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Occurrence, Distribution and Characterization of Rice Yellow Mottle Virus Isolates Genus *Sobemovirus* in Southwest Nigeria

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ABSTRACT

Rice Yellow Mottle Virus (RYMV) genus *Sobemovirus* is a highly variable pathogen that is very infectious to rice plant. This variability hinders rice breeding for durable resistance to the virus and effective deployment of improved cultivars in Southwest Nigeria. Disease surveys in 5 Southwest states (Lagos, Oyo, Ogun, Ekiti and Ondo) revealed RYMV disease incidence of between 15-70% in farmers' fields and serological indexing confirmed 92% of collected leaf samples positive to RYMV with 24% from rice and 76% from weeds. The weed with 76% RYMV positive suggests being the main reservoir of RYMV in Southwest Nigeria. Biological test on collected fields leaf samples identified 3 groups (GroupA, GroupD and GroupE) of Resistance Breaking (RB) RYMV isolates and 2 groups (GroupB and GroupC) of normal isolates. Pathotyping 20 RYMV isolates against 10 differential varieties identified 17 isolates as Highly Pathogenic Isolates (HPI) and 3 as Mildly Pathogenic Isolates (MPI) while 4 rice varieties were Highly Resistant (HR), 2 were Moderately Resistant (MR) and 4 were susceptible. HPI isolates present in five states and MPI isolates in two states. Serological study using the same 20 RYMV isolates revealed two major Nigeria serogroup (NSg1 and NSg2) and four subgroups (NSg1a, NSg1b, NSg2a and NSg2b). NSg1a and NSg1b comprised both normal and RB isolates while NSg2a and NSg2b were typical of RB isolates only. This information would assist rice breeding programs to develop durable resistant cultivars to RYMV disease in Southwest Nigeria.

Key words: Rice yellow mottle virus, disease surveys, disease incidence, farmers fields, serological indexing, biological test, pathogenic isolates, serotypes, Southwest Nigeria

INTRODUCTION

Rice (*Oryza* spp.) is grown widely in many parts of the world. It is the major food source for about 40% of the world's human population (Ortiz, 2011). Rice has become a major staple cereal in most countries of Africa especially West Africa where it accounted for more than 25% of the cereals consumed (Africa Focus, 2004). In Nigeria, the importance of rice in the diet of the people is steadily on the increase. The annual consumption of the staple for an average Nigerian is as high

as 24.8 kg of rice which represents 9% of the total calorie intake (Akpokodje *et al*., 2001). In spite of the efforts made in increasing rice area under cultivation, yields remained very low, thus the production has not been able to meet the consumption level of the growing population. Rice production in Africa is seriously affected by diseases. Rice Yellow Mottle Virus (RYMV) constitutes a major constraint to rice production in Africa (Sere *et al*., 2008), particularly in the lowland and irrigated rice ecologies (Banwo *et al*., 2004). RYMV belongs to the genus *Sobemovirus* (Tamm and Truve, 2000). The virus which is indigenous to Africa is widely spread in almost all the West African countries including Nigeria (Banwo *et al*., 2004). RYMV is environmentally stable and highly infectious to rice (Banwo *et al*., 2004). The disease is characterized by mottle and yellowing symptoms of varying intensities depending on genotype and this could be mistaken for iron or nitrogen deficiency (Onasanya *et al*., 2006; Gnanamanickam, 2009). Gnanamanickam (2009) stated that infected plants had pale yellow mottle leaves, stunted, reduced tillering, non-synchronous flowering, poor panicle exertion, spikelet discoloration of grains which could be as a result of secondary infection by fungi and crinkling of new leaves. But in mechanically inoculated plants, the first symptoms are few yellow-green spots on the youngest leaves, although more resistant cultivars may show no distinctive symptoms (Gnanamanickam, 2009). Yield losses of between 4-90% have reported which depend on genotype and infection time (Onwughalu *et al*., 2010, 2011). RYMV is transmitted through mechanical contact and inoculations as well as by insect-vectors such as beetles and long-horned grasshoppers (Nwilene *et al*., 2009). It has been established that cows, donkeys and grass rats transmit the virus in irrigated rice fields (Sarra and Peters, 2003), however, the disease is not transmitted through seed or nematode (Abo *et al*., 2004). The virus has also been observed on some grasses, besides cultivated rice, including wild rices (Abo *et al*., 2000). RYMV came to limelight in Nigeria with the introduction of exotic rice varieties from Southeast Asia coupled with the introduction of cropping practices without dry season gaps (Abo *et al*., 2000). The disease has since become a limiting factor to rice production in Nigeria and rice is becoming increasingly important in Nigeria. For this reason, the government is investing heavily in the development of the domestic rice sector. However, the expensive efforts to increase rice production by the development of irrigation schemes where water and water management are available, allowing double cropping and promotion of productive varieties from Asia were hindered by the spread of RYMV (Banwo *et al*., 2004).

RYMV strains in the field differ in their pathogenicity (N'Guessan *et al*., 2000). The isolate variability of the virus accounts for the observation that rice variety resistant to RYMV in one location could be susceptible in another location. Therefore, there is the need to identify and characterize these strains biologically and serologically. Besides, the knowledge of the serological relationships between RYMV isolates is valuable in diagnostic work and may prove to be important in epidemiological studies and disease control. The existence of different RYMV strains in the field that are different in their pathogenicity is often a matter of considerable practical importance. Therefore reliable criteria are needed for distinguishing, identifying and pathotyping these strains. Screening for durable resistance need to take into consideration the existence of these different strains, as most often the breakdown in resistance is attributed to a poor prerelease challenge with appropriate pathogen population (Mekwatanakarn *et al*., 2000). In previous studies, RYMV isolates have been pathotyped in some West African countries (Onasanya *et al*., 2004, 2006) except Nigeria. Besides, the knowledge of the serological relationships between RYMV isolates is valuable in diagnostic work and may prove to be important in epidemiological studies and disease control. Also in some studies, African RYMV isolates were serotyped using double immunodiffusion gel assay

(Sere *et al.*, 2005). Phylogenetic analysis of some RYMV isolates based on sequences of the coat protein gene has been reported by Traore *et al.* (2005). However, because of relative expensiveness of sequencing many isolates of RYMV, few sequence data are available for phylogenetic analysis. Besides, few data are available on RYMV serodiversity, disease ecology and interactions between different RYMV strains in Southern Nigeria. However, Serological Differentiation Index (SDI) data has been reported using the phylogenetic analysis of plant viruses serological classification (Sere *et al.*, 2007) but never been used for RYMV serodiversity in Southern Nigeria. For a better understanding of the RYMV pathogen population structure in Southern Nigeria, it will be very important to characterize this pathogen in order to reveal information on the diversity in RYMV pathogen population in terms of virulence, pathotypes, serotypes, cultivar resistance, disease epidemics and geographical distributions of pathogenic isolates. The present study aimed to assess RYMV disease occurrence and distribution, to characterize RYMV isolates serologically and biologically and to evaluate the RYMV population structure in terms of Resistance Breaking (RB) isolates in lowland and irrigated rice ecosystems in Southwest Nigeria. This approach will provide useful information on integrated management of RYMV disease for sustainable deployment of resistant varieties and development of suitable breeding strategies based on identification of donors for resistance to the Southern Nigeria RYMV isolates population.

MATERIALS AND METHODS

Research location: The laboratory and screen-house studies were carried out at Plant Pathology Unit, Africa Rice Center, Cotonou, Benin Republic. The RYMV disease survey and leaf sampling study was carried out in Southwest Nigeria with Africa Rice Center, Nigeria Station, Ibadan, Oyo State, Nigeria. All the research studies were conducted between April to December 2010.

RYMV disease survey and sampling: RYMV disease survey and sampling (Abo *et al.*, 2002) was conducted in five states in South-west Nigeria where rice cultivation is predominant. The five states were Oyo, Ogun, Ondo, Ekiti and Lagos. Lowland and irrigated rice fields were visited to evaluate RYMV disease incidence in farmers' fields. Leaf samples were collected from rice varieties that showed RYMV typical symptoms and also from weed in the surrounding rice fields. Collected leaf samples were stored in ice-box during collection and later transferred into freezers in the laboratory for analysis.

Serological tests on collected leaf samples: Enzyme-linked Immunosorbent Assay (ELISA) test using RYMV polyclonal antibody was carried out on the leaf samples collected from both rice varieties and weed on farmers' fields to detect the virus. Indirect-antigen coated-plate Enzyme-linked Immunosorbent Assay (ACP-ELISA) was performed as described by Sere *et al.* (2007).

Biological test on collected leaf samples

RYMV propagation: All the RYMV leaf samples obtained both from rice varieties and weed during the survey were propagated on six rice varieties (two RYMV susceptible varieties and four RYMV resistant varieties) (Table 1) using method adopted by Onasanya *et al.* (2004).

Measurement of parameter: Plant height, chlorophyll was carried out by SPAD meter, disease incidence and severity were estimated at 21 Days after Inoculation (DAI) (Onasanya *et al.*, 2004, 2006).

Table 1: Identity of rice varieties used for RYMV leaf sample propagation

Code	Genotype	RYMV resistance status
V_1	IR 64	Susceptible
V_2	Bouake 189	Susceptible
V_3	Gigante	Resistant
V_4	TOG 5681	Resistant
V_5	WAC 117	Moderately resistant
V_6	TOG 5672	Resistant

Table 2: List of Southwest RYMV isolates selected after biological test and were used for both serotyping and pathotyping studies

Code	Isolate code	Locality	State	Primary host	Propagated host	*Status
I1	NG1-RB-Ogun1	Abeokuta	Ogun	Ofada rice	IR64	RB
I2	NG1-RB-Ogun2	Abeokuta	Ogun	Ofada rice	WAC117	RB
I3	NG2-RB-Ogun3	Abeokuta	Ogun	Ofada rice	IR64	RB
I4	NG3-RB-Lagos1	Itoikin	Lagos	Ofada rice	IR64	RB
I5	NG3-RB-Lagos2	Itoikin	Lagos	Ofada rice	WAC117	RB
I6	NG4-RB-Lagos3	Itoikin	Lagos	Ofada rice	IR64	RB
I7	NG5-RB-Oyo1	Oyo	Oyo	Weed	WAC117	RB
I8	NG6-RB-Oyo2	Oyo	Oyo	Weed	Bouake 189	RB
I9	NG6-RB-Oyo3	Oyo	Oyo	Weed	WAC117	RB
I10	NG7-N-Ondo1	Ogbese	Ondo	Weed	IR64	N
I11	NG8-RB-Ondo2	Ogbese	Ondo	Weed	WAC117	RB
I12	NG9-RB-Ondo3	Ogbese	Ondo	Weed	IR64	RB
I13	NG9-RB-Ondo4	Ogbese	Ondo	Weed	WAC117	RB
I14	NG10-N-Ekiti1	Igbemo Ekiti	Ekiti	Weed	IR64	N
I15	NG11-N-Ekiti2	Igbemo Ekiti	Ekiti	Weed	IR64	N
I16	NG12-RB-Ekiti3	Igbemo Ekiti	Ekiti	Weed	IR64	RB
I17	NG13-RB-Ekiti4	Igbemo Ekiti	Ekiti	Weed	IR64	RB
I18	NG13-RB-Ekiti5	Igbemo Ekiti	Ekiti	Weed	Bouake 189	RB
I19	NG14-RB-Ekiti6	Igbemo Ekiti	Ekiti	Weed	IR64	RB
I20	NG15-N-Ekiti7	Igbemo Ekiti	Ekiti	Weed	IR64	N

*RB: Resistance breaking isolate; N: Normal isolate

Harvesting of RYMV isolates: Leaves with typical RYMV symptoms on susceptible IR 64 were harvested as isolate at 21 DAI after data collection and stored in freezer for use in serotyping and pathotyping analyses.

Pathotyping characterization analysis of different RYMV isolates

RYMV isolates: Twenty RYMV isolates (Table 2) harvested during biological test were used in the pathotyping study.

Experimental design: Randomized Complete Block Design (RCBD) with 3 replications was used. Twenty RYMV isolates (Table 2) were used to screen 10 rice varieties (Table 3) inside the screen house at Africa Rice Center (Africa Rice), Cotonou, Benin Republic. Two liters-plastic pots were used. Rice grains were pre-germinated in sterile Petri dishes under sterilization condition. One plastic pot per variety per isolate in three replications was used.

Table 3: Identity of rice varieties used for pathotyping characterization analysis of different RYMV isolates

Code	Genotype	RYMV resistance status
V_1	IR 64	Susceptible
V_2	Bouake 189	Susceptible
V_3	BG 90-1	Susceptible
V_4	Gigante	Resistant
V_5	TOG 5681	Resistant
V_6	WAC 116	-
V_7	TOG 5674	Resistant
V_8	TOG 5672	Resistant
V_9	TOG 7291	Resistant
V_{10}	WAC 117	-

Inoculation of rice varieties: The ten young rice varieties were inoculated mechanically (Onasanya *et al*., 2004) with the selected isolates in the screen house 21 days after transplanting in 3 replicates. Another set of same ten young rice varieties in 3 replicates not inoculated were used as controls.

Measurement of parameter: Plant height, chlorophyll content (SPAD), disease incidence and severity at 21 and 42 Days after Inoculation (DAI) were estimated (Onasanya *et al*., 2004, 2006). Total yield was measured at maturity for both inoculated and control rice varieties.

Serotyping characterization analysis of different RYMV isolates

YMV isolates: The twenty RYMV isolates (Table 2) harvested during biological test used in pathotyping characterization study were also used for serotyping characterization analysis for result comparison purpose.

Polyclonal antibody used for serotyping: Twenty-six RYMV polyclonal antibodies (Table 4) were used for the serotyping characterization study. The polyclonal antibodies were obtained from Plant Pathology Unit, Africa Rice Center (Africa Rice), Cotonou, Benin.

ACP-ELISA: Indirect-antigen coated-plate enzyme-linked immunosorbent assay (ACP-ELISA) was performed as described by Sere *et al*. (2007).

Data analysis: Biological test and pathotyping characterization data analysis as well as Additive Main Effect and Multiplicative Interaction (AMMI) analysis were carried out using IRRISTAT version 4.3 (Zhu and Kuljaca, 2005; Ebdon and Gauch, 2002b).

In the serotyping characterization analysis, the Optical Density (OD) values from ACP-ELISA were transformed into a binary character matrix ("1" for a positive polyclonal antibody detection of each RYMV isolate and "0" for negative none detection of each RYMV isolate by a polyclonal antibody). Pairwise distance matrices were compiled by the numerical taxonomy system (NTSYS-pc 2.0) software (Rohlf, 2000) using the Jaccard coefficient of similarity (Ivchenko and Honov, 1998). Dendrogram was created by Unweighted Pair-group Method Arithmetic (UPGMA) cluster analysis (Jako *et al*., 2009).

Table 4: Identity of 26 RYMV polyclonal antibodies used for the RYMV isolates serotyping characterization study

Antiserum code	Region	Country
RYMV-Pab1	Niono4	Mali
RYMV-Pab2	Niono8	Mali
RYMV-Pab3	M'Peniesso	Mali
RYMV-Pab4	Molodo	Mali
RYMV-Pab5	Longorola	Mali
RYMV-Pab6	Kayo macina	Mali
RYMV-Pab7	Selingue	Mali
RYMV-Pab8	Kogoni K7	Mali
RYMV-Pab9	Banzon	Burkina Faso
RYMV-Pab10	Kafirguela	Burkina Faso
RYMV-Pab11	IITA	Nigeria
RYMV-Pab12	Saga	Niger
RYMV-Pab13	Kollo	Niger
RYMV-Pab14	Kirkissaye	Niger
RYMV-Pab15	Bonfeba	Niger
RYMV-Pab16	SayI	Niger
RYMV-Pab17	Diomana	Niger
RYMV-Pab18	M'be	Cote d'Ivoire
RYMV-Pab19	Danane	Cote d'Ivoire
RYMV-Pab20	Gagnoa -L	Cote d'Ivoire
RYMV-Pab21	Gagnoa-U	Cote d'Ivoire
RYMV-Pab22	Guehiebli	Cote d'Ivoire
RYMV-Pab23	Tapeguia	Cote d'Ivoire
RYMV-Pab24	Odienne	Cote d'Ivoire
RYMV-Pab25	Sakassou	Cote d'Ivoire
RYMV-Pab26	Sassandra	Cote d'Ivoire

RESULTS

RYMV disease survey and sampling: RYMV disease survey and sampling were successfully carried out in five states (Ogun, Lagos, Oyo, Ondo and Ekiti) in Southwest Nigeria. A total of 50 RYMV leaf samples were collected from farmers' fields in 6 localities in the five states in Nigeria (Table 5). Lowland and irrigated rice ecologies were covered during the survey. Out of the 50 RYMV leaf samples collected 14 were from cultivated rice varieties and 36 were from weeds. RYMV disease incidence was between 15-70% in farmers' fields across the five states with highest disease incidence from Oyo state and the least disease incidence from Ekiti state.

Serological tests on collected leaf samples: The serological test conducted on 50 RYMV leaf samples was carried out successfully. The serological test using ACP-ELISA and RYMV polyclonal antibody was carried out to confirm the presence of RYMV in these leaf samples. Out of 50 leaf samples tested 46 (92%) were RYMV positive (Table 5). Out of these 46 (92%) tested positive to RYMV, 11 (24%) were from cultivated rice varieties and 35 (76%) were from weeds.

Biological test on collected leaf samples: The biological test was successfully conducted on all the RYMV leaf samples inside the screen-house. This comprised of the propagation of 50 RYMV leaf

Table 5: RYMV leaf samples collected and RYMV indexing status

			No of sample collected and RYMV diagnosis					
Locality	State	Ecology	Rice	RYMV*	Weed	RYMV*	Total sample	Total RYMV*
Alamala-Abeokuta	Ogun	Lowland	3	3	6	6	9	9
Itoikin	Lagos	Lowland	6	3	4	4	10	7
Ibadan	Oyo	Irrigated	5	5	4	4	9	9
Oyo	Oyo	Lowland	-	-	4	4	4	4
Ogbese-Akure	Ondo	Lowland	-	-	9	9	9	9
Igbemo Ekiti	Ekiti	Lowland	-	-	9	8	9	8
Total			14	11	36	35	50	46

*Number of leaf samples positive to RYMV in ELISA test

Table 6: List of RYMV isolates obtained after propagation and resistance breaking status

				Propagation hosts						
Isolate	Sample No.	Locality	Primary host	IR 64	Bouake 189	Gigante	TOG 5681	WAC 117	TOG 5672	*Status
NG1	2	Abeokuta	Ofada rice	+	+	-	-	+	-	RB
NG2	10	Abeokuta	Ofada rice	+	+	-	-	+	-	RB
NG3	12	Itoikin	Ofada rice	+	+	-	-	+	-	RB
NG4	18	Itoikin	Ofada rice	+	+	-	-	+	-	RB
NG5	30	Oyo	Weed	-	-	-	-	+	-	RB
NG6	31	Oyo	Weed	+	+	-	-	+	-	RB
NG7	34	Akure	Weed	+	-	-	-	-	-	N
NG8	39	Ogbese	Weed	-	-	-	-	+	-	RB
NG9	41	Ogbese	Weed	+	+	-	-	+	-	RB
NG10	42	Igbemo Ekiti	Weed	+	-	-	-	-	-	N
NG11	43	Igbemo Ekiti	Weed	+	+	-	-	-	-	N
NG12	44	Igbemo Ekiti	Weed	+	+	-	-	+	-	RB
NG13	46	Igbemo Ekiti	Weed	-	+	-	-	+	+	RB
NG14	47	Igbemo Ekiti	Weed	+	+	-	-	+	-	RB
NG15	48	Igbemo Ekiti	Weed	+	+	-	-	-	-	N

*RB: Resistance breaking isolate, N: Normal isolate, +: Positive RYMV symptom, -: No symptom

samples from Southwest Nigeria using 6 rice varieties and identification of resistance breaking RYMV isolates. From the study, out of 50 RYMV leaf samples propagated 15 RYMV isolates were recovered from which 4 were normal isolates and 11 were Resistance Breaking (RB) isolates (Table 6). Resistance breaking RYMV isolates were isolates that attacked RYMV resistant variety giving rise to a typical RYMV symptom. None of the RB isolates was able to attack Gigante and TOG5681. Using the six propagating rice hosts, the relationship and diversity among RB and Normal (N) RYMV isolates was revealed by AMMI cluster analysis (Fig. 1, 2). Out of 15 RYMV isolates analyzed, a total of 5 groups (GroupA, GroupB, GroupC, GroupD and GroupE) were obtained at 40% similarity coefficient (Fig. 1). RB RYMV isolates were clustered into 3 different groups (GroupA, GroupD and GroupE) while the normal RYMV isolates clustered into 2 different groups (GroupB and GroupC) (Fig. 1). The study was able to reveal that, normal RYMV isolate is different from resistance breaking RYMV isolate and diversity is higher among RB isolates than among the normal isolates. Among the six propagating rice hosts used, some relationship and

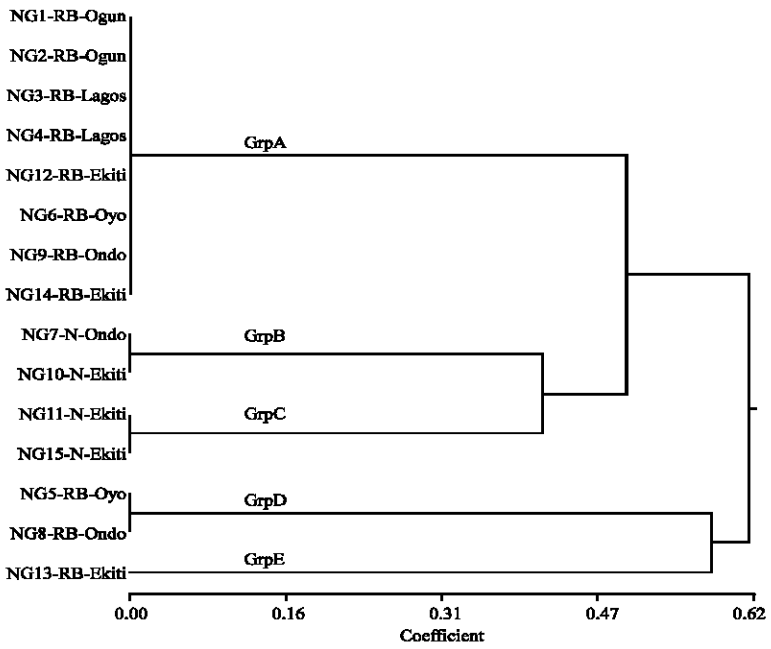

Fig. 1: Cluster showing relationship and diversity among Resistance Breaking (RB) and normal (N) RYMV isolates from Southwest Nigeria using six propagating rice hosts as revealed by AMMI analysis

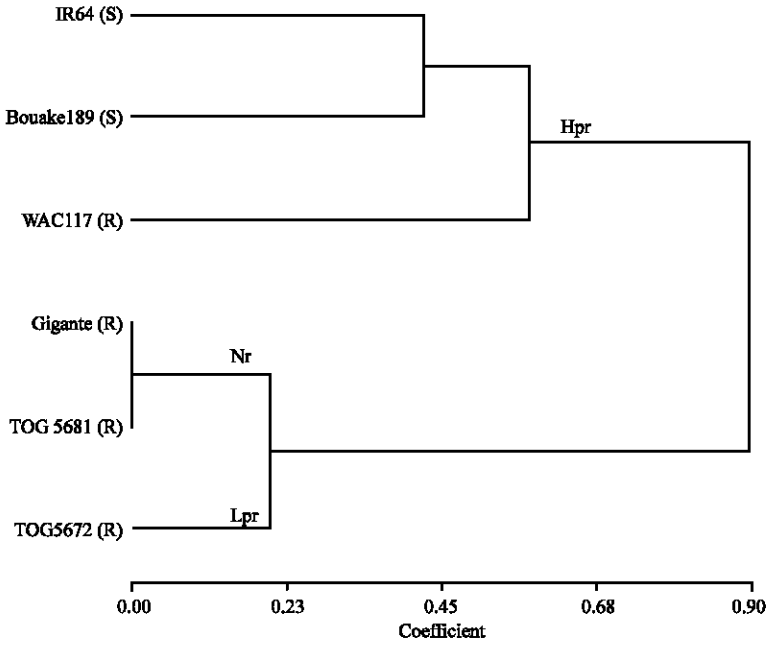

Fig. 2: Cluster showing relationship and diversity among two susceptible (S) and four resistant (R) in response to Resistance Breaking (RB) and Normal (N) RYMV isolates as revealed by AMMI analysis

diversity among two Susceptible (S) and four Resistant (R) in response to RB and Normal (N) RYMV isolates were revealed by AMMI cluster analysis (Fig. 2). At 90 and 20% similarity coefficient, three clusters formed (high propagating reactive host, Hpr; low propagating reactive host, Lpr; none reactive host, Nr) that revealed the propagating characteristics of the six rice hosts (Fig. 2). The rice varieties IR64, Bouake189 and WAC 117 were characterized as High Propagating Reactive (Hpr) hosts, TOG 5672 as Low Propagating Reactive (Lpr) host and Gigante and TOG 5681 as None Reactive (Nr) host (Fig. 2). On the basis of High Propagating Reactive (Hpr) hosts, 20 RYMV isolates (Table 2) from Southwest Nigeria were selected and were used for both serotyping and pathotyping studies.

Pathotyping characterization analysis of different RYMV isolates: Considerable diversity was observed in the reactions of 10 rice genotypes to 20 RYMV isolates from 5 states in Southwest Nigeria in terms of chlorophyll reduction, height reduction, yield reductions, disease incidence and disease severity (Table 7, 8). On the basis of ANOVA results, there was significant different ($p = 0.01$) in variety by isolate interaction and variety by days after inoculation in terms of chlorophyll reduction, height reduction, yield reductions, disease incidence and disease severity, meaning that diverse interactions exist between RYMV isolates and rice varieties and RYMV disease development was not the same at 21 and 42 days after inoculation (Table 7, 8). Percentage chlorophyll reduction ranged between 14.4-39.9% (Table 9) while% height reduction was between 9.1-34.9% (Table 10). While disease incidence ranged between 11.3-60.4% (Table 11), disease severity was between 0.4-96.1% (Table 12) and (%) yield reduction was in the range of 35.7-91.7% (Table 13). According to AMMI analysis, all the 20 RYMV isolates were responsible mainly for unfavorable interactive conditions leading to significant chlorophyll reduction, height

Table 7: Analysis of variance for percentage chlorophyll reduction, percentage height reduction, disease incidence and disease severity

		F RATIO			
Source	DF	%SPADR	%HR	%DI	%SEV
Rep (R)	2	3.73ns	5.40*	<1	<1
Variety (V)	9	124.01**	257.99**	2007.03**	1233.56**
Isolate (I)	19	16.78**	9.94**	1.79*	1.33ns
Days after inoculation (D)	1	2.22ns	4.38*	43.62**	4.02*
VxI	171	2.78**	4.47**	2.39**	2.20**
VxD	9	38.66**	41.62**	24.19**	6.38**
IxD	19	4.24**	1.10ns	2.11**	3.18**
VxIxD	171	2.71**	2.17**	2.28**	1.89**

**: Significant at 1% level; *: Significant at 5% level; ns: Not significant, SPADR: Chlorophyll reduction; HR: Height reduction; DI: Disease incidence; SEV: Disease severity

Table 8: Analysis of variance for percentage yield reduction

Source	DF	SS	MS	F
Rep (R)	2	14.61	7.30	3.96*
Variety (V)	9	981.88	109.10	59.19**
Isolate (I)	19	190.75	10.04	5.45**
VxI	171	555.14	3.25	1.76**

**: Significant at 1% level, *: Significant at 5% level

Table 9: Variety by isolate interaction means comparison for percentage chlorophyll reduction due to RYMV disease

Isolate (I)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	I-Mean
D = D21											
I1	44.8c-h	32.4def	44.8abc	26.1bcd	16.6a-d	2.5f	17.8abc	14.2ef	16.5a-e	38.0d-g	25.4
I2	48.1b-f	37.6c-f	42.8a-d	27.9bcd	4.9bcd	11.2def	10.8bc	22.8b-f	4.9cde	38.8d-g	25.0
I3	65.4a	23.4ef	46.4abc	24.2bcd	14.7a-d	16.1b-f	19.6abc	27.8b-e	20.4abc	60.4a	31.8
I4	45.8b-g	30.5def	23.4fgh	18.3cd	1.5d	2.3f	12.7bc	10.2f	7.3b-e	58.7ab	21.0
I5	31.5g-k	38.6b-e	29.9d-g	17.9cd	8.6a-d	30.8a	20.5ab	18.6c-f	12.9a-e	34.5e-h	24.4
I6	34.7f-j	30.1def	36.6b-f	43.1a	15.6a-d	24.3a-d	19.8abc	70.7a	19.8a-d	39.3d-g	33.4
I7	19.7k	23.6ef	13.3h	16.3d	7.6bcd	10.0def	4.8c	16.7def	7.4b-e	28.7gh	14.8
I8	51.5a-e	36.0c-f	36.5b-f	22.0bcd	16.3a-d	9.3def	31.5a	26.9b-e	18.8a-e	41.5d-g	29.0
I9	58.7abc	31.1def	51.6a	17.1cd	10.0a-d	9.7def	18.7abc	18.1c-f	21.1ab	28.7gh	26.5
I10	52.7a-d	60.0a	32.6c-g	23.6bcd	20.2ab	18.6a-e	9.9bc	36.1b	24.3a	50.1a-d	32.8
I11	34.8f-j	30.8def	25.1e-h	32.4abc	19.1abc	13.3c-f	13.3bc	24.0b-f	9.3a-e	51.8a-d	25.4
I12	42.3d-h	31.8def	38.9a-e	17.5cd	4.0cd	8.5ef	21.5ab	22.8b-f	10.7a-e	43.6c-f	24.1
I13	30.9h-k	41.4bcd	26.4e-h	22.7bcd	13.6a-d	7.0ef	15.9bc	13.4ef	4.7de	47.1a-e	22.3
I14	51.2a-e	52.4ab	48.7ab	24.3bcd	14.5a-d	13.2c-f	15.4bc	26.9b-e	16.6a-e	56.1abc	31.9
I15	59.8ab	52.0ab	42.4a-d	21.2cd	8.1bcd	9.5def	16.8bc	29.6bcd	16.1a-e	48.4a-e	30.4
I16	23.7ijk	33.5def	19.1gh	24.0bcd	17.5abc	4.7ef	21.5ab	18.5c-f	5.7cde	21.6hi	18.9
I17	48.8b-f	37.1c-f	49.7ab	23.2bcd	17.6abc	15.9b-f	13.3bc	10.9f	7.8b-e	44.7b-f	26.9
I18	37.7e-i	48.9abc	25.9e-h	36.9ab	7.4bcd	18.5a-e	10.5bc	24.6b-f	11.7a-e	12.7i	23.5
I19	22.7jk	22.4f	52.0a	22.6bcd	11.5a-d	28.6ab	16.0bc	30.6bcd	5.7cde	23.0hi	23.5
I20	45.4b-h	34.8c-f	34.9b-f	36.9ab	23.6a	27.7abc	12.1bc	32.4bc	3.8e	31.5fgh	28.3
D = D42											
I1	38.3cd	29.6bc	26.7ab	11.8bcd	20.2a-d	13.8c-f	14.6abc	22.3ab	26.7ab	32.5b-e	23.6
I2	36.6cd	33.7bc	31.6ab	24.9ab	30.9ab	20.2b-f	13.3abc	20.8ab	32.1ab	18.9efg	26.3
I3	57.6b	33.6bc	35.4ab	27.9a	21.0a-d	18.6b-f	14.6abc	19.4ab	21.3ab	56.0a	30.5
I4	31.7cde	29.1bc	31.2ab	28.2a	25.9abc	26.9a-d	13.7abc	22.5ab	27.8ab	28.5c-f	26.5
I5	34.2cde	37.7bc	29.9ab	27.4a	15.5bcd	30.2ab	17.1abc	18.0ab	28.9ab	39.8bc	27.8
I6	20.9efg	32.5bc	29.5ab	24.3ab	19.3a-d	12.7def	9.8c	13.6b	20.3b	36.8bcd	22.0
I7	11.5g	34.0bc	21.7bc	8.5cd	14.3cd	7.4f	13.1bc	24.7ab	30.6ab	3.4h	16.9
I8	55.3b	39.5ab	35.1ab	25.8ab	26.4abc	25.5a-e	8.7c	18.2ab	30.3ab	24.2d-g	28.9
I9	30.3cde	26.6bcd	29.8ab	20.5abc	16.8bcd	19.4b-f	25.4ab	23.8ab	29.5ab	26.5c-f	24.8
I10	74.8a	51.6a	36.3ab	24.1ab	25.3a-d	28.1abc	6.5c	23.9ab	23.2ab	37.2bcd	33.1
I11	41.2c	30.3bc	33.3ab	22.3abc	33.3a	38.4a	28.1a	21.0ab	29.7ab	46.2ab	32.4
I12	30.3cde	29.5bc	25.6bc	25.3ab	23.6a-d	25.2a-e	6.4c	17.4ab	26.5ab	26.6c-f	23.6
I13	25.4def	25.4b-e	41.5a	20.6abc	20.0a-d	16.5b-f	8.8c	15.2ab	28.8ab	27.9c-f	23.0
I14	36.3cd	52.3a	34.4ab	31.5a	30.7ab	30.5ab	17.6abc	29.8a	36.6a	31.0c-f	33.0
I15	39.9cd	12.2e	27.3ab	28.3a	21.8a-d	21.5b-f	11.3bc	22.9ab	31.8ab	36.1bcd	25.3
I16	15.2fg	13.6de	12.0c	18.8abc	18.4a-d	14.3c-f	5.4c	19.3ab	28.0ab	19.2efg	16.4
I17	34.6cde	34.2bc	25.4bc	25.1ab	23.2a-d	25.5a-e	9.5c	20.4ab	30.8ab	35.0bcd	26.3
I18	28.2c-f	22.4cde	25.0bc	5.0d	10.2d	23.1b-e	4.6c	21.2ab	25.5ab	11.9gh	17.7
I19	65.6ab	33.1bc	25.6bc	23.5ab	25.1a-d	10.5ef	12.0bc	16.0ab	31.7ab	16.7fg	26.0
I20	39.8cd	32.3bc	24.1bc	22.0abc	19.9a-d	25.2a-e	14.9abc	20.3ab	29.4ab	24.6d-g	25.2
V-Mean	39.9	34	32.5	23.6	17.3	17.9	14.4	22.6	20.3	34.4	25.7

In a column under each D, means followed by a common letter are not significantly different at the 5% level by DMRT. D: Day after inoculation; D42 = 42 day after inoculation; D21 = 21 day after inoculation

reduction, disease incidence, disease severity and yield reductions in all the rice cultivars (Fig. 3-5). Based on cluster dendrogram classification for isolates pathogenic and genotypes viral

Table 10: Variety by isolate interaction means comparison for percentage height reduction due to RYMV disease

Isolate (I)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	I-Mean
D = D21											
I1	19.8ef	29.0b-e	21.9cde	2.6b	2.7fg	27.1ab	11.5c	3.1b	13.5cde	9.6fg	14.1
I2	27.8a-e	24.5c-f	20.6de	9.2ab	13.9b-g	31.1ab	18.6bc	7.5ab	13.7cde	20.0c-g	18.7
I3	28.3a-e	12.0f	30.1bcd	12.2ab	6.8d-g	36.3a	16.7bc	4.1ab	23.4a-e	25.0b-e	19.5
I4	31.6a-e	28.5b-e	30.8bcd	12.2ab	4.8d-g	19.7b	17.4bc	18.2a	17.8b-e	32.1bcd	21.3
I5	27.1b-e	19.9def	14.7e	14.3ab	17.1b-e	27.7ab	12.8c	14.1ab	20.3a-e	23.2cde	19.1
I6	41.2a	40.2ab	35.1bc	5.8ab	24.3b	25.3ab	10.3c	4.1ab	20.6a-e	45.8a	25.2
I7	36.5abc	32.4a-d	27.9b-e	3.2b	36.7a	29.1ab	16.7bc	9.6ab	30.3ab	25.2b-e	24.7
I8	34.1a-d	30.9a-d	28.7bcd	10.3ab	13.4b-g	33.5ab	19.3bc	14.4ab	20.6a-e	21.9c-f	22.7
I9	28.7a-e	33.4a-d	29.9bcd	6.0ab	7.0d-g	35.8a	67.4a	6.2ab	22.7a-e	30.0bcd	26.7
I10	26.4b-e	21.4def	25.2b-e	10.1ab	3.4d-g	32.9ab	15.4c	4.3ab	23.2a-e	20.0c-g	18.2
I11	19.6ef	23.5c-f	26.2b-e	6.9ab	10.0c-g	33.8ab	16.3c	3.7b	16.7b-e	26.5b-e	18.3
I12	34.7a-d	16.7ef	25.6b-e	13.4ab	3.3efg	28.4ab	17.6bc	13.2ab	22.2a-e	8.0g	18.3
I13	11.5f	32.5a-d	29.5bcd	11.2ab	22.6bc	25.2ab	13.5c	7.2ab	10.2e	13.0efg	17.6
I14	24.3c-f	35.7abc	31.8bcd	3.2b	21.5bc	29.1ab	13.3c	2.1b	27.3abc	33.2bc	22.1
I15	21.7def	29.7b-e	26.0b-e	8.1ab	16.4b-f	24.6ab	8.0c	10.9ab	21.8a-e	13.4efg	18.0
I16	38.6ab	43.9a	30.3bcd	6.4ab	7.4d-g	20.5b	16.0c	9.4ab	24.2a-e	36.9ab	23.3
I17	23.1c-f	35.7abc	27.7b-e	5.5ab	8.1d-g	27.8ab	7.4c	6.6ab	17.5b-e	9.7fg	16.9
I18	33.2a-e	44.0a	36.7b	17.6a	17.3bcd	29.4ab	29.7b	11.1ab	25.8a-d	32.3bcd	27.7
I19	19.8ef	35.7abc	59.5a	9.5ab	6.5d-g	23.7ab	16.7bc	4.1ab	32.5a	19.3d-g	22.7
I20	24.3c-f	31.7a-d	29.1bcd	8.4ab	1.6g	26.7ab	20.9bc	9.8ab	13.0de	13.4efg	17.9
D = D42											
I1	26.6e	38.1a-d	37.5bc	2.7c	3.1d	11.2b-e	10.1ab	5.6cd	20.1c-f	33.9def	18.9
I2	37.0a-e	35.7a-d	39.4bc	11.0bc	12.0bcd	15.4b-e	3.4ab	7.0cd	1.6g	38.8b-e	20.1
I3	41.1a-d	27.6cde	36.3bc	4.5c	4.5d	23.9abc	1.9b	6.2cd	19.8c-f	40.8b-e	20.6
I4	42.8a-d	34.9a-d	42.5bc	4.2c	8.4cd	10.6b-e	14.0ab	33.9a	11.7fg	50.7ab	25.4
I5	30.3de	35.7a-d	34.6bc	21.0ab	4.2d	12.2b-e	8.9ab	19.3bc	17.6c-f	40.9b-e	22.5
I6	42.0a-d	43.0ab	40.6bc	8.2bc	21.6ab	9.9cde	7.1ab	10.5cd	28.5bc	61.1a	27.2
I7	45.9ab	39.0abc	40.0bc	6.5c	26.2a	12.1b-e	8.8ab	5.4cd	26.4bcd	37.7b-f	24.8
I8	40.8a-d	32.3a-d	45.4abc	10.8bc	13.7bcd	24.7ab	11.0ab	13.3cd	13.3d-g	37.5b-f	24.3
I9	34.2b-e	40.5abc	32.4c	3.1c	3.9d	34.1a	8.0ab	31.7a	44.9a	20.8gh	25.3
I10	37.8a-e	31.8bcd	40.8bc	5.9c	4.2d	6.4e	14.1ab	13.8cd	11.8fg	43.3b-e	21.0
I11	30.7cde	33.6a-d	42.3bc	6.4c	4.2d	9.2de	10.9ab	25.9ab	12.8efg	46.3bcd	22.2
I12	46.4ab	25.5de	41.1bc	11.2bc	1.5d	19.9b-e	3.5ab	10.2cd	21.3c-f	25.0fgh	20.5
I13	42.0a-d	41.7ab	42.6bc	9.1bc	7.8cd	14.8b-e	7.5ab	8.6cd	2.8g	42.2b-e	21.9
I14	35.7b-e	45.7ab	45.2abc	4.9c	18.7abc	20.7bcd	8.4ab	6.8cd	31.0bc	48.8b	26.6
I15	37.9a-e	18.3e	46.5abc	7.4c	5.2d	11.7b-e	1.5b	7.1cd	11.3fg	34.9c-f	18.2
I16	44.3a-d	46.2a	16.9d	31.2a	5.1d	15.1b-e	10.9ab	7.2cd	36.6ab	48.5bc	26.2
I17	33.2b-e	42.2ab	42.9bc	4.2c	1.3d	13.7b-e	16.3a	3.5d	2.7g	30.9efg	19.1
I18	44.5abc	42.7ab	39.7bc	13.8bc	27.4a	18.1b-e	12.9ab	8.4cd	26.1b-e	15.3h	24.9
I19	50.3a	43.2ab	56.8a	14.0bc	4.6d	15.6b-e	12.9ab	2.2d	14.6d-g	37.7b-f	25.2
I20	38.4a-e	41.8ab	47.2ab	6.9c	4.2d	17.0b-e	17.1a	5.2cd	11.8fg	34.6def	22.4
V-Mean	33.3	33.5	34.9	9.1	10.6	22.1	13.8	9.9	19.6	30.7	21.7

In a column under each D, means followed by a common letter are not significantly different at the 5% level by DMRT. D: Day after inoculation; D42 = 42 day after inoculation; D21 = 21 day after inoculation

resistance levels, 17 RYMV isolates were classified as Highly Pathogenic Isolates (HPI) and 3 RYMV isolates were classified as Mildly Pathogenic Isolates (MPI) (Fig. 4) while 4 rice varieties

Table 11: Variety by isolate interaction means comparison for disease incidence due to RYMV disease

Isolate (I)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	I-Mean
D = D21											
I1	54.7cde	62.3a	57.8a	11.1a	11.1b	11.1a	11.1b	18.5a	11.1b	48.9abc	29.8
I2	56.4b-e	51.0a-e	55.4a	11.1a	11.1b	11.1a	11.1b	11.1a	11.1b	52.8abc	28.2
I3	61.1bcd	25.1g	50.1a	11.1a	11.1b	11.1a	18.9b	11.1a	11.1b	44.0bc	25.5
I4	64.4abc	52.1a-e	55.1a	11.1a	11.1b	11.1a	11.1b	11.1a	11.1b	50.6abc	28.9
I5	43.1f	57.8abc	53.7a	13.0a	11.1b	11.1a	11.1b	13.0a	11.1b	49.6abc	27.5
I6	64.5abc	52.4a-e	52.7a	11.1a	11.1b	11.1a	11.1b	11.1a	11.1b	59.4a	29.5
I7	73.1a	48.2cde	35.3b	11.2a	11.1b	11.1a	13.0b	11.1a	20.0b	32.4d	26.6
I8	61.0bcd	49.7b-e	56.4a	11.1a	11.1b	11.1a	11.7b	11.1a	11.1b	51.7abc	28.6
I9	46.6ef	45.4def	57.7a	14.5a	11.1b	19.5a	14.8b	18.5a	11.1b	54.1abc	29.3
I10	57.8bcd	54.7a-e	48.8a	11.1a	11.1b	11.1a	11.1b	11.1a	11.1b	48.7abc	27.6
I11	43.9f	59.9ab	59.1a	11.1a	11.1b	11.1a	11.1b	11.1a	11.1b	54.6ab	28.4
I12	60.5bcd	44.2ef	50.7a	18.2a	11.1b	11.1a	20.6b	11.1a	11.1b	51.0abc	28.9
I13	51.8def	56.4a-d	55.8a	11.1a	11.1b	11.1a	11.1b	11.1a	11.1b	52.6abc	28.3
I14	67.0ab	50.9a-e	57.6a	11.1a	14.8ab	11.1a	14.8b	11.1a	11.1b	53.2abc	30.3
I15	55.2cde	55.3a-e	50.1a	11.1a	11.1b	11.1a	11.1b	11.1a	11.1b	47.5bc	27.5
I16	54.9cde	36.9f	53.1a	11.1a	11.1b	11.1a	11.1b	11.1a	11.1b	53.7abc	26.5
I17	54.8cde	49.5b-e	50.7a	11.1a	11.1b	11.1a	11.1b	11.1a	11.1b	42.7c	26.4
I18	58.3bcd	53.5a-e	31.0b	19.0a	22.2a	11.1a	11.1b	11.1a	14.3b	29.3d	26.1
I19	51.8def	58.1 abc	56.1a	11.1a	20.7ab	11.1a	14.4b	11.1a	17.1b	53.3abc	30.5
I20	54.7cde	25.4g	52.9a	11.1a	11.1b	11.1a	43.9a	11.1a	44.5a	51.1abc	31.7
D = D42											
I1	71.7a	67.3ab	68.7abc	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	66.2abc	34.0
I2	63.3abc	63.1ab	63.1abc	11.1b	18.5a	11.1a	11.1a	11.1a	11.1a	58.4abc	32.2
I3	69.3ab	65.7ab	65.1abc	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	62.5abc	32.9
I4	64.6abc	60.1ab	67.8abc	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	54.8c	31.4
I5	62.6abc	64.1ab	71.8a	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	69.3a	33.4
I6	61.9abc	58.7ab	50.3d	20.7a	11.1a	11.1a	11.1a	11.1a	19.0a	61.6abc	31.7
I7	57.6c	56.8b	60.6a-d	11.1b	14.8a	11.1a	11.1a	11.1a	11.1a	40.7d	28.6
I8	65.4abc	59.1ab	57.4cd	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	62.7abc	31.1
I9	65.7abc	60.7ab	65.3abc	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	60.9abc	31.9
I10	64.6abc	63.3ab	62.6abc	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	65.0abc	32.2
I11	63.9abc	60.9ab	63.7abc	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	63.6abc	31.9
I12	64.2abc	65.6ab	65.1 abc	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	62.7abc	32.4
I13	62.8abc	61.8ab	60.1 bcd	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	61.0abc	31.2
I14	70.5ab	63.3ab	62.0abc	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	59.4abc	32.2
I15	72.3a	63.3ab	62.0abc	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	66.9ab	33.1
I16	59.3bc	59.8ab	35.4e	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	60.5abc	28.2
I17	59.5bc	68.9a	70.8ab	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	66.1abc	33.2
I18	69.3ab	60.7ab	71.4ab	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	55.4bc	32.3
I19	46.3d	65.1ab	60.3a-d	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	43.2d	28.2
I20	64.6abc	63.3ab	58.4cd	11.1b	11.1a	11.1a	11.1a	11.1a	11.1a	57.3bc	31.0
V-Mean	60.4	56	57	11.8	12	11.3	12.7	11.5	12.6	54.5	30.0

In a column under each D, means followed by a common letter are not significantly different at the 5% level by DMRT. D: Day after inoculation; D42 = 42 day after inoculation; D21 = 21 day after inoculation

Gigante, TOG5672, TOG5674, TOG5681) were Highly Resistant (HR), 2 rice varieties (TOG6691, WAC116) were Moderately Resistant (MR) and 4 rice varieties (BG90-1, Bouake189, WAC117,

Table 12: Variety by isolate interaction means comparison for disease severity due to RYMV disease

Isolate (I)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	I-Mean
D = D21											
I1	96.8a	88.7ab	97.5a	0.0a	0.0c	0.0a	0.0c	33.4a	0.0d	89.8a	40.6
I2	100.0a	90.8ab	96.7a	0.0a	0.0c	0.0a	0.0c	0.0b	0.0d	95.7a	38.3
I3	97.8a	40.5d	91.3a	0.0a	0.0c	0.0a	16.7bc	0.0b	0.0d	84.5a	33.1
I4	97.6a	89.7ab	92.2a	0.0a	0.0c	0.0a	0.0c	0.0b	0.0d	86.5a	36.6
I5	100.0a	94.5ab	100.0a	8.4a	0.0c	0.0a	0.0c	8.4b	0.0d	92.7a	40.4
I6	98.4a	54.2cd	96.1a	0.0a	0.0c	0.0a	0.0c	0.0b	0.0d	97.4a	34.6
I7	93.7a	83.4ab	94.7a	0.5a	0.0c	0.0a	8.4bc	0.0b	40.0b	78.2a	39.9
I8	97.0a	81.6ab	96.7a	0.0a	0.0c	0.0a	2.1 c	0.0b	0.0d	89.7a	36.7
I9	82.1 a	80.5ab	94.7a	15.3a	0.0c	16.7a	16.7bc	16.3ab	0.0d	100.0a	42.2
I10	100.0a	100.0a	92.4a	0.0a	0.0c	0.0a	0.0c	0.0b	0.0d	84.2a	37.7
I11	83.3a	100.0a	100.0a	0.0a	0.0c	0.0a	0.0c	0.0b	0.0d	96.8a	38.0
I12	96.0a	70.3bc	82.9a	16.2a	0.0c	0.0a	28.5b	0.0b	0.0d	83.4a	37.7
I13	85.6a	97.2ab	98.4a	0.0a	0.0c	0.0a	0.0c	0.0b	0.0d	93.6a	37.5
I14	93.5a	94.8ab	100.0a	0.0a	16.7c	0.0a	16.7bc	0.0b	0.0d	94.2a	41.6
I15	96.4a	92.0ab	88.1a	0.0a	0.0c	0.0a	0.0c	0.0b	0.0d	79.3a	35.6
I16	90.9a	95.0ab	45.9b	0.0a	0.0c	0.0a	0.0c	0.0b	0.0d	89.0a	32.1
I17	90.6a	91.1ab	92.5a	0.0a	0.0c	0.0a	0.0c	0.0b	0.0d	77.1a	35.1
I18	98.2a	97.2ab	83.4a	2.7a	83.3a	0.0a	0.0c	0.0b	9.5cd	40.2b	41.5
I19	85.8a	96.3ab	100.0a	0.0a	43.2b	0.0a	14.8bc	0.0b	26.7bc	85.8a	45.3
I20	92.7a	32.6d	95.2a	0.0a	0.0c	0.0a	66.7a	0.0b	66.7a	100.0a	45.4
D = D42											
I1	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	40.0
I2	100.0a	100.0a	100.0a	0.0a	16.7a	0.0a	0.0a	0.0a	0.0b	100.0a	41.7
I3	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	40.0
I4	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	83.3a	38.3
I5	92.0ab	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	39.2
I6	100.0a	97.5a	83.3a	14.4a	0.0a	0.0a	0.0a	0.0a	16.7b	100.0a	41.2
I7	100.0a	100.0a	100.0a	0.0a	4.2a	0.0a	0.0a	0.0a	0.0b	100.0a	40.4
I8	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	40.0
I9	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	40.0
I10	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	40.0
I11	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	40.0
I12	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	40.0
I13	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	40.0
I14	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	40.0
I15	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	40.0
I16	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	50.0a	94.8a	44.5
I17	100.0a	100.0a	50.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	35.0
I18	100.0a	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	33.4b	33.3
I19	76.4b	100.0a	100.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	37.6
I20	100.0a	100.0a	50.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0b	100.0a	35.0
V-Mean	96.1	91.7	93	1.4	4.1	0.4	4.3	1.4	5.2	91.2	38.9

In a column under each D, means followed by a common letter are not significantly different at the 5% level by DMRT. D: Day after inoculation; D42 = 42 day after inoculation; D21 = 21 day after inoculation

IR64) were susceptible (Fig. 3). The pathotyping study has also revealed the distribution, movement and population structure of RYMV isolates across the five Southwest states in Nigeria

Table 13: Variety by isolate interaction means comparison for percentage yield reduction due to RYMV disease

	Variety (V)										
Isolate (I)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	I-Mean
I1	89.0a	74.0a	79.7a	64.5ab	49.3a-d	53.3a	49.6a-d	48.7a-d	64.8ab	60.5abc	63.4
I2	91.1a	77.0a	87.5a	42.8ab	47.5a-e	93.3a	28.4cd	48.0a-e	1.8e	59.0abc	57.6
I3	91.7a	73.1a	69.1a	45.6ab	23.8ef	66.7a	39.3bcd	38.0cde	30.1cd	69.8abc	54.7
I4	96.1a	79.6a	80.5a	58.4ab	28.3def	53.3a	47.0a-d	60.7abc	15.1d	98.1a	61.7
I5	93.7a	77.9a	96.5a	70.2a	64.4ab	73.3a	57.6abc	68.7ab	46.1abc	73.3abc	72.2
I6	91.0a	77.0a	73.9a	64.1 ab	30.1 c-f	79.3a	43.9a-d	24.0def	36.5a-d	84.1ab	60.4
I7	92.3a	71.4a	82.7a	60.1 ab	50.7a-d	76.7a	72.4a	20.0ef	49.3a-d	41.0cd	61.7
I8	81.5a	58.0a	54.9a	63.3ab	44.8a-e	66.0a	49.9a-d	6.7f	1.4e	17.9d	44.4
I9	95.3a	54.1a	81.1a	66.7ab	33.8b-e	81.3a	52.5a-d	38.0a-e	36.5a-d	71.8abc	61.1
I10	90.7a	53.3a	63.5a	30.2b	16.9f	73.3a	43.9a-d	38.0cde	36.5bcd	54.8bc	50.1
I11	93.5a	79.6a	71.5a	47.9ab	47.0a-e	74.0a	56.6abc	45.3a-e	39.3a-d	73.9abc	62.9
I12	90.6a	73.6a	84.3a	63.1ab	40.6a-e	72.7a	44.2a-d	37.3a-e	36.1bcd	70.8abc	61.3
I13	94.1a	73.6a	77.9a	58.8ab	52.1a-d	82.0a	38.0a-d	68.7ab	15.1de	61.0abc	62.1
I14	97.2a	64.1a	82.1a	68.3a	58.4abc	78.3a	59.2abc	75.3a	74.0a	73.3abc	73.0
I15	94.5a	87.0a	75.7a	64.7ab	41.6a-e	78.7a	35.9bcd	32.0cde	24.2cd	72.3abc	60.7
I16	90.7a	86.6a	68.0a	57.8ab	42.9a-e	74.1a	66.2ab	59.3abc	52.1abc	67.2abc	66.5
I17	92.9a	79.6a	85.6a	53.5ab	44.7a-e	74.5a	30.5bcd	58.7abc	16.0de	79.5abc	61.5
I18	93.3a	80.5a	79.7a	69.0a	73.5a	78.3a	32.0bcd	36.0b-e	40.2a-d	92.3abc	67.5
I19	84.3a	74.0a	85.1a	50.9ab	40.2b-f	78.7a	29.2d	30.7cde	59.4ab	20.5d	55.3
I20	90.8a	51.9a	64.3a	56.8ab	25.6def	78.5a	38.3a-d	27.3def	39.3a-d	55.9abc	52.9
V-Mean	91.7	72.3	77.2	57.8	42.8	74.3	45.7	43.1	35.7	64.9	60.5

In a column means followed by a common letter are not significantly different at the 5% level by DMRT

Table 14: Distribution of the RYMV isolates pathotypes across the five Southwest states in Nigeria

Pathotype		RYMV isolate distribution and movement					
Main group	Sub group	Lagos	Oyo	Ogun	Ekiti	Ondo	% Occurrence
HPI	HPIa			3	2	4	45
	HPIb	3	2		3		40
MPI			1		2		15

(Table 14). Pathotype HPIa has 45% occurrence in three states (Ogun, Ekiti and Ondo), HPIb has 40% in three states (Lagos, Oyo and Ekiti) and MPI has 15% in two states (Oyo and Ekiti). Consequently HPI isolates were present in all the five states while MPI isolates were found only in two states (Oyo and Ekiti) (Table 14). At state level, Ogun and Ondo have only HPIa RYMV isolates, Lagos has only HPIb, Oyo has HPIb and MPI and Ekiti has HPIa, HPIb and MPI (Table 14).

Serotyping characterization analysis of different RYMV isolates: Serotyping characterization analysis of 20 RYMV isolates (Table 2) using 26 polyclonal antibodies (Table 4) in ACP-ELISA has been carried out. Serological relationship among 20 RYMV isolates from Southwest Nigeria has been revealed which comprised of two major serogroups (NSg1 and NSg2) and four subgroups (NSg1a, NSg1b, NSg2a and NSg2b) (Fig. 6). NSg1a and NSg1b serogroups comprised of both normal and RB RYMV isolates while NSg2a and NSg2b serogroups were typical

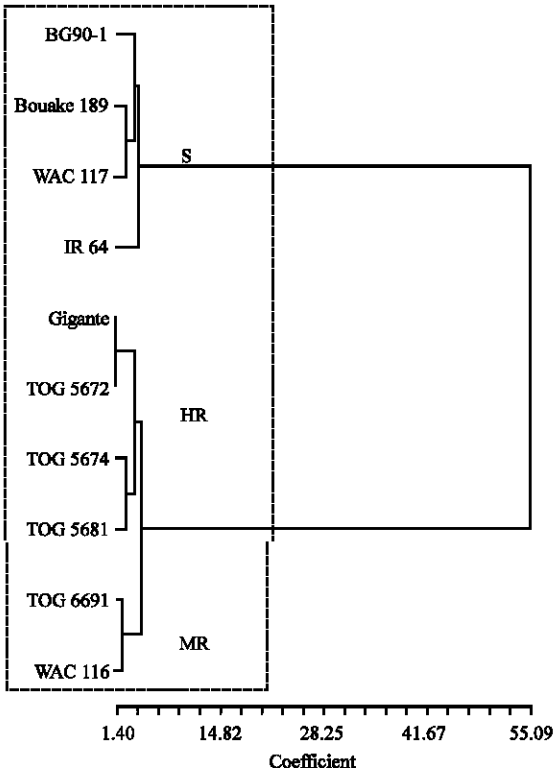

Fig. 3: Cluster dendrogram showing classification of genotype (cultivar) level of resistance to environment (isolate) using Additive Main effects and Multiplicative Interaction (AMMI) analysis. HR: Highly resistant; MR: Moderately resistant; S: Susceptible

Table 15: Distribution of the RYMV isolates serogroups across the five Southwest states in Nigeria

Serogroup		RYMV isolate distribution and movement					
Main group	Subgroup	Lagos	Oyo	Ogun	Ekiti	Ondo	% Occurrence
NSg1	NSg1a	3		3	4	2	60
	NSg1b		2		1	1	20
NSg2	NSg2a		1			1	10
	NSg2b				2		10

of RB RYMV isolates only. This serotyping study has further revealed the diversity-complex nature among the RB RYMV isolates from Southwest Nigeria and the possibility that some normal RYMV isolates seemed to possess RB characteristics. The serotyping study has also revealed the distribution, movement and population structure of RYMV isolates across the five Southwest states in Nigeria (Table 15). Serogroup NSg1a has 60% occurrence in four states (Lagos, Ogun, Ekiti and Ondo), NSg1b has 20% in three states (Oyo, Ekiti and Ondo), Nsg2a has 10% in two states (Oyo and Ondo) and NSg2b has 10% in one state (Ekiti). Consequently NSg1 isolates were present in all the five states while NSg2 isolates were found only in three states (Oyo, Ekiti and Ondo). At state level, Ogun and Lagos have only NSg1a RYMV isolates, Oyo has NSg1b and NSg2a, Ekiti has NSg1a, NSg1b and NSg2b and Ondo has NSg1a, NSg1b and NSg2a. The pathotyping study would therefore target the development of durable resistant rice varieties to Nsg1 and Nsg2

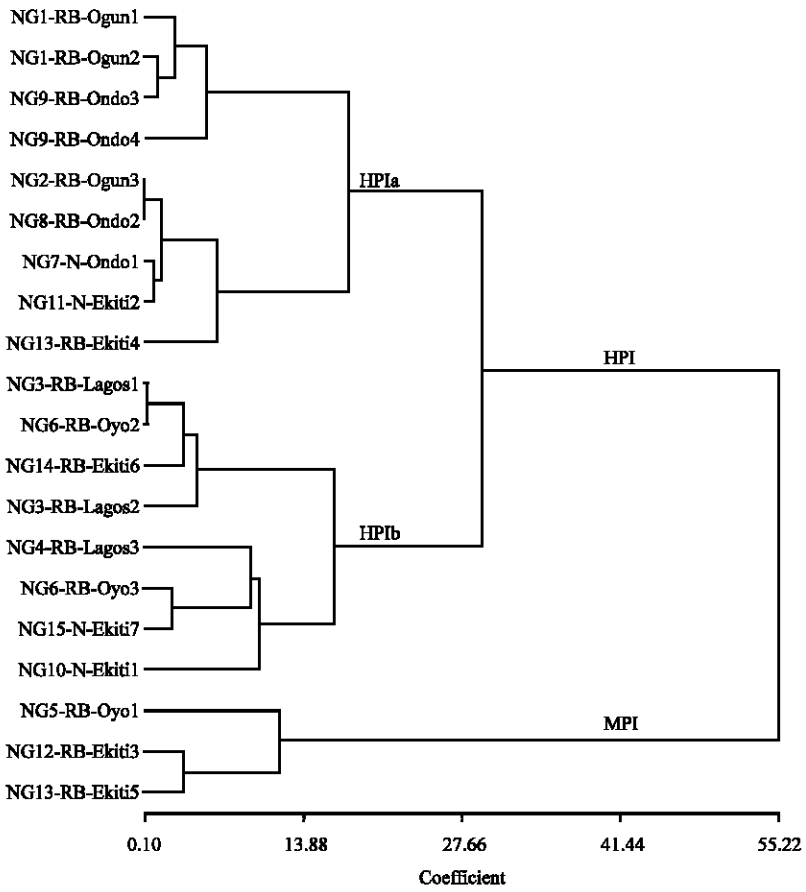

Fig. 4: Cluster dendrogram showing classification of environment (isolate) pathogenic level to genotype (cultivar) using Additive Main effects and Multiplicative Interaction (AMMI) analysis

isolates serotypes and to determine any possible linkage between RYMV pathotype and serotype in Southwest Nigeria. All the 26 polyclonal antibodies were separated into two major serogroups (PSg1 and PSg2) while PSg2 was further separated into three subgroups (Psg2a, Psg2b and PSg-2c) (Fig. 7). According to the composition of each serogroup, some polyclonal antibodies raised from isolates collected in different West African countries appeared to be similar. Besides, polyclonal antibodies produced from isolates collected in a same country were also different. Polyclonal antibodies belonging to PSg1, PSg2a, PSg2c and PSg-2b serogroups have diagnostic potential of 30, 55-65, 75-80 and 85-100%, respectively, for all the 20 RYMV isolates analyzed (Table 16).

Linkage between RYMV pathotype and serotype: At the state level, the identified RYMV NSg1a serotype in Ogun and Lagos are carrying HPIa and HPIb pathotype, respectively, Oyo with NSg1b and NSg2a serotypes formed HPIb and MPI pathotypes, all NSg1a, NSg1b and NSg2a serotypes in Ondo formed HPIa pathotype only and NSg1a and NSg1b serotypes in Ekiti were characterized as HPIa, HPIb and MPI (Table 17). Thus Ekiti has the highest number of serotypes with corresponding highest number of pathotypes (Table 17).

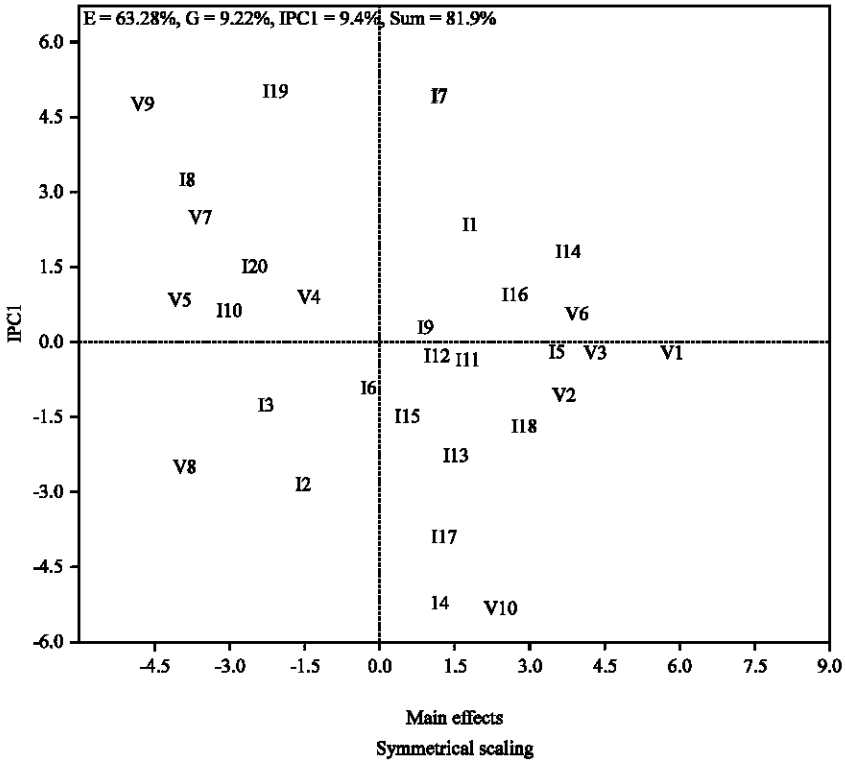

Fig. 5: Genotype (cultivar) by environment (isolate) interaction effects on yield reduction using Additive Main effects and Multiplicative Interaction (AMMI) analysis

Table 16: Percentage diagnostic potential and diversity among 26 RYMV polyclonal antibodies

Polyclonal antibody	% Diagnostic potential	Sero group
RYMV-Pab1	30	PSg1
RYMV-Pab6		
RYMV-Pab9	55-65	PSg2a
RYMV-Pab2		
RYMV-Pab13		
RYMV-Pab20		
RYMV-Pab15	75-80	PSg2c
RYMV-Pab21		
RYMV-Pab23		
RYMV-Pab22		
RYMV-Pab24		
RYMV-Pab10	85-100	PSg2b
RYMV-Pab25		
RYMV-Pab3		
RYMV-Pab16		
RYMV-Pab17		
RYMV-Pab18		
RYMV-Pab7		
RYMV-Pab11		
RYMV-Pab12		

Table 16: Continued

Polyclonal antibody	% Diagnostic potential	Sero group
RYMV-Pab26		
RYMV-Pab4		
RYMV-Pab5		
RYMV-Pab8		
RYMV-Pab14		
RYMV-Pab19		

Table 17: Relationship between RYMV isolates serotype and pathotype population structure across five Southwest states in Nigeria

			RYMV isolate distribution and movement					
Typing	Main group	Sub group	Lagos	Oyo	Ogun	Ekiti	Ondo	% Occurrence
Serotype	NSg1	NSg1a	3		3	4	2	60
		NSg1b		2		1	1	20
	NSg2	NSg2a		1			1	10
		NSg2b				2		10
Pathotype	HPI	HPIa			3	2	4	45
		HPIb	3	2		3		40
	MPI			1		2		15

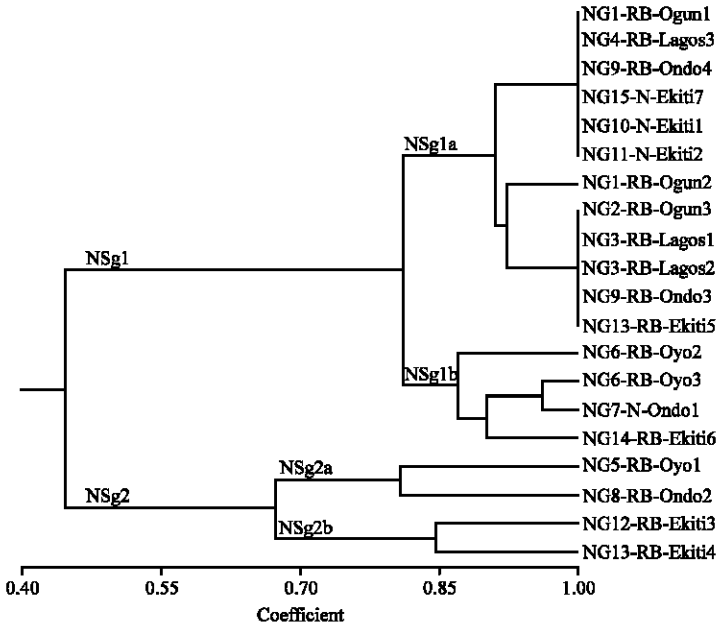

Fig. 6: Serological relationship among 20 RYMV isolates from Southwest Nigeria as revealed by 26 RYMV polyclonal antibodies in ACP-ELISA

DISCUSSION

Periodic disease survey and sampling as well as accurate serological indexing method are prerequisites into understanding the present status, movement and epidemiology of RYMV disease in upland, lowland and irrigated rice ecologies in Africa (Onasanya *et al*., 2004, 2006). In the current study, RYMV disease survey revealed RYMV disease incidence was between 15-70% in

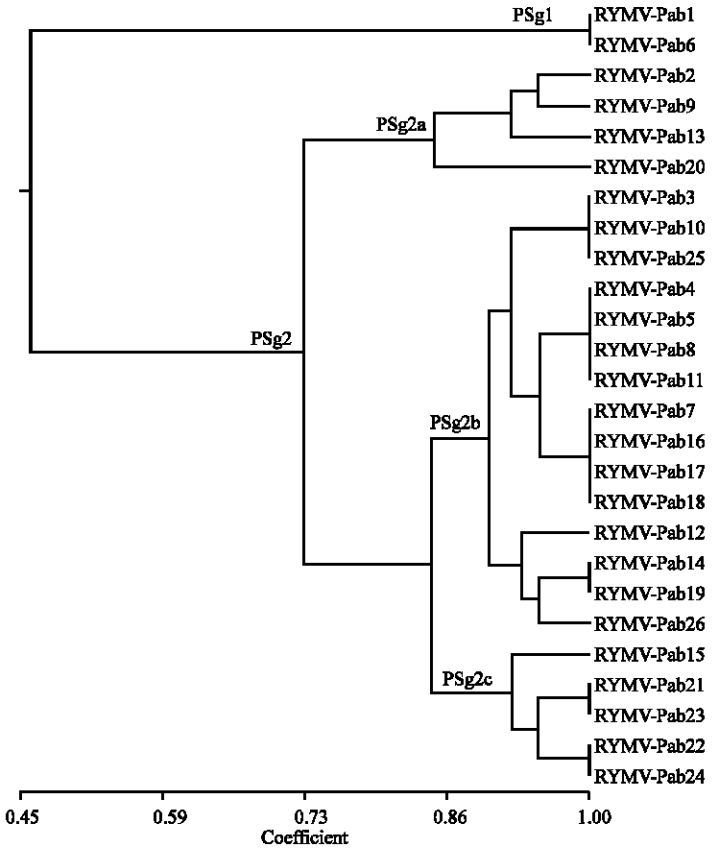

Fig. 7: Serological relationship among 26 RYMV polyclonal antibodies in their reaction with 20 RYMV isolates from Southwest Nigeria in ACP-ELISA

farmers' fields across the five Southwest states in Nigeria with highest disease incidence from Oyo state and the least disease incidence from Ekiti state. Besides, serological indexing confirmed 92% of collected leaf samples were RYMV positive out of which 24% were from cultivated rice varieties and 76% were from weeds. The weed having the greater 76% RYMV positive by serological indexing suggests possibility of being the main reservoir of RYMV in Southwest Nigeria and may responsible for season-to-season source of RYMV inoculums and spread in farmers' fields across rice ecologies (Onasanya *et al*., 2004, 2006). Previous study has revealed the importance of natural reservoir hosts in the persistence spread of RYMV under fields conditions (Abo *et al*., 2002).

RYMV is a variable virus with many pathological and serological variants and its unlimited number of pathological and virulence characters and lack of standardization of pathological conditions and virulence tests among different researchers have led to confusion and uncertainty in the characterization of this pathogen from rice (N'Guessan *et al*., 2000; Onasanya *et al*., 2004, 2006). Resistance Breaking (RB) RYMV isolates are isolates that attacked RYMV resistant variety giving rise to typical RYMV symptoms (Onasanya *et al*., 2006). The present study identified RB and normal RYMV isolates. Although this was the first time RB isolates identified in southwest Nigeria, it has been identified in the West and Central Africa (Hebrard *et al*., 2006; Traore *et al*., 2006). The study, however, revealed that normal RYMV isolate is different from RB RYMV isolate and diversity is higher among resistance breaking isolates than among the normal isolates in Southwest Nigeria.

The application and use of pathotyping methods to study distribution, movement and population structure of RYMV isolates has led to the identification of two pathotypes of RYMV isolates in Southwest Nigeria. The Additive Main Effect and Multiplicative Interaction (AMMI) analysis seemed effective in understanding and explaining complex Genotype by Environment (GE) interactions between the rice genotypes and RYMV pathotypes (Ebdon and Gauch, 2002a; Onasanya *et al*., 2004, 2006). Such interactions could generate complex data sets difficult to understand with Ordinary Analysis of Variance (ANOVA). In the current study, 20 RYMV isolates used covered major rice ecologies from five Southwest states in Nigeria leading to very high RYMV interactions among rice genotypes. The existence of HPI and MPI RYMV pathotypes obtained in this study have led to differential interactions among genotypes with heavy implications on the genotype resistance and yield stability. In previous studies, two virulent pathotypes of RYMV isolates have been known to exist in Mali and Cote d'Ivoire and these virulent pathotypes were present in different parts of the countries (Onasanya *et al*., 2004, 2006).

As revealed by this study, genotypes pathogenic resistance to HPI and MPI RYMV pathotypes first occurs at the level of the individual and involves physiological or behavioral tolerance or adaptability. Subsequent response to increasing viral pathogenicity may involve survival only of the better-adapted genotypes HPI pathotypes which consist of 17 isolates, could be described as possessing both stable and high level of virulence affecting genotypes resistance to RYMV across 5 Southwest states in Nigeria. Under different rice ecologies in Southwest Nigeria, four varieties (Gigante, TOG5672, TOG5674 and TOG5681) possessed heterogenous viral resistance characteristics making them to be more stable, adaptable and more resistant to stress induced by HPI pathotypes originated from different states. Genotypes that have adapted to endure variable isolates or strains infestations are more likely to tolerate an independent stress compared to those genotypes that are only adapted to a fixed isolate or strain (Ebdon and Gauch, 2002a, b).

As RYMV isolates population increases, there is probability that HPI pathotypes population will be more than that of MPI and possible interactions between these two pathotypes could lead to the emergence of new RB strains. The use of highly resistant varieties (Gigante, TOG5672, TOG5674 and TOG5681) will potentially reduce HPI and MPI pathotypes population and their interactions. There is probability that the four resistant varieties (Gigante, TOG5672, TOG5674 and TOG5681) obtained in this study will survive and evolve through combinations of genes present in the population since population resistance is enhanced by genes polymorphism that may result in short-term selection of more tolerant genotypes in stressful viral environments (Ebdon and Gauch, 2002a,b).

The classification of the 20 RYMV isolates into two main serogroups (NSg1 and NSg2) and four subgroups (NSg1a, NSg1b, NSg2a and NSg2b) indicates the existence and levels of serodiversity among RYMV isolates in Southwest Nigeria. The serodiversity patterns displayed by the two serogroups and four subgroups shown that they differed by specific combination of epitopes. This conformed to the earlier study of existence of several serotypes of RYMV isolates (N'Guessan *et al*., 2000; Sere *et al*., 2007). The serotyping study has further revealed the diversity-complex nature among the resistance breaking RYMV isolates from Southwest Nigeria and the possibility that some normal RYMV isolates seemed to possess resistance breaking characteristics. Besides, the serotyping study has also revealed the distribution, movement and population structure of RYMV isolates across the five Southwest states in Nigeria. Many isolates emanating from same locality, field and host were observed to be serologically different and this explains the fact that within a set of isolates of related strains in the same host plant, many possibilities of interaction exist

(Onasanya *et al*., 2006). This possible isolate and host plant interaction varies between one locality to another thus account for diverse serological variability that exist among different isolates of RYMV in Southwest Nigeria. The serological similarities observed between isolates within the same and different states confirm the great cross-infection potential of RYMV transmitted under natural conditions by different insect vectors (Sere *et al*., 2007; Nwilene *et al*., 2009).

In this study, there were indications of localized micro variation among Southwest states isolates with the emergence of NSg2b serogroup in Ekiti and NSg2a serogroup in Ondo and Oyo that were not found among Ogun and Lagos isolates. This is practically important for various RYMV identifications and further strengthens the need for deployment of durable rice varieties in the region. Besides, serogroup NSg1a has 60% occurrence in four states (Lagos, Ogun, Ekiti and Ondo), NSg1b has 20% in three states (Oyo, Ekiti and Ondo), NSg2a has 10% in two states (Oyo and Ondo) while NSg2b has 10% in one state (Ekiti). Consequently NSg1 isolates were present in all the five states while NSg2 isolates were found only in three states (Oyo, Ekiti and Ondo). This indicates that isolates belonging to NSg1 serogroup are the most widely distributed in Southwest Nigeria and could be responsible for wide distribution RYMV infections of rice plants with up to 35.7-91.7% yield losses in the region. The evolution of NSg2 serogroup could be as a result of possible interaction and co-existence between NSg1a and NSg1b serotypes. It was suggested that, the successive transmission by beetles and other insects of NSg1a and NSg1b mixtures might have lead to interaction and co-existence among the serotypes from which NSg2 came into existence. It was hypothesized that such possibilities of interaction within a set of isolates of related strains might lead to frequent occurrence of mutants which might be responsible for the high level of serological variation among the isolates (Sere *et al*., 2005, 2007). More research involving the use of molecular techniques is needed to confirm if further sub-groupings would be appropriate, since there was a good correspondence between serological and molecular diversity of RYMV isolates (N'Guessan *et al*., 2000; Fargette *et al*., 2002).

One implication of our findings to the practical management of RYMV disease is that control measures will have to target different strains of the pathogen in different localities. Present results demonstrate that Serological Data Index (SDI) generated from ACP-ELISA has great potential for serological identification and classification of RYMV isolates in Southwest Nigeria. The specific distinction pattern of each isolate SDI and its phylogeny are consistent, repeatable and reliable (Sere *et al*., 2007). The definition of specific distinct pattern for each isolate or strain should be a simple and straightforward task. Obviously, for these distinctions to have a practical meaning for the rice breeder, specific distinct pattern for each isolate must be related to the degree of virulence present. For example, in the present study the identified RYMV NSg1a serotype in Ogun and Lagos were HPIa and HPIb pathotypes respectively, Oyo with NSg1b and NSg2a serotypes formed HPIb and MPI pathotypes, all NSg1a, NSg1b and NSg2a serotypes in Ondo formed HPIa pathotype only while NSg1a and NSg1b serotypes in Ekiti were characterized as HPIa, HPIb and MPI. Thus Ekiti has the highest number of serotypes with corresponding highest number of pathotypes. This was achieved by a systematic comparison of distinct serotyped isolates or strains contrasting to their degree of virulence to rice. A similar approach has been used to determine the serological relationships of RYMV isolates in Cote d'Ivoire (Sere *et al*., 2007). The phylogeny of RYMV isolates, using serological and pathological methods, should be useful for its surveillance in rice growing regions, in epidemiological studies to assess its identity and interaction as well as assist breeding programs aiming at the effective development of cultivars with durable resistant to RYMV.

CONCLUSION

RYMV is a variable virus with many pathological and serological variants. Periodic disease survey and sampling as well as accurate serological indexing method are prerequisites into understanding the present status, movement and epidemiology of RYMV disease in upland, lowland and irrigated rice ecologies in Southwest Nigeria. Two RYMV pathotypes and serotypes confirmed present in Southwest Nigeria. The serotyping and pathotyping studies have further revealed the diversity-complex nature among the resistance breaking RYMV isolates from Southwest Nigeria and the possibility that some normal RYMV isolates seemed to possess resistance breaking characteristics. Besides, the serotyping and pathotyping studies have also revealed the distribution, movement and population structure of RYMV isolates across the five Southwest states in Nigeria. The pathotyping study has been useful in the identification of four resistant rice varieties to RYMV disease in Southwest Nigeria. The phylogeny of RYMV isolates, using serological and pathological methods, should be useful for its surveillance in rice growing regions, in epidemiological studies to assess its identity and interaction as well as assist breeding programs aiming at the effective development of cultivars with durable resistant to RYMV disease.

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