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REACTION OF RICE CULTIVARS TO A VIRULENT RICE YELLOW MOTTLE VIRUS STRAIN IN UGANDA

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ABSTRACT

Rice (*Oryza* spp.) has long been an important food staple for many traditional rice growing communities. Key developments in efforts to improve rice production such as the development of NERICA varieties have been widely recognised. Nonetheless, emerging diseases such as the Rice Yellow Mottle Virus sobemovirus (RYMV) undermine dissemination of new technologies and sustained productivity of the crop. The RYMV is highly variable and several resistance-breaking strains have been identified. Appropriate sources of resistance to RYMV should be identified and characterised in order to pyramid genes for both complete and partial resistance. In this study, reaction of nine (9) rice cultivars to RYMV virulent strain in Uganda was determined. Four upland and three lowland NERICA varieties, an *O. Sativa* lowland variety, Gigante and IR64 were observed. Isolates of RYMV were collected from three "hot-spots" in Uganda (Lira, Luweero and Iganga). The isolate from Iganga was most virulent on RYMV susceptible cultivar (IR64), and thus used to constitute study treatment evaluated in the study. Cultivars were potted and raised in a screenhouse experiment arranged in a randomised complete block design (RCBD) with three replicates. Significant differences for relative area under disease progress curve (RAUDPC) ($P \leq 0.01$) were detected among the cultivars. Similarly, significant positive correlations ($P \leq 0.05$) were observed between mean disease score and percentage reduction in grain weight and between percentage grain weight and reduction in plant height. Susceptible cultivars were characterised based on premature death, and significantly reduced grain weight and plant height ($P \leq 0.01$ and $P \leq 0.05$), respectively. These results demonstrated the aggressive nature of the specific RYMV strain, and provided evidence with regard to the importance of grain weight and plant height in evaluating RYMV disease resistance.

Key Words: Grain weight, NERICA, plant height, *Oryza sativa*, susceptible cultivars

RÉSUMÉ

Le riz (*Oryza* spp.) a été depuis longtemps un aliment de base pour plusieurs communautés cultivatrices de riz. Les développements-clés dans l'effort d'améliorer la production rizicole, tel que le développement des variétés NERICA, ont été largement reconnus. Néanmoins, l'apparition des maladies telle que le virus de la panachure jaune de type *sobemovirus* contraint la dissémination des nouvelles technologies et la productivité soutenue de la culture. Le virus de la panachure jaune du riz est très variable et plusieurs souches virulentes ont été identifiées. De sources appropriées de résistance à la maladie de la panichure jaune du riz devront être identifiées et caractérisées afin de pyramider les gènes pour une résistance complete et/ou partielle. Dans cette étude, la réaction de 9 cultivars de riz au virus virulent de la panachure jaune en Ouganda était déterminée. Quatre variétés NERICA de montagne et trois variétés NERICA des bas-fonds, ainsi qu'une variété des bas-fonds de type *O.sativa*, Gigante et IR64, étaient observées. Les isolats du virus de la panachure jaune du riz étaient collectés des "zones dangereuses" de l'Ouganda, notamment Lira, Luwero et Iganga. L'isolat de l'Iganga était le plus virulent au cultivar IR64 susceptible au virus de la panachure jaune et donc était utilisé pour constituer le traitement évalué dans cette étude. Les cultivars étaient cultivés en pots dans une serre et arranges en blocs aléatoires complets avec trois répétitions. Des différences significatives pour la surface relative sous la courbe progressive de la maladie ($P \leq 0.01$) étaient détectées parmi les cultivars. De façon similaire, des corrélations positives significatives (P≤

0.05) étaient observées entre les moyennes des cotations de la maladie et le pourcentage de réduction du poids des grains, et entre le pourcentage du poids des grains et la réduction de la hauteur de plant. Les cultivars susceptibles étaient caractérisés sur base de la mort prématurée, et la réduction significative du poids des grains et la hauteur de plant (P≤0.01 et P≤0.05), respectivement. Ces résultats ont démontré la nature aggressive du virus de la panachure jaune et fournissent l' évidence de l'omportance du poids en grains et la hauteur de plants dans l'évaluation de la résistance à la maladie de la panachure jaune du riz.

Mots Clés: Poids en grains, NERICA, hauteur de plants, *Oryza sativa*, cultivars susceptibles

INTRODUCTION

Rice (*Oryza* ssp.) is an important food security crop which has in the recent past received considerable research attention specifically focusing on crop improvement efforts. These efforts have resulted in the development and dissemination of the NERICA rice varieties (Kijima *et al.*, 2006; Somado *et al.*, 2008) and the golden rice which represents a genetic engineering concept for developing of nutrient dense staple crops with potential to support efforts to reduce malnutrition in developing countries (Potrykus, 2003). Several countries are now benefiting from these developments in rice production. In Uganda for instance, the NERICA varieties have been widely adopted by smallholder farmers (Kijima *et al.*, 2008). Despite improvements in rice production in Uganda, several resistance breaking strains to RYMV have been identified and this necessitates further crop improvement efforts.

In light of this, screening of elite materials for resistance to RYMV is important. In order to ensure high disease pressure without the risk of causing an epidemic, screening for disease resistance is often done in isolation, in a screenhouse, which also protects both inoculated and uninoculated (control) plants from possible outside infection. Although the use of visual assessment as a diagnostic method of RYMV symptoms has been adequate to determine rice accessions that are highly susceptible to RYMV, precise assessment based on symptomatology alone is unreliable for resistant accessions (Thottappilly and Rossel, 1993). Therefore, both visual assessment based on the Standard Evaluation System (SES) (developed by IRRI in 1988 on a scale of 1-9) and a practical score chart for RYMV, based on morphological characteristics and serological diagnostics (IITA 1986; John and Thottappilly, 1987) have been developed. Also, RYMV can be detected in any condition with a rapid diagnostic kit that uses nitrocellulose membranes employing RYMV specific antibodies (Ayassi *et al.*, 1995).

In this study, the reaction of selected rice cultivars to a prevalent local RYMV strain in Uganda was assessed with a view of generating data on choice of parents in future breeding programmes for resistance to the virulent RYMV strain.

MATERIALS AND METHODS

Research site. The research was conducted at the National Crops Resources Research Institute (NaCRRI), located at 00 32'' N and 32 37'' E, in the bimodal rainfall region of central Uganda. It is 27 km north of Kampala at an elevation of 1150 metres above sea level. It has tropical wet and mild dry periods with slightly humid conditions (averaging 65%), and an average rainfall of 1200mm/year. The vegetation is wooded savannah with tall trees and tall grasses dominated by *Pennisetum purpureum* and *Parnicum maximum* (NARO, 2005).

Plant materials. As a follow-up to previous studies for resistance to RYMV at NaCRRI, nine cultivars were selected (Lamo, 2010) for screening as shown in Table 1. Eight of these cultivars including; NERICA-4-lowland, NERICA-6-lowland, NERICA-10-lowland, IR64-lowland, NERICA-8-upland, NERICA-11-upland, NERICA-12-upland, and NERICA-13-upland, are farmer preferred cultigens in Uganda, while Gigante is a resistant check.

Inoculum preparation and application. Inoculum of RYMV was collected from three main rice growing areas in Uganda that represent "hot-

TABLE 1. Origin and pedigree of selected cultivars[1, 2]

Genotype	Reaction to RYMV	Pedigree	Origin/source
Gigante-Lowland	Resistant	Local accession from Mozambique	WARDA
NERICA -8-Upland	Resistant	WAB 450-1-BL1-136-HB	WARDA
NERICA-11 –Upland	Resistant	WAB 450-16-2-BL2-DV1	WARDA
NERICA-13 –Upland	Resistant	WAB 880-1-38-20-28-P1-HB	WARDA
NERICA-12-Upland	Resistant	WAB 880-1-38-20-17-P1-HB	WARDA
IR64-Lowland	Susceptible	IR 18348-36-3-3	IRRI
NERICA -4 –Lowland	Susceptible	WAS 122-IDSA 11-WAS 8-2	WARDA
NERICA -6 –Lowland	Susceptible	WAS 122-IDSA 13-WAS 10-FKR 1	WARDA
NERICA -10-Lowland	Susceptible	WAS 122-IDSA-10-WAS-7-2-FKR 1-TGR 89	WARDA

[1]Symptom rating on a scale of 1–9: 1-1.5=highly resistant (HR), 1.6-4.5 = resistant(R), 4.6–6.5 = moderately resistant (MR), 6.6-8.5 = susceptible(S), 8.6-9 = highly susceptible (HS)
[2]Reaction to RYMV was based on previous screening studies at NaCRRI (Lamo, 2010)

spots" for RYMV disease, according to Lamo (2010). These included Iganga in Eastern Uganda, Lira in Northern Uganda, and Luweero in Central Uganda. Isolates were tested on the susceptible cultivar IR64 and the most virulent isolate was identified as that from Iganga (in Eastern Uganda). Presence of RYMV was confirmed by the enzyme-linked immunosorbent assay method (ELISA) and the symptoms recorded. A highly virulent virus isolate was maintained on cultivar IR64 after mechanical inoculation at 14 days post-emergence, when plants had attained a 3-leaf stage. A mixture of 100 g of infected leaves was collected and ground, using sterile mortars and pestles in 10 ml of double distilled water. The virus solution was then rubbed onto leaves of test plants from the leaf-base to the tip with fingers moistened with prepared inoculum.

Cultivar reaction to RYMV in Uganda. Selected cultivars were potted and raised in a screenhouse at NaCRRI in a randomised complete block design (RCBD) with three replicates. Seedlings were thinned to three plants per pot. Test plants were confirmed to be visibly free of RYMV symptoms before inoculation. Thereafter, inoculation was achieved by rubbing test plants at 14 days post-emergence (when most plants had achieved a 3-leaf stage) from the leaf-base to the tip with fingers moistened with prepared inoculum. The non-inoculated plants of the nine test materials were used as controls. The inoculation was repeated after a week to avoid any escapes. Reaction of individual plants to RYMV was scored at one, two, three and four weeks after the initial inoculation, based on the intensity of visual foliar symptoms. The mean disease severity scores in plant populations were obtained using the IRRI standard evaluation system for rice (IRRI, 1988) where;

1= no visible symptoms,
3= green leaves with sparse dots of streaks and < 5% of height reduction,
5= green leaves or pale green leaf with mottling and 6-25% height reduction; flowering slightly delayed,
7= leaves pale yellow to yellow, with 26-75% height reduction; flowering delayed,
9= leaves turn yellow or orange, >75% height reduction; no flowering or some plants dead.

A scale developed by Zouzou *et al.* (2008) was used with slight modification to classify the mean scores for disease severity. Values between 1.0 and 1.5 were assigned a score of 1 (highly resistant), 1.6-4.5 were rated as 3 (resistant), 4.6-6.5 as 5 (moderately resistant), 6.6-8.5 as 7 (susceptible), and 8.6-9 as 9.0 (highly susceptible).

Based on the weekly average score for severity, the area under disease progress curve (AUDPC) was calculated for each cultivar, as described by Campbell and Madden (1990). The AUDPCs were normalised to relative AUDPCs (RAUDPCs) by dividing them by the total area included in the graph. The RAUDPCs were plotted on a bar graph for comparison among the cultivars, and further subjected to analysis of variance (ANOVA) using GenStat software (GenStat, 2010), where significant treatments were detected from the ANOVA, treatment means were separated using Fisher's Protected LSD at the 5% level.

RYMV impact on yield components. To assess the overall impact of RYMV, mean values of plant height (measured on the tallest tiller following anthesis from ground level to the tip of the panicle), number of active tillers (vegetative branches that develop panicles), days to heading (time when 50% of the panicles have at least partially exerted from the boot), and grain weight per plant (measured from 100 grains dried to 13% moisture content) were compared for inoculated and non-inoculated of well established seedlings of each cultivar. The impact of the disease on yield-related traits was assessed based on the following formula:

Impact (%) = (Ni - I) x 100 / Ni

Ni = mean values on the uninoculated seedlings, and I = mean values on the inoculated seedlings (Zouzou *et al.,* 2008).

RESULTS AND DISCUSSION

Following inoculation, all cultivars were found to be sensitive to RYMV at varying levels of severity (Fig. 1). Distinctive symptoms of RYMV were observed one week post-inoculation, with the intensity of symptoms varying among cultivars. Typical symptoms of RYMV included sparse elongated yellow spots, mottled green or pale leaves, and pale yellow, yellow or orange leaves.

At 3-4 weeks post-inoculation, most cultivars had attained maximum disease scores (Table 2). Cultivars displayed different levels of incidence and severity, as shown by significantly different RAUDPCs ($P \leq 0.01$) (Table 3).

Based on the mean disease scores at 4 weeks (Table 2), and based on the RYMV disease scoring scale modified by Zouzou (2008) with slight modification, cultivars were grouped as follows: Gigante was resistant (R), N-11 upl, N-8 upl, and N-13 upl were moderately resistant (MR), N-12 upl was susceptible (S), and N-4 lwl, IR64 lwl, N-6 lwl and N-10 lwl were highly susceptible (HS) (Table 2).

Reaction of test plants indicated that no cultivar escaped infection or expressed immunity to RYMV. This differed from results of previous

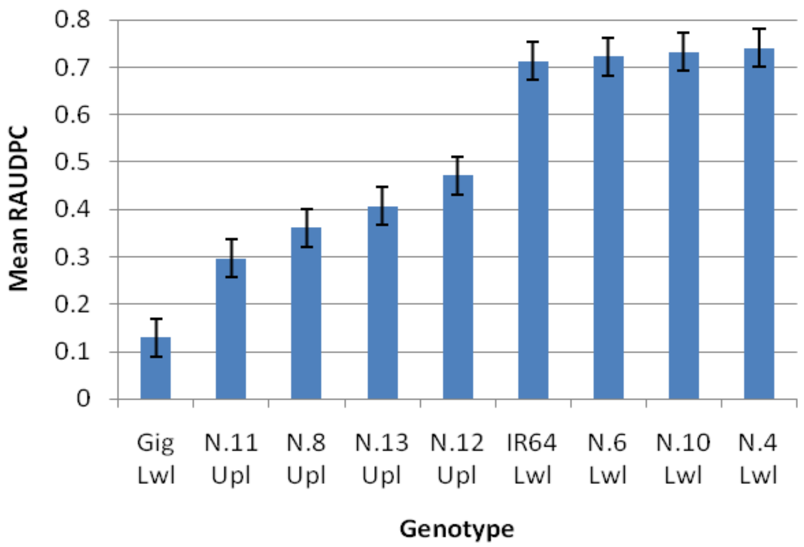

Figure 1. Mean RAUDPC for resistance to RYMV in selected rice cultivars. Error bars indicate a 0.04 standard error of cultivar means.

TABLE 2. Mean disease severity scores across 4 weeks [1, 2]

Genotype	Weeks				Reaction to RYMV
	1	2	3	4	
Gigante lwl	1.0	1.7	1.7	1.7	R
N-11 upl	1.7	3.0	4.3	5.0	MR
N-8 upl	1.7	3.7	5.7	5.7	MR
N-13 upl	2.3	5.0	5.7	5.7	MR
N-12 upl	3.0	5.0	7.0	7.0	S
IR64 lwl	6.3	9.0	9.0	9.0	HS
N-6 lwl	7.0	9.0	9.0	9.0	HS
N-10 lwl	7.7	9.0	9.0	9.0	HS
N-4 lwl	8.3	9.0	9.0	9.0	HS
Mean	4.3	6.0	6.7	6.8	
LSD 5%	2.0	1.3	1.9	1.6	
CV (%)	26.6	9.0	11.5	9.8	

[1]Symptom rating on a scale of 1–9: 1-1.5=highly resistant (HR), 1.6-4.5 = resistant(R), 4.6–6.5 = moderately resistant (MR), 6.6-8.5 = susceptible(S), 8.6-9 = highly susceptible (HS)
[2]LSD and CV for weeks 2, 3 and 4 were calculated from Error Mean Square (EMS) of an ANOVA that excluded the cultivars with a consistent mean disease score of 9

TABLE 3. ANOVA for RAUDPCs for selected rice cultivars in Uganda[1]

Source	d.f	m.s
Replicate	2	0.002^{ns}
Genotype	4	0.051^{**}
Error	8	0.004

** Significant at $P \leq 0.01$; ns Non-significant at $P \leq 0.05$
[1] ANOVA excluded cultivars that died of RYMV prior to week 2 scoring

studies in which all the selected upland NERICA cultivars were resistant to RYMV (Lamo, 2010). Thus, this suggested a more aggressive RYMV strain than the one used in the previous study or that the test conditions in this study were more conducive to severe disease development. Premature death of lowland cultivars indicated a high rate of viral multiplication and a highly virulent virus strain. Furthermore, Kouassi (2005) reported that rice plants infected within 20 days after planting exhibit most of the typical RYMV symptoms, may stop growing, and eventually die. More rapid disease development in lowland than upland cultivars suggested that either the isolate is more aggressive on lowland rice in general, or that the NERICA lowland cultivars had susceptibility genes in common. Also, late development of disease symptoms in upland cultivars is suggestive of a low multiplication rate of RYMV particles, which would therefore require a longer incubation period for the virus to reach damaging levels. These results are in agreement with observations by Zouzou *et al*. (2008) and other authors (Thottappilly and Rossel, 1993; Albar *et al*., 1998; N'guessan, 1999; Mamadou, 1999; Konate *et al*., 2006). They indicated that *Oryza sativa indica* and *O. sativa japonica*, are sensitive to RYMV, with the former being highly susceptible.

Susceptible cultivars were characterised by premature death, and significantly reduced grain weight and plant height ($P \leq 0.01$ and $P \leq 0.05$), respectively (Tables 4).

Positive significant correlations were observed between mean disease score and percentage grain weight reduction ($P \leq 0.05$; $R^2=0.37$), between percentage reductions in grain weight and plant height ($P \leq 0.05$; $R^2=0.30$), and between percentage reductions in number of tillers and days to heading ($P \leq 0.1$; $R^2=0.23$) (Table 5).

TABLE 4. ANOVA for RYMV impact on yield related traits[1]

Source	d.f	Mean squares for impact			
		Plant height	Tiller #	Grain weight	Days to heading
Replicate	2	0.5[ns]	96.9[ns]	78.3[ns]	22.7[ns]
Genotype	4	174.3[*]	983.1[ns]	869.9[**]	12.7[ns]
Error	8	26.8	365.4	63.0	31.1

[ns] Non-significant at $P \leq 0.05$; [**,*] Significant at $P \leq 0.01$ and $P \leq 0.05$
[1]Cultivars that died of RYMV prior to week 4 scoring were excluded from the ANOVA

TABLE 5. Correlation of yield-related traits[1]

	% reduction in tillers #	Mean disease score	% grain weight reduction	% days to heading reduction
Mean disease score	0.27			
% Grain weight reduction	0.18	0.61[*]		
% Days to heading reduction	0.48[+]	0.13	0.05	
%Plant height reduction	-0.30	0.30	0.55[*]	-0.20

[*,+] Significant at $P \leq 0.05$ and at $P \leq 0.1$ respectively (df=13).
[1] Correlation excluded genotypes that died of RYMV

Significant correlations ($P \leq 0.05$) observed between mean disease score and percentage reduction in grain weight, and between percentage grain weight and reduction in plant height suggest the importance of grain weight and plant height in evaluating disease resistance. Zouzou *et al.* (2008) also observed a strong correlation between the mean disease score and reduction in grain weight. Similar results were also reported by Albar *et al.* (1998). These results suggest a virulent RYMV strain, and demonstrate the importance of grain weight and plant height in evaluating RYMV disease resistance.

CONCLUSION

This study sought to characterise rice cultivars' reaction to a virulent RYMV isolate in Uganda in order to recommend appropriate sources of resistance for RYMV breeding strategies in Uganda. An aggressive RYMV strain from Iganga (Eastern Uganda) was used and none of the tested resistant RYMV cultivars escaped infection or expressed an immune reaction to RYMV. Further research is recommended to characterise the pathogenicity of RYMV variants in Uganda and to determine their individual importance in rice yellow mottle epidemics. This study recommends cultivars; Gigante (lowland), NERICA 8 (upland), NERICA 11 (upland), and NERICA 13 (upland), as potential sources of resistance to RYMV. There is also an apparent need for additional sources of resistance with different genetic determinants in breeding for RYMV resistance to broaden the genetic base.

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REFERENCES

Albar, L., Lorieux, M., Ahmadi, N., Rimbault, I., Pinel, A., Sy, A., Fargette, D. and Ghesquière, A. 1998. Genetic basis and mapping of the resistance to rice yellow mottle virus. I. QTLs identification and relationship between resistance and plant morphology *Theoretical and Applied Genetics* 97:1145-1154.

Ayassi, M.H., Grainer, M., Peterschmitt, M., Chatenet, M. and Notteghem, J.L. 1995. A rapid diagnostic kit for RYMV detection. Paper presented at the 1st International Symposium on RYMV, 18-22 Sept. 1995, WARDA, Bouake, Cote d'Ivoire.

Campell, C.L. and Madden, L.V. 1990. Introduction to plant disease epidemiology. John Wiley and Sons, New York.

GenStat. 2010. Lawes Agricultural Trust. GenStat for Windows Discovery, 12th Edition. International Ltd.

International Institute for Tropical Agriculture (IITA). 1986. *Oryza glaberrima* as a source of resistance to rice yellow mottle virus. pp. 84-85. *Annual Report and Research Highlights*. IITA, Ibadan, Nigeria.

International Rice Research Institute (IRRI). 1988. Standard Evaluation System (SES) for Rice. Los Banos, Laguna, The Philippines. 3rd Edition, pp. 23.

John, V.T. and Thottappilly, G. 1987. A scoring system for rice yellow mottle virus. *International Rice Research Newsletter* 12: 26.

Kijima, Y., Sserunkuma, D. and Otsuka, K. 2006 How revolunary is the "NERICA revolution"?: Evidence from Uganda. *The Developing Economics, XLIV* 2:252 - 67.

Kijima, Y., Otsuka, K. and Sserunkuma, D. 2008. Assessing the impact of NERICA on income and poverty in central and western Uganda. *Agricultural Economics* 38:327- 337.

Konaté, G., Traoré, O., Traoré, M.D. and Fargette, D. 2006. Rice seedbeds as a source of primary infection by Rice yellow mottle virus. *European Journal of Plant Pathology* 115(2): 181-186.

Kouassi, N.K., N'Guessan, P., Albar, L., Fauquet, C.M. and Brugidou, C. 2005. Distribution and Characterization of Rice yellow mottle virus: A Threat to African Farmers . The American Phytopathological Society. *Plant Disease* 89 (2):124-133.

Lamo, J. 2010. Genetic Studies on Drought Tolerance and Grain Shattering in Rice. PhD Thesis, University of KwaZulu-Natal. pp. 205.

Mamadou, M.C. 1999. Etude de la résistance du riz (*Oryza* sp.) au virus de la panachure jaune du riz (RYMV). Thèse de Doctorat 3ème cycle. Université de Ouagadougou. 121pp.

National Agricultural Research Organisation (NARO), 2005. Responding and Contributing to the Modernisation of Agriculture through Research, Kampala, Uganda.

N'guessan, K.P. 1999. Diversité moléculaire et sérologique du virus de la panachure jaune du riz (RYMV) en Afrique et aspects épidémiologiques en Côte d'Ivoire. Thèse de l'Ecole Nationale. Supérieure Agronomique de Montpellier. 118pp.

Potrykus, I. 2003. Nutritionally enhanced rice to combat malnutrition disorders of the poor. *Nutrition Reviews* 61:6-11.

Somado, E., Guei, R. and Keya, S.O. 2008. NERICA (New Rice for Africa) - a compendium: Africa Rice Center.

Thottappilly, G. and Rossel, H.W. 1993. Evaluation of resistance to rice yellow mottle virus in Oryza species. *Indian Journal of Virology* 9: 65-73.

Zouzou, M.,Kouakou, T.H., Kone, M. and Issaka, S. 2008. Screening rice (*Oryza sativa L.*) varieties for resistance to rice yellow mottle virus. *Scientific Research and Essay* 3(9):416-424.