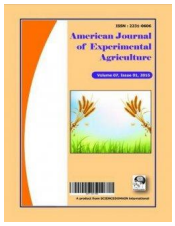

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Screening of Rice Accessions For Resistance to Rice Yellow Mottle Virus

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VSET and MDA made the collection of rice accessions. Author VSET conducted the experiments. Authors VEG and SKO designed the study. Authors OT and VSET drafted the manuscript. Authors OT and VSET made the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Rice yellow mottle virus (RYMV) is responsible for the most damaging virus disease of rice in Africa. The objective of this study was to assess the reaction of rice accessions to RYMV, for better control of the virus. Rice accessions including landraces and collections from research institutes were collected from 2010 to 2013 in Burkina Faso and Ghana. Two viral inoculums composed of non-resistance-breaking RYMV isolates (inoculum-1) on the one hand and of resistance-breaking isolates (inoculum-2) on the other hand were used for the screening experiments in the greenhouse. A subset of rice accessions were exposed to field isolates under field conditions of virus transmission. Experimental designs were randomized complete blocks with three replicates. Of 117 rice accessions challenged with inoculum-1, 69.2% were susceptible to RYMV and expressed disease symptoms between 10 and 13 days post-inoculation (DPI). Partial resistance

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was found in 30.7% of the accessions which expressed symptoms between 15 and 17 DPI. When inoculum-2 was used, the proportion of susceptible accessions was higher (84.6%) and symptoms appeared earlier (7-10 DPI). High resistance was not found in any accession. Leaf virus content allowed a clear distinction between susceptible, partially resistant and highly resistant accessions. Altogether, these results indicated that the choice of virus isolates is critical when screening rice germplasm for resistance to RYMV. Non-resistance-breaking isolates should be used for successful detection of resistance in screened accessions.

Keywords: Rice germplasm collection; landrace; farmers' preferred varieties; leaf virus content.

1. INTRODUCTION

Rice is a major crop in West Africa with a production estimated at 11.94 million tons in 2012, which represented 45.8% of the whole African production [1]. The necessity for disease management in rice has come to the foreground of crop production since the green revolution. Several damaging outbreaks occurred with all the main virus diseases of rice including rice yellow mottle, white leaf ('hoja blanca'), rice grassy stunt and rice ragged stunt [2]. Rice yellow mottle disease is endemic to Africa where it is confined. It is induced by Rice yellow mottle virus (RYMV) which is considered as the most damaging rice pathogen on the continent. Yield losses often vary from 25 to 100% [3]. RYMV is easily transmitted mechanically but field dissemination is done by a number of vectors among which beetles are likely the most important.

The main control methods of rice yellow mottle disease include the use of resistant genotypes and application of insecticides to control the vector of RYMV. The use of pesticides in modern agriculture has contributed to improved world food supply through the achievement of better plant growth and yield. However, pesticides and particularly insecticides are often hazardous and their indiscriminate use for controlling pests in crops has been associated with several drawbacks such as resurgence of resistant insect populations, poisoning of farmers and environmental pollution [4]. Pesticides, therefore, need to be used in a more responsible manner in order to preserve the environment [5].

Host plant resistance to biotic stresses can play a pivotal role in crop protection [6,7]. Use of resistant varieties has been considered as an attractive and effective means to control diseases. It requires no additional cost other than that of seeds of resistant genotypes and it is environmentally safe [8]. Moreover, unlike other disease management technologies, resistant varieties can easily be adopted by farmers and widely disseminated. These considerations are particularly applicable to the context of rice growing systems in Africa where almost all farmers are smallholders.

Many rice accessions including *O. sativa*, *O. glaberrima* and wild species *O. longistaminata* and *O. barthii* were screened at the International Institute of Tropical Agriculture and at Africa Rice Center (AfricaRice) using either mechanical inoculation of the virus or direct field exposure [9,10,11]. Several national research institutions have also screened local accessions for resistance to RYMV [12,13,14,15]. Consistent results were not always found between these studies. As shown in Table 1, consistency in varietal reaction between authors was observed in a few cases such as the high resistance in Gigante, Bekarosaka, Tog5672, Tog5674, Tog5681 and Tog7291. By contrast, conflicting reactions were observed in several cases. For instance, rice accessions such as Moroberekan and OS6 were found highly resistant or even immune in some studies [10,15] but only partially resistant in others [11]. Coulibaly et al. [12] reported OS6 as a susceptible accession. More strikingly, cv. Moroberekan showed different reactions when it was grown under irrigated versus rainfed conditions [15]. Inconstancies in reactions to RYMV across accessions likely reflect the fact that RYMV isolates differed. Therefore, accessions reported as resistant in a given area were susceptible elsewhere.

The genetic basis of resistance to RYMV found in most resistant rice accessions was determined. Partial resistance found in cv. Azucena is polygenic [16]. High resistance which is monogenic and recessive is conferred by two genes RYMV1 and RYMV2. Four alleles of RYMV1 gene have been identified in resistant rice accessions [17]. These are rymv1-2 in cv. Gigante or cv. Bekarosaka, rymv1-3 in cv. Tog5681, rymv1-4 in cv. Tog5672 and rymv1-5, in Tog5674. RYMV2 was found in cv Tog7291 but its allelic pattern remained unknown.

Table 1. Some reactions of rice accessions to rice yellow mottle virus

Resistance level	Accession	References
Immune	TOG 5672	[12]
	ExDoko, Tob5689, Tob5701, Tob7382, Tog5379, Tog5674, Tog5681, Tog7235, Tog7291 Tol12, Tol268	[11]
	ITA235, ITA257, IDSA6, FAROX299, IAC164, Itame Nembeika, Azi, Toubabou, Gnonkonsoka, Moroberekan, OS6	[10,15]
High resistance	Gigante, Bekarosaka, Tog5681, Tog7235, Tog7291, Tog5675, Tog5674, Tog7226, Tog7238, VL6, VL123	[12,14,18,19]
	IRAT156, ITA 315, IR50, IR56, IRAT170, ITA128, IRAT161, IRAT104, ITA305, ITA303, BPT1235, W1263, GEB24, PY2, Kalinga2, Kannagi, IR9830-26-3-3	[10]
Partial resistance	IRAT104, Moroberekan, FKR33	[12]
	OS6, Moroberekan, LAC23, CT19, IRAT110. ITA-235, ITA257, ITA303, ITA305, ITA307, ITA313, ITA315	[11]
	MRC603-303. Ratna, Tnau175, TKM9, MTU15, KAU I675. Kaohsiung-Senyu, IR29, IR46, PVRI, UPR254-21-1,IR9802-31-2,IITA, FR77068-2, IR 19473-461-2-3-3-2	[10]

RYMV isolates are known to display a high diversity according to their geographical and ecological origins [20,21]. In West Africa alone, three major RYMV strains, S1, S2 and Sa, were found based on the coat protein variability. Another layer of complexity is that each strain exhibits different pathogenic features. The occurrence of resistance-breaking isolates [20] is a serious threat for the durability of resistances in the field. Crosses between a few *O. glaberrima* accessions have indicated the existence of additional potential resistance genes [22].

In this study we assessed the reaction of rice accessions collected from Burkina Faso and Ghana using all major RYMV strains occurring in West Africa.

2. MATERIALS AND METHODS

2.1 Germplasm Collection

Rice varieties were collected from national research systems including the Institute of Environment and Agricultural Research (INERA) in Burkina Faso and the Crop Research Institute of Kumasi in Ghana. Farmer's landraces were also collected mainly from lowland rice cultivation areas in different localities of the western region of Burkina Faso and from the Volta region of Ghana. Germplasm collected from INERA included a subset of ten top farmers' preferred rice varieties identified from a participatory rural appraisal. Collected rice accessions were stored in a cold room at 10-15°C.

2.2 Sources of Inoculum

All virus isolates used in the experiments originated from West African countries, namely Burkina Faso, Ghana, Mali and Niger. They were part of INERA plant virus collection maintained at the Laboratory of Plant Virology and Biotechnology. In a first experiment, 11 non-resistance breaking isolates (nRB) were included in virus inoculum-1. Six of these isolates were of strain S1 and the remaining isolates belonged to strain S2. Leaf samples infected by corresponding isolates were mixed at equal weights. In a second experiment, another inoculum (virus inoculum-2) was made of nine resistance-breaking (RB) isolates of RYMV strains S1 (4 isolates), S2 (4 isolates) and Sa (1 isolate). A third experiment involved 20 RYMV field isolates collected from main rice cultivation perimeters in Burkina Faso, distinct from those used in the two previous experiments. These field isolates were used singly to screen 23 rice accessions.

2.3 Plant Inoculation and Data Analysis

All virus isolates were first propagated in susceptible rice cultivar BG90-2 using mechanical inoculation in an insect-proof greenhouse. Infected leaf samples were ground with sterile pestles and mortars in 0.05 M potassium phosphate buffer, pH 7.0 at the ratio of 1 g of leaf for 10 ml of buffer. Carborundum (600 mesh) was added to the extracts which were subsequently rubbed onto the leaves of rice seedlings 21 days post-germination (DPG).

Leaves from plants infected with each isolate that showed clear visible symptoms were harvested two weeks post-inoculation and used as inoculum sources.

Oryza sativa cv.BG90-2 was used as susceptible control along with resistant rice genotypes including *O. sativa japonica* cv. Azucena as partial resistance control. The high resistance controls were made of *O. sativa indica* cultivars Gigante and Bekarosaka, as well as *O. glaberima* cultivars Tog5681, Tog5672 and Tog7291. All rice accessions were screened in the greenhouse by mechanically inoculating the virus to five plants of each accession. Virus inoculation was done 21 days post germination (DPG). Symptoms development was monitored for 45 days post-inoculation (DPI). Leaves of inoculated plants were collected at 14 DPI for leaf virus content assessment. Leaf virus content was assessed in leaf extracts by double antibody sandwich Enzyme-linked immunosorbent assays (DAS-ELISA) using a broad spectrum polyclonal antibody [23].

Data were analyzed using Statistica software ver.6 (http://www.statsoft.com). One-way analysis of variance (ANOVA) was used to test differences in the mean number of days for symptom appearance between accessions. Data from each accession was compared to the control BG90-2 using Dunnett's test [24]. ANOVA was also used to test for significant differences between leaf virus contents in rice accessions.

3. RESULTS

3.1 Rice Accessions Collected

In total, 125 rice accessions were collected from 16 locations in Burkina Faso and Ghana. Accessions were predominantly from research institutes (46 accessions from INERA including the eight checks and 45 accessions from CSIR-CRI). Most of these accessions were released after varietal improvement which did not consider rice mottle disease management. Thus, apart from varieties used as checks, the accessions had never been screened for resistance to the RYMV disease. Out of 34 accessions collected from farmers in both countries, 21 were landraces that belonged to *O. glaberrima* species and 13 were of *O. sativa* species.

3.2 Reactions of Rice Accessions to Inoculums of RYMV Isolates

The reactions of rice accessions to the RYMV isolates are summarized in Table 2. Days to symptom appearance varied among the accessions inoculated with virus inoculum-1. Symptoms on the leaves of the susceptible control BG90-2 were observed as early as 10 DPI and all inoculated plants showed symptoms at 13 DPI. Partially resistant control Azucena showed symptoms between 15 and 17 DPI. When using nRB isolate inoculum, no symptom was observed in highly resistant rice accessions until 45 DPI when the experiment was terminated.

Analysis of variance of the number of days for symptom appearance indicated a significant rice accession effect ($F=45.38$; $P<0.001$, $df=118$), which confirmed differences in reactions among the rice accessions. Post-hoc analysis using Dunnett's test and taking BG90-2 as control group indicated that, apart from accessions used as checks, all accessions could be grouped in two categories. Accessions which did not differ significantly from BG90-2 were susceptible to RYMV. They represented the largest group (69.2%). They were assigned to the susceptible (S) group. Varieties preferred by most farmers belonged to this group. The second group (30.7%) included accessions which showed symptoms significantly later than BG90-2. Accessions in this category belonged to the partially resistant (PR) phenotype. Only two farmers' preferred varieties (TS2 and FKR28) exhibited the PR phenotype.

Reactions of rice accessions after inoculation with RYMV inoculum-2 resulted in the expression of symptoms in BG90-2 earlier than with inoculum-1. Symptoms appeared in some plants after 7 DPI and all plants were symptomatic at 10 DPI. By contrast, inoculated plants of the partially resistant accession Azucena showed symptoms between 14 and 18 DPI. Inoculated plants of all highly resistant checks, apart from Tog5672, were symptomatic at 17 DPI.

Symptoms were visible on plants of highly resistant accessions Bekarosaka, Gigante and Tog5681 between 13 and 17 DPI. By contrast, in Tog5674 and Tog7291, inoculated plants showed symptoms between 8 and 9 DPI. Differences in reactions of rice accessions following inoculation with RYMV isolates inoculum-2 were found significant in one-way ANOVA ($F=42.03$; $P<0.001$; $df=123$). As with virus inoculum-1, Dunnett's post-hoc test resulted in three distinct groupings of accessions. Susceptible accessions formed the largest group (84.6%) while partially resistant accessions

represented only 15.4%. As with inoculum-1, no accession showed high resistance phenotype.

As shown in Fig. 1, the proportion of resistant accessions identified after inoculation was significantly less (χ^2 =7.43; P=0.006) when virus inoculum-1 was used than with inoculum-2. Up to 14.4% of accessions identified as partially resistant following inoculation with virus inoculum-1 were susceptible after inoculation with inoculum-2.

3.3 Virus Accumulation in Inoculated Plants

Assessment of the levels of virus multiplication in plants, expressed as optical densities, indicated that rice accessions could be grouped based on the leaf virus content. Following inoculation with virus inoculum-1, three groups of accessions were distinguished (Fig. 2A). The first group consisted of all accessions identified as highly resistant (HR) when assessing the time for symptom appearance. No virus could be detected in these accessions because they reacted as the healthy control leaf extract giving a background reaction only. A second group included the susceptible check BG90-2 and accessions of the S phenotype. As indicated by the high absorbance values, accessions of the second group supported high virus multiplication. The third group included accessions of the PR-phenotype and Azucena. In this group, ELISA reactions indicated relatively low virus titres. There was a large variation in reactions of PR pathotypes as indicated by the higher standard deviation.

Assessment of virus titre in leaf extracts infected by virus inoculum-2 resulted in a different pattern (Fig. 2B). High virus titre was found in Tog5672 as well as in another group of accessions including BG90-2, Tog7291, Tog5674 and all S-phenotype accessions. Lower virus titre was obtained from PR-phenotype accessions as well as Tog5681, Gigante, Bekarosaka and Azucena.

3.4 Reactions of Rice Accessions to Field RYMV Isolates

Following inoculation with individual field RYMV isolates that had not been characterized, the susceptible check BG90-2 developed symptoms with all virus isolates (Table 2). Partially resistant check cv. Azucena displayed PR phenotype with almost all virus isolates and only isolate VII was able to overcome its partial resistance. Similarly, two accessions (Gh1577 and FKR33) showed the PR phenotype with almost all isolates but were susceptible to isolate III. The highly resistant check cv. Gigante remained symptomless after inoculation with six of the 10 virus isolates, therefore displaying a high resistant (HR) phenotype. However, it developed symptoms similarly to cv. BG90-2 to four isolates, indicating S phenotype. Consequently, the six isolates which could not overcome resistance in Gigante were non-resistance breaking isolates. Alternatively, the four other isolates which induced symptoms on Gigante were RB isolates. Half of the 20 rice accessions tested showed the PR phenotype, regardless of the isolate used. The remaining accessions displayed the S phenotype in most cases, particularly with virus isolates that were able to overcome resistance in cv. Gigante. With non-resistance breaking isolates, all accessions except cv. CG14 showed resistance (PR phenotype).

4. DISCUSSION

Part of the rice germplasm (16.8%) collected during the surveys consisted of accessions of the African rice *O. glaberrima* held by farmers. This indicates that some farmers continue to grow *O. glaberrima* varieties despite the fact that most rice varieties grown in West Africa belong to *O. Sativa* species. The African rice has a low yield potential compared to its Asian counterpart, but it is used by some communities for food, rituals and herbal medicine [25,26]. Cultivation of *O. glaberrima* by smallholder farmers may also be due its better adaptation to stresses caused by pests, diseases and abiotic constraints [27].

Screening of the collected rice accessions for resistance to RYMV indicated that virus-host interactions strongly depended on the virus isolates. Up to 45.9% of rice accessions expressed the PR phenotype with virus inoculum-1. They were found to be susceptible when inoculum-2 was used. Consequently, virus inoculum-1, composed of non-resistance breaking isolates, was more effective in the identification of resistance in rice accessions. Virus inoculum-2 was able to overcome resistance in highly resistant accessions used as controls. However, some of these accessions displayed partial resistance even though the high resistance was no longer effective. These results suggest that the mechanisms for overcoming partial and high resistance are distinct. Previous studies clearly indicated that high resistance and partial resistance have different genetic bases

[18,28]. Therefore, the ability of virus inoculum-2 to overcome the partial resistance in some of the PR accessions to inoculum-1 was expected. Possibly, inoculum-2 also included virulent isolates distinct from those which overcame the high resistance conferred by the RYMV1 gene. This was apparent in the breakdown of resistance conferred by RYMV2 gene in Tog7291.

Altogether, screening rice accessions for resistance to RYMV indicated that most rice accessions were susceptible to RYMV, which is consistent with previous studies [12,15]. No new highly resistant rice accession was identified in this study even in *O. glaberrima* species in which most sources of resistance to RYMV have been found. Additional high resistance genes are to yet to be found in rice, particularly the African rice [22]. Therefore, screening rice germplasm for resistance to disease, particularly RYMV, needs to be continued in order to identify suitable resistance sources. Efforts are continuously to collect and preserve rice germplasm at both national and international levels. More than 200,000 rice accessions are reported in 40 National and International Rice Gene Banks [29]. Most accessions in these collections have not been screened for disease resistance. The present study contributed to the characterization of national rice collections to identify partially resistant accessions which can be used in breeding programmes for rice yellow mottle disease management. Conflicting results attributed to the effect of environment have been frequently reported in screening experiments conducted for the identification of resistance sources to RYMV [15,30]. Indeed, the environmental conditions may have some effects on the virus-host interactions but our results suggest that most screening experiments failed to take into account the virus dimension adequately. The use of virus inoculum-1 and inoculum-2 composed of nRB and RB isolates, respectively, led to inconsistent identification of PR-phenotype rice accessions. This result was confirmed when field isolates of the virus were used for screening. Moreover, isolates which did not overcome RYMV1 resistance gene in Gigante gave inconsistent virus-host interactions in CG14 (Table 3).

Overall, screening for resistance to RYMV should be based on a good knowledge of the virus diversity. The identification of sources resistant to the virus requires the use of well characterized nRB isolates. Although virus inoculum-1 and individual nRB isolates led to similar results in the identification of PR-phenotype accessions, inoculum consisting of an inoculum of virus isolates may drive to synergic effects in overcoming some potential sources of partial resistance to RYMV. Indeed the biological effects of interactions between RYMV isolates are poorly known. In mixed infections of rice plants, S2 isolates dominated over S1 isolates for virus accumulation but there was no evidence of interaction in the virus accumulation between either types of isolates and S4 isolates [31].

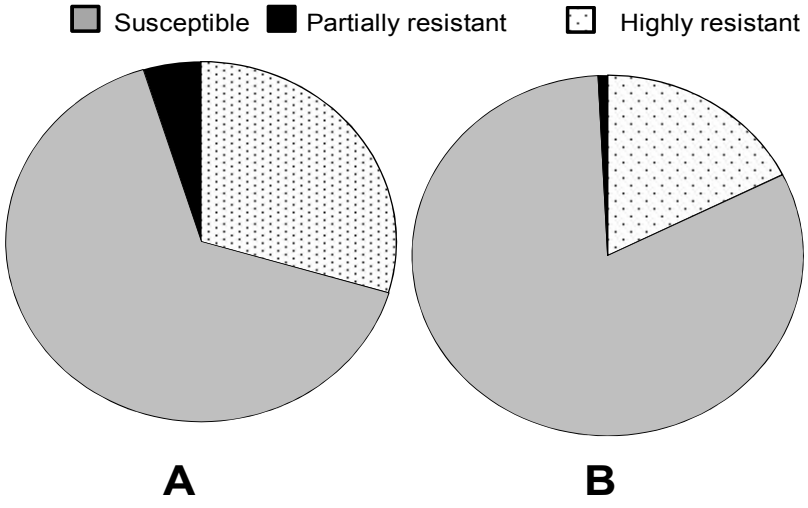

Fig. 1. Proportions of susceptible, partially resistant and highly resistant rice accessions identified after inoculation of RYMV isolates inoculum-1 (A) and inoculum-2 (B)

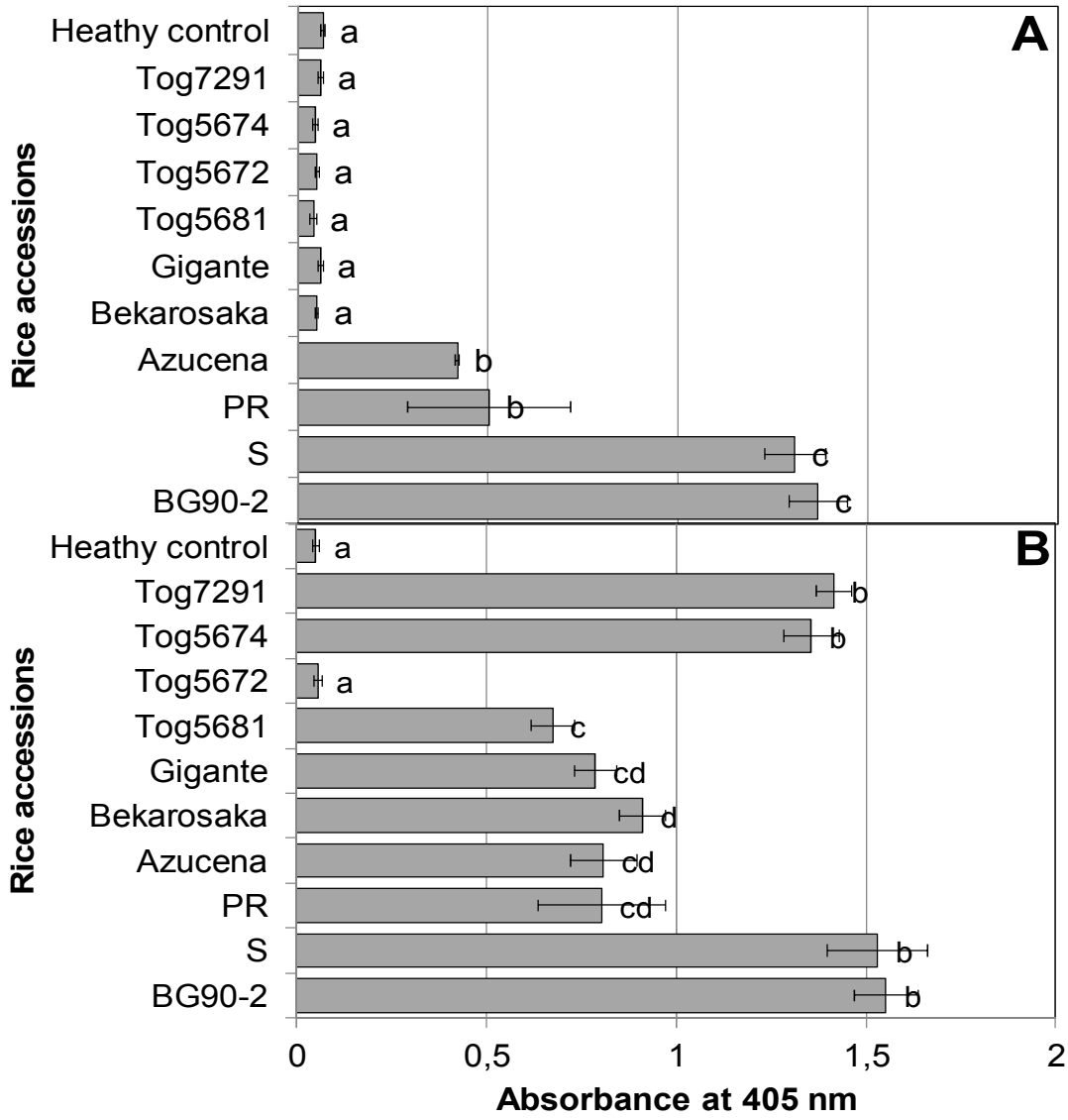

Fig. 2. Mean virus titres in leaves of rice accessions inoculated with inoculum-1 (A) and inoculum-2 (B) of RYMV isolates. Data from susceptible (S) and partially resistant (PR) accessions were pooled, respectively. Means associated with the same letter(s) did not differ significantly according to Fisher's LSD test at P=0.05. Error bars indicate standard deviation of the mean

Table 2. Reactions of rice accessions to inoculation of two inoculums of RYMV isolates

N°	Rice accessions[a]	Number of days for symptom appearance[b]			
		Virus inoculum-1		Virus inoculum-2	
(HR) control	Gigante	NS	(HR)	15.2±1.8	(PR)
(HR) control	Bekarosaka	NS	(HR)	14.2±1.1	(PR)
(HR) control	Tog5681	NS	(HR)	16.6±0.5	(PR)
(HR) control	Tog5672	NS	(HR)	NS	(HR)
(HR) control	Tog5674	NS	(HR)	8.6±0.5	(S)

N°	Rice accessions[a]	Number of days for symptom appearance[b]			
		Virus inoculum-1		Virus inoculum-2	
(HR) control	Tog7291	NS	(HR)	8±0	(S)
(HR) control	Azucena	16±1	(PR)	16.6±1.7	(PR)
(S) control	BG90-2	11.6±1.3	(S)	8.2±1.6	(S)
1	FKR14	9±0	(S)	7.2±0.4	(S)
2	FKR16	9±0	(S)	6.6±0.5	(S)
3	FKR18	9±0	(S)	7±0	(S)
4	FKR19	11.6±0.5	(S)	7.4±0.9	(S)
5	FKR45N	9.8±1.8	(S)	8.6±0.5	(S)
6	FKR56N	9±0	(S)	7±0	(S)
7	FKR62N	9±0	(S)	7±0	(S)
8	FKR60N	10±0	(S)	7.4±0.9	(S)
9	FKR2	7.8±1.1	(S)	8.2±1.6	(S)
10	Adaisi	9±0	(S)	10.2±1.8	(S)
11	Alcame-Femelle	9±0	(S)	7±0	(S)
12	Alcame-Male	9±0	(S)	7.8±1.1	(S)
13	Basmati370	9.2±0.4	(S)	8.2±1.6	(S)
14	Boning kari	9±0	(S)	8.6±0.5	(S)
15	Bouake189	9.8±0.8	(S)	7.6±0.5	(S)
16	Chinoire maalo	10.2±1.3	(S)	6±0	(S)
17	Chinois	10.2±1.3	(S)	8.4±0.9	(S)
18	Djineve	10.2±1.1	(S)	9.4±2.2	(S)
19	Cv"Fao"	8.6±0.5	(S)	8.2±1.1	(S)
20	FKR35	10.2±1.1	(S)	9.4±2.2	(S)
21	FKR39	10±0	(S)	9.4±1.3	(S)
22	FKR42	9±0	(S)	7.4±0.5	(S)
23	FKR50	10±0	(S)	7.4±0.5	(S)
24	FKR58N	13±0.7	(S)	8.8±0.4	(S)
25	GH 4008	9 ± 0	(S)	9.2±0.8	(S)
26	GH1571	8.8±0.4	(S)	7.4±0.5	(S)
27	FKR48	9±0	(S)	7.2±0.4	(S)
28	GH1584	7.2±0.4	(S)	8±0	(S)
29	GH1584 bis	9±0	(S)	8.2±0.4	(S)
30	GH1585	7.6±0.5	(S)	8.4±0.5	(S)
31	GH1589	7±0	(S)	6.2±0.4	(S)
32	GH1796	9±0	(S)	8±0	(S)
33	GH1801	10.2±1.3	(S)	8±0	(S)
34	GH1835	8.4±0.5	(S)	8.8±0.4	(S)
35	GH4008	10±0	(S)	9.4±0.5	(S)
36	GR18	8.4±1.3	(S)	7.2±0.4	(S)
37	IR5	9±0	(S)	7.2±1.1	(S)
38	IR64	8.6±0.5	(S)	6.6±0.5	(S)
39	IR67908-5-1	9.8±0.4	(S)	9.4±1.3	(S)
40	IR70445-146-3-3	8.8±1.1	(S)	9.4±1.3	(S)
41	IR70445-229-4-1	9±0	(S)	10.6±0.9	(S)
42	IR71137-184-3-2-3-3	11±1.2	(S)	10±0	(S)
43	IET6279	9.8±0.4	(S)	8.8±1.6	(S)
44	IR72870-120-1-2-2	9.2±0.4	(S)	8.2±1.6	(S)
45	ITA320	9.8±0.4	(S)	10.6±0.5	(S)
46	ITA324	9±0	(S)	10.4±0.5	(S)
47	Jasmine85	9.8±0.4	(S)	10±0	(S)
48	KRC-Baika	10.2±0.4	(S)	9.4±1.3	(S)
49	Maalobo	11.2±0.8	(S)	6.4±0.5	(S)
50	Maalowouleen	9.6±0.5	(S)	6.4±0.9	(S)
51	Malina	9±0	(S)	8.2±1.1	(S)
52	Maloba	9±0	(S)	6.8±1.1	(S)

N°	Rice accessions[a]	Number of days for symptom appearance[b]			
		Virus inoculum-1		Virus inoculum-2	
53	Maloboo	9±0	(S)	6.8±1.1	(S)
54	Marobou	10.4±1.9	(S)	7.6±0.9	(S)
55	Marshall	9.2±0.4	(S)	7±0	(S)
56	Moobou	9.8±0.8	(S)	7.6±0.9	(S)
57	Moui	9±0	(S)	6±0	(S)
58	Mouikwin1	9±0	(S)	7.6±0.9	(S)
59	Mouikwin2	9±0	(S)	7.6±0.9	(S)
60	Mouikwin3	8.4±0.5	(S)	7.6±0.9	(S)
61	Mouikwin5	9±0	(S)	7.6±0.9	(S)
62	Mouiplaa	9±0	(S)	7.2±1.1	(S)
63	Nerica16	9±0	(S)	8.8±1.1	(S)
64	NERICA2	12.8±1.6	(S)	9±0	(S)
65	Napone	8.4±0.5	(S)	8±0	(S)
66	NERICA3	12±0	(S)	7.2±0.4	(S)
67	Nerica54	9±0	(S)	9±0	(S)
68	Orodara	9.2±0.4	(S)	6±0	(S)
69	P38	13.2±1.5	(S)	8.4±0.9	(S)
70	Paroyente	8±0	(S)	8±0	(S)
71	Perfum-rice	10.2±0.8	(S)	8±0	(S)
72	Rox-cv	9.2±0.4	(S)	9±1.4	(S)
73	Sikamoo	9±0	(S)	8.6±2.2	(S)
74	Soomalo	10.2±1.1	(S)	6.4±0.9	(S)
75	TanghinI	9±0	(S)	7±0	(S)
76	TanghinII	9±0	(S)	7.8±0.4	(S)
77	Tiefagamalo	9±0	(S)	7.2±0.8	(S)
78	Tox728-1	9.2±0.4	(S)	8.6±1.2	(S)
79	Viwonor tall	13.8±0.8	(S)	7.6±1.3	(S)
80	Wita7	9.8±0.4	(S)	7.6±1.3	(S)
81	Woussou	9±0	(S)	7±0	(S)
82	Viwonor short	17.2±2.9	(PR)	10.4±0.5	(S)
83	FKR37	15.2±1.3	(PR)	9.4±0.5	(S)
84	Dissi	16.2±1.3	(PR)	6.4±0.9	(S)
85	CRI38 NERICA 5	17.8±2.7	(PR)	7.2±0.4	(S)
86	FKR1	15.2±1.8	(PR)	7±0	(S)
87	FKR49	19±0	(PR)	10.4±0.5	(S)
88	FKR47N	21.8±1.8	(PR)	10.6±1.3	(S)
89	IR71138-49-2-2-1-2	14.6±1.3	(PR)	10.2±0.4	(S)
90	Kumazuce	16.4±0.5	(PR)	7.8±1.1	(S)
91	Maalo-gwai	21.8±4	(PR)	6±0	(S)
92	Maaloteliman	17.2±2	(PR)	7.6±0.9	(S)
93	Mouikwin4	17.6±1.7	(PR)	7.2±1.1	(S)
94	N28K	19.6±0.5	(PR)	10.8±0.4	(S)
95	NERICA1	16±1.7	(PR)	10±0	(S)
96	Nerica23	19.8±0.4	(PR)	9.2±0.4	(S)
97	Nerica28	16.8±2.2	(PR)	7.4±0.5	(S)
98	NERICA4	16±0	(PR)	8.4±0.5	(S)
99	FKR28	19±2	(PR)	10.2±1.8	(S)
100	TS2	17.6±1.5	(PR)	17.2±4.1	(PR)
101	FKR21	17.2±1.6	(PR)	17.4±2.2	(PR)
102	FKR29	21.2±2.5	(PR)	16±1.4	(PR)
103	FKR33	14.4±0.9	(PR)	18.4±0.9	(PR)
104	FKR41	14.4±3.6	(PR)	15±0	(PR)
105	FKR43	21.4±5	(PR)	15±0	(PR)
106	Nerica7	15.4±0.5	(PR)	16.6±3.1	(PR)
107	Nerica9	19±1.2	(PR)	14.8±0.4	(PR)

N°	Rice accessions[a]	Number of days for symptom appearance[b]			
		Virus inoculum-1		Virus inoculum-2	
108	Nerica24	19±2.2	(PR)	13.8±1.6	(PR)
109	Nerica-pluvial	14±5.2	(PR)	13.8±1.6	(PR)
110	IDSA85	15.2±1.1	(PR)	16.6±2.6	(PR)
111	GH1577	19±0	(PR)	16.4±0.5	(PR)
112	GH1520	21±5	(PR)	21.4±3.6	(PR)
113	Aromatic	15.2±0.8	(PR)	15.8±1.1	(PR)
114	Aromatic-short	16±1	(PR)	18.2±3.5	(PR)
115	Beauty	18±3	(PR)	19.6±0.5	(PR)
116	Digang	16±2.5	(PR)	17±1.7	(PR)
117	CG14	16.4±1.3	(PR)	12.6±4.3	(PR)

[a]Farmer's ten top preferred rice varieties are indicated in boldface

[b]mean number of days for symptom appearance after virus inoculation ± standard deviation (n=5) with virus inoculum-1 and inoculum-2; no symptom (NS) was observed in some cases;

[c] Reaction phenotypes (indicated in parentheses) were attributed to accessions after one-way ANOVA of the number of days for symptom appearance followed by Dunnett's test (P < 0.05), taking BG90-2 as control group: S, susceptible; PR, partially resistant; HR, highly resistant

Table 3. Reactions of 20 rice accessions to inoculation with 10 RYMV field isolates[a]

Rice accessions	RYMV isolates									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Digang	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
TS2	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
CG14	PR	S	S	S	PR	S	S	S	PR	S
GH1577	PR	PR	S	PR	PR	PR	PR	PR	PR	PR
FKR21	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
Aromatic short	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
FKR28	PR	PR	S	S	PR	PR	PR	PR	PR	PR
FKR29	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
Beauty	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
Dissi	PR	S	S	S	PR	PR	PR	PR	PR	PR
FKR49	PR	PR	S	S	PR	PR	PR	PR	PR	PR
FKR33	PR	PR	S	PR	PR	PR	PR	PR	PR	PR
FKR43	PR	PR	S	S	PR	PR	PR	PR	PR	PR
FKR47N	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
IDSA 85	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
Maalo-teliman	PR	PR	S	S	PR	PR	PR	PR	PR	PR
Moui kwin4	PR	S	S	S	PR	PR	PR	PR	PR	PR
Viwonor short	PR	PR	S	S	PR	PR	S	PR	PR	PR
NERICA 1	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
CRI38 NERICA 5	PR	PR	PR	PR	PR	PR	PR	PR	PR	PR
BG90-2	S	S	S	S	S	S	S	S	S	S
Azucena	PR	PR	PR	PR	PR	PR	S	PR	PR	PR
Gigante	HR	S	S	S	HR	HR	S	HR	HR	HR

[a]For each RYMV isolate, reaction phenotypes were attributed to rice accessions after one-way ANOVA of the number of days for symptom appearance followed by Dunnett's test (P <0.05), taking susceptible (S) variety BG90-2 as control group. Cultivars Azucena and Gigante were used as partially resistant (PR) and highly resistant (HR) checks

5. CONCLUSION

The use of nRB RYMV isolates on the one hand and RB isolates on the other to screen rice accessions clearly showed that the choice of inoculum sources is critical. If RB isolates are used, most resistance sources are likely to get undetected. For this reason, field isolates of the virus are not suitable for the screening tests as large discrepancies may occur between locations due to differences in RYMV variability. Inoculum made of nRB isolates should be used to be able to detect partial and high resistance sources. Both resistances are biologically detected by

monitoring symptom appearance after inoculation. However, leaf virus content assessment is also suitable for distinguishing between susceptibility as well as partial and high resistance to RYMV.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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