Research Journal of
Applied Science
MEDWELL PUBLICATIONS

Determination of Pathogenicity Relationships of Rice Yellow Mottle Virus (RYMV) Isolates on Near Isogenic Lines (NILs) and Some Released *Oryza* Species in Nigeria

[1, 2]J.T. Onwughalu, [1, 2]M.E. Abo, [2]A.O. Nwankiti, [2]J.K. Okoro and [3]D. Silue
[1]*National Cereals Research Institute (NCRI), Badeggi, Niger State, Nigeria*
[2]*Department of Crop and Environmental Protection, University of Agriculture Makurdi, Benue State, Nigeria*
[3]*Africa Rice Center (Africa Rice), Cotonou, Benin Republic*

Key words: Pathogenicity, RYMV isolates, rice genotypes, near isogenic lines, investigation

Corresponding Author:
J.T. Onwughalu
National Cereals Research Institute (NCRI), Badeggi, Niger State, Nigeria

Page No.: 27-35
Volume: 15, Issue 1, 2020
ISSN: 1815-932x
Research Journal of Applied Sciences

Abstract: The study was carried out to investigate pathotypes relationship of six Rice Yellow Mottle Virus (RYMV) isolates from North Central Zone of Nigeria in 2016 using some released *Oryza* species and Near Isogenic Lines (NILs). The experiment was laid out in a split-split plot design in the screen house at Badeggi, Niger State, Nigeria. Yield and agronomic data were subjected to statistical analysis using CropStat Version 7.2. The result showed a non-significant interaction effect of rice genotypes, disease isolates and inoculation regimes on spikelets sterility (%) and a highly significant effect ($p = 0.01$) on yield per plant (g) at harvest. The impact of the virus on the number of panicles per plant at maturity was significantly higher on test plants inoculated at active tillering stages which consistently produced lower mean panicle numbers across the levels of virus isolates. The result also showed that NIL 54 produced high resistance to the virus which is not due to immunity as the leaf extract from inoculated plant was infectious on susceptible FKR 28 during back inoculation test. NIL 54 did not also record significant reduction on the average leaf length (cm) per plant at maturity with the virus isolates; neither did it express the characteristic symptoms of the virus with the six isolates. Obubu-Ofu and Makurdi isolates circumvented the resistance in Gigante and showed obvious yellow mottle symptoms. The serological analysis and RYMV isolates characterization indicated that the six virus isolates belonged to sero groups 1 and 2 (S1 and S2). NIL 54 is recommended for further pathogenicity investigation with more isolates of RYMV in Nigeria or elsewhere, since, no obvious symptom of the virus was observed on the plant when challenged with S2 and S1 isolates in the present study.

INTRODUCTION

Rice has become a regular item in the Nigerian diet, largely because of the convenience in which it can be prepared, however, its culture is threatened by biotic and abiotic stresses which includes virus diseases[1-5]. Over 30 viruses are reported to infect rice through experimental tests and in nature with 25 of them having direct economic impact on rice production[6, 7]. Majority of these viruses occur in countries of Asia and the America, whereas only 5 (Rice stripe necrosis furovirus, rice crinkle disease, Maize streak gemini virus strain A, African cereal streak virus and rice yellow mottle sobemovirus) are known to infect rice in nature in Africa and its neighboring islands[8]. The Rice Yellow Mottle Virus (RYMV) is the most economically important viral disease of rice in Africa where it causes severe yield losses[11-15]. The virus belongs to *Sobemovirus* group, a single stranded positive sense RNA. Since, it was first reported in Nigeria in 1975[9]; the virus has spread to other lowland rice growing areas in the country, having been reported in rice fields at tillering and panicle initiation stages in Niger, Kano, Bauchi, Gombe and Benue States in the North Central Zone of Nigeria between 2000 and 2008[16, 17] and also States of Lagos, Oyo, Ogun, Ekiti and Ondo[18] as well as Akwa-Ibom, Ebonyi and Sokoto States[2]. It was also reported in Zamfara State[19] and other southeastern States of Delta, Imo and Anambra[20].

Most rice cultivars, especially those of the *Oryza sativa indica* species, are susceptible to RYMV and suffer yield losses depending on genotype, viral strain and age of plant at time of infection among other factors[21, 22]. The rice (*O. sativa*) cultivar Gigante and the African rice (*O. glaberrima*) cultivars Tog5681, Tog5672, Tog 5674 and Tog5675 are known to be highly resistant to RYMV[23, 24]; hence, negligible yield losses were reported when resistant cultivars Gigante and Tog 5672 were challenged with 15 RYMV isolates in field trials[25]. During the past years, several NILs (BC_3F_5) with rymv1-2 resistant allele from Gigante were developed by the Biotechnology Unit at Africa Rice Center (Africa Rice) and they have been evaluated for resistance to RYMV in the Republics of Mali and Guinea[26] as well as Nigeria[27]. Enzyme-Linked-Immuno Sorbent Assay (ELISA) test also revealed 36 NILs to be resistant to RYMV with low virus content with 22 lines showing introgression of rymv1-2 allele through gene marker selection[26]. However, RYMV isolates were capable of breaking down the high resistance of Gigante and Tog 5681 in a natural infestation condition[13] or after serial inoculations[21]. The existence of different RYMV strains in the field that differ in their pathogenicity has therefore become a matter of considerable practical importance[18]. The reports of Resistance Breaking (RB) isolates of RYMV[28, 29] and the wide geographical distribution and high frequencies of RB isolates according to Traore *et al*.[14] represent a high risk for the durability of resistance to RYMV. The implication is that if such RB isolates are frequent in field conditions, it would undermine the stability of resistance to RYMV existing in some rice cultivars as well as the Near Isogenic Lines (NILs) developed for RYMV resistance in Africa.

As a result of this growing economic importance of RYMV, and reports of emerging resistance breaking virus isolates in some countries of Africa[28, 29, 14], it has become necessary to investigate the pathotypes and pathogenicity of isolates in Nigeria. Pathogenic variability determination and validation of molecular traits for increasing RYMV resistance in rice crop is pivotal to the rice value chain development in Nigeria. This will greatly facilitate breeding programs geared towards development of durable resistance which remains the most reliable control measure against the virus disease.

MATERIALS AND METHODS

The experiment was conducted in the screen house at the National Cereals Research Institute (NCRI) Badeggi between May and October, 2016. Rice genotypes used in the experiment included four Near Isogenic Lines (NILs)-NILs 2, 130, 16 and 54, two checks (Gigante-Resistant Check (RCK) and FKR 28-Susceptible Check (SCK)) obtained from the Africa Rice Center (AfricaRice) Cotonou, Benin Republic and two conventional farmers' choice varieties, FAROs 44 and 52 sourced from the genetic resource unit of NCRI, Badeggi, Nigeria.

The experiment was laid out in a split-split plot design with the virus isolates, ricegenotypes and inoculation regimes constituting the three factors. The virus isolates were given the village names where they were collected on rice fields as follows: Mararaba-Obi (Nasarawa State), Gulu, Wuya, Edozhigi (Niger State), Makurdi (Benue State) and Obubu-Ofu (Kogi State). Virus inoculation regimes included a control (zero inoculation) according to Little and Hills[30] with the other inoculation regimes at active tillering (42 Days After Transplanting, (DAT)) and booting stages of the plants. The 6×8×3 factorial (144 treatment combinations) was replicated thrice and treatment allocation was at random using the paper disc method[31, 32].

Rice seedlings were raised in the nursery box and transplanted, one seedling per bucket when they were 3 weeks old. Soil media (fresh fadama soil) was collected from NCRI lowland research field at Badeggi, sun-dried and later moistened with tap water before filling the buckets with 3 kg fadama soil. The buckets were constantly supplied with fresh tap water in the mornings and evenings until maturity (i.e., 2 weeks before harvest). 3.4 g of N: P: K 20:10:10 was applied to the plants at

seedling stage (21 DAT) to enhance tiller establishment; and followed later by split application of Urea (2 g per bucket) at 45DAT and at early flowering stages to ensure optimal growth and yield.

Leaves of RYMV infected plants in the screen house were cut with sterilized razor blade and ground in an electric blender (6 g leaf-tissue 100 mL^{-1} of distilled water, i.e., 6% w/v). An abrasive, carborundum powder (600 mesh) was added to the inoculums at the rate of 5 mg mL^{-1}[13] to aid virus penetration into leaf tissues. Virus extracts were finger-rubbed on upper and lower leaves of the test plants at active tillering stage (42 DAT) and booting stages. In order to avoid possible escapes from infection, all test plants were re-inoculated twice at 2-day intervals according to Thottappilly and Rossel[33]. RYMV symptom expression was scored based on Standard Evaluation Scale (SES) developed by IRRI (20) for rice at 35 Days After Inoculation (DAI). Plants assessed were classified as Highly Resistant (HR), Moderately Resistant (MR) and Highly Susceptible (HS) using the SES score.

ELISA techniques: Antigen Coated-Plate Enzyme Linked Immunosorbent Assay (ACP-ELISA) was carried out at the Plant Pathology Unit of the AfricaRice. Leaf samples earlier collected from the virus inoculated rice plants at tillering and booting stages in the screen house at Badeggi were used to check the virus content. Leaf samples were ground in coating buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$, 20 g DIECCA, pH 9.6) at 1:10 (0.1 g to 1000 µL) dilution. 100 µL virus sap extract was added to duplicate wells of microtitre plate and incubated for 1 hour at 37°C in a humid chamber. The plates were then washed with phosphate buffer saline-Tween 20 (PBS-T), which constituted (8 g NaCl, 0.2 g KH_2PO_4, 1.1 g Na_2HPO_4 and 0.2 g KCl) plus Tween 20 (500 µL) in 3 washes with a washing bottle for 3 min. Plates were then dried by tapping upside down on tissue paper and later loaded with 200 µL of 3% Bovine Serum Albumin (BSA) to block each well. The plate was incubated at 37°C for 1 h, after which the solution (BSA) was poured off and dried by tapping on tissue paper with no washing. Polyclonal antiserum (1 µL of serum in 10 mL of conjugate buffer) was diluted and incubated at room temperature for 1 h with gently shaking using an electronic shaker. Then, 100 µL of the solution was dispensed into each well of the ELISA plate, covered and incubated in a humid chamber at 37°C for 1 h. Plates were washed with PBS-T and dried using the same method above. After washing 100 µL of goat anti-rabbit alkaline phosphate (Sigma), a commercially purchased enzyme diluted 1:15,000 in conjugate buffer (PBS-T+2% PVP-40 polyvinyl pyrrolidone+0.2% egg albumin (Sigma A-5253) was added to each well and incubated at 4°C overnight.

After overnight incubation, the plates were washed again thrice in 3 min with 3 changes of PBS-T. 100 µL of freshly prepared substrate (1 mg mL^{-1} of p-nitrophenylphosphate [Sigma] in substrate buffer (diethanolamine, sodium azide (NaN_3, H_2O) pH 9.8) was then added to each well and incubated at room temperature for 1 h. Results were assessed by visual observation and reading of samples with spectrophotometer measurement at absorbance (OD 405 nm) after 3 h. Colour change was accepted as positive when the reading was at least greater or equal to twice the mean absorbance of the non-infected control rice sample. The serological profiles of the virus isolates were also determined using monoclonal antibodies (MAbs A, G and D) in the Triple-Antibody Sandwich-Enzyme Linked Immunosorbent Assay (TAS-ELISA) as described by Koenig and Paul[34] and modified by AfricaRice Center to determine strain diversity. The wells of ELISA plates were coated with 100 uL/well of polyclonal antibodies raised in rabbits against RYMV at 1/1000 dilution in coating buffer (1.59 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 L) and incubated at 37°C for 2 h. 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, 1.1 g dibasic sodium phosphate and 0.2 g potassium chloride dissolved in 900 mL H_20 and adjusted to pH 7.4 with HCl to make up to 1 L+0.5 mL Tween 20 per L) and tapped dry. The sites on the well where antibodies were not adsorbed were blocked with 200 µL of 3% w/v solution of skimmed milk dissolved in distilled water and incubated at 37°C for 1 h. The plates were then washed once with PBS-T for 3 min and tapped to dry on tissue paper. Then 100 µL of sap macerated from 1 g leaf (using mechanical blender) in 10 ml PBS-T were put in each well and incubated at 37°C for 2 h. The plates were again washed three times with PBS-T in 3 min and tapped dry on a tissue paper upside down. About 100 µL of Monoclonal antibody (Mab) reared against RYMV at a working dilution of 1:8000 diluted in PBS-T was added to each well of the plates and incubated overnight at 4°C. The plates were then washed with PBS-T titre in 3 min and tapped dry.

Then, 100 µL of goat anti-rabbit alkaline phosphatase (Sigma) diluted to 1:1000 in conjugate buffer (i.e., PBS-Tween+2% egg albumin) was added to each well and incubated at 37°C for 2 h. The plates were again washed three times with PBS-Tween and tapped dry on tissue paper. Then, 200 µL of freshly prepared substrate (1 mg mL^{-1} of p-nitrophenyl phosphate dissolved in substrate buffer (i.e., 97 mL diethanolamine, 800 mL H_2O, 0.2 g NaN_3, adjusted to PH 9.6 with HCl and made up to 1 L with H_2O) was added to each well and incubated in the dark at room temperature for 1 h. The colour

change in the substrate assessed by spectrophotometric measurement with ELISA reader (DYNEX MR ELISA) at absorbance (OD 405 nm) after 3 h was accepted as positive when the reading was at least greater or equal to twice the mean absorbance of the non-infected control rice sample.

Back inoculation test for virus isolates was conducted using leaf extract from test plants earlier inoculated at booting stage with the six virus isolates. Extract preparation and inoculation procedures followed the steps described above and the inoculation was done on the RYMV susceptible FKR 28 at 21 Days After Planting (DAP). Symptom expression of RYMV was observed and scored after 5 weeks from inoculation date based on SES[35].

Growth and yield parameters such as plant height (cm), number of panicle per plant at maturity and 1000 grain weight per plant at harvest were measured. Percent spikelets sterility (%) per plant at maturity and yield (g/plant) were also determined. Plant yield (g/plant) was determined by weighing (using sensitive electronic scale), the quantity of rice spikelets produced at harvest per plant. Measured parameters were analyzed using Cropstat Windows Version 7.2[36].

RESULTS

The main effect of rice genotypes treatments means on plant height (cm), number of leaves per plant and average leaf length (cm) per plant at maturity indicated that NIL 54 outperformed the other test plants, producing a significantly (p = 0.01) highest mean height of 93.33 cm, 29.83 tillers and average leaf length of 39.82 cm at maturity (Table 1). Obubu-Ofu isolate gave the highest height reduction with a mean value of 82.31 cm, whereas the highest impact on tiller production was recorded by Edozhigi isolate, which produced the lowest mean number of tillers per plant at maturity as 23.99.

The treatment main effect of inoculation regimes indicated that plants inoculated at booting stages produced lower mean effect on number of panicles per plant at harvest as well as the average panicle length (cm) per plant at maturity (Table 2). However, disease severity (%) was higher on test plants inoculated at active tillering stages (I_1), which gave 0.391% with Makurdi isolate recording the highest mean disease severity of 0.245% (Table 2).

Table 3 indicated that the main effect of the three factors produced a highly significant effect (p = 0.01) on spikelets sterility (%), 1000 grain weight (g) and yield (g) per plant at harvest. NIL 54 gave the lowest mean value of 31.34% for the spikelets sterility at harvest and also highest yield of 18.69g at harvest which was significantly higher (p = 0.01) than yield records obtained by other rice genotypes. Moreover, the ELISA result (Table 4) indicated that all test plants contained varying levels of the virus, with the RYMV indexing showing that virus content varied across rice genotypes and inoculation stages. The result also showed that the virus multiplied more in plants inoculated at active tillering stages which gave higher virus contents (Table 4). Irrespective of the levels of symptom expression on rice genotypes,

Table 1: Main effect of virus isolates, rice genotypes and inoculation regimes on measured agronomic parameters at maturity

	Parameters			
Treatments	Plant height (cm) plant^{-1}	Number of tillers plant^{-1}	Number of leaves plant^{-1}	Average leaf length (cm) plant^{-1}
Virus isolates				
Mararaba-Obi	89.53	23.47	67.97	85.34
Gulu	86.25	26.32	71.40	32.39
Makurdi	85.17	32.06	72.13	36.53
Wuya	87.29	24.79	67.74	40.13
Edozhigi	83.62	23.99	67.88	39.22
Obubu-Ofu	82.31	24.15	60.88	42.24
LSD (P = 0.05)	2.577**	1.971**	5.284**	1.466**
Rice genotypes				
NIL 130	84.07	25.96	69.69	36.74
NIL 2	90.43	32.19	66.28	38.69
NIL 16	80.50	22.91	70.54	37.34
NIL 54	93.33	29.83	68.72	39.82
Gigante (RCK)	84.26	25.76	65.48	36.81
FKR 28 (SCK)	87.69	23.74	69.50	37.99
FARO 44	79.70	22.24	66.19	37.47
FARO 52	85.57	23.74	67.04	36.29
LSD (p = 0.05)	2.976**	2.276**	6.102NS	1.692**
Inoculation regimes				
I_1	88.79	26.99	87.34	37.90
I_0 (Control)	97.03	30.81	88.83	39.90
I_2	71.26	19.59	27.62	35.13
LSD (p = 0.05)	1.822**	1.394**	3.748**	1.036**

** = Highly significant (p = 0.01); NS = Non Significant; LSD = Fisher's Least Significant Difference; I_1 = Inoculated at tillering stage; I_2 = Inoculated at booting stage; I_0 = un-inoculated control entries; SCK = Susceptible Check; RCK = Resistant Check

Table 2: Main effect of virus isolates, rice genotypes and inoculation regimes on some yield components and disease severity at 42 Days After Inoculation (DAI) in the screen house condition

	Parameters				
Treatments	Date to 100% flowering plant^{-1}	Percent productive tillers plant^{-1}	Number of panicles^{-1} at harvest	Average panicle length (cm) plant^{-1}	Disease severity (%) at 42 DAI
Virus isolates					
Mararaba-Obi	96.04	48.89	12.04	21.29	0.182
Gulu	100.10	48.27	12.96	21.07	0.208
Makurdi	99.71	59.26	11.85	20.88	0.245
Wuya	101.74	50.85	11.54	20.09	0.193
Edozhigi	105.86	52.81	11.93	20.99	0.172
Obubu-Ofu	104.04	54.72	11.04	19.91	0.219
LSD (p = 0.05)	0.319**	4.463**	0.777**	0.839**	NS
Rice genotypes					
NIL 130	108.33	65.50	8.30	21.60	0.225
NIL 2	103.11	64.01	11.06	20.93	0.152
NIL 16	88.39	44.25	12.22	21.58	0.200
NIL 54	99.17	40.97	16.54	20.36	0.048
Gigante (RCK)	88.06	38.03	15.44	20.40	0.270
FKR 28 (SCK)	109.24	62.16	9.04	19.20	0.304
FARO 44	95.54	43.20	13.07	21.11	0.170
FARO 52	118.15	61.61	9.48	20.46	0.256
LSD (p = 0.05)	0.368**	5.153**	0.897**	0.969**	0.511**
Inoculation regimes					
I_1	100.23	53.66	11.81	21.54	0.391
I_0 (Control)	91.44	46.23	16.33	22.17	0.000
I_2	112.08	57.52	7.54	18.40	0.219
LSD (p = 0.05)	0.226**	3.156**	0.549**	0.593**	0.031**

** = Highly significant (p = 0.01); * = Significant (p = 0.05); I_1 = Inoculated at tillering stage; I_2 = Inoculated at booting stage; I_0 = un-inoculated control entries; SCK = Susceptible Check; RCK = Resistant Check

Table 3: Main effect of virus isolates, rice genotypes and inoculation regimes on spikelets sterility (%), 1000 grain weight (g) and yield (g) per plant

	Parameters		
Treatments	Spikelets sterility (%)	1000 grain weight per plant	Yield (g) per plant
Virus isolates			
Mararaba-Obi	32.91	19.24	18.64
Gulu	42.37	20.43	14.14
Makurdi	43.09	17.61	13.88
Wuya	43.41	18.58	12.83
Edozhigi	41.64	19.82	12.19
Obubu-Ofu	44.72	19.67	10.80
LSD (P = 0.05)	5.336**	1.448**	0.989**
Rice genotypes			
NIL 130	46.01	18.81	10.58
NIL 2	32.24	19.87	11.94
NIL 16	35.95	20.44	16.41
NIL 54	31.34	20.30	18.69
Gigante (RCK)	32.15	20.43	17.65
FKR 28 (SCK)	55.78	15.22	8.16
FARO 44	45.21	20.22	15.39
FARO 52	52.19	18.50	11.16
LSD (p = 0.05)	6.162**	1.672**	1.142**
Inoculation regimes			
I_1	41.82	19.61	14.63
I_0 (Control)	27.38	21.42	18.88
I_2	54.87	16.66	7.73
LSD (p = 0.05)	3.773**	1.024**	0.700**

** = Highly significant (p = 0.01); LSD = Fisher's Least Significant Difference; I_1 = Inoculated at tillering stage; I_2 = Inoculated at booting stage; I_0 = un-inoculated control entries; SCK = Susceptible Check; RCK = Resistant Check

leaf extracts of all earlier inoculated rice genotypes were highly infectious on Back Inoculation Test (BIT) and elicited characteristic symptoms of RYMV at SES score of 5 (MR) with the susceptible FKR 28. Leaf extracts from NIL 54 with negative RYMV indexing similarly was infectious and elicited RYMV symptoms on the highly susceptible FKR 28 (Table 4).

The result of the serological test (Table 5) indicated that RYMV was detected with monoclonal antibodies of Mabs A, Mabs G and Mabs D. Four isolates from Obubu-Ofu, Wuya, Makurdi and Mararaba-Obi showed the characteristic feature of Ser.I strain of the virus, whereas two isolates from Edozhigi and Gulu were characterized as Ser 2 strain of the virus (Table 5).

Table 4: Reaction levels of the rice genotypes to Rice Yellow Mottle Virus (RYMV) as determined by SES score, ELISA result and Back Inoculation Test (BIT)

		Visual (SES) score		ELISA values (OD 405 nm, 3h)		RYMV Indexing		
Disease isolates	Rice genotypes	I_1	I_2	I_1	I_2	I_1	I2	BIT
Mararaba-Obi	NIL 130	7	5	0.907	0.195	+++	-	+++
	NIL 2	1	1	0.187	0.183	-	-	+++
	NIL 16	3	3	1.620	0.203	++++++	-	+++
	NIL 54	1	1	0.180	0.182	-	-	+++
	Gigante (RCK)	7	5	1.163	0.160	+++	-	+++
	FKR 28 (SCK)	9	9	1.600	1.063	++++++	2	+++
	FARO 44	7	5	0.713	0.186	+++	0	+++
	FARO 52	7	7	0.492	0.505	1	0	+++
Gulu	NIL 130	7	5	1.087	0.183	+++	-	+++
	NIL 2	3	1	0.184	0.169	-	-	+++
	NIL 16	7	3	1.855	0.228	+++++	-	+++
	NIL 54	1	1	0.171	0.159	-	-	+++
	Gigante (RCK)	7	7	1.646	0.152	+++++	-	+++
	FKR 28 (SCK)	7	7	1.366	0.165	++++	-	+++
	FARO 44	5	5	1.395	1.847	++++	+++++	+++
	FARO 52	7	5	1.24	1.474	++++	++++	+++
Makurdi	NIL 130	5	3	0.971	0.251	+++	-	+++
	NIL 2	1	1	0.173	2.260	-	++++++	+++
	NIL 16	7	5	2.154	0.183	++++++	-	+++
	NIL 54	1	1	0.176	0.156	-	-	+++
	Gigante (RCK)	7	5	1.698	0.149	+++++	-	+++
	FKR 28 (SCK)	9	7	0.877	0.172	+++	-	+++
	FARO 44	7	7	1.743	0.189	+++++	-	+++
	FARO 52	7	5	0.796	1.010	0	+++	+++
Wuyya	NIL 130	7	5	1.15	0.254	+++	-	+++
	NIL 2	1	1	0.152	0.150	-	-	+++
	NIL 16	5	5	0.148	0.172	-	-	+++
	NIL 54	1	1	0.164	0.159	-	-	+++
	Gigante (RCK)	9	7	1.985	1.187	++++++	+++	+++
	FKR 28 (SCK)	9	9	1.758	2.193	+++++	++++++	+++
	FARO 44	9	7	1.726	1.455	+++++	++++	+++
	FARO 52	5	5	0.334	0.181	-	-	+++
Edozhigi	NIL 130	1	1	0.161	0.190	-	-	+++
	NIL 2	1	3	0.173	0.183	-	-	+++
	NIL 16	7	5	1.446	0.204	++++	-	+++
	NIL 54	1	1	0.150	0.146	-	-	+++
	Gigante (RCK)	7	5	1.890	0.151	+++++	-	+++
	FKR 28 (SCK)	7	9	1.289	1.671	++++	+++++	+++
	FARO 44	7	5	1.768	0.188	+++++	-	+++
	FARO 52	3	5	0.171	0.221	-	-	+++
Obubu-Ofu	NIL 130	5	3	1.117	0.160	+++	-	+++
	NIL 2	5	3	0.778	0.181	0	-	+++
	NIL 16	7	3	0.171	1.878	-	+++++	+++
	NIL 54	3	1	0.176	0.169	-	-	+++
	Gigante (RCK)	5	5	0.365	0.176	0	-	+++
	FKR 28 (SCK)	9	7	1.796	0.232	+++++	-	+++
	FARO 44	7	5	1.335	0.275	++++	-	+++
	FARO 52	5	5	0.228	0.189	-	-	+++

SES: Standard Evaluation Scale for rice at 5 weeks after inoculation (IRRI, 1996); where, 1 - 3 = R (resistant), 5 = MR (Moderately Resistant), 7 = MS (Moderately Susceptible) and 9 = HS (Highly Susceptible); ELISA: Enzyme Linked Immunosorbent; BIT = Back Inoculation Test; + = ≥positive control 405 nm, 3h) = 0.482, - = ≤ Negative control (OD 405 nm, 3h) = 0.173; I_1 = Inoculated at tillering stage; I_2 = Inoculated at booting stage.

Table 5: Serological analysis and characterization of RYMV isolates collected from six locations of Mararaba-Obi, Gulu, Makurdi, Wuya, Edozhigi and Obubu-Ofu.

	OD (405nm, 3h) using Monoclonal antibodies (Mabs)			
RYMV isolate	Mabs A	Mabs G	Mabs D	Serological group
Mararaba-Obi	0.807^{+}	3.232^{+}	0.553^{-}	S1
Gulu	0.374^{-}	3.169^{+}	0.474^{-}	S2
Makurdi	0.605^{+}	3.210^{+}	0.458^{-}	S1
Wuya	0.744^{+}	3.196^{+}	0.475^{-}	S1
Edozhigi	0.566^{-}	3.213^{+}	0.469^{-}	S2
Obubu-Ofu	0.758^{+}	3.222^{+}	0.481^{-}	S1

OD ≤ 0.31 ≤ 0.6 = 1 (negative); OD ≤0.61≤1.2 = 2 (positive); OD ≤ 1.21 ≤ 1.8 = 3 (positive); OD ≤ 1.81 = 4 (positive). If, Mabs A (+ve) and Mab D (-ve) = S1, Mab A (+ve) and Mab G (+ve) = S1, Mabs A (-ve) and Mabs D (+ve) = S2, Mab A (-ve) and Mabs G (+ve) = S2

DISCUSSION

Yields obtained by inoculated NILs were lower than yield values obtained with the control un-inoculated entries across the levels of virus isolates. Bakker[37] had also reported a yield loss of over 50% in rice varieties inoculated at a vigorous stage (active tillering stage) in a screen house experiment. The varying effects produced by the virus isolates on growth and yield components of the rice genotypes might be an indication of virus isolates pathogenicity differences. Obvious RYMV symptoms were not observed on NIL 54 across the levels of virus isolates. Ndjiondjop *et al*.[38] reported that high resistance behaves as a monogenic recessive genetic trait and is associated with lack of symptom development and blockage of virus movement. Bailiss and Senananyake[39] reported that virus infection could lead to decreased assimilate input into the inflorescence, pod or seed of plants, leading to degeneration of pollen and drying up of stigma. Therefore, spikelets of highly susceptible varieties infected by RYMV are often sterile and empty at harvest[27]. Differences observed among the NILs in terms of percent spikelets sterility (%) and yield performances might also be a result of innate individual differences since they contain similar transgenes.

Virus isolates from Obubu-Ofu and Makurdi were observed to break the resistance in Gigante by showing symptoms. There are reports that some RYMV isolates were capable naturally and after serial inoculations[28] of breaking down the high resistance of Gigante and Tog 5681. The present study has also confirmed that the resistance in NIL 54 is not similar to immunity as the virus was infectious on susceptible FKR 28 during back inoculation test and SES score of 1 and 3 for RYMV Resistance (R) were recorded for NILs 2 and 54 in a consistent manner at both inoculation regimes for Mararaba-Obi, Gulu, Makurdi, Wuya and Edozhigi isolates (Table 4).

It is that virulence and cultivar age also interplayed in the symptom expression on these genotypes. In a previous experiment, RYMV symptoms appeared 31 and 33 dpi respectively on the resistant cultivars - Gigante and Tog 5681; whereas symptoms were induced in a shorter time interval in BG 90-2 than in the resistant cultivars (6-10 dpi versus 19 to 42 dpi)[19]. This might explain why NIL 130 and NIL 16 that recorded positive virus indexing at tiller stage inoculation was however, shown to have a negative value for the test plant inoculated at booting stage at all levels of virus isolates. Subsequent expression of typical symptoms of RYMV on back inoculation test, and the detection of the virus on ELISA (OD 405 nm) are clear indications that immunity is still not found in the elite rice cultivars. There is however hope with NIL 54 due to the low virus quantity recovered on ELISA test and also a symptomless expression of the virus disease on the plant with the six virus isolates.

The result of the serological analysis and RYMV isolates characterization indicated that the six virus isolates belonged to serogroups 1 and 2. Isolates from Obubu-Ofu, Wuya, Makurdi and Mararaba-Obi were classified as S1, whereas isolates from Edozhigi and Gulu were characterized as S2 (Table 5). Wuya isolate (Ser 1) had been recovered from a weed host-*Echinochloa colona* L. found on a rice field at Wuya village in the same Local Government Area of Gbako, where Edozhigi isolate belonging to Ser. 2 was found. Presence of two serotypes within the same State of Kaduna was earlier reported[21], indicating that the States might possibly become hot-spots of the virus in the nearest future. Edozhigi (S2) and Wuya (S1) isolates were obtained on rice fields barely 3 km apart. Similar result was obtained by Salaudeen *et al*.[21] who reported that SA and SB from Gangara and Tudun Iya in Kaduna State respectively, were less than 2 km apart. N'Guessan *et al*.[25] also discovered two different serotypes of the virus co-exiting in the nearby fields in Cote d'Ivoire with S2 predominating overwhelmingly in surveyed locations. S2 dominance was however not due to higher pathogenicity of S2 isolates whereas in tested cultivars, pathogenicity of the isolates was also not strain specific[25].The present study appears to have recorded a dominance of S1 (four isolates) over S2 (two isolates) in contrast with[25]; however, virus pathogenicity did not also appear to be strain specific as test plants produced varying effects with the same isolates. The serological similarities observed between isolates within the same different states confirm the great cross-infection potential of RYMV transmitted under natural conditions by different insect vectors[40, 41].

CONCLUSION

The result of this study has confirmed that the six virus isolates belonged to serogroups1 and 2 (S1 and S2). Four virus isolates from Obubu-Ofu, Wuya, Makurdi and Mararaba-Obi were classified as S1 whereas isolates from Edozhigi and Gulu were characterized as S2. The study has confirmed the presence of two serotypes of RYMV in the same State of Niger in the North Central Nigeria. The development of RYMV resistant cultivars adaptable in the zone should therefore, take into account the presence of the two strains of the virus for durable resistance in the field. NIL 54 is also recommended for pathogenicity studies involving more isolates of RYMV in Nigeria as no obvious symptom was observed on the plant when challenged with S2 and S1 isolates in the present study. More so, the breeding management activity involving the transfer of the RYMV resistant alleles in NIL 54 to elite susceptible rice cultivars in the North Central Nigeria is highly recommended for efficient management of RYMV in the area.

ACKNOWLEDGEMENT

The corresponding author wishes to acknowledge the pathology Unit of Africa Rice Center (AfricaRice), Cotonou, Benin Republic for providing facilities, technical and material assistance for the serological characterization of the virus isolates.

REFERENCES

01. Breniere, J., 1983. The principal insect pests of rice in West Africa and their control. West African Rice Development Association, Monrovia, Liberia.
02. Singh, B.N., S. Fagade, M.N. Ukwungwu, C. Williarn and S.S. Jagtap *et al*., 1997. Rice growing environments and biophysical constraints in different ecological zones in Nigeria. Met. J., 2: 35-44.
03. Konate, G., S. Sarra and O. Traore, 2002. Rice yellow mottle virus is seed-borne but not seed transmitted in rice seeds. Eur. J. Plant Pathol., 107: 361-364.
04. Sarra, S., P. Oevering, S. Guindo and D. Peters, 2004. Wind-mediated spread of rice yellow mottle virus (RYMV) in irrigated rice crops. Plant Pathol., 53: 148-153.
05. Kouassi, N.K., P. N'Guessan, L. Albar, C.M. Fauquet and C. Brugidou, 2005. Distribution and characterization of Rice yellow mottle virus: A threat to African farmers. Plant Dis., 89: 124-132.
06. Ou, S.H., 1985. Rice Disease. 2nd Edn., Kew Commonwealth Mycological Institute, England, UK., Pages: 368.
07. Abo, M.E., A.A. Sy and M.D. Alegbejo, 1998. Rice Yellow Mottle Virus (RYMV) in Africa: Evolution, distribution, economic significance on sustainable rice production and management strategies. J. Sustainable Agric., 11: 85-111.
08. Abo, M.E. and A.A. Sy, 2007. Rice virus diseases: Epidemiology and management strategies. J. Sustainable Agric., 11: 113-134.
09. John, V.T., G. Thottappilly and V.A. Awoderu, 1984. Occurrence of rice yellow mottle virus in some Sahelian countries in West Africa. FAO Plant Protect. Bull., 32: 86-87.
10. Fomba, S.N., 2007. Screening for seedling resistance to rice yellow mottle virus in some rice cultivars in Sierra Leone. Plant Dis., 72: 641-642.
11. Taylor, D.R., A.S. Fofie and M. Suma, 1990. Natural infection of rice yellow mottle virus disease (RYMV) on rice in Sierra Leone. Int. Rice Res. Newslett., 15: 19-19.
12. Abo, M.E., M.D. Alegbejo, A.A. Sy and Y. Sere, 2004. Field incidence of rice yellow mottle virus, genus sobemovirus on rice and a weed species in the field in Cote d'Ivoire. Acta Agronomica Hungarica, 50: 85-89.
13. Konate, G., O. Traore and M. Coulibaly, 1997. Characterization of rice yellow mottle virus isolates in Sudano-Sahalian areas. Arch. Virol., 142: 1117-1124.
14. Traore, O., M.D. Traore, D. Fargette and G. Konate, 2006. Rice seedbeds as a source of primary infection by Rice yellow mottle virus. Eur. J. Plant Pathol., 115: 181-186.
15. Salaudeen, M.T., O.O. Banwo, B.D. Kashina and M.D. Alegbejo, 2008. Preliminary studies on the serotypes and sero-distribution of rice yellow mottle Sobemovirus in Northern Nigeria. Biol. Environ. Sci. J. Trop., 5: 23-26.
16. Abo, M.E., M.N. Ukwungwu and A. Onasanya, 2002. The distribution, incidence, natural reservoir hosts and insect vectors of Rice Yellow Mottle Virus (RYMV), genus Sobemovirus in Northern Nigeria. Tropicultura, 20: 198-202.
17. Onwughalu, J.T., M.E. Abo and J.K. Okoro, 2009. Preliminary investigation on the occurrence of Rice Yellow Mottle Virus (RYMV) and its insect vectors in Benue State, Nigeria. Nig. J. Plant Prot., 23: 44-52.
18. Onasanya, R.O., D.B. Olufolaji, A. Onasanya, Y. Sere, F.E. Nwilene, M. Wopereis and P. Kiepe, 2011. Occurrence, distribution and characterization of rice yellow mottle virus isolates genus Sobemovirus in Southwest Nigeria. Trends Applied Sci. Res., 6: 1301-1323.
19. Alegbejo, M.D., B.A. Raji, I.U. Abubakar and O.O. Banwo, 2006. Rice yellow mottle virus disease, a new disease of rice in Zamfara, Nigeria. Int. Rice Res. Notes, 31: 39-39.
20. Awoderu, V.A., 2008. Rice yellow mottle virus in West Africa. Int. J. Pest Manage., 37: 356-362.
21. Salaudeen, M.T., O.O. Banwo, B.D. Kashina and M.D. Alegbejo, 2008. Effect of Rice yellow mottle Sobemovirus on yield in some rice cultivars. Biol. Environ. Sci. J. Trop., 5: 27-34.
22. Onwughalu, J.T., M.E. Abo, J.K. Okoro, A. Onasanya and Y. Sere, 2010. The effect of rice yellow mottle virus infection on the performance of rice (Oryza sativa L.) relative to time of infection under screenhouse condition. J. Applied Sci., 10: 1341-1344.
23. Ndjiondjop, M.N., L. Albar, D. Fargette, C. Fauquet and A. Ghesquiere, 2007. The genetic basis of high resistance to Rice Yellow Mottle Virus (RYMV) in cultivars of two cultivated rice species. Plant Dis., 83: 931-935.
24. AfricaRice, 2011. Recent research on rice diseases in Africa. AfricaRice is a CGIAR Research Center, Africa.

25. N'guessan, P., A. Pinel, A.A. Sy, A. Ghesquiere and D. Fargette, 2007. Distribution, pathogenicity and interactions of two strains of rice yellow mottle virus in forested and savanna zones of West Africa. Plant Dis., 85: 59-64.
26. Jaw, A., M.N. Ndjionndjop, R. Akromah and Y. Sere, 2012. Identification of near-isogenic lines resistance to Rice yellow mottle virus. Afr. Crop Sci. J., 20: 163-168.
27. Abo, M.E., M.N. Ukwungwu, J.D.A. Hughes and S.M. Misari, 2001. Possible sources of resistance to Rice Yellow Mottle Virus (RYMV), genus sobemovirus in some rice genotypes in Nigeria. J. Agric. Environ., 2: 263-270.
28. Fargette, D., A. Pinel, O. Traore, A. Ghesquiere and G. Konate, 2003. Emergence of resistance-breaking isolates of Rice yellow mottle virus during serial inoculations. Eur. J. Plant Pathol., 108: 585-591.
29. Hebrard, E., A. Pinel-Galzi, A. Bersoult, C. Sire and D. Fargette, 2006. Emergence of a resistance-breaking isolate of rice yellow mottle virus during serial inoculations is due to a single substitution in the genome-linked viral protein Vpg. J. Gen. Virol., 87: 1369-1373.
30. Little, T.M. and F.J. Hills, 1978. Agricultural Experimentation: Design and Analysis. John Wiley and Sons, New York, Pages: 350.
31. Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. 2nd Edn., John Wiley, Hoboken, New Jersey, Pages: 680.
32. Obi, I.U., 2002. Statistical Methods of Detecting Differences between Treatment Means and Research Methodology Issues in Laboratory and Field Experiments. 2nd Edn., AP Express Publishing Company Ltd., Nsukka, Nigeria, Pages: 116.
33. Thottappilly, G. and H.W. Rossel, 1993. Evaluation of resistance to rice yellow mottle virus in Oryza species. Ind. J. Virol., 9: 65-73.
34. Koenig, R. and H.L. Paul, 1982. Variants of elisa in plant virus diagnosis. J. Virol. Methods, 5: 113-125.
35. IRRI., 2007. Cropstat for windows version 7.2 2007.3. International Rice Research Institute, Philippines.
36. IRRI., 1996. The International Rice Testing Program-Standing Evaluation System (ITP-SES) for Rice. 4th Edn., International Rice Research Institute, Philippines,.
37. Bakker, W., 1974. Characterization and ecological aspects of rice yellow mottle virus in Kenya. Agric. Res. Rep., 829: 1-152.
38. Ndjiondjop, M.N., C. Brugidou, S. Zang, D. Fargette, A. Ghesquiere and C. Fauquet, 2002. High resistance to rice yellow mottle virus in two cultivated rice cultivars is correlated with failure of cell to cell movement. Physiol. Mol. Plant Pathol., 59: 309-316.
39. Bailiss, K.W. and S. Senananyake, 1984. Virus infection and reproductive losses in faba bean (Vicia faba L.). Plant Pathol., 33: 185-192.
40. Sere, Y., A. Onasanya, K. Akator, A. Afolabi and M.E. Abo, 2007. Serological differentiation indices and phylogenetic analysis ofRice yellow mottle virus isolates in cote d`Ivoire. J. Boil. Sci., 7: 1147-1154.
41. Nwilene, F.E., Y. Sere, M.N. Ndjiondjop, M.E. Abo and A.K. Traore et al., 2008. Rice Yellow Mottle Virus (RYMV) and its Insect Vectors, Ecology and Control. Blackwell Science Ltd, Oxford, England, Pages: 550.