

ISSN 1996-0719

International Journal of
Plant
Pathology

Survey on Incidences and Severity of Rice Yellow Mottle Virus Disease in Eastern Uganda

^{1,2}Dennis Ochola and ²Geoffrey Tusiime

¹Cereals Research Program, National Crops Resources Research Institute, Uganda

²Department of Crop Science, Faculty of Agriculture, Makerere University, Uganda

Corresponding Author: Dennis Ochola, Cereals Research Program, National Crops Resources Research Institute, Uganda Tel: +256712301290

ABSTRACT

The present study was conducted to assess the incidences and severities of *Rice Yellow Mottle Virus* (RYMV) disease in Eastern Uganda. In September 2009, a field survey was conducted in 11 rice-growing districts by randomly selecting rice fields along main roads and occasionally on feeder roads. Symptoms assessment and Enzyme-Linked Immunosorbent Assay (ELISA) were used as tools of disease diagnosis. The collected 49 samples showed a strong positive reaction with polyclonal antiserum raised against an S4 isolate from Madagascar. Narrow serological and biological variability among samples confirmed that the S4 strain was prevalent in all surveyed lowland rice-growing districts. Disease incidence and severity were high with mean values of 72% and 2.3 respectively but did not vary significantly ($p = 0.05$) among districts, varieties, altitudes and cultivation ecologies. Bugiri recorded the highest disease incidence (80%) and severity (2.5) respectively, while Namutumba had the lowest incidence (62%) and severity (2.1), respectively. Moreover, disease was most severe in districts closest to the Lake Victoria Basin. The farmer-preferred varieties showed varying degrees of susceptibility. The highest levels of disease occurred in variety K5, whereas the variety K85 had the lowest levels of disease. The results indicate that the continuous cultivation of susceptible varieties has lent impetus for the widespread of a highly infectious RYMV strain in Eastern Uganda. Plant breeders should provide significant agronomical protection against adverse effects of the virus by identification of different sources of durable resistance genes to be pyramided into the farmer preferred lowland varieties that possess good culinary traits.

Key words: Degrees of susceptibility, agronomic protection, durable resistance, lake victoria basin, farmer-preferred varieties

INTRODUCTION

Rice Yellow Mottle Virus (RYMV) an emergent virus in Africa is a major rice health constraint endemic to the continent (Abo *et al.*, 1998; Fargette *et al.*, 2002; Banwo *et al.*, 2004). RYMV was first detected 45 years ago in Otonglo near Kisumu along the shore of the Kivorondo Gulf of Lake Victoria (Abo *et al.*, 2000). Ever since then, this notorious disease has spread to all major rice growing zones of sub-Saharan Africa and to the neighboring Islands of Zanzibar and Madagascar (Banwo, 2002; Reckhaus and Adriamasintseheno, 1997). Outbreaks causing variable yield losses have been reported in irrigated lowland and water swamped rice production systems (Traore *et al.*, 2001). Reductions in yield ranging from 58-68% were reported in the Republic of

Niger; 54-97% in Sierra Leone; 20-45% in Burkina Faso; 64-100% in Mali; 67-84% in Cote d' Ivoire; 25-100% in Tanzania (Taylor *et al.*, 1990; Sere, 1991; Sy *et al.*, 1993; Luzi-Kihupi *et al.*, 2000; Kouassi *et al.*, 2005). A notable example was the devastating epidemic in Marovoay and Lake Aloatra, Madagascar (Reckhaus and Randrianangaly, 1990) and in Zamfara State, Nigeria (Alegbejo *et al.*, 2001) that resulted in rice farmers abandoning their fields for cattle grazing. Abundant evidence suggests that in an attempt to increase rice production, cultivation practices encouraged creation of conditions that favored the widespread of RYMV and its vectors, while restricting opportunities necessary for mutual adaptations (Fargette *et al.*, 2006). Just recently, Pinel-Galzi *et al.* (2006) produced the first report of the occurrence of RYMV in Uganda. It is most likely that the disease was present in the country for a number of years being confused with nitrogen deficiency. However, because no quantifiable information is readily available on the incidence and severity of the virus, there is a wide knowledge gap of its epidemiology and transmission among researchers, extension officers and farmers in Uganda, yet such information is a necessity for management approaches for the disease to be developed. This study aimed at generating some of such fundamental information, like the prevalence, severity and incidence of RYMV in Eastern Uganda.

MATERIALS AND METHODS

Field survey: A field survey was conducted in September and October 2009 covering 11 rice-growing districts in Uganda, namely Butaleja, Mbale, Budaka, Pallisa, Namutumba, Iganga, Mayuge, Bugiri, Bukedea, Soroti and Lira (Fig. 1). The criteria for selection of districts was based on emerging reports of the persistent yellowing and stunting in rice fields that were irreversible by the application of nitrogen fertilizers. Rice fields were randomly selected in each rice-growing area along main roads and occasionally on feeder roads. Fifteen plants were randomly sampled per rice

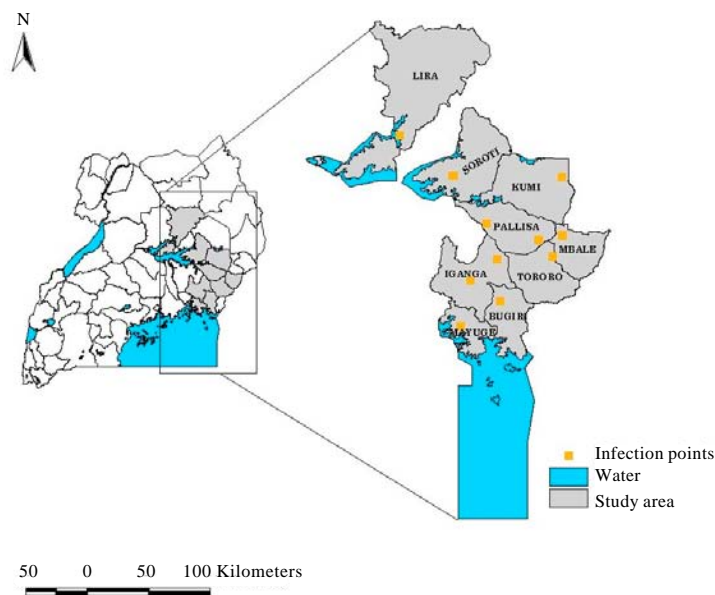


Fig. 1: Map of Uganda showing surveyed districts in Eastern Uganda







Symptom appearance	Scale [†]	Scale ^{††}	Descript	Aggressiveness
	0	0	No symptom	0%
	1	1	Sparse yellow spots	Trace to 5%
	1.5	3	Oblong yellow linear spots	6% to 25%
	2	5	Elongated spots parallel to veins	26% to
	2.5	7	Dark orange patches/mild wilt	76% to 95%
	3	9	Extreme wilt and death of rice plants	96% to 100%

Fig. 2: Field and Screen house evaluation scales of rice yellow mottle virus developed based on the reaction of the virus on susceptible variety IR64. [†]Modification of evaluation scale by Raymundo *et al.* (1979); ^{††} Modification of the evaluation scale by John and Thottappilly (1987)

field in a two-way diagonal pattern. However, fields with young transplanted rice plants and those with re-growths were most preferred, since they facilitated clear identification of RYMV infected plants. Disease incidence was assessed as a visual estimate of the percentage coverage of plants infected in each field. Disease severity was assessed using a modification of an earlier scale by Raymundo *et al.* (1979). The modification was done by the addition of intermediate scores to capture a wider magnitude of disease presence, so that 0 = 0% (no infection); 1 = less than 5%; 1.5 = 6-25%; 2 = 26-75%; 2.5 = 76-95%; 3 = 96-100% (Fig. 2). The altitudes, longitudes and latitudes were recorded for each sampled field using the GPS handset, Geko[®] 301 Personal Navigator (Garmin International Inc., Olathe, Kansas, USA). The collected data were used to generate a map of sampled points by interpolating the surface from GPS points and the associated field severity data using the inverse distance, from which mean values were calculated for all factors measured for each field and rice growing area using ARC-VIEW 3.3[®].

Serological detection and infectivity of rice yellow mottle virus: Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Clark and Adams, 1977) was used to test for the presence of RYMV in 49 leaf samples collected from Eastern Uganda. A polyclonal antiserum produced against a Madagascan S4 isolate was used as the primary antibody in this study. A Benchmark[®] microplate reader (BIO-RAD Laboratories, Hercules, California, USA) measured the optical density (A405 nm) after a substrate hydrolysis for 1, 12 (overnight) and 24 h. Samples considered positive were those with optical density values greater than twice the value for the healthy control. Positive ELISA reactions were classified as weak (+), intermediate (++) , strong (+++) and very strong (++++). Regeneration of RYMV from leaf samples was conducted to propagate and ascertain the infectivity of each isolate on the highly susceptible cultivar IR64. Seeds of IR64 were soaked in water for 24 h and incubated for 48 h at room temperature (25°C) to facilitate sprouting. Three pre-sprouted seeds were sowed in 40 cm diameter buckets. Two weeks later, inoculum was prepared by squashing 1 g of finely sliced infected leaf tissue in a drop of double distilled water using sterile mortars and pestles prior to dilution with 10 mL of double distilled

water. The resulting homogenate was filtered with cheesecloth and preserved at 4°C in a refrigerator. Mechanical inoculation was done using the finger-rub technique (Raymundo *et al.*, 1979). During this process, pieces of cotton wool were dipped in inoculum mixed with fine sand and subsequently rubbed onto leaves ensuring maximum wetting and formation of mild bruises that would act as infection passageways. Appearance of symptoms was monitored from 7 to 20 days post inoculation (dpi) using the scale 0-9 modified from John and Thottappilly (1987).

Data analysis: Based on the data collected during the survey, variance and mean comparisons of disease severity scores and percentage incidence were performed. The effects of type of rice variety, cultivation environment and elevation on the observed disease severities and incidence were analyzed using GENSTAT 13 statistical software (VSN International, UK).

RESULTS

Virus incidence and severity in Eastern Uganda: Forty-nine rice fields in the altitude ranges 1083-1154 meters above sea level (m.a.s.l) were surveyed in 11 rice-growing districts in 2009. Rice yellow mottle virus was present in all rice fields surveyed in Butaleja, Mbale, Budaka, Pallisa, Namutumba, Iganga, Mayuge, Bugiri, Bukedea, Soroti and Lira (Table 1). Disease incidence and severity were high with mean values of 72% and 2.3, respectively but did not vary significantly ($p = 0.05$) among districts, varieties, altitudes and cultivation environments. The highest incidences were registered in Bugiri (80%) followed by Mayuge and Pallisa with 78 and 76% respectively. On the other hand, the lowest incidences occurred in Soroti (69%), Lira (66%) and least in Namutumba (62%). Moderate to severe symptoms were prevalent in sampled rice fields ranging between 2.1 and 2.5 in each district. Moreover, disease was most severe in districts closest to the Lake Victoria Basin, namely Bugiri and Mayuge (Table 1). Differences in cultivation environment between irrigated and rainfed lowland fields did not significantly influence disease incidence and severity (Table 2). The farmer-preferred varieties showed varying degrees of susceptibility. The highest incidence of disease occurred in variety K5, whereas the variety K85 had the lowest

Table 1: Altitude, severity and incidence of *rice yellow mottle virus* disease in eleven districts of Eastern Uganda surveyed in 2009

District	Altitude (m.a.s.l)*	Severity	Incidence (%)
Butaleja	1083	2.3	70
Mbale	1086	2.3	72
Budaka	1081	2.3	73
Pallisa	1068	2.4	76
Namutumba	1112	2.1	62
Iganga	1083	2.3	71
Mayuge	1154	2.5	78
Bugiri	1129	2.5	80
Bukedea	1131	2.3	72
Soroti	1087	2.2	69
Lira	1093	2.2	66
Mean	1101	2.3	72
% CV	2.5	11.2	14.5
LSD 0.05	24.1	0.2	13.8
F-test [†]	ns	ns	ns

[†]ns: Not significant, * m.a.s.l: Meters above sea-level

Table 2: Effect of rice ecology on severity and incidence of rice yellow mottle virus

Rice ecology	Severity	Incidence (%)
Irrigated lowland	2.3	71
Rainfed lowland	2.3	73
Mean	2.3	72
% CV	11	13.9
LSD 0.05	0.17	6.63
F-test†	ns	ns

†ns: Not significant

Table 3: Effect of rice variety on severity and incidence of rice yellow mottle virus

Rice variety	Severity	Incidence (%)
K5	2.4	75
K85	2.3	70
Supa	2.3	71
Mean	2.3	72
% CV	11.1	13.8
LSD 0.05	0.2	8.0
F-test†	ns	ns

†ns: Not significant

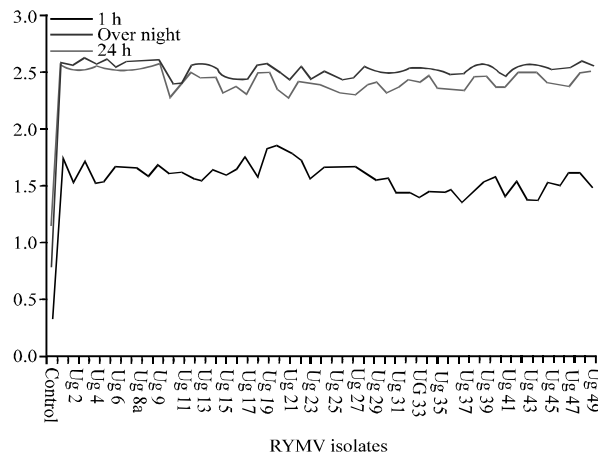


Fig. 3: Narrow variability of optical density readings of 49 rice yellow mottle virus isolates recorded after 1, 12 (overnight) and 24 h of substrate hydrolysis

incidence (Table 3). Disease severities in the range 2.3 to 2.4 revealed the absence of resistance among the encountered varieties in the surveyed districts (Table 3). This result indicates that the continuous cultivation of susceptible varieties has lent impetus for the widespread of this highly infectious viral disease in Eastern Uganda.

Serological detection and infectivity of rice yellow mottle virus: All leaf samples collected during the survey showed a strong positive reaction with polyclonal antiserum raised against an S4 isolate from Madagascar. Narrow serological variability in Optical Density (OD) readings among isolates (Fig. 3) confirmed that the S4 strain was prevalent in all surveyed lowland rice-growing districts. The isolate Ug 20 originating from Pallisa had the highest OD reading of 1.83 while

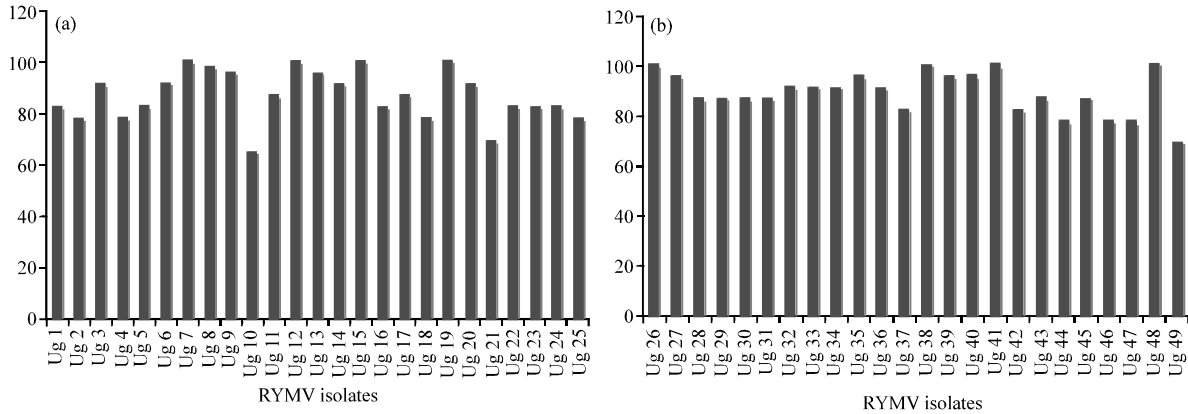


Fig. 4: Variations in percentage infectivity of susceptible variety IR64 by 49 rice yellow mottle virus isolates from Eastern Uganda

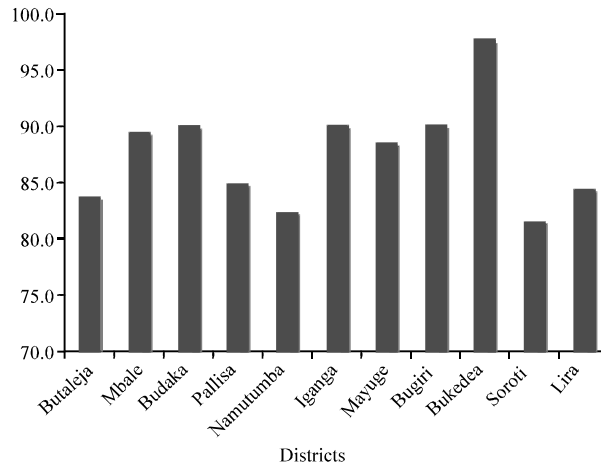


Fig. 5: Percentage infectivity of susceptible variety IR64 by 49 isolates averaged according to their districts of origin in Eastern Uganda

Ug 30 from Mayuge had the lowest OD of 1.37 after 1 h substrate hydrolysis. However, no significant differences in OD readings were observed after 12 h (Overnight) and 24 h substrate hydrolysis (Fig. 3). Biological tests of infectivity of the highly susceptible check IR64 by 49 isolates was characterized by early disease initiation between 7 to 10 dpi, rapid disease development and highest mean disease severities. However, it did not reveal significant differences ($p = 0.05$) in the capability of 49 isolates to induce disease on the highly susceptible cultivar IR64 which supports the possible lack of pathotype variability among the existent S4 strains in Eastern Uganda. Variations in the levels of isolate aggressiveness ranging between 64 and 100% were observed. The most aggressive isolates, namely Ug 7, Ug 12, Ug 15, Ug 19, Ug 38 and Ug 48 led to high mortality of IR64 plants (Fig. 4). After virus regeneration and maintenance on rice cultivar IR64, an assessment of aggressiveness of isolates by district of origin showed that the most aggressive isolates originated from the districts of Iganga (90%) and Bukedea (98%) (Fig. 5).

DISCUSSION

The serological detection of Rice Yellow Mottle Virus (RYMV) in the 49 collected field samples revealed a narrow variability in absorbance readings among isolates and confirmed that S4 strains were prevalent in Eastern Uganda. This is consistent with an earlier report by Pinel-Galzi *et al.* (2006) who found four samples collected from a subsistence rice field northeast of Lake Victoria in Uganda to contain isolates of the East African S4, a strain which is also found in Madagascar, Kenya and Tanzania. However, since all isolates were serologically the same, our results seemed to contradict the findings of Sere *et al.* (2007) who asserted that isolates emanating from the same locality, field or host were serologically different. Biological tests did not reveal significant differences in the capability of 49 isolates to induce disease on the highly susceptible cultivar IR64. Thus, suggesting a narrow serological and biological variability of the existent S4 strain in Eastern Uganda. This result supports the finding by Traore *et al.* (2005) who noted that strain distribution was not linked to pathogeny, host species or agroecology. However, it contradicted the view that biological tests of RYMV were more sensitive than serological detection of the virus by Traore *et al.* (2008). Nevertheless, Onasanya *et al.* (2006) asserts that as isolate populations increase in the future, there is a probability for pathotypes to interact leading to the emergence of new highly virulent strains. Fortunately, our results indicated limited pathotype variability among Eastern Uganda strains. Notwithstanding, plant viruses are a salient biotic constraint causing serious productivity losses of a wide range of economically important crops worldwide (Bhyan *et al.*, 2007) to be taken lightly. The potential impact of RYMV disease on the rice industry in Uganda was reflected by its occurrence in the 11 surveyed rice-growing districts in Eastern Uganda. This represents the capability of this emergent plant virus to cause serious epidemics in irrigated lowland and water swamped rice production systems across the region (Traore *et al.*, 2001; Fargette *et al.*, 2006). This study shows that the highest disease incidences and severities occurred in the districts of Mayuge and Bugiri which are located on the periphery of the Lake Victoria Basin. Based on the analysis of historical precedents of rice cultivation in Uganda by Wilfred (2006), the establishment of large-scale rice schemes in Eastern Uganda circa 1960 was indeed contemporaneous with the first detection of RYMV in Western Kenya along the shores of the Kavirondo Gulf of Lake Victoria (Banwo *et al.*, 2004; Fargette *et al.*, 2006). Due to the close proximity of Eastern Uganda to Western Kenya, there is consistent indication that the virus was present in the northeastern plains of Lake Victoria in Uganda, where it survived on wild grass hosts until when changes in rice cultivation practices created conditions that favored its spread (Pinel-Galzi *et al.*, 2006; Sere *et al.*, 2008). This observation is reconcilable with earlier and recent reports that natural sources of virus infection for the spread of the disease to rice crops were present in a few water-dependent wild grasses, such as *Echinochloa colona* and *Panicum repens* (Abo *et al.*, 2000; Nwilene *et al.*, 2009). The intensive usage of irrigation in rice cultivation is implicated to have enhanced growth and continuity of a wide range of weeds that acted as alternative hosts and reservoirs from which the viral inoculum spreads to healthy rice crops (Konate *et al.*, 1997; Traore *et al.*, 2009). For this reason, our study had anticipated higher disease incidences in irrigated rather than in rainfed lowlands fields, for the most part because high humidity of soils is associated with high incidences of several virus diseases (Pourrahim *et al.*, 2007). Unfortunately, our findings indicated that differences in source of water required for rice cultivation (irrigated or rainfed) did not significantly influence disease incidence and severity in the lowlands of Eastern Uganda. However, the results indicated the absence of resistant varieties as the proximate cause for the widespread of RYMV disease in the surveyed districts. All the encountered farmer-preferred

varieties, namely K5, K85 and Supa were unable to withstand the incursion by isolates of the virus found in the different districts. This supports findings of Abo *et al.* (1998) implicating the introduction of more vulnerable new varieties from Asia with the erosion of several advantages associated with heterogeneity of traditional landraces in restricting the spread of virus diseases. It is likely that earlier forms of rice culture practiced in Eastern Uganda achieved great levels of stability against RYMV by the direct sowing of a mixture of traditional landraces such as Cakala, Matama, Kawemba, Kigaire and Seena in the same field (Wilfred, 2006). RYMV vectorial transmission is notably by leaf feeding beetles belonging to the Chrysomelidae family (Banwo *et al.*, 2001). Yet, in view of the prevailing number of beetles, short retention time of the virus within its vectors and the absence of seed-borne transmission, there is a need to elucidate modes of transmission that must have facilitated the East-North dispersal of RYMV across Uganda (Konatè *et al.*, 2001). Therefore, this study asserts that farmers' operations readily contributed to the widespread of the virus in Eastern Uganda. Among the cropping practices that have facilitated the build-up of the virus inoculum and the perpetuation of RYMV disease in the surveyed districts is the incorporation of rice stubble back into soil during land preparation. This observation is in consonant with earlier reports by Reckhaus and Andriamasintseho (2001) and Traore *et al.* (2008). The latter authors associated this practice with the release of large inoculum supply that contaminates soil and provides the initial foci of infection in succeeding crops especially if the stubble was heavily infected. Apparently, survival and regeneration of stubble after harvest is facilitated by the abundance of irrigation water (Abo *et al.*, 2000). During this present study, we also noticed that farmers grazed their livestock, particularly cattle on rice regenerated from infected stubble during the off-season. Moreover, Sara (2005) reported that as livestock graze from field-to-field, viral inoculum was transferred from infected fields to the neighboring uninfected fields, thus further perpetuating the disease. In addition, Sarra and Peters (2003) reiterated that the number of infected plants increases 5-to-10 fold whenever animals have access to plots with infected plants. Furthermore, Ugandan rice farmers frequently use overage-etiolated seedlings which they trimmed to half the normal length prior to transplanting. The cultural practice of trimming overage rice seedlings with contaminated farm implements was implicated by Tsuboi *et al.* (1995) to transfer virus inoculum to healthy seedlings (Abo *et al.*, 2000). Apparently, together with bruises caused to root tissues in the process of uprooting seedling from seedbeds, such injuries act as avenues for primary infection by inoculum present in contaminated soils (Traore *et al.*, 2006). For this reason, this study presents the likelihood for transplantation of infected seedlings as the most efficient means of mechanical dissemination of the virus, responsible for its widespread in rice-growing areas of Eastern Uganda.

CONCLUSION

Prior to this study, there was no available data on the incidences and severity of RYMV in Uganda. We have provided plausible evidence that a highly infectious S4 strain has spread beyond its renowned hotspots in Butaleja to as far as the northwestern districts of Lira and Soroti. The introduction of irrigated rice cultivation practices coupled with the adoption of susceptible varieties and a drift from direct seeding in favor of transplantation of seedlings must have increased the importance of this hitherto minor virus into a continental problem. In particular, there is a consistent indication that the continuous cultivation of susceptible varieties in the surveyed rice-growing districts has lent impetus for the widespread of RYMV disease in Eastern Uganda. In this respect, breeding of rice varieties with durable resistance is recommended as a main component

of an integrated pest management system for controlling RYMV spread in rice fields. Therefore, the onus is on plant breeders to provide significant agronomical protection against adverse effects of disease throughout the commercial life of the cultivar. However, in view of the narrow genetic base of the available rice germplasm in Uganda, there is an urgent need for the identification of different sources of durable resistance genes to be pyramided into the farmer preferred lowland varieties that possess good culinary traits. A potentially useful breeding strategy should employ a combination of RYMV strains with different levels of aggressiveness that will exert sufficient prerelease challenge to avoid the future breakdown of then deployed resistant cultivars.

ACKNOWLEDGMENTS

Special thanks to R. Edema for the logistical assistance during the field surveys; Mr. R. Obonyo for the serological work; and Mr. N. Anguzu for maintenance of infectivity experiment.

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