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Research Article

ASSESSMENT OF A MINI-CORE COLLECTION OF RICE CULTIVARS FOR RESISTANCE/TOLERANCE TO RICE YELLOW MOTTLE VIRUS (RYMV) IN BURKINA FASO, WEST AFRICA

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A B S T R A C T

Rice yellow mottle virus (RYMV), an emerging sobemovirus, is the cause of one of the most devastating rice diseases in Africa. In Burkina Faso, yield losses up to 100% have been reported. As the large majority of cultivated rice varieties are susceptible to this virus, the main alternatives for the control of the disease are the use of genetic resistance and the chemical control of the insect vector.

Here, 84 rice germplasm from INERA collection were screened for RYMV resistance/tolerance in order to support the development of other alternatives for disease control. Screening against three RYMV isolates was performed in a greenhouse. Resistance to infection was evaluated by RYMV inoculation followed by symptom assessment and ELISA. Disease incidence and severity were recorded. Among 84 genotypes, only three (3.57%) [NIL 130, WAB 2098 R, NIL 16] was resistant and four (4.76 %) [Tog5672, Tog5674, Tog5681, and Tog7291] highly resistant to infection with RYMV. Seven teen genotypes (20.24%) were classified as moderately resistant, 55 (65.48%) as susceptible, and five (5.95%) as highly susceptible [IR 841, Bouake 189, FKR 55, FKR 42, BG 90-2].

The resistant genotypes identified are good candidates for a breeding program for RYMV-resistant cultivars. Moderate resistant genotypes could be used by producers in cultivation under integrated production systems.

INTRODUCTION

Rice is one of the most important food crops for people in low- and lower-middle-income countries. In sub-Saharan Africa, where most of the population falls within the category of low incomes, the demand for rice has increased considerably since 1982 (FAOSTAT, 2018). In Burkina Faso, rice is a staple crop ranking at fourth position after sorghum, millet and maize in terms of production and area of cultivation.

However, rice production is being subverted by abiotic and biotic factors, including plant diseases. Among these factors limiting to rice production in Burkina Faso, viruses appear to be significant production constraints. Among these viruses, *Rice yellow mottle virus* (RYMV) causes severe/ economic yield reduction in rice (Sérémé *et al*., 2016; Savary *et al*., 2019).

Depending on the rice genotype, viral strain, stage of infection and ecology, RYMV yield losses range from 10 to 100% (Awoderu, 1991, Konaté *et al.*, 1997; Kouassi *et al.*, 2005). Plants that display severe symptoms during seedling and early vegetative stages often result in plant death (Bakker, 1974; Sérémé *et al*., 2016).

RYMV is a member of the genus Sobemovirus (Hull et Fargette, 2005), which was reported first in 1966 in Kenya (Bakker, 1974) and subsequently in virtually all other rice-producing regions in Africa, but not elsewhere (Traoré *et al*., 2005). Accordingly, the virus is categorized as emergent (Anderson *et al*., 2004).

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The natural host range of RYMV is restricted to wild (*Oryza longistaminata, Oryza barthii*) and cultivated (*Oryza sativa, Oryza glaberrima*) rice species. In addition, natural infection of a few wild grasses (*Echinocloa colona, Panicum repens)* has been reported (Konaté *et al*., 1997), but their role as sources of inoculum is unclear. Recently, some wild accessions from the primary gene pool (AA genome), including *O. glumaepatula, O. breviligulata, O. meridionalis, O. rufipogon,* and *O. nivara*, have also shown susceptibility in screening experiments (Allarangaye *et al*., 2007; Odongo *et al*., 2019).

RYMV is transmitted by contact and by animal vectors. Transmission by seeds has not been detected in either cultivated or wild rice species (Abo *et al*., 2004; Bakker, 1974; Fauquet and Thouvenel, 1977; Konaté *et al.*, 2001; Allarangaye *et al.*, 2006). Seedbed nurseries and infected crop stubble also serve as auxiliary sources of the virus (Traoré *et al*., 2006; Uke *et al*., 2014).

RYMV management is difficult and have been addressed through adapting the virus control measures to local situations. This includes the use of varietal mixtures and pesticide sprays, early planting, destruction of previous plants, ratoons and volunteer crops, removal of infected plants, and crop rotation (Odongo *et al*., 2021). These practices aim at disrupting the life cycle of the virus and improving crop health (Traoré *et al*., 2009). However, the application of these measures is limited and still ineffective in epidemic context. Hence, the use of resistant varieties is the most cost-effective and eco-friendly way of controlling the disease. Recent advances in genetics and molecular biology have contributed to the characterization of natural sources of resistance in the two cultivated rice species, i.e., *O. sativa* (Asian rice) and *O. glaberrima*.

High resistance, associated with a lack of symptoms, undetectable virus content and no yield losses upon field infection, is very rare and only reported in two *O. sativa indica* varieties and a few *O. glaberrima* rice accessions (Ndjiondjop *et al*. 1999; Rakotomalala *et al*. 2008). On the contrary, partial resistance, characterized by a delay in symptom expression and virus accumulation, is widespread in *O. sativa japonica* upland varieties including cultivar (*cv.*) Azucena and involves several quantitative trait loci (QTLs) (Boisnard *et al*. 2007).

Unfortunately, the resistant varieties are limitedly available and the resistance is not stable due to the appearance of new virulent strains of the pathogen. Indeed, due to RYMV rapid evolution (Fargette *et al*., 2008), resistance-breaking variants have been observed across Sub-Saharan Africa (Traoré *et al*., 2006) and the emergence of virulent isolates after serial passages in resistant cultivars has been reported (Fargette *et al*., 2002).

So much work remains to be done to identify the best strategies to limit the prevalence and yield losses caused by RYMV. Interestingly, a good deal of research work has been directed to identify resistant/tolerant sources under diverse environmental conditions and continuing screening of available genotypes and new germplasm, which constitutes the basis of this work, has been suggested by several research workers (Ashfaq *et al*., 2014; Sérémé *et al*., 2016). Therefore, to evaluate sources of RYMV resistant/tolerant genotypes, 84 local and foreign rice accessions from INERA collection were screened by mechanical inoculation. The level of resistance/tolerance to RYMV accumulation in rice leaf tissues was evaluated using a combination of visual symptom observations and enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Plant material

A total of 84 accessions from the germplasm collection of the Institute of Environment and Agricultural Research (INERA), Burkina Faso, were tested for resistance/tolerance to RYMV. Gigante, Azucena and IR64 cultivars were included in the assay as resistant, tolerant and susceptible controls respectively.

RYMV source and propagation

A mixture of three different RYMV isolates, representative of RYMV diversity in Burkina Faso was used based on their different origin, strain, and aggressiveness. RYMV isolate BF710 was isolated from rice plants at Dedougou (western region). Henceforth in this paper, it is denoted as a representative of the serotype Ser-Sa of RYMV. RYMV isolate BF716 was isolated from rice plants at Banzon (Western region). Hence, within this text it is denoted as a representative of the serotype Ser1 of RYMV. Isolate BF1 was isolated at Karfiguela and was used as the aggressive isolate. It belongs to the S2 strain of RYMV. The virus was propagated and maintained in the highly susceptible rice variety IR 64 plants in an insect-proof glasshouse.

Mechanical inoculation

To obtain the inoculum, RYMV infected leaves of the susceptible rice cv. IR 64 were crushed with a mortar and pestle and homogenized in 0.01 M phosphate buffer pH 7.0 at the ratio of 1:10 (w/v). Extract was dusted with carborundum (600 mesh) (Sérémé *et al* 2016). 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. Mock-inoculated control plants were inoculated with phosphate buffer at the same stages.

Experimental design

Twenty seeds of each accession were sown in small clay pots filled with sterilised soil under greenhouse conditions. Once germinated, seedlings were transplanted to the pots (7 seedlings per pot). Fourteen plants (two pots) per accession were inoculated as described by Sérémé *et al*. (2016). Six seedlings per accession, corresponding to the rest of twenty seedlings, were kept as controls: three as a non-inoculated control and another as a mock-inoculated control. Controls were kept in order to avoid infection with RYMV. All plants were maintained in an insect-proof greenhouse at 25 to 30°C and 80 to 90% relative humidity and monitored for symptom development. The distance between the pots did not allow contact between plants. Leaves were collected at 21 days post inoculation (dpi) due to the fact that most infected IR 64 plants started drying 3 weeks after inoculation. The experiment was repeated two times.

Serological assay

Leaves from the same accession were pooled and assayed by serology for virus presence. Leave extracts of each pool sample were tested by double antibody sandwich-enzyme-

linked immunosorbent assay (DAS-ELISA) (Clark & Adams 1977), using polyclonal antibody against RYMV (N'Guessan *et al.*, 2000; Sérémé *et al*., 2016).

Table 1 Characteristics of the 84 rice accessions screened for rice yellow mottle disease resistance

	Genotypes	Pedigree	Group	Ecology	Origin
1	ADNY 11		Indica	Irrigated	AfricaRice
2	ARC 1-432-B-1				India
3	ARC 1-448-B-1				India
4	ARICA 2	WAB-2056-2-FKR 2-5-TGR 1-B	Indica	Irrigated	AficaRice
5	ART 346-10-2-1				
6	ART 347-9-1-1				
7	ART 348-4-1-1				
8	ART 349-1-1-1				
9	AZUCENA (Tolerant check)	Traditional landrace	Japonica	Irrigated	IRRI
10	BG 90-2	Peta 3 * TN 1) / Remadja	Indica	Irrigated	AfricaRice
11	Bouake 189				Côte d'Ivoire
12	C101A51		Indica	Irrigated	AfricaRice
13	F6 53		Indica	Irrigated	AfricaRice
14	F6 57		Indica	Irrigated	AfricaRice
15	FKR 19	TOX 728-1 (Local-Nigeria)	Sativa	Irrigated/Lowland	IITA
16	FKR 2	GAMBIAKA	Indica	Irrigated/Lowland	Gambie
17	FKR 28	IITA 123	Indica	Irrigated/Lowland	IITA
18	FKR 33		Japonica	Irrigated/Lowland	Burkina Faso
19	FKR 42	IR 5657-33-2-1/IR 2061-465-1-5-5	Indica	Irrigated	IRRI
20	FKR 45N	WAB880-1-38	Nerica	Rain-fed	Burkina Faso
21	FKR 47N	WAB 881-10-37	Nerica	Rain-fed	Burkina Faso
22	FKR 49N	WAB 880-1-38	Nerica	Rain-fed	Burkina Faso
23	FKR 50	4456 - IR 1529-680-3	Sativa	Irrigated/Lowland	Burkina Faso
24	FKR 51		Sativa	Rain-fed	Burkina Faso
25	FKR 53		Sativa	Rain-fed	Burkina Faso
26	FKR 54	WABIR12979	Sativa	Irrigated/Lowland	Burkina Faso
27	FKR 55	WAB450-I-BL-1-736-HB	Nerica	Rain-fed	AficaRice/INERA
28	FKR 56N	WAB450-I-BL-1-736-HB	Nerica	Irrigated/Lowland	Burkina Faso
29	FKR 58N	WAS 191-9-3	Nerica	Irrigated/Lowland	Burkina Faso
30	FKR 59	WAS191-9-5-3-2	Japonica	Rain-fed	Burkina Faso
31	FKR 60N	WAS 122-IDSA-1-WAS-1-1-B	Nerica	Irrigated/Lowland	Burkina Faso
32	FKR 61	WABC165	Japonica	Rain-fed	Burkina Faso
33	FKR 62N	WAS 122-IDSA-1-WAS-6-1	Nerica	Irrigated/Lowland	Burkina Faso
34	FKR 64	Taiwan Selection 2	Indica	Irrigated/Lowland	Burkina Faso
35	FKR 66	WAT1046-B43	Indica	Irrigated/Lowland	Burkina Faso
36	FKR 68	IR75866-2-7-1-WAB1	Indica	Irrigated/Lowland	Burkina Faso
37	FKR 70	IR 75-884-12-12	Indica	Irrigated/Lowland	Burkina Faso
38	FKR 72		Indica	Irrigated/Lowland	Burkina Faso
39	FKR 74	WAB 2094-WAC 2-TGR2-B	Indica	Irrigated	AfricaRice
40	FKR 76		Sativa	Irrigated/Lowland	Burkina Faso
41	FKR 78		Sativa	Irrigated/Lowland	Burkina Faso
42	FKR 80		Sativa	Irrigated/Lowland	Burkina Faso
43	GIGANTE (Resistant check)	Traditional Landrace	Indica	Irrigated	AfricaRice
44	IR 47	TOG5681/3*IR64	Indica	Irrigated	IRRI
45	IR 64 (Susceptible check)	IR 5657-33-2-1/IR 2061-465-1-5-5	Indica	Irrigated	IRRI
46	IR 67013-58-1-2		Indica	Irrigated	IRRI
47	IR 75884-12-12-14 AB1		Indica	Irrigated	IRRI
48	IR 841		Indica	Irrigated	IRRI
49	KOGONI		Indica	Irrigated	IRRI
50	MOROBEREKAN		Japonica	Irrigated	Côte d'Ivoire
51	NERICA 2	IRGC 96892 Gambia	Nerica	Irrigated	AfricaRice
52	NIL 130	IR64/Gigante(BC3Fn)	Indica	Irrigated	AficaRice
53	NIL 16	Sahelika/Gigante(BC3Fn)	Indica	Irrigated	AfricaRice
54	NIL 2	IR67/Gigante(BC3Fn)	Indica	Irrigated	AficaRice
55	NIL 54	IR47/Gigante(BC3Fn)	Indica	Irrigated	AfricaRice
56	NIPPONBARE		Japonica	Irrigated	AficaRice
57	ORYLUX 3	WAB 2066-6-FKR4-WAC1-TGR1-B	Indica	Irrigated	AficaRice
58	ORYLUX 4	WAB 2066-23-FKR3-5-TGR3-3	Indica	Irrigated	AficaRice
59	ORYLUX 5	WAB 2066-14-FKR3-1-TGR1-1	Indica	Irrigated	AficaRice
60	ORYLUX 6	WAB 2066-12-FKR4-5-TGR1	Indica	Irrigated	AfricaRice
61	PNA 647F456		Indica	Irrigated	AficaRice
62	ROCK 25		Indica	Irrigated	AfricaRice
63	SAHEL 177	IR 31851-96-2-3-2-1 /IR 66231-37-1-2	Indica	Irrigated	AficaRice
64	SAHEL 328	Sahel 134 / IR 66231-37-1-2	Indica	Irrigated	AfricaRice
65	SAHEL 329	Jaya / Basmati 370	Indica	Irrigated	AficaRice
66	Tog5672		Glaberrima	Irrigated	IITA
67	Tog5674		Glaberrima	Irrigated	IITA
68	Tog5681		Glaberrima	Irrigated	IITA
69	Tog7291		Glaberrima	Irrigated	IITA
70	TS2	Taiwan Selection 2-2-2	Indica	Irrigated	Chine Taiwan
71	WAB 2066-6-FKR4WAC1-TGR1-B-WAT-B1	WAB 2066-6-FKR4WAC1-TGR1-B-WAT-B1	Indica	Irrigated	AficaRice
72	WAB 2066-6-FKR4WAC1-TGR1-BWAT-B16	WAB 2066-6-FKR4WAC1-TGR1-BWAT-B16	Indica	Irrigated	AfricaRice
73	WAB 2066-6-FKR4WAC1-TGR1-B-WAT-B6	WAB 2066-6-FKR4WAC1-TGR1-B-WAT-B6	Indica	Irrigated	AficaRice
74	WAB 2066-6-FKR4WACI-TGR1-BWAT-B11	WAB 2066-6-FKR4WACI-TGR1-BWAT-B11	Indica	Irrigated	AfricaRice
75	WAB 2066-6-FKR4WACI-TGR1-BWAT-B9	WAB 2066-6-FKR4WACI-TGR1-BWAT-B9	Indica	Irrigated	AficaRice

76	WAB 2066-WAT20-1-B-1-TGR1	WAB 2066-WAT20-1-B-1-TGR1	Indica	Irrigated	AfricaRice
77	WAB 2066-WAT20-1-B-1-TGR3	WAB 2066-WAT20-1-B-1-TGR3	Indica	Irrigated	AficaRice
78	WAB 2066-WAT21-1-B-1-1	WAB 2066-WAT21-1-B-1-1	Indica	Irrigated	AfricaRice
79	WAB 2066-WAT21-1-B-1-3	WAB 2066-WAT21-1-B-1-3	Indica	Irrigated	AficaRice
80	WAB 2066-WAT21-1-B-1-TGR1	WAB 2066-WAT21-1-B-1-TGR1	Indica	Irrigated	AfricaRice
81	WAB 2066-WAT21-1-B-1-TGR2	WAB 2066-WAT21-1-B-1-TGR2	Indica	Irrigated	AficaRice
82	WAB 2066-WAT21-1-B-1-TGR3	WAB 2066-WAT21-1-B-1-TGR3	Indica	Irrigated	AfricaRice
83	WAB 2098	WAB 2098	Indica	Irrigated	AficaRice
84	WAB 2138-WACB-2-TGR2-WAT5-1	WAB 2138-WACB-2-TGR2-WAT5-1	Indica	Irrigated	AfricaRice
85	WAB 638-1	WAB 638-1	Indica	Irrigated	AfricaRice
86	WAS 183-B-6-2-4	WAS 183-B-6-2-4	Indica	Irrigated	AficaRice
87	WAT 1046-B-43-2-2-2	WAT 1046-B-43-2-2-2	Indica	Irrigated	AficaRice

For each accession, are indicated the pedigree, the genetic group, the production ecology, and the country or organism of origin, and, based on a priori knowledge.

All buffer system and incubation times were as previously described (Konaté *et al.*, 1997). Samples were mashed in extraction buffer at a dilution of 1:10 (w/v) and centrifugation at 8,000 × g for 10 min. 0.1 ml of the extract obtained was analysed. The mean absorbance value (A_{405} nm) from healthy controls plus three times the standard deviation was taken as the negative-positive threshold.

Symptoms evaluation and plant analysis

Severity. Throughout the inoculation assays, the symptoms shown by each of the inoculated plants were recorded and compared to the non-inoculated controls. Symptoms appearance was monitored from 2 to 31 days post inoculation (dpi) according to Sérémé *et al*. (2016). A slightly modified standard evaluation system described by Sérémé *et al*., (2016) 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.

Data analysis. Data recorded were analysed as described by Sérémé *et al*. (2016). Briefly, data for each accession 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 on rice genotypes were subjected to Analysis of variance (ANOVA) by using Statistica version 6 (StatSoft Inc., 2003). The treatment means were compared by the Least Significant Difference (LSD) test at 5% level (Gomez and Gomez, 1984). Genotypes were compared for disease incidence and severity

RESULTS

Results on reaction of rice germplasm consisting of 84 genotypes against rice yellow mottle virus (RYMV) under controlled conditions are given in Table 2.

Symptom expression and dpi

Single inoculations in rice accessions with RYMV isolates that represent the diversity of Burkinabe strains produced symptoms that were scored according to the scales from 0 to 9, as defined in the Materials and Methods. A large majority of the 84 genotypes tested, expressed pronounced symptoms after inoculation (Table 2). Eighty of 84 genotypes showed various systemic symptoms of RYMV. The major genotypes showed mosaic, mottle, streaks, spot, sparse, necrosis, and yellowing of leaves. At the 21 days follow-up, four genotypes (4.76%) had no symptom and three (3.57%) mild symptoms with score comprised between 2 and 3, 25 (29.77%) moderate symptoms with score ranking between 4 to 5, and 52 (60.90%) severe presenting score superior or equal to 6.

The time of appearance of these symptoms in individual plants was variable, but most of the replicate plants of each genotype showed similar symptoms at 21 dpi (Fig.1). The major genotypes developed symptoms between 7- and 10-days post inoculation (dpi) while other genotypes exhibited symptoms between 11 and 25 dpi (Fig.1).

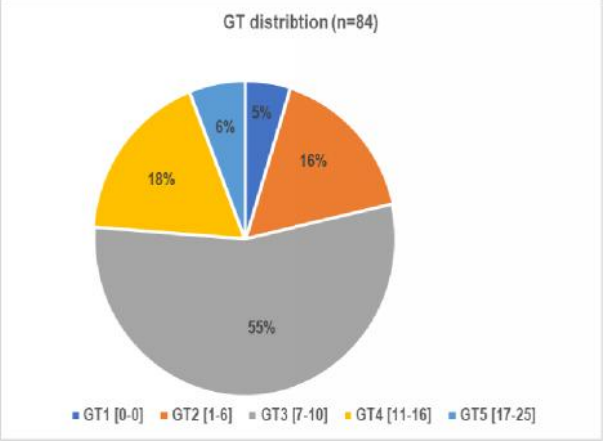

Fig 1 Distribution of genotype related to day post-inoculation
GT1 [1-6] in the pie graph corresponds to genotypes having a dpi comprised between 1 to 6 days

All of these genotypes exhibited 57.14-100 % RYMV infection on the basis of incidence with relatively high titre (> 1.0) detection in the upper symptomatic leaves, so considered all of these susceptible to RYMV.

Genotypes reaction

The present study results demonstrated varying responses of rice genotypes to RYMV. Not all of the 90 accessions germinated in all two replications, and data were obtained for 84 accessions. The most resistant and most susceptible genotypes are presented here, along with checks and genotypes (Table 2).

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

Code	Genotype	Dpi	Type of Symptoms	ELISA	Mean plants positive/Total plants	Incidence	Mean severity	Reaction to RYMV
G34	GIGANTE	0	no symptoms	-	0/14	0.00[e]	1	HR
G57	Tog5672	0	no symptoms	-	0/14	0.00[e]	1	HR
G58	Tog5674	0	no symptoms	-	0/14	0.00[e]	1	HR
G59	Tog5681	0	no symptoms	-	0/14	0.00[e]	1	HR
G60	Tog729	0	no symptoms	-	0/14	0.00[e]	1	HR
G4	BG 90-2	6	Spot, yellowing	++++	14/14	100.00[a]	9.00	HS
G12	FKR 42	6	mottling, death plants	++++	14/14	100.00[a]	9.00	HS
G36	IR 64	6	Mottling, death plants	++++	14/14	100.00[a]	9.00	HS
G39	IR 841	6	Spot, yellowing	+++	14/14	100.00[a]	5.87	HS
G86	FKR 55	6	Mottling, necrotic	+++	14/14	100.00[a]	7.24	HS
G87	Bouake 189	6	Mottling, yellowing	+++	14/14	100.00[a]	7.09	HS
G1	ADNY 11	12	Spot, yellowing	++	3/14	21.43[c]	4.70	MR
G3	AZUCENA	14	Spot, yellowing	++	4/14	28.57[c]	4.79	MR
G6	F6 53	17	Chlorotic spot	++	4/14	28.57[c]	4.87	MR
G7	F6 57	13	Chlorotic spot	++	3/14	21.44[c]	4.70	MR
G17	FKR 51	12	Chlorotic spot	++	4/14	28.57[c]	4.97	MR
G27	FKR 66	15	Chlorotic spot	++	3/14	21.49[c]	4.76	MR
G30	FKR 72	12	Spot, yellowing	++	4/14	28.57[c]	5.00	MR
G35	IR 47	11	Yellowing	++	3/14	21.45[c]	4.70	MR
G41	MOROBEREKAN	13	Chlorotic spot	++	3/14	21.46[c]	4.70	MR
G42	NERICA 2	12	Streaks	++	4/14	28.57[c]	4.87	MR
G46	NIL 54	11	Mosaic	++	3/14	21.47[c]	4.73	MR
G47	NIPPONBARE	11	Streaks	++	4/14	28.57[c]	4.88	MR
G75	WAB 638-1	12	Spot, yellowing	++	3/14	21.48[c]	4.74	MR
G80	ART 346-10-2-1	14	Mottling, necrotic	++	4/14	28.57[c]	4.94	MR
G81	WAB 2138-WACB-2-TGR2-WAT5-1	13	Mottling	+++	4/14	28.58[c]	5.04	MR
G82	ART 348-4-1-1	18	Mottling	++	3/14	21.50[c]	4.77	MR
G84	ART 349-1-1-1	16	Necrotic	++	4/14	28.57[c]	4.97	MR
G85	ART 347-9-1-1	14	Mottling	++	3/14	21.51[c]	4.77	MR
G43	NIL 130	25	Mild mosaic	+	2/14	14.28[d]	2.76	R
G44	NIL 16	22	Sparse dot	+	2/14	14.29[d]	3.66	R
G74	WAB 2098 R	17	Sparse dot	+	2/14	14.30[d]	3.00	R
G2	FKR 74	9	Spot, yellowing	+++	11/14	78.57[b]	7.76	S
G5	C101A51	7	yellowing	+++	11/14	78.57[b]	5.83	S
G8	FKR 19	8	Mosaic	+++	11/14	78.57[b]	5.83	S
G9	FKR 2	7	Yellowing	+++	12/14	85.71[b]	6.14	S
G10	FKR 28	6	Yellowing	+++	12/14	85.71[b]	7.23	S
G11	FKR 33	9	Streaks, mottling	+++	13/14	85.71[b]	7.60	S
G13	FKR 45N	6	Chlorotic	+++	8/14	57.14[b]	6.60	S
G14	FKR 47N	9	Streaks	+++	8/14	57.15[b]	6.87	S
G15	FKR 49N	9	Streaks	+++	9/14	64.29[b]	7.40	S
G16	FKR 50	6	Streaks	+++	10/14	71.43[b]	7.66	S
G18	FKR 53	5	Chlorotic spot	+++	13/14	85.71[b]	7.87	S
G19	FKR 54	7	Spot, yellowing	+++	12/14	85.71[b]	6.16	S
G20	FKR 56N	5	Mottling	+++	12/14	64.30[b]	7.43	S
G21	FKR 58N	7	Yellowing, Necrotic	+++	10/14	71.44[b]	7.68	S
G22	FKR 59	6	Chlorotic spot	+++	8/14	57.16[b]	6.87	S
G23	FKR 60N	10	Chlorotic spot	+++	12/14	85.71[b]	6.23	S
G24	FKR 61	6	Chlorotic spot	+++	10/14	71.45[b]	7.68	S
G25	FKR 62N	8	Necrotic	+++	10/14	71.46[b]	7.68	S
G26	FKR 64	10	Streaks, mottling, yellowing	+++	11/14	78.57[b]	7.00	S
G28	FKR 68	6	Spot, yellowing	+++	13/14	85.71[b]	7.87	S
G29	FKR 70	6	Spot, yellowing	+++	12/14	85.71[b]	6.26	S
G31	FKR 76	8	Spot, yellowing	+++	11/14	78.57[b]	6.17	S
G32	FKR 78	7	Spot, yellowing	+++	11/14	78.57[b]	5.87	S
G33	FKR 80	10	Spot, yellowing	+++	9/14	64.31[b]	7.46	S
G37	IR 67013-58-1-2	7	Spot, mottling	+++	9/14	64.32[b]	7.47	S
G38	IR 75884-12-12-14 WAB-1	8	Spot, yellowing	+++	10/14	71.47[b]	7.70	S
G40	KOGONI	7	Necrotic	+++	8/14	57.17[b]	7.04	S
G45	NIL 2	9	Streaks	+++	10/14	71.48[b]	7.70	S
G48	ORYLUX 3	8	Spot, yellowing	+++	9/14	64.33[b]	7.49	S
G49	ORYLUX 4	8	Spot, mottling	+++	9/14	64.34[b]	7.53	S
G50	ORYLUX 5	10	Necrotic, yellowing	+++	10/14	71.49[b]	7.73	S
G51	ORYLUX 6	8	Spot	+++	11/14	78.57[b]	5.87	S
G52	PNA 647F456	7	Necrotic, yellowing	+++	12/14	85.71[b]	7.28	S
G53	ROCK 25	8	Mottling, necrotic	+++	9/14	64.35[b]	7.53	S
G54	SAHEL 177	7	Mosaic, mottling	+++	8/14	57.18[b]	7.09	S

G55	SAHEL 328	8	Spot, yellowing	+++	9/14	64.36[b]	7.53	S
G56	SAHEL 329	7	Spot, yellowing	+++	8/14	57.19[b]	7.24	S
G61	TS2	10	Streaks, mottling	+++	11/14	78.57[b]	5.93	S
G62	WAB 2066-6-FKR4WAC1-TGR1-B-WAT-B1	7	Spot, yellowing	+++	9/14	64.37[b]	7.54	S
G63	WAB 2066-6-FKR4WAC1-TGR1-BWAT-B16	8	Spot, yellowing	+++	12/14	85.71[b]	7.30	S
G64	WAB 2066-6-FKR4WAC1-TGR1-B-WAT-B6	8	Necrotic, yellowing	+++	12/14	85.71[b]	7.39	S
G65	WAB 2066-6-FKR4WACI-TGR1-BWAT-B11	7	Necrotic, yellowing	+++	13/14	85.71[b]	7.90	S
G66	WAB 2066-6-FKR4WACI-TGR1-BWAT-B9	9	Spot, yellowing	+++	10/14	71.50[b]	7.73	S
G67	WAB 2066-WAT20-1-B-1-TGR1	7	Spot, yellowing	+++	11/14	78.57[b]	5.95	S
G68	WAB 2066-WAT20-1-B-1-TGR3	8	Necrotic, yellowing	+++	11/14	78.57[b]	5.97	S
G69	WAB 2066-WAT21-1-B-1-1	8	Spot, mottling	+++	8/14	57.20[b]	7.24	S
G70	WAB 2066-WAT21-1-B-1-3	8	Mottling, yellowing	+++	9/14	64.38[b]	7.54	S
G71	WAB 2066-WAT21-1-B-1-TGR1	7	Spot, yellowing	+++	12/14	85.71[b]	7.19	S
G72	WAB 2066-WAT21-1-B-1-TGR2	8	Spot, yellowing	+++	12/14	85.71[b]	7.47	S
G73	WAB 2066-WAT21-1-B-1-TGR3	7	Spot, yellowing	+++	11/14	78.57[b]	6.13	S
G76	WAS 183-B-6-2-4	7	Spot, yellowing	+++	9/14	64.39[b]	7.57	S
G77	WAT 1046-B-43-2-2-2	10	Chlorotic spot	+++	10/14	71.51[b]	7.74	S
G78	ARICA 2	7	Mottling	+++	8/14	57.21[b]	7.49	S
G79	ARC 1-448-B-1	8	Mottling, necrotic	+++	9/14	64.40[b]	7.58	S
G83	ARC 1-432-B-1	9	Mottling, yellowing	+++	9/14	64.41[b]	7.60	S

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

Based on the 1- 9 disease rating scale, days post-inoculation and ELISA assay, the genotypes were classified as highly resistant, resistant, moderately resistant, susceptible, and highly susceptible to infection with RYMV. According to these criteria established, when plants of different genotypes were inoculated with RYMV, four genotypes (4.76%) were classified as highly resistant to infection [Tog5672, Tog5674, Tog5681, and Tog7291] and three genotypes (3.57%) as resistant [NIL 130, WAB 2098 R, NIL 16]. The first group manifests no symptoms while the second one has exhibited sparse symptom as well as RYMV detection in relatively low titer (< 0.09, data not showed) in the upper leaves. Seventeen genotypes (20.24%) were classified as moderately resistant, 55 (65.48%) as susceptible, and five (5.95%) as highly susceptible [IR 841, Bouake 189, FKR 55, FKR 42, BG 90-2] (Fig. 2.).

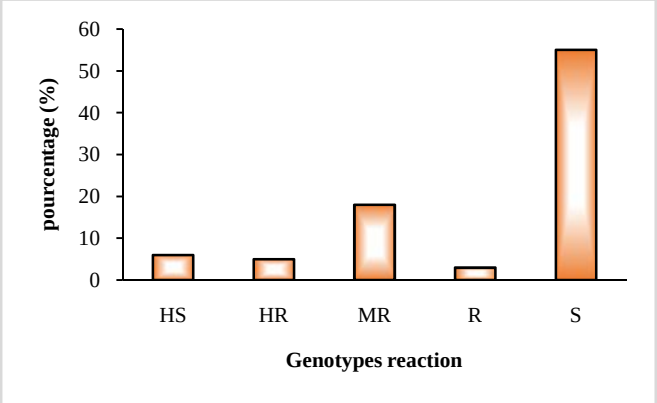

Fig 2 Distribution of genotypes related to their reaction to rice yellow mottle virus inoculation, assessed 31 dpi by their symptom intensity. *HR: Highly resistant, R: Resistant, MR: Moderately resistant, S: Susceptible, HS: Highly susceptible*

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). The high susceptibility of the cv. IR 64 was also confirmed, as it obtained the highest incidence score, reaching 100%. Azucena, chosen as the tolerant control, displayed relative low incidence (28.57 %) with 4.79 as mean severity, its tolerance was therefore confirmed.

DISCUSSION

Rice yellow mottle disease is one of the most important diseases of rice in Africa. Development of genetic resistance is the simplest and most operative method of controlling virus diseases and is especially appropriate for RYMV. Extensive

effort was deployed to develop rice resistance for RYMV, and although tolerance has been described in some varieties (Kam *et al* 2008; Sérémé *et al*., 2016; Traoré *et al*., 2015 et Asante *et al*., 2020), it was not extensively utilized in many cultivated varieties. The genetic control of resistance is complex, and development of RYMV resistance is still a major goal of rice breeding. In the field, occurrence of RYMV is difficult to predict as it may occur in combination with other viruses (Konaté *et al*, 1997; Sérémé, 2010). Hence, screening of rice germplasm against RYMV should be carried out through symptomatology and serology (DAS-ELISA). In this study we systematically evaluated the rice germplasm held at INERA genebank for RYMV resistance/tolerance and identified genotypes that could be used by producers and in rice breeding programs.

Symptom assessments based on both disease rating scale and ELISA tests were conducted at 31 dpi to optimize the method of assessment. Disease severity scores correlated with ELISA values (Table 2) and all the plants that exhibited symptoms (score > 3) in greenhouse were positive by ELISA indicating that symptom assessment is almost reliable. No asymptomatic lines were identified in this study. ELISA result has confirmed the visual observation based on evaluation scale. Indeed, genotypes that showed conspicuous yellow mottle symptoms in the highly susceptible group contained high virus titres. Moderately resistant (MR) genotypes exhibited low disease severity and low ELISA values after RYMV inoculation. These results are consistent with those mentioned by Thottappilly and Rossel (1993), Sérémé *et al*., (2016), Munganyinka *et al*. (2016) and Asante *et al*., (2020).

The susceptible check used for this study was IR 64, a widely available reference cultivar. However, we identified accessions having similar susceptibility to RYMV. Those accessions would make excellent susceptible checks because they have the same incidence of 100 % compared with IR 64 (Table 2). Any of the following accessions could be used as susceptible checks: Bouake 189, FKR 55, BG-90-2, and IR 841.

Our results showed that, excepted *Oryza glaberrima* accessions, none of the 80 rice genotypes screened were immune to RYMV. The majority of the genotypes (78) showed a RYMV incidence of 21.43 to 100%, while only three genotypes had an incidence less than 20%. The symptom severity of RYMV was the lowest in NIL 130 among the genotypes tested. This genotype expressed no mosaic-to-mild mosaic symptoms after RYMV infection, while the rest of the genotypes exhibited characteristic symptoms like mosaic, mottling, yellowing, streaks, and plants death depending upon tested genotypes. Positive reaction was revealed by ELISA, when extracts from symptomatic plants were tested. These results are apparent from those reported by several authors that the large majority of rice germplasm is susceptible to RYMV infection (Rossel and Thottappilly, 1993; Konaté *et al*., 1997; Kouassi *et al*., 2005; Mogga *et al*., 2012; Kam *et al*, 2028, Sérémé *et al*., 2016; Asante *et al* 2020, Traoré *et al*., 2015). This shows the overall susceptibility of the rice germplasm to RYMV in West Africa and underlines the high potential damage that this virus can cause to rice, especially when young plants are infected.

Instead, the use of a mixture of RYMV isolates for inoculation, none of the four *O. glaberrima* accessions [Tog5672, Tog5674, Tog5681, Tog7291] tested were susceptible to RYMV, confirming the strong resistance of these genotypes previously reported by several authors. Indeed, Tog5681, Tog5672, Tog5674, and Tog7291 have the resistance allele *RYMV1-3*, *RYMV1-4*, *RYMV1-5*, and *RYMV2* respectively (Ndjiondjop *et al*., 2001; Albar *et al*., 2006; Thiémélé *et al*.,2010).

Interestingly, results of the present investigation prove that natural resistance or tolerance exists in tested rice genotypes against RYMV. Resistance to RYMV has been reported only in a few rice varieties including *O. glaberrima* accessions (Ghesquière *et al*., 1997; Ndjiondjop *et al*., 1999; Rakotomalala *et al*., 2008, Sérémé *et al*., 2016, Kam *et al*., 2018). So, it is desirable to find new sources of resistance to diversify the genetic basis of the resistance. Thus, these tolerant varieties could be an important component in integrated RYMV disease management. Our results are also consistent with previous study indicated that natural resistance to RYMV was present in NIL 130 and that the variety had minimum incidence from the disease compared to others (Sérémé *et al*., 2016).

Differential response to RYMV inoculation could be observed as a result of the mixture of three isolates used and the difference in their aggressiveness and pathogenicity. This is explained by isolate and host interaction indicating that the response of rice to isolates is genotype as well as isolate dependent. This results is supported by the findings of N'Guessan *et al*. (2001) and Konaté *et al*. (1997). This differential resistance levels and the number of resistance/tolerance genotypes observed in the present study and those of previous studies may also reflect variation in resistance mechanism presents in tested rice genotypes.

CONCLUSION

The present results revealed significant variation in RYMV severity and incidence. Four genotypes were highly resistant, three were resistant, seventeen genotypes were moderately resistant; 60 genotypes were susceptible-to high susceptible to rice yellow mottle disease.

The genotypes with the resistance to RYMV should be used to develop varieties with the highest possible resistance for use in developing resistant genotype. Genotype with moderate resistance could be used by producers in cultivation under integrated production systems.

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