	*0.0
10	0.0 (0.7)	0.0
15	6.7c (14.8)	28.7c
20	90.3a (72.3)	51.6a
25	13.3b (21.3)	31.4b
30	0.0 (0.57)	0.0
35	0.0 (0.57)	0.0
40	0.0 (0.57)	0.0
45	0.0(0.57)	0.0
50	0.0 (0.57)	0
LSD (5%)	3.5	0.2

* Significant different at 10 %level of significance (P<0.1

Morphological characterization

Morphological characterization of rust causing fungi collected from the field

The urediniospore collected from the four different genotypes MG4, Piata, Xareas and Marandu were compared for morphological variations and observations were recorded for size of urediniospores. The isolates cock tail showed variation from mostly ellipsoid, yellowish to orange and scattered germ pores. In microscopic examinations, shape of urediniospores was found similar in all genotypes but slight variation was noticed in size. Out of four different genotypes, the urediniospores collected from the four genotype showed mean lengths and widths which did not significantly vary i.e. MG4 (25.7 x 22.7 µm), Piata (25.9 x 23.0 µm) and Xareas (25.3 x 21.8) µm, whereas Marandu had larger sized urediniospores (26.4 x 22.6 µm) (Table 5).

Table 5: Morphological variation in rust causal agent infecting genotypes of bracharia

Genotype	Length (µm)	Width (µm)
MG4	25.7 (21-31)	22.7 (17-25)
Piata	25.9 (17-31)	23.0 (17-28)
Xareas	26.4 (23-30)	22.6 (17.25)
Marandu	25.3 (22-30)	21.8 (17-25)

Morphological characterization of isolates collected after artificial incubation

Under the growth chamber the isolates were collected from each plant in the four replicates and characterized morphologically for sizes, colour, shape and the presence of germ tube. It was established that isolates from MG4 ranged in size of 34 µm to 68 µm, globose to oval in shape and yellow in colour with two isolate showing the presence of germ tube. Piata isolates ranged between 20 µm to 34 µm with two being oval and globose in shape, three showed the absence of germ tube and one absent. Xareas had the largest spore size of 52 µm

to 79 µm and were oval in shape and yellow in colour with two having presence of germ tube. Marandu isolates had a spore size range between 45 µm to 65 µm and they were all yellow in colour and germ tube was observed in two isolates as show in the Table 6 below.

Table 6: Morphological characterization of rust causing fungi in bracharia

Isolate number	Spore Size	Spore Shape	Spore Colour	Germ tube
Isolate-1 Piata	23 µm	Oval	Yellow	Absent
Isolate-1 Piata	34 µm	Oval	Yellow	Absent
Isolate-1 Piata	20 µm	Globose	Yellow	Absent
Isolate-1 Piata	30 µm	Globose	Yellow	Present
Isolate-2 MG4	34 µm	Oval	Yellow	Present
Isolate-2 MG4	55 µm	Oval	Yellow	Present
Isolate-2 MG4	35 µm	Globose	Yellow	Absent
Isolate-2 MG4	68 µm	Globose	Yellow	Absent
Isolate-3 Xareas	79 µm	Oval	Yellow	Absent
Isolate-3 Xareas	52 µm	Oval	Yellow	Present
Isolate-3 Xareas	60 µm	Oval	Yellow	Present
Isolate-3 Xareas	53 µm	Oval	Yellow	Absent
Isolate-4 Marandu	65 µm	Oval	Yellow	Absent
Isolate-4 Marandu	45 µm	Oval	Yellow	Present
Isolate-4 Marandu	60 µm	Oval	Yellow	Present
Isolate-4 Marandu	50 µm	Globose	Yellow	Absent

DISCUSSION

The occurrence of rust fungus

MG4 genotype under field, glasshouse and growth chamber conditions was found to be the most susceptible genotype, and Piata was found to be more tolerant when compared with the four genotypes studied. These results indicate high level of tolerance of Piata genotype to leaf rust causing fungi; therefore it can be a possible source of resistance genes. Similar results were observed by Gravert; *et al.*, (2002) in their screening of genotypes which can offer potential sources of genetic exploitation for rust management.

These results are also in tandem with Nzioki *et al.*, (2016), though they reported that Xareas was most infected under growth chamber. In the current study MG4 was most infected followed by Xareas. Similarly under field conditions MG4 was also severely infected indicating the high susceptibility of this genotype to rust disease. This implies therefore that the genotype need not be grown under rust prone environment.

Pathological characterization of leaf rust fungi and growth condition of rust

The results showed that isolates of rust from MG4 were more virulent on MG4 genotype than isolates obtained from other Bracharia genotypes followed by isolates from Xareas, which caused high disease severity on MG4. These indicate that isolates tend to be more virulent to the plant where they were initially parasitizing. This phenomenon could be due to the high presence of germ tube producing isolates from these bracharia genotypes and also

the high growth rate shown by these isolates. Isolates from Piata showed low germ tube presence and shorter germ tube length, thus can explain the low virulence shown in the current study, Kelemu *et al*., (1995); Lenné and Trutmann, (1994) also reported similar phenomenon.

The growth conditions of rust pustules were determined by exposing into different regimes of relative humidity, temperature and time to germ tube development were established under rust ideal environmental conditions. All bracharia cultivars tested (MG4, Piata, Xareas and Marandu) were susceptible to rust diseases in the field as well as in controlled environment. In the field the percent disease incidence of 100% was recorded while disease severity was higher in MG4 (7.5 and 8.5) in 2016 and 2017, respectively in a disease severity scale of 0 to 9. The disease was least severe in Piata (2.5) in 2016 but in 2017 Marandu was least infected. Under artificial inoculation and controlled growth conditions MG4 proved more susceptible (6.5) while, Piata (1.7) was less severe in glasshouse but under growth chamber Xareas was most affected with diseases score of 6.5. The isolate from MG4 was more virulent on MG4 than isolates obtained from other bracharia cultivars followed by isolates from Xareas that caused disease severity of (3.5) on MG4. When the isolates were subjected to different environmental conditions it was established that optimum relative humidity of 75% and temperature of between 20-25^{0}C was ideal for germ tube germination; this agrees with what was reported by Lenné and Trutmann, (1994).

CONCLUSION

There was high incidence of rust disease in all the four Bracharia genotypes collected from the different regions of Kenya and were planted at BECA- ILRI trial plots. Similarly the disease severity was also high in all the bracharia genotypes. MG4 genotype under field, glasshouse and growth chamber conditions were found to be the most susceptible genotype, but Piata was found to be more tolerant when compared with the four genotypes studied. On pathological characterization the isolates from MG4 were more virulent on MG4 than isolates obtained from other bracharia genotypes followed by isolates from Xareas and Marandu but isolates from Piata were least virulent. The relative humidity of 75% and temperature of between 20^{o}C – 25^{o}C was optimal and ideal for spore germination and germ tube development. Morphologically the isolates varied from spore size of 20µm to 79µm and spore shape was oval and globose but all were yellow in colour

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