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The reaction of intraspecific and interspecific rice cultivars for resistance to rice yellow mottle virus disease

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ABSTRACT

A study was conducted in a green house of National Crop Resource Research Institute, Uganda to determine the reaction for resistance to rice yellow mottle virus (RYMV) in nine rice genotypes. Four were interspecific (N-1, N-4, N-6 and Naric 1) and five intraspecific (two locals K5 and K85, and three introduced WAC 116, WAC117 and Gigante). The screening of these parental materials for resistance to RYMV was done by artificial inoculation with a virulent isolate from Iganga. For among the materials tested, none was found to be immune. The results revealed four patterns of reaction to RYMV among the cultivars: three resistant (WAC 116, WAC 117 and Naric1), four moderately resistant (Nerica 6, Nerica 4, Nerica 1 and Gigante) and two susceptible (K85 and K5). In the current study, Gigante was recorded to have the severity of RYMV symptoms. Furthermore, lines WAC 116, WAC 117 and Naric1 were identified as sources of resistance to RYMV, and therefore candidates for use as parents to improve resistance in susceptible preferred local rice varieties.

Key words: Genes, inoculation, rice, screening, symptoms

INTRODUCTION

Breeding for resistance against diseases such as Rice yellow mottle virus disease is becoming an important part of many improvement programs [14]. In such programs, a plant breeder focuses part of his effort on selection and development of resistant plant lines [17]. The probability of identifying resistant lines is dependent on the availability of an effective screening methodology and an environment favorable to artificially creating the disease [15]. Screening for disease resistance is often done under controlled conditions in a screen house where both inoculated and non-inoculated plants are protected from undesirable infection [12]. Artificial inoculation of test genotypes is necessary to obtain a more uniform disease pressure than would occur under natural conditions [12]. Screening for resistance to RYMV typically uses mechanical inoculation [11], though [13] reported the potential for using insect vectors. It was reported that despite some differences, both methods screen the varieties in the same way and can be used effectively [13].

The rice improvement programme of National Crops Resources Research Institute -Uganda has recently introduced new materials including interspecific and intraspecific to broaden the genetic base of germplasm in the country.

However, the reaction of most of these materials to key rice constraints (biotic and abiotic) needs to be determined in order to fully utilize these genotypes in breeding programs. Previous studies by [8] , and [7] assessed the response of some lowland and upland rice varieties to RYMV disease and identified some sources of resistance, including Gigante as resistant and Nerica 8, Nerica 11 and Nerica13 as moderately resistant. To further broaden this resistance, the current study aimed to screen farmer-preferred local varieties (intraspecific) and new improved varieties (interspecific) for resistance to RYMV.

MATERIALS AND METHODS

Genetic Plant Material

Nine genotypes were evaluated for reaction to RYMV infection. Detailed descriptions of those materials are presented in Table 1 below. Also included was the genotype, Gigante, used as resistant check and two cultivars (IR64 and SUPA) added as checks for susceptibility.

Table 1. Characteristics of rice genotypes used in the screening

Genotype	Pedigree	Origin
WAC 116 - lowland	IRIS 251- 42131	Africa Rice
WAC 117 - lowland	IRIS 251- 42157	Africa Rice
Naric 1 - upland	IRAT 257	IITA Nigeria
Nerica 1 - upland	WAB 450-I-B-P-38-HB	Africa Rice
Nerica 4 - upland	WAB 450-I-B-P-91-HB	Africa Rice
Nerica 6 - upland	WAB 450-I-B-P-160-HB	Africa Rice
K5 – lowland	Unknown	Local- Uganda
K85 – lowland	Unknown	Local- Uganda
Gigante – lowland (R ck)	Local accession from Mozambique	Africa Rice
IR64 - lowland (S ck)	IR 18348-36-3-3	IRRI
SUPA -lowland (S ck)	Unknown	Local- Uganda

(S ck)=Susceptible check, (R ck) = Resistant check

Site

The research was conducted under an artificial environment in a green house at the National Crops Resources Research Institute (NaCRRI) at Namulonge. This Research Station is located at 0º 32" N and 32º 37" E, in the bimodal rainfall region of central Uganda. It is at an elevation of 1150 meters above sea level with an average rainfall of 1200mm/year.

Inoculum used

Inoculum of RYMV was collected from two main rice growing areas in Uganda that represent “hot-spots” for RYMV disease according to survey reports [10]. These were Iganga in Eastern Uganda, and Lira in Northern Uganda. The virulence of these isolates was confirmed by mechanically inoculating the susceptible genotypes, SUPA, K5 and K85 with the two isolates (Iganga and Lira isolates).

To prepare the inoculum one gram of infected leaf tissue was first crushed in a drop of doubly- distilled water using sterile mortars and pestles until 80% of the leaf tissue material was macerated. The resultant leaf extract was diluted 10 times by additing10 ml of doubly- distilled water. Inoculations commenced 14 days after transplanting using a mixture of carborundum powder to aid the infection. The inoculation was performed by rubbing the mixture onto the leaves from the base to the top using pieces of cotton wool. Since test inoculations conducted in both the study [8] and the current study confirmed the isolate from Iganga to be more virulent than that from Lira, this isolate was thus used in all subsequent trials, and maintained on K5 plants.

Evaluation of the reaction of rice cultivars to the Iganga-isolate of RYMV

The genotypes evaluated were raised in 10 liter plastic buckets in a split-plot design with three replications. The inoculation was considered as the main-plot and the variety as the sub-plot. The accessions IR 64, SUPA and Gigante were included to assess the disease pressure and degree of resistance. After germination the seedlings were thinned to two plants per pot. Plants were also supplied with NPK 17-17-17 at a rate of 2 gm/pot to avoid confusion between the yellowing associated with disease development and that due to malnutrition. Inoculation of test plants was done 14 days post-emergence, and repeated one week later to ensure success. Non-inoculated plants were used as controls.

Data collection

Disease scoring

Plants were inspected for any symptoms of RYMV disease, and scored at one, two, three and four weeks after inoculation. Scoring was done on individual plants, using a 1- 9 standard scale [3]. In this scale:
1= No symptoms observed.
3= Leaves green, but with sparse dots or streaks, and less than 5% reduction of height.
5= Leaves green or pale green with mottling, 6%-25% height reduction, flowering slightly delayed.
7= Leaves pale yellow or yellow, 26-75% height reduction, flowering delayed.
9= Leaves yellow or orange, more than 75% height reduction, no flowering or some plants dead.

To asses severity of the disease, a modified scale developed by Zouzou *et al.* (2008). Values from 1 to 1.5 were given a score of 1 = highly resistant, 1.6 - 4.5 were assigned a score of 3 = resistant, 4.6 - 6.5 were rated as 5 = moderately resistant, 6.6 - 8.5 as 7 = susceptible, and 8.6 - 9 as 9 = highly susceptible.

Components of the yield

Data on important agronomic traits was collected. This included the plant height (measured from the soil surface to the tip of the shoot in cm), the number of tillers per plant (recorded for each hill) and the 1000 seed weight (in gm). These data were used to assess both inoculated and non-inoculated seedlings of each variety, and thus the impact of the disease on growth of the rice. In each case, mean values were calculated and the impact of the disease was assessed using the following formula:

Impact (%) = (Ni-I) X100/Ni **[18]**

Where
Ni = mean values on the seedlings not inoculated
I= mean values on the seedlings inoculated

Data analysis

Data were subjected to analysis of variance (ANOVA) using GenStat software (14th edition) to obtain the mean squares and differences in the mean disease severity. The mean severity scores across four weeks were used to evaluate the reaction of the materials to RYMV.

RESULTS

Reactions of rice varieties to inoculation

Results on reaction of rice genotypes against rice yellow mottle virus (RYMV) disease showed a highly significant difference ($P \leq 0.001$) for resistance to RYMV among the genotypes across 4 weeks (Table 2). All the nine genotypes tested showed symptoms of rice yellow mottle virus after inoculation, expressing different levels of susceptibility and resistance.

Table 2. Mean squares for RYMV severity scores of eleven rice genotypes (8 parents plus 3 checks) over four weeks after artificial inoculation in screen house

SOV	d.f.	Week 1	Week 2	Week 3	Week 4
Rep	2	0.16	0.47	0.18	0.10
Genotype	10	3.95***	8.72***	10.82***	11.32***
Error	20	0.14	0.18	0.12	0.10

**** Significant at P ≤ 0.001*

In general, distinctive symptoms of RYMV were observed by the second week after inoculation. The symptoms observed included sparse and elongated yellow spots, mottled green or pale leaves, and pale yellow, yellow or orange leaves. At 3-4 weeks post-inoculation most genotypes had attained maximum disease scores (Table 3). Based on the severity of their symptoms and in the modified scale for RYMV disease scoring [18], the genotypes WAC 116, WAC 117 and Naric 1 displayed better resistance than did the resistant check, and were rated resistant. Interspecific lines N-6, N-4 and N-1 were moderately resistant and N-6 showed better resistance than did the check. Compared to the susceptible checks, the local intraspecific lines K5 and K85 were more highly susceptible than SUPA, but slightly better than IR64.

Table 3. Mean squares of scores for RYMV severity of eleven rice genotypes (8 parents plus 3 checks) over four weeks after artificial inoculation in screen house

Genotype	Week 1	Weeks Week 2	Week 3	Week 4	Reaction to RYMV
Parents					
WAC116	1.00a	1.00a	2.58a	3.17a	R
WAC 117	1.08a	1.58a	2.83a	3.33a	R
Naric 1	1.08a	1.67a	3.08a	3.42ab	R
N-6	1.42ab	1.67a	3.17a	3.92b	MR
N-4	1.33ab	3.00b	4.33b	4.67c	MR
N-1	1.82b	2.92b	4.25b	4.83c	MR
K85	3.67c	5.25cd	7.17c	7.50d	S
K5	3.58c	5.33cd	7.00c	7.58d	S
Res. Check					
Gigante	1.58ab	3.33b	3.83b	4.58c	MR
Susc. Checks					
SUPA	3.17c	4.75c	6.75c	7.17d	S
IR 64	3.75c	5.58d	7.25c	8.33e	HS
Grand mean	2.1	3.28	4.8	5.3	
LSD 5%	0.6	0.72	0.6	0.5	
CV%	17.2	12.9	7.4	5.8	

Means in the same column followed by same letters do not differ significantly at P = 0.05

Effect of RYMV infection on yield components

The mean squares for the effect of RYMV disease on rice performance are presented in Table 4. The results revealed highly significantly differences (P<0.001) in the means (non-inoculated plus inoculated) of the measured variables. Similarly, the effect of RYMV inoculation on yield components differed from inoculated to non-inoculated genotypes at P < 0.01. However, no significant interactions of genotype and inoculation rates were observed (P > 0.05).

Table 4. Mean squares for infection of eleven rice genotypes with RYMV and effect on yield components during the season A 2012 at the National Crops Resources Research Institute (NaCRRI)-Namulonge

SOV	d.f.	Plant height	# tillers	1000 grain weight
Inoculation (Main –plot)	1	674.56**	286.46**	91.00**
Main-plot error	2	1.23	1.25	0.23
Genotype (Sub-plot)	10	1747.24***	341.80***	113.68***
Genotype.inoc	10	31.83^{ns}	11.34^{ns}	1.60^{ns}
Sub-plot error	40	15.80	12.75	2.79

*** and *** Significant at 0.01 and 0.001 probability levels, respectively*

Table 5. Effect of RYMV disease on the components of the yield for 11 rice genotypes after artificial infection under screen-house condition during the season A 2012 at the National Crops Resources Research Institute (NaCRRI)-Namulonge

			Impact		
Varieties	Mean score at 4 wks	Resistance group	Height reduction%	Tiller # reduction%	1000 Grain Weight reduction%
WAC116	3.17a	R	2.0	4.84	4.3
WAC 117	3.33a	R	2.5	5.9	4.0
Naric 1	3.42ab	R	2.5	16.3	5.2
N-6	3.92b	MR	3.0	12.8	4.6
N-4	4.67c	MR	3.7	27.3	9.2
N- 1	4.83c	MR	2.9	23.6	8.2
K85	7.50d	S	15.8	17.9	14.3
K5	7.58d	HS	17.8	25.7	20.9
Res. check					
Gig	4.58c	MR	6.8	9.7	10.0
Susc. Checks					
SUPA	7.17d	S	12.0	30.6	15.6
IR 64	8.33e	HS	20.3	28.0	14.8

Means in the same column followed by same letters do not differ significantly at P≤ 0.05

Results presented in table 5 show the disease effect on yield components. Results showed that RMYV infection reduced plant height between 2.0 to 20.3% depending on genotype whereas tiller number per plant varied from 4.84 to 30.6 % and 1000 grain weight varied from 4.0 to 20.9% (Table 5). All the resistant (R) and moderately resistant

(MR) genotypes showed a small reduction of 1000 grain weight compared to the resistant check. Of the susceptible varieties, K5 lost 20.9% of its 1000 grain weight in contrast to the two susceptible checks: IR4 and SUPA, which lost 15.6% and 14.8%, respectively (Table 5)

Significant correlation was found between disease severity and % reduction in plant height (b = 3.45***), tiller number (b = 3.47***) and 1000 grain weight (b = 2.68**) (Table 6).

Table 6 Mean squares from analysis of variance for regression of reduction in plant height, number of tillers and 1000 grain weight on RYMV disease severity

SOV	df	Plant height reduction	Tiller number reduction	1000 grain weight reduction
Regression	1	453.74***	455.27***	271.50**
Residual	9	4.01	44.02	5.03
Total	10			
‘’b’’		3.45	3.47	2.68
R^2		0.93	0.53	0.86
S.E		2.00	6.63	2.24

***, *** significant at 0.01, 0.001 probability levels respectively*

DISCUSSION

None of the varieties tested was immune to infection by RYMV. All the genotypes developed the typical symptoms at 2-4 weeks post-inoculation which confirmed the virulence of the RYMV isolate used and the reliability of the mechanical inoculation method. Similar results were reported [7; 18] in materials tested in their studies. Similarly, [6] reported that rice plants infected within 20 days after planting exhibited most of the typical RYMV symptoms.

On the basis of the symptoms developed, genotypes were classified into four classes: three (3) were resistant (i.e., WAC 116, WAC 117 and Naric1), four (4) moderately resistant (N-6, N-4, N-1 and Gigante) and two susceptible (K85 and K5). These results showed that the intraspecific lines (*Indica* species) were highly susceptible. Similar results were reported by other authors [1; 7; 18; 16] who indicated that *Oryza sativa indica* were sensitive to RYMV. A work carried out in Uganda [9] also supports the present study and identified lines WAC116 and WAC117 suitable donors for durable resistance. Furthermore our results showed that Gigante is moderately resistance which is contrary to the results reported [7] and [2] that Gigante is resistant and highly resistant respectively. This contradictory result could be explained either by differences in aggressiveness between the isolates used, but mostly by the breakdown of resistance in Gigante because RYMV is characterized by a large number of variants which are able to break the varietal resistance in time and space [4; 5].

The effect of RYMV disease on yield components differed among varieties, as evidenced by a highly significant regression of disease score on % reduction in height, tiller number, and grain weight. This effect was not evident in the genotype x inoculation interaction in ANOVA, indicating that inherent genotype differences obscured the differential effect of the disease in ANOVA, even though the effect was strongly indicated in the regression. These results are similar to the findings [18], in which he stated that the behavior of the tested varieties is not homogeneous in relation to the disease, but varies by variety and the parameter selected. Analysis of the relationship revealed negative corrections with RYMV infection.

CONCLUSION

The highly farmer preferred rice varieties were mostly susceptible to rice yellow mottle virus. The introgression of resistance in these varieties would improve the yield. The identified sources of resistance to RYMV in this study are recommended to be used as parents for the introgression of genes for resistance into susceptible preferred local rice varieties. Furthermore, the breakdown of resistance in Gigante suggests the high genetic diversity of RYMV strains. Thus gene pyramiding could help in breeding for resistance to RYMV disease.

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