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Screening Improved Rice Varieties (*Oryza spp*) for their Resistance / Tolerance to *Rice Yellow Mottle Virus* in West Africa

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***Abstract* – Rice yellow mottle virus (RYMV) is the most important rice-infecting virus in Africa. In the present study, sixteen improved rice varieties from Africa Rice and from the Environment and Agricultural Research Institute (INERA) of Burkina Faso were screened for their reaction to RYMV under greenhouse conditions. Rice seedlings were mechanically inoculated with anon-resistance-breaking but highly severe RYMV isolate. Screening was conducted by visual symptom scoring and virus-assessment through ELISA tests 31 dpi. The response to RYMV of 16 rice accessions was assessed. Disease incidence and severity were recorded. According to differences in these measured traits control cultivars IR 64, Azucena and Gigante were proved to be the most susceptible, tolerant and resistant ones, respectively. The result showed that five of the thirteen tested rice genotypes, were moderately to resistant to RYMV. The others eight were susceptible. The results suggested that rice germplasms from Africa Rice and INERA contain valuable germplasms resistant to RYMV, and the screened germplasms in the present study could provide promising resistance sources for rice breeding. These resistant and moderate resistant genotypes could be used by farmers in cultivation under integrated production systems and by breeders in developing new rice hybrid resistant genotypes to RYMV.**

***Keywords* – Rice Yellow Mottle Virus, Resistant, ELISA, Symptoms.**

I. INTRODUCTION

As one of the most important diseases affecting rice in Africa, yellow mottle disease has become the most serious and prevalent diseases in West Africa in recent years. The causal agent is Rice yellow mottle virus (RYMV) of the plant virus genus *Sobemovirus* with five open reading frames (ORF) [1], [2]. RYMV was first detected in 1966 at Kisumu in Western Kenya near Lake Victoria [3]. It has since been observed and detected in almost all rice-producing countries of sub-Saharan Africa [4].

Symptoms induced by the virus are highly variable including leaf mottling and yellowing, stunting, reduced tillering, non-uniform flowering, and plant death. Disease severity depends on the rice genotype, the virus strain, the age of a plant at infection, and the climatic factors [3]. Today, six strains have been identified with specific geographical adaptations. Three strains, namely S1, S2, and S3, are found in West and Central Africa whereas S4, S5, S6 are present in East and Southern Africa [5],[6].

RYMV has a narrow host range restricted to the two cultivated rice species *Oryza sativa* and *O. glaberrima*, the wild rice species *O. longistaminata* and *O. barthii*, and a few other wild Poaceae species [3].Field studies have revealed the significant role of rice nurseries in the propagation of RYMV [7]. Movement of the virus over short distances by mammals has been reported [8] [9]. RYMV is transmitted by beetle species of the family Chrysomel idae [3]. Biological tests indicated that RYMV is not transmitted through seeds of rice [3], [10], [11], [12] and of wild host species [13].Crop damage by RYMV can be devastating causing variable yield losses that may reach 100% depending on the rice genotypes, infectious strain, stage of infection and environment [14], [15].

Management of RYMV is difficult and the common control practices are based on reducing vector population mainly by pesticides or physical barriers. Due to the large populations of insect vectors vector seclusion is not an ideal way of controlling the spread and damage induced by RYMV. Hence, development of genetic resistance in the rice host is the best solution for any virus problem such as RYMV, since it requires no chemical input and/or plant seclusion and may be stable and long-lasting. Current investigations have revealed three types of resistance to RYMV: partial natural resistance, high natural resistance, and resistance obtained through genetic transformation [16]. High resistance is associated with lack of symptom development, undetectable virus content and blockage of virus movement [17]. This resistance was reported in two *O. sativa indica* varieties and a few *O. glaberrima* of the Tog series [18], [19]. Partial resistance, characterized by a delay in virus accumulation and in symptom development is present in rice *Oryza japonica* cultivar (cv.) Azucena and a few other japonica cultivars [20]. Control of the disease through the development of transgenic plants has been investigated and some transgenic lines with a partial

level of RYMV resistance have been obtained [21]. Up to now, three resistance gene namely RYMV1, RYMV2 and RYMV3 have been identified ([18], [22], [23].

Unfortunately, there have been reports of the natural occurrence and prevalence of resistance-breaking RYMV isolates in West Africa [7], [14] and of the emergence of virulent isolates after serial passages in resistant cultivars [24]. The sources of resistance to RYMV are still limited, although extensive efforts in evaluating existing rice germplasm sources may lead to the discovery of additional resistance sources. The purpose of this study was to identify rice varieties displaying disease tolerance or resistance in West Africa. Results from these studies will provide essential information to initiate a breeding program for resistance or tolerance to RYMV in rice.

II. Materials and Methods

A. RYMV Source and Propagation

A non-resistance-breaking RYMV isolate belonging to S1 strain, highly aggressive, from Burkina Faso [25] was used as virus source for mechanical inoculation. The virus was propagated and maintained in the highly susceptible rice variety IR 64 plants.

B. Plant material

Sixteen different rice genotypes used in this study were obtained from Africa Rice Center (formerly WARDA) and from the germplasm collection of the Environment and Agricultural Research Institute (INERA), Burkina Faso (Table 1). Twenty seeds of each genotype were sown in small clay pots filled with soil under greenhouse conditions. At 2-leaf stage the rice seedlings were transplanted to plastic pots (6 seedlings per pot). Two pots were used per variety. The rest of twenty seedlings (8 in total) was used as the non-inoculated control.

Table 1. Background information of rice genotypes tested against RYMV

Accession	Pedigree	Parents	Source	Species or subspecies*
Azucena	Traditional Landrace	Traditional Landrace	Philippines	Japonica
FKR 19	TOX 728-1	Mashuri/IET 1444	WARDA	*sativa*
FKR 2	Gambiaka	Traditional landrace	Burkina	Indica
FKR 28	ITA123 (FKR 28)	Mut. OS6	WARDA	Japonica
FKR 45N	WAB880-1-38-20-17-P Hb (Nerica 12)	CG14/WAB56-50	WARDA	Interspecific
FKR 50	4416 XIR1523-680-3	4416/IR1523-680-3	Burkina	*sativa*
FKR 58N	(Nerica-L 60)	IR64/TOG5681/4*IR64	WARDA	Interspecific
FKR 62N	WAS 122-IDSA-1-WAS-6-1 (Nerica-L 19)	TOG5681/3*IR64	WARDA	Interspecific
Gigante	Traditional Landrace	Traditional Landrace	Mozambique	Indica
IR 47	IR4570-83-3-3	-	IRRI	Indica
IR 64	IR 18348-36-3-3	-	IRRI	Indica
NIL 130	IR64/Gigante (BC3Fn)	IR64/Gigante	WARDA	Indica
NIL 16	SAHELIKA/Gigante (BC3Fn)	Sahelika/Gigante	WARDA	Indica
NIL 2	FRK28/Gigante/FKR28/FKR28/FKR28 BC3Fn	FKR28/Gigante	WARDA	Indica
NIL 54	IR47/Gigante (BC3Fn)	FKR28/Gigante	WARDA	Indica
WAB 2098-	WAB2098-WAC1-FKR2 (IRGC 96892 Gambia)	Nerical-20/Nerica-l41	WARDA	Interspecific

**interspecific rice genotypes were obtained from crosses between Oryza indica x O.glaberrima species*

C. Mechanical Inoculation

RYMV infected leaves of the susceptible rice cv. IR 64 were collected and homogenized in 0.01 M phosphate buffer pH 7.0 at the ratio of 1:10 (w/v). Carborundum (600 mesh) was added to the extract to aid the penetration of the virus into leaf tissues during mechanical inoculation. The extract was rubbed onto the leaves of 2-week-old seedlings which were subsequently rinsed with distilled water. Non-inoculated plants of each test genotype were maintained as control.

D. Experimental Design

Twenty plants per variety were used for the experiment. Among these seedlings, twelve per variety were inoculated as described above. All plants were grown in 1-litre plastic buckets, maintained in an insect-proof greenhouse at 25 to 30°C and 80 to 90% relative humidity, and observed for symptom development. Rice accessions were compared to Cvs IR64, Azucena and Gigante, which showed, susceptibility, partial and high resistance to RYMV, respectively. Because most infected IR 64 plants started drying 3 weeks postinoculation, leaves were collected at 21 days postinoculation (dpi). The experiment was repeated two times. Leaves from the same variety were pooled and assayed by serology for virus presence.

E. Serological assay

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to investigate the presence of virus in leaf samples [26]. A polyclonal antibody that reacts strongly and similarly with all the RYMV isolates of West and Central Africa was used as the coating antibody [27]. The same antibody was coupled to alkaline phosphatase and used as conjugate. All buffer system and incubation times were as previously described [14]. For detection of RYMV in the samples, analyses were done directly on extracts obtained after grinding the leaves (1 g in 10 ml of buffer) and centrifugation at 8,000 × g for 10 min. The mean absorbance value (A_{405} nm) from healthy controls plus three times the standard deviation was taken as the negative-positive threshold.

F. Observations and Measurements

The measured parameters were disease incidence and severity for the different varieties.

Severity. Appearance of symptoms and disease progress was monitored for each inoculated genotype from 2 to 31 days post inoculation (dpi). A slightly modified standard evaluation system based in the one described by [14] was used to evaluate the severity of RYMV symptoms, as following : highly resistant (HR) for score 1 (no symptoms with 0-10% infection), resistant (R) for score 3 (sparse dots or streaks with 11-20% infection), moderately resistant (MR) for score 5 (mottling with 21-30% infection), susceptible (S) for score 7 (yellowing and stunting with 31-85 % infection), and highly susceptible (HS) for score 9 (necrosis and sometimes plant death with >85 infection).

Incidence. Incidence was calculated using the following formula:

$$I\ (\%) = \frac{PA \times 100}{PT}$$

Where I: disease incidence; PA: number of infected or dead plants (a plant was considered as infected as soon as a visible symptom was observed); PT: total number of plants inoculated.

G. Data Analysis

The data recorded for each of the varieties were used to detect the resistant, tolerant, or susceptible varieties, based on the 1-9 disease rating scale and ELISA assay. Because disease incidence data are not suitable for ANOVA analysis, data were log transformed to meet the assumptions for that analysis. Mean disease incidence in rice genotypes were compared by Analysis of variance (ANOVA). Analyses were performed using the statistical software as in [28]. Varieties were compared for disease incidence and severity.

III. Results

A large majority of the sixteen accessions tested, expressed pronounced symptoms after inoculation (Fig. 1). Indeed, 75 % of the tested accessions presented symptom score superior or equal to 5. Moreover, a significant amount of virus was detected in 93.75 % of the 16 accessions tested by ELISA, 30 dpi.

Results on the reaction of rice germplasm, against *Rice yellow mottle virus* (RYMV) under controlled conditions are given in Table 2. Fifteen of 16 genotypes showed systemic symptoms of RYMV including sparse dots, mosaic, mottling, necrosis, yellowing and death of plants (Table 2).Individual plants of FKR 45N, FKR 50, IR 64, FKR 2, FKR 28, FKR 58N, NIL 2, FKR 19, FKR 62Nshowed mosaic, mottle, necrosis, yellowing and death of plants, and symptoms developed at 9 dpi while other genotypes exhibited symptoms between 11 and 25 dpi.All of these nine genotypes exhibited an important RYMV infection on the basis of 1-9 disease rating scale with relatively high titre (> 1.0; data not showed) detection in the upper symptomatic leaves, so considered all of these susceptible to RYMV Burkinabe isolate. Similarly, on the basis of disease rating scale (1-9) and ELISA tests, only three genotypes IR 47, NIL 54, and Azucena, the tolerant control, were grouped as moderate resistant.

On the other hand, the three genotypes, NIL 16, NIL 130 and WAB 2098 have manifested sparse symptom as well as RYMV detection in relatively low titer (< 0.18, data not showed) in the upper leaves and therefore catalogued as resistant against RYMV. Similarly, the control cv. Gigante did not manifest any symptom based on both disease rating scale and ELISA tests, confirming its high resistance (Table 2).

According to disease incidence, substantial variability was found for the 16 varieties tested (Tables 3). Disease incidence varied from 0 to 100%. The high susceptibility of the cv. IR 64 was confirmed, as it obtained the highest incidence score, reaching 100%. However, some varieties seemed just as susceptible, such as varieties FKR 19, FKR 62N, FKR 2, FKR 28, FKR 58N, FKR 45N, FKR 19 and NIL 2 with incidence score reaching between 66.67 and 83.33 %. Azucena, chosen as the tolerant control, displayed relative low incidence (25 %); its tolerance was therefore confirmed.

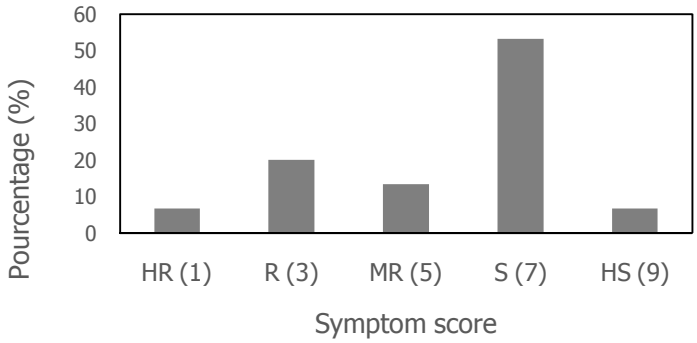

Fig.1. Population distribution according to their response to virus inoculation of 16 rice cultivars assessed 31 dpi by their symptom intensity using the 1-9 standard evaluation system: HR (1) Highly resistant, R (3) Resistant, MR (5) Moderately resistant, S (7) Susceptible, HS (9) Highly susceptible

Table 2. Type of symptoms and incidence of RYMV disease under greenhouse

Varieties	Mean number of plants positive/total plants tested	Incidence (%)
IR 64	12/12	100.00a
FKR 19	9/12	75.00c
FKR 62N	10/12	83.33b
NIL 2	9/12	75.00c
IR 47	2/12	16.67ef
FKR 28	9/12	75.00c
FKR 2	9/12	75.00c
FKR 58N	8/12	66.67cd
FKR 45N	10/12	83.33b
FKR 50	10/12	83.33b
Azucena	3/12	25.00e
NIL 54	3/12	25.00e
NIL 16	1/12	8.33g
NIL 130	1/12	8.33g
WAB 2098*	1/12	8.33g
Gigante	0/12	0.00h

Table 3. Symptom scoring and virus assessment in ELISA test of the response of 10 cultivars to inoculation with RYMV isolate

Varieties	Days to symptom appearance	Mean of symptom severity	Type of symptoms observed	ELISA	Reaction to RYMV
IR 64	6	9.00	Plant death, yellowing	+++	HS
FKR 19	9	6.80	Mottling	+++	S
FKR 62N	9	6.40	Mottling, necrotic	+++	S
NIL 2	8	6.40	Mottling	+++	S
IR 47	11	5.24	Yellowing	++	MR
FKR 28	6	7.40	Yellowing	+++	S
FKR 2	7	7.10	Yellowing	+++	S
FKR 58N	7	6.8	Mottling, yellowing	+++	S
FKR 45N	6	7.00	Chlorotic	+++	S
FKR 50	6	7.62	Mottling	+++	S
Azucena	11	5.00	Mottling	++	MR
NIL 54	11	480	Mosaic	++	MR
NIL 16	22	2.80	sparse dot	+	R
NIL 130	25	2.50	sparse dot	+	R
WAB 2098	12	3.00	Streaks	+	R
Gigante	0	1.00	no symptoms	-	HR

Resistance: HR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible
Serodiagnosis: (-) = negative reaction; (+) = weak reaction; (++) = weak, but clear reaction; (+++) = strong reaction

IV. Discussion

This study aimed at identifying sources of natural resistance to RYMV in improved rice varieties in West Africa. Because RYMV epidemic occurs randomly, screening for resistance under field condition is difficult. Thus 16 rice genotypes were evaluated for resistance to RYMV by mechanical inoculation under greenhouse conditions. Inoculated rice lines were evaluated based on the observational symptom severity during the 4-5 weeks after inoculation. In order to evaluate virus content and to detect RYMV in asymptomatic plants, an ELISA test was performed at 31 dpi. No asymptomatic line was identified in this study. ELISA result is confirmed the visual rating based on SES scale. Indeed, varieties that showed conspicuous yellow mottle symptoms in the highly susceptible group contained high virus titres. This result is consistent with those mentioned by [29].

The genotypes were classified into five reaction groups based upon % infected plants and ELISA test. These were: highly resistant, resistant, moderately resistant, susceptible and highly susceptible. The proportions of rice genotypes falling in each category were6.25, 18.75, 12.50, 56.25 and 6.25 respectively. It is apparent from the above results that the majority of the tested genotypes were susceptible to RYMV infection. This shows the overall susceptibility of the rice germplasm to RYMV in West Africa and underlines the threat posed by the virus. Thus, the screening of rice germplasm revealed that immunity/resistance against RYMV disease is scarce in the germplasm. Such scarcity of resistance has earlier been reported [14], [15], [29], [30]. This scarcity of resistance

in rice genotypes against RYMV disease calls for introduction of resistance foreign lines/cultivars from foreign international sources and breeding their resistant into the existing commercial cultivars through conventional breeding procedures or development of resistant sources through mutation breeding using radiation or chemical mutagens.

Although, no accessions tested in this screen showed strong resistance to RYMV infection, there appeared to be some promising levels of resistance/tolerance to RYMV infestation even among cultivars that could be used in breeding programs. Indeed, two lines (IR 47, NIL 54) and three ones (NIL 16, NIL 130, WAB 2098) exhibited moderately and resistant reaction respectively. These observations confirmed the resistance/tolerance status of NIL 130 as reported by [31], [35]. WAB 2098 was obtained from the cross of two NERICA (New Rice for Africa)varieties which shared in common *Oryza glaberrima* Tog 5681 (African rice) as parents. Tog 5681 displays the resistant gene *RYMV1-3*. So, an explanation would be that the observed tolerance of WAB 2098 is inherited from this resistant parent, *O. glaberrima*. Indeed, African rice is known to be a rich reservoir of genes of interest for the control of many diseases including RYMV[32], [33].Interestingly, these five varieties could be an important component in integrated RYMV disease management.

Some similar studies carried out simultaneously in 3 West African countries (Benin, Burkina Faso, and Côte d'Ivoire), using the same varieties, showed different performances for these varieties, which could vary from one country to another. For example, NIL 2 was susceptible in Burkina Faso (This study), resistant in Benin [34], [35] and highly resistant in Cote d'Ivoire [31]. Several other examples of differential responses to RYMV infection are suspected to be isolate dependent[4], [36], [37]. This discrepancy suggests that pathogen pressure was not the same everywhere; in addition, strong genetic variability may exist in the virus in a same country [14], [27], with possible variability in virulence and aggressiveness [7], [14]. Thus, the responses to RYMV depends on the genotype, screening conditions (plant age, RYMV isolate, climatic conditions), the evaluation method and the ecology [15], [30], [35], [38]. Mogga et al. [30] and Oludare et al. [38] showed that upland genotypes are mostly resistant to RYMV while those of lowland and irrigated ecologies ones are mostly susceptible. Altogether, the concept of a susceptible, tolerant or resistant variety therefore has to be considered with considerable caution because of different environmental conditions. These environmental conditions affect varietal performance, hence the reaction of plants to the virus.

V. Conclusion

The results presented here confirmed the results of previous studies [14], [15], [30], indicating that rice is susceptible to RYMV and typical symptoms of rice yellow mottle disease were produced. It views of these results, it would also be advisable to undertake a larger scale search for tolerance/resistance to RYMV because no clear highly resistant accession was identified in this study. The present study also demonstrated the existence of resistance sources (NIL 16, NIL 130, WAB 2098) against RYMV within West African rice lines that could potentially be used by farmers in cultivation under integrated production systems and by breeders in developing new rice hybrid resistant genotypes.

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