The Resistance of Farmers' rice Varieties to Rice Yellow Mottle Virus (RYMV) at Badeggi, Nigeria

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Summary

Forty-eight farmers' rice varieties and 12 improved and released varieties were screened in the screenhouse at the National Cereals Research Institute (NCRI) Badeggi, Nigeria by mechanical sap inoculation for their resistance to Rice Yellow Mottle Virus (RYMV). The rice varieties were categorized into 4 groups: highly susceptible, moderately susceptible, moderately resistant and resistant, based on standard evaluation scale (SES) for rice. Back-inoculation tests to a highly susceptible variety, Bouake 189 and enzyme linked immunosorbent assay (ELISA) showed that none of the varieties was immune to RYMV. The highly susceptible varieties displayed conspicuous yellow, mottle and stunting symptoms of RYMV. Many varieties were highly susceptible to RYMV and elicited high virus titre. Most of these farmers' varieties are either highly susceptible or moderately susceptible to RYMV.

Résumé

La résistance des variétés traditionnelles de riz aux virus de la panachure jaune de riz (RYMV) à Badeggi, Nigeria

Quarante-huit variétés traditionnelles de riz et douze variétés améliorées de riz vulgarisées, ont été criblées dans la serre de l'Institut national de recherche des céréales (INRC) à Badeggi au Nigeria par inoculation mécanique de la sève pour leur résistance aux virus de la panachure jaune du riz (RYMV). Les variétés de riz testées ont été classées par catégorie dans 4 groupes: très sensible, modérément sensible, modérément résistant et résistant, sur base de l'échelle standard d'évaluation (ESE) pour le riz. La rétroinoculan sur une variété très sensible, Bouaké 189, et le test ELISA ont montré qu'aucune des variétés testées n'était immune au RYMV. Les variétés très sensibles ont présenté des symptômes typiques de la panachure jaune (jaunissement et rabougrissement). Plusieurs de ces variétés très sensibles au RYMV ont également montré un taux élevé de virus. La plupart des variétés de ces fermiers sont très sensibles ou modérément sensibles au RYMV.

Introduction

The *Oryza* species *O. glaberrima* Steud., *O. sativa* L., *O. longistaminata* Chev. and Roehr, *O. barthii* Chev. and *O. punctata* Kotsky and Steud are found in west African rice ecology (21). *O. glaberrima* is said to be indigenous to west Africa (24) and has been in cultivation for the past 3500 years (16, 23). *O. sativa* was introduced into west Africa in about 1890 (12, 30). Another report had it that *O. sativa* was first brought to Madagascar from Indonesia and then to east and west Africa in the 1950s (22).

Rice Yellow Mottle Virus (RYMV) was first noticed in November 1966 along the shores of the Kavirondo Gulf of Lake Victoria, Kenya where the disease had probably been present for a number of years on grass hosts (10). It was noticed in west Africa in 1975 (25) and was subsequently detected in Nigeria in the 1980s (26). The disease is widespread in Africa including Nigeria (3, 5).

RYMV is transmitted by mechanical contact and inoculation of sap (1, 2, 10). The virus is also transmitted by beetles and insects with chewing and biting mouthparts (2, 3, 10). It belongs to the sobemovirus group (17, 27) and is very stable and highly infectious to rice (14). The virus causes a severe disease of rice in most rice growing countries in Africa and its adjourning islands (3). Yield loss ranges from 25 to 100% depending on the date and time of infection as well as the genotype (9).

It has been documented that RYMV is indigenous to Africa (15) and it came to the limelight with the introduction of exotic rice varieties from southeast Asia coupled with intensification of cropping practices without dry season gaps (29). The area originally affected in Kenya was part of a new irrigation project which had led to an increase in rice cultivation due to the availability of water for sequential planting throughout the year (10, 29). It was under similar conditions that RYMV was reported on rice in west Africa in 1975 (25). This situation as well as lack of extensive adaptive testing of the exotic rice varieties in their new environments, led to the disruption of apparent equilibrium established between host local

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rice and RYMV (13). Much traditional African rice such as *O. glaberrima* has been found to have higher level of resistance to RYMV than *O. sativa* (7, 23).
Many improved rice varieties have been released to farmers by National Cereals Research Institute (NCRI) in Nigeria (16). However, the constant cultivation of supposed landraces with local names alongside some new introductions released to or held on by the farmers during on-farm trials has thrown a doubt as to true identity of these landraces. It has also been noticed that most farmers named varieties after either the person or organization that introduced them (6). It is generally believed that local landraces should be more tolerant to stresses than newly introduced exotic varieties (7, 23) because they have co-evolved and became adapted to the environments (29).
The objective of this study is to evaluate the resistance levels of farmers' rice varieties collected from farmers' rice fields in some states in central zone of Nigeria.

Materials and methods

The source of improved and local rice varieties

The varieties with local names were collected from farmers' fields at harvest time while some released improved varieties were obtained from the Genetic Resources Unit of Rice Division, National Cereals Research Institute (NCRI) Badeggi.

The source and maintenance of RYMV isolate

The virus isolate was obtained from infected rice plants in a farmer's field at Edozhigi near Bida, Niger state, and was maintained on Bouake 189, a highly susceptible rice variety, in the screenhouse at Badeggi by serial sap inoculations at the seedlings stages. The virus was designated as "Edozhigi RYMV strain".

Preparation of virus extracts and inoculation procedure

For serial sap inoculations, virus extracts were prepared from virus infected leaves of rice plants. Infected leaves were ground in an electric blender (6 g leaf-tissue/100 ml of distilled water i.e 6% w/v). The virus extracts were finger rubbed on test rice varieties previously dusted with carborundum (600 mesh) to allow virus penetration into leaf tissues. In order to avoid possible escapes from infection, all plants were re-inoculated twice at 2-day intervals as described by Thottappilly and Rossel (28). Twenty-five seedlings of each test variety were first inoculated at 45 days after seeding (DAS) in the screenhouse at Badeggi. Thirty cm diameter plastic pots were filled with 2 kg Fadama topsoil, and three pots were used for each treatment. The seedlings were thinned to five plants per pot. About 3.4 g of NPK (25-10-10) fertilizer was dispensed in each pot at seedling stage when they were 5 weeks old. Some improved rice varieties with known levels of resistance to RYMV (4, 8, 28) were included in the test to serve as reference checks (See footnote on table 2).

Scoring for RYMV

The Standard Evaluation Scale (SES) of 1-9 (18) for RYMV was used to rate the entries at 80 days after planting (DAP). The rating was based on height reduction, mottle and yellow symptoms of infected leaves where 1-3 represents green leaves with sparse dots or streaks and 5 represents green leaves or pale green leaves with mottling. A score of 7 represents pale yellow or yellow leaves whereas 9 represents yellow or orange leaves and some plants dead.

Back-inoculation test

The back-inoculation on Bouake 189, a highly susceptible variety, was carried out at 35 days after inoculation (80 DAP). Sap from leaves of infected plants of every test entry extracted in distilled water as described previously was inoculated to five carborundum dusted 25 days old seedlings of Bouake 189. The leaves were dusted with carborundum prior to inoculation to aid virus penetration into leaf tissues. The back-inoculation tests were rated by ELISA (13, 20).

Enzyme linked immunosorbent assay (ELISA) procedure

ELISA of leaf samples was carried out to evaluate and determine the virus titre in the inoculated rice plant (28). The indirect triple antibody sandwich (TAS) ELISA as described by Koenig and Paul (20) and modified by Virology Unit International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria was followed. The wells of ELISA plates were coated with 100 ul/well of polyclonal antibodies raised in rabbits against RYMV at $^{1}/_{500}$ dilution in coating buffer (1.5 g sodium carbonate, 2.93 g sodium bicarbonate, 0.20 g sodium azide dissolved in 900 ml H_20 and adjusted to pH 9.6 with HCl to make up to 1 litre) and incubated at 37 °C for 2 hours. The plates were then washed three times with phosphate buffered saline-Tween (PBS-T) (8.0 g sodium chloride, 0.2 g monobasic potassium phosphate, 1.15 g dibasic sodium phosphate, 0.2 g potassium chloride, 0.2 g sodium azide dissolved in 900 ml H_20 and adjusted to pH 7.4 with HCl to make up to 1 litre + 0.5 ml Tween 20 per litre) and tapped dry. The sites on the well where antibodies were not adsorbed were blocked with 200 ul of 5% w/v solution of non-fat milk (Marvel, UK) dissolved in distilled water and incubated at 37 °C for one hour. The plates were inverted and allowed to drain. Then 100 ul of sap macerated from 1 g leaf in 10 ml PBS-T + 2% w/v polyvinyl pyrollidone (PVP) were put in each well and left overnight in the refrigerator at 4 °C. The plates were again washed three times with PBS-T and 100 ul of monoclonal antibody (Mab) reared against RYMV at a working dilution of 1:1000 diluted in PBS-T was added to each well of the plates and incubated at 37 °C for 2 hours. The plates were washed further three times with PBS-T. Then 100 ul of goat anti-mouse IgG alkaline phosphotase diluted in conjugate buffer was added per well and incubated at 37 °C for 2 hours. It was

further washed three times with PBS-T and 200 ul of 1 mg/ml of p.nitrophenyl phosphate substrate tablets dissolved in substrate buffer (97 ml diethanolamine 600 ml H_20, 0.2 g sodium azide adjusted to pH 9.6 with HCl and make up to 1 liter with H_20) was added to each well. The plates were incubated at 37 °C and the colour change in the substrate quantified at A405 nm with a DYNEX MR ELISA micro-reader after 1 hour. Absorbance values (A405 nm) were accepted as positive when the reading was greater or equal to twice the mean absorbance of the non-infected control rice sample.

Table 1
Morphological characteristics, visual scores and ELISA values of some farmers and researchers' varieties

S/No	Varietal names	Location/ Village	States	Agronomic Characteristics			RYMV score	ELISA Values (A405 nm)
				Days to 50% Flow.	Plant height (cm)	Pan/m ^{2}		
1	Tomawawagi	Gubata	Niger	89	102	260	7(MS)	1.19
2	Ebangichi	Badeggi		88	89	250	9(HS)	1.61
3	Saganuwangi	Kanko		93	91	200	9(HS)	1.96
4	Tomako	Kusotachin		102	97	240	9(HS)	1.84
5	Ladanci	Doko		89	101	165	7(MS)	1.48
6	Ebangichi	Kusotachin		86	88	220	7(MS)	1.92
7	Nasara	Bidakowangi		65	105	205	5(MR)	0.72
8	Gyanako	Kusotachin		113	105	225	5(MR)	0.87
9	Ebangichi	Gbadafu		103	127	275	7(MS)	1.51
10	Toma	Doko		89	98	325	9(HS)	2.16
11	Egwazawunkpa	Doko		86	103	225	9(HS)	1.25
12	Finiko	Gbadafu		98	113	315	7(MS)	1.70
13	Philippines	New Bussa		85	87	200	9(HS)	1.84
14	Danmale	New Bussa		89	70	310	5(HS)	1.83
15	Gargaza	New Bussa		82	87	195	7(MS)	1.49
16	Mass	Dwarfu		106	103	215	7(MS)	1.77
17	Ebangichi	Edozhigi		83	88	205	7(MS)	1.69
18	Jufangi	Kanko		82	68	185	9(HS)	1.97
19	Faro-Sipi	Gbadafu		96	98	220	9(HS)	1.83
20	Somazhigi	Doko		86	102	285	5(MR)	1.28
21	Gabaci	Gbadafu		82	138	160	5(MR)	0.83
22	Dokoci	Doko		97	104	190	5(MR)	0.59
23	Manbeci	Kanbari		107	119	210	3(R)	0.35
24	Shankuyagi	Kusotachin		86	119	190	7(MS)	1.22
25	Ndawodzufanci	Sheshi Audu		89	116	145	9(HS)	1.90
26	Bokuchi	Doko		75	122	150	7(MS)	1.62
27	Gyanako	Chanchaga		105	124	200	9(HS)	1.68
28	Shagari	Gubata		75	122	220	7(MS)	1.44
29	Ebangichi	Kanko		82	113	190	5(MR)	0.83
30	Bisalane Yakolo	Chanchaga		103	78	270	2(R)	0.26
31	Eyewawagi	Kusotachin		100	117	180	2(R)	0.31
32	Nnakashi Kpanti	Dwarfu		114	118	195	2(R)	0.29
33	Mambechi	Edozhigi		82	117	140	2(R)	0.26
34	Ndacelegbo	Dwarfu		101	134	180	2(R)	0.37
35	Dubbu 1	Ndabissan		86	104	190	2(R)	0.35
36	Faran Kaura	Birnin Kebbi	Kebbi	98	134	205	3(R)	0.30
37	Akpuruka	Ndabissan	Niger	100	88	275	7(MS)	1.66
38	Jarankaura	Birnin Kebbi	Kebbi	79	106	150	5(MR)	1.17
39	Ndabisangi	Ndabissan	Niger	98	97	125	7(MS)	1.66
40	Kpuruga	Gaza		75	98	170	5(MR)	1.14
41	Danboto	Birnin Kebbi		82	88	165	5(MR)	0.74
42	Gbagudu	Tufa		107	123	270	9(HS)	1.90
43	Pasankunya	Tufa		76	122	285	2(R)	0.28
44	Janiri	Birnin Kebbi	Kebbi	105	137	175	7(MS)	1.55
45	Manbekochi	Ndabissan	Niger	82	102	250	7(MS)	1.55
46	Bubanfari	Birnin Kebbi	Kebbi	98	83	235	5(MR)	1.43
47	Ebangichi	Gadza	Niger	82	90	270	5(MR)	0.36
48	Dubu 2	Ndabissan		101	93	155	5(MR)	1.50
49	Nasarawa 1	Lafia	Nasarawa	96	88	190	7(MS)	1.95
50	Ndachele	Ndabissan	Niger	107	131	140	2(R)	0.30
51	Maiada	Birnin Kebbi	Kebbi	103	132	175	5(MR)	0.51
52	FARO 44	NCRI, Badeggi	Niger	92	96	228	9(HS)	1.83
53	LAC 23			93	118	100	2(R)	0.31
54	Suakoko 8			115	119	245	2(R)	0.28
55	FARO 52 (WITA 4)			97	102	175	7(MS)	1.61
56	FARO 27			99	98	288	5(MR)	1.48

HS= Highly Susceptible, MS= Moderately Susceptible, MR= Moderately resistant, R= Resistant, According to IRRI (1996)
ELISA= Enzyme linked immunosorbent Assay. Numbers 1 - 51 are varieties with local names while numbers 52 - 56 are varieties with researchers names.

Results and discussion

Some of the agronomic characteristics, RYMV score and ELISA values of the test varieties are presented in table 1. It indicated high diversity in days to fifty percent flowering, ranging from early to very late maturing. Many entries are dwarf to intermediate (70 to 120 cm) in height while few exhibited tall height (about 130 cm). The number of panicles per square meter ranged from 100 to 325. It was significant to note that all the tested cultivated rice varieties harbored the virus and none was immune although many of these varieties are cultivated widely in northern part of Nigeria (5), hence the spread of RYMV.

The results of the screening showed that 13 varieties were highly susceptible, 17 moderately susceptible, 14 moderately resistant and 12 resistant to the virus (Table 2). The highly susceptible varieties displayed conspicuous yellow, mottle and stunting symptoms of RYMV. Other varieties in this group exhibited stunted growth and eventually died. The local landraces exhibited similar symptoms of RYMV to those exhibited by the improved varieties such as Bouake 189, IR5, and FARO 44 (Sipi 692033) classified in the same group. As mentioned by Thottappilly and Rossel (28), the ELISA result corresponded with the visual rating based on SES scale. The varieties that showed conspicuous yellow mottle symptoms in the highly susceptible group contained high virus titre. Some varieties in moderately resistant group such as Nasara, Somazhigi, Ebangichi (Kanko), Dubu 1 and Dubu 2 however contained high virus titre in ELISA yet they exhibited mild and less conspicuous visual symptoms.

It was found that many farmers' varieties had similar agronomic characteristics and groupings to the improved released varieties (Tables 1 and 2). It is possible that most of these varieties with local names are not actually landraces but improved and released varieties which have lost their identity over time through the deliberate re-naming of such varieties by farmers. It is also possible that the farmers' variety called "Philippines" might have been a lowland indica introduced from southeast Asia. The indica

Table 2
Resistance levels of farmers' varieties with local names to Rice Yellow Mottle Virus (RYMV) as determined by visual evaluation scale, ELISA and back-tests in the screenhouse at Badeggi, Niger State, Nigeria

Groupings of varieties into categories	Reaction rating (SES) [1]	ELISA (A405 nm)	Back-test inoculation test[2]
Highly susceptible (HS)			
Toma, Faro-Sipi, Ndawodzufanchi, Ebangichi (Badeggi), Philippines, Gyanako (Chanchaga), Danmale, Egwazawunkpa, Saganuwangi, Tomako, Bouake 189, IR5, FARO 44 (Sipi 692033)	9 (HS)	+++	+++
Moderately susceptible (MS)			
Tomawowagi, Landaci, Ebangichi (Kusotachin), Ebangichi (Gbadafu), Ebangichi (Edozhigi), Finiko, Gargaza, Mass, Shankuyagi, Bokuchi, Akpuruka, Ndabisangi, Janiri, Manbekochi, Nasarawa1, FARO 52, FARO 29 (BG90-2)	7 (MS)	+++	+++
Moderately Resistant (MR)			
Nasara, Gyanako (Kusotachin), Somazhigi, Gabaci, Dokoci, Ebangichi (Kanko), Kpuruga, Danboto, Bubanfari, Ebangichi (Gadza), Dubu 2, FARO 27, FARO 40, Suakoko 8	5 (MR)	++	+++
Resistant (R)			
Manbechi (Kanbari), BisalaneYakolo, Eyewawagi, Dubbu1, Maiada (Birnin Kebbi), Nnakashi kpanti, Manbeci (Edozhigi), Ndacelegbo, Faran Kaura (Birnin Kebbi), Ndachele, Moroberekan, LAC23	1-3 (R)	+	+++

[1]IRRI(1996) Standard Evaluation Scale (SES) for rice
[2]Back-inoculation test to a Highly Susceptible rice variety, Bouake 189
ELISA= Enzyme linked immunosorbent assay
Researchers' varieties used as checks in different groupings: Highly Susceptible (HS)= Bouake 189, IR5, FARO 44 (Sipi 692033); Moderately Susceptible (MS)= FARO 52, FARO 29 (BG90-2); Moderately Resistant (MR)= FARO 27, FARO 40, Suakoko 8; Resistant (R)= Moroberekan, LAC 23.

type varieties are highly susceptible to RYMV (3, 4, 9). Therefore, further classification of these varieties with local names should be made to ascertain their real identity so that they could be used as donors for breeding for resistance to RYMV in Nigeria.

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