

Analysis of Serological Variability and Hierarchical Distribution of Rice yellow mottle Sobemovirus Isolates in Northern Nigeria

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ABSTRACT

A panel of four monoclonal antibodies (MAbs) was used to study the immunological profile of *Rice yellow mottle virus* (RYMV) genus Sobemovirus. Serological profiles of 35 representative isolates of RYMV from Borno, Gombe, Kaduna, Kano, Niger, Sokoto and Zamfara states in Northern Nigeria. All the RYMV isolates were classified into three major serogroups (SG1, SG2 and SG3) and further separated into six subgroups (Sg1a, Sg1b, Sg2a, Sg2b, Sg3a and Sg3b). The results demonstrate a significant serological variability among RYMV isolates in Northern Nigeria. The hierarchical analysis of the serological profiles data revealed high viral load in Kano, Kaduna and Gombe states, these show they are suitable locations for strategic RYMV diagnostic and field epidemiological studies.

1. INTRODUCTION

Different serotypes (Sere, *et al.*, 2007) and Pathotypes (Onasanya *et al.*, 2004) of *Rice yellow mottle virus* (RYMV) genus Sobemovirus (Fargette and Hull, 2005) isolates are known to exist. The virus is indigenous to the African continent (Abo, *et al.*, 1998; Banwo, *et al.*, 2004) and its neighbouring islands (Kouassi *et al.*, 2005; Raymundo and Buddenhagen 1976). It was first discovered in Kenya in the 1960s (Bakker, 1974). The virus is now found in all sub-Saharan African countries and in Madagascar in the Indian Ocean (Raymundo and Buddenhagen, 1976; Abo, *et al.*, 1998). It is characterized by high genetic variability due to its intrinsic high rates of mutation which is evident from the detection of several strains of the pathogen in some countries of Africa (Pinel *et al.*, 2000; N'Guessan *et al.*, 2001; Traore *et al.*, 2005; Pinel-Galzi and Fargette, 2006). Five major serotypes have been identified, 3 in West and Central Africa (Ser1, Ser 2, Ser 3) and the other 2 in East Africa (Ser 4 and Ser 5) (Kanyeka *et al.* 2007). So far six strains of the virus have been reported, 3 in West and Central Africa (S1, S2 and S3) and 3 in East Africa (S4, S5 and S6) (Pinel *et al.*, 2000; Fargette and Konate, 2004; Kanyeka *et al.*, 2007).

RYMV is transmitted through mechanical contact, sap inoculation, insect vectors, animals and culture practices (Abo *et al.*, 2002; Sarra and Peters, 2003; Sarra *et al.*, 2004; Nwilene *et al.*, 2009). Symptoms are quite variable and depend on the particular isolate–host combination but frequently include mottling, yellowing or orange leaf discolouration, reduced tillering, stunting of plants and sterility of flowers (Bakker, 1974; Fargette *et al.*, 2002). Serious epidemics of RYMV have been reported in all rice ecological systems throughout Africa (Abo *et al.*, 1998). Yield losses as high as 100% have often been reported, depending on the rice cultivars, time of infection, rice cropping systems and the isolates considered (Abo *et al.*, 1998; Luzi-Kihupi *et al.*, 2000). RYMV causes considerable damage in rice production. Early infection can lead to death of the plant in varieties which are highly susceptible (N'Guessan *et al.*, 2001).

The diversity among RYMV isolates was reported immunologically with polyclonal and monoclonal antibodies (MAbs) (Fauquet and Thouvemel, 1977; Mansour and Baillis 1994; Konate

et al., 1997; N'Guessan, *et al.*, 2000; Alkali *et al.*, 2012). Both serological and molecular analyses showed large virus diversity and geographical distribution of the strains, with a split between isolates from East and West Africa, and with different strains in the savanna and forested regions of West Africa (Traore *et al.*, 2001). Both virulent and avirulent isolates were found among the strains, indicating that phytopathological traits of an isolate are not hereditary or confined to a specific strain or a particular region, but occurs naturally in different lineages in different parts of Africa (Sorha *et al.*, 2005). Due to the coordinated efforts of African Scientists and their collaborators, representative isolates from most of the countries where RYMV causes economic losses have now been characterized (Abubakar *et al.*, 2003). However, the significance of different isolates of RYMV in northern Nigeria has not extensively been studied. Thus, more study is needed for distinguishing and identifying serological profiles of RYMV, because of existence of different RYMV strains in the field. The study was carried out to determine the status and examine the variability of RYMV isolates found in the rice field in northern Nigeria

2. MATERIALS AND METHODS

2.1. Field Survey and Sampling

Surveys were carried out in rice fields during the wet and dry seasons in the year 2008 to 2010. Seven states (Borno, Gombe, Kaduna, Kano, Niger, Sokoto and Zamfara) were surveyed. Hotspots and epidemic areas within those states were surveyed. The surveys were carried out at the tillering and panicle initiation stages (Abo *et al.*, 2002). In each field surveyed, three quadrants, 4 × 4 m² in size were randomly chosen (Alegbejo and Kashina, 2000). Infected rice leaves with and without RYMV-like symptoms were randomly sampled on the edges of the field, within the rice field and in the vicinity of the RYMV-infected rice plants using the visual diagnostic symptoms of RYMV (IRRI, 1996). Samples were collected using separate polyethylene bags, labeled, kept in ice box and transported to the Virology laboratory, Department Crop Protection, ABU, Zaria.

2.2. RYMV Propagation

The virus was recovered from the infected samples by back inoculation to the highly susceptible rice cultivars Bouake 189 (Onasanya *et al.*, 2004). For inoculation tests, leaves from each specimen plant were pooled and then ground with a sterilized pestle and mortar, 1g of the infected leaves in 10 ml of 0.1M phosphate buffer (pH 7.4) (Luzi-Kihupi *et al.*, 2000; (Sarra *et al.*, 2004)), containing carborundum powder (600 mesh) to act as an abrasive to facilitate virus entry into the plant. The extracts were used in inoculating 5 seedlings of Bouake 189 cultivar, planted in plastic pot filled with, sterilized soil to each isolate by mechanical (sap) inoculation. In order to avoid possible escapes from infection, all plants were re-inoculated twice at two day intervals as described by Thottappilly and Russell (1993). Uninoculated seedlings were included to serve as reference check. The test plants were kept for 3 weeks in the screenhouse and monitored for symptom(s) expression. Leaves of the test plants showing symptoms of RYMV were harvested and later used as sources of inocula during the serotyping and molecular characterization.

2.3. Determination of Serological Profiles of RYMV

Leaves of the test plant showing symptoms of RYMV inoculated from rice leaf samples were harvested and used for serological profile determination. The Triple Antibody Sandwich Enzyme Linked Immunosorbent Assay (TAS-ELISA) as outlined by Clark and Adams (1977) was used in the determination of serotypes of RYMV at the Virology laboratory, Department of Crop Protection, ABU, Zaria, Nigeria. Four monoclonal antibodies (AS-0478/11, AS-0478/12, AS-0478/21 and AS-0478/22) used were obtained from plant virus collection centre, DSMZ (German collections of Microorganism and cell cultures) Braunschweig, Germany. Polyclonal antibody was also used as non-discriminative with the same affinity to each RYMV isolate to detect virus independently of the serological properties of the isolates as a reference control. DAS-ELISA method as described by Clark and Adams (1977) was used. Colour change was measured with ELISA microreader (GF. M3000 Microplate reader, B.Bran Scientific & Instrument Company,

England). Negative- positive threshold were set at two times the mean of healthy control sample's absorbance values (A405 nm). Reaction was considered strong positive (+++), mild (++) or weak (+) when absorbance value ≥ 4 times, $3 \leq 4$, $3 \leq 2.5$ or $2 \leq 2.5$ of healthy control samples absorbance values, respectively. Hierarchical cluster dendrogram analysis was also generated from the data obtained with the four monoclonal antibodies reactivity from the TAS ELISA results. Using average linkage between isolates by rescaled distance cluster combine analysis (Graph pad instat statistical analysis package software).

3. RESULTS

3.1. Serological Profile of RYMV Isolates

Several variants of serological profiles were distinguished by their unusual reactions with four discriminating monoclonal antibodies (MAbs) panel (AS 0478/11, AS 0478/12, AS0478/21 and AS 0478/22) raised against Nigerian isolates of RYMV. Twenty isolates from Kano, Kaduna and Gombe states showed strong positive reaction with all the MAbs and sixty five isolates from Gombe, Kano, Kaduna, Niger, Sokoto and Zamfara states showed mild to weak positive reactions with all the MAbs (Serotype A). Nine isolates from Borno and Gombe states showed mild reaction with MAbs 4 and weak reaction with MAbs 2 and MAbs 3. Similarly eight isolates from Kaduna and Kano states showed weak reaction with MAbs 2, MAbs 3 and MAbs 4 (serotype B). Five isolates from Gombe state showed mild positive reaction with MAbs 1, MAbs 2 and MAbs 3 (serotype C). Three isolates from Borno state showed weak to mild positive reactions with MAbs 3 and MAbs 4 while ten isolates from Sokoto and Zamfara states showed weak reactions with MAbs 3 and MAbs 4. (Serotype D). Four isolates from Borno and Sokoto states showed weak and mild positive reactions with MAbs 3, respectively (serotype E). Twelve isolates from Kano, Niger and Sokoto states showed weak positive reactions with MAbs 4 (serotype F).

3.2. Hierarchical Cluster Dendrogram Analysis of RYMV Serological Profile

There were considerable diverse serological relationships obtained among 35 representatives RYMV isolates serological profiles from seven states in northern Nigeria (Figure 1). The rescaled distance cluster combine, using average linkage between groups by hierarchical cluster dendrogram analysis, give rise to 0.25 to 1.00 coefficient similarity ranges. At 0.25 coefficient similarity levels, all the isolates were separated into three major serogroups (SG1, SG2 and SG3). While at 0.75 coefficient similarity levels SG1, SG2 and SG3 were further separated into two subgroups (Sg1a and Sg1b), (Sg2a and Sg2b) and (Sg3a and Sg3b), respectively. Similarly, based on the rescaled distances among the isolates linkage analysed at 1.00 coefficient similarity levels, Some isolates were serologically identical at subgroups Sg1a (14), Sg1b (5), Sg2a (5), Sg2b (6), Sg3a (5) and Sg3b (5) respectively (Figure 1).

Table 1: Serological profile of RYMV isolates detected with monoclonal antibodies in Triple antibody sandwich enzyme linked immunosorbent assay (TAS ELISA)

S/no	State	Site of collection	Number of isolates	Monoclonal antibodies			
				M1	M2	M3	M4
1	Borno	Konduga 1	4	-	+	+	++
2		Konduga 2	4	-	-	+	-
3		Madurari	3	-	-	+	+
4		Malari	2	-	-	+	++
5	Gombe	Dadinkowa1	5	+++	+++	+++	+++
6		Dadinkowa 2	5	++	++	++	-
7		Hinna 1	5	++	+++	+++	+
8		Hinna 2	4	-	++	++	+
9		Hinna 3	4	+++	+++	+++	+

10	Kaduna	Bomo	5	-	+	+	++
11		Kurmi Bomo	3	+++	+++	+++	+++
12		Sayen Gobirawa	5	+	++	++	++
13		Nasarawan Buhari	4	+	+	++	+
14		Shika	2	++	++	++	++
15		Giwa	2	-	+	+	+
16		Anguwan Sogiji	4	+++	+++	+++	+++
17		Zabi	2	+	+	+	+
18		Hayen Sambo	3	+	+	++	+
19		Wusasa	2	+	+	++	+
20		Zango Shanu	2	-	+	+	+
21	Kano	Kura	5	+++	+++	+++	+++
22		Chiromawa	4	++	++	+++	+++
23		Kadawa	5	+	+	++	+++
24		Dakastalle	4	-	-	-	+
25		Kwanan Yari	4	-	+	+	+
26		Rano	3	+++	+++	+++	+++
27	Niger	Wushishi	4	-	-	-	+
28		Badeggi	5	+	+	++	+++
29	Sokoto	Goronyo 1	5	++	++	+++	+++
30		Goronyo 2	4	-	-	+	+
31		Sokoto	4	-	-	-	+
32	Zamfara	Talata Mafara 1	5	-	-	+	+
33		Talata Mafara 2	4	-	-	++	-
34		Rini	5	+	++	++	+++
35		Birnin Tudu	4	-	-	+	+

Strong positive reaction = +++

Positive reaction = ++

Weak positive reaction = +

Negative reaction = - -

M1= Monoclonal antibody AS-0478/11

M2 = Monoclonal antibody AS-0478/12

M3= Monoclonal antibody AS-0478/21

M4= Monoclonal antibody AS-0478/22

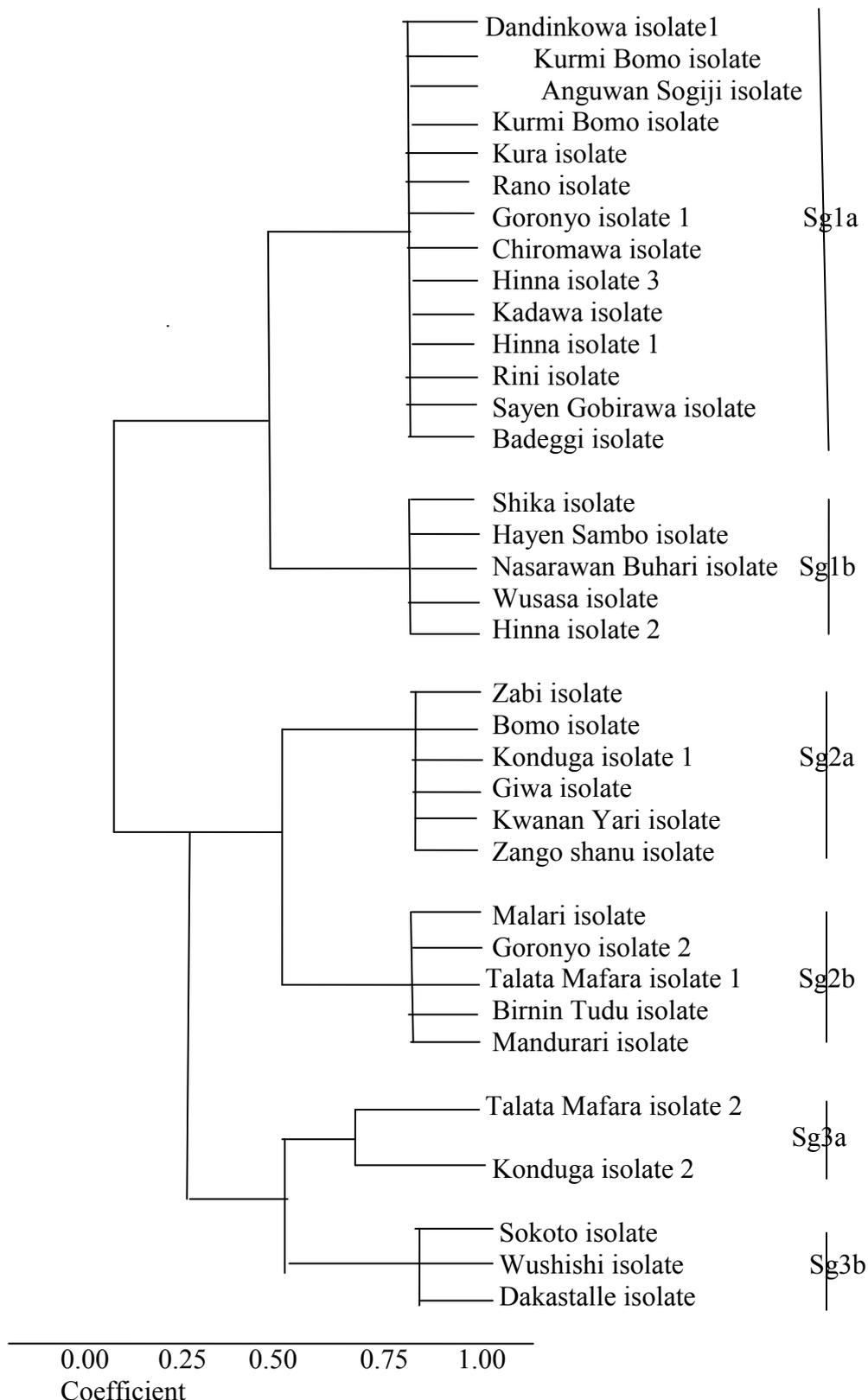


Fig. 1 Hierarchical relationship dendrogram of RYMV isolates of seven states in northern Nigeria derived from 35 serological profiles data using four monoclonal antibodies in TAS-ELISA

4. DISCUSSION

The occurrence of immunological diversity of the RYMV isolates assessed with panel of monoclonal antibodies in the surveyed areas, suggested that several serotypes co-exist in the surveyed ecological zones of northern guinea savanna (Kaduna state), southern guinea savanna (Niger state) and sudan savanna (Borno, Gombe, Kano, Sokoto and Zamfara states) in northern

Nigeria. This result is consistent and complementary to the findings of N'Guessan *et al.* (2000) and Saluadeen *et al.* (2008) who reported that several serotypes of RYMV can co-exist in the same country. In addition to the serological relationship found in the surveyed areas, differential reactivity was observed among isolates collected from a few metres apart in the same area, but similar to an isolate from a different area. This explains the fact that within a set of isolates of related strains in the same host plant, many possibilities of interaction exist (Mathews, 1991). It also indicates the existence and levels of serodiversity among RYMV isolates in the surveyed areas. This is consistent with the earlier reports by Fargette *et al.* (1995) and N'Guessan *et al.* (1995) that isolates from the same areas could be quite different among each other and close to an isolate from a remote country. Sere *et al.* (2007) also reported that many isolates of RYMV emanating from same locality, field and host were observed to be serologically different. The serological similarities observed between isolates within the same and different localities in the surveyed areas, confirm the great cross-infection potential of RYMV transmitted under natural conditions by different insect vectors (Bakker, 1971; Hammond *et al.*, 1999). This possibly accounted for the different serological profiles obtained among different RYMV isolates collected in the surveyed areas. RYMV has been described as a variable virus with many pathological and serological variants (Konate *et al.*, 1997; Sasaya *et al.*, 1997; N'Guessan *et al.*, 2000 and Sere *et al.*, 2007; Onasanya *et al.*, 2011). This result are in agreement with the observed prevalence of several variants of the serological profiles recorded in this study, distinguished by their unusual reactions with the same MAbs tested in some isolates collected across the surveyed areas. This information implies that weak and strong reaction recorded in this study by some of the isolates tested with the panel of MAbs, can probably be ascribed to weak reaction with the virus isolates rather than low concentration of the virus in the infected tissue, because a conspicuous symptom was observed after propagating the virus in susceptible rice cultivar BOUAKE 189 (to most of the isolates used.). Similar results were reported by Mansour and Baillis (1994) who used RYMV isolates collected from rice in Cote d'Ivoire, Kenya, Niger, Nigeria and Sierra Leone.

Based on the reaction pattern to the panel of MAbs in TAS-ELISA six serological profiles or groups have been identified (Sep A to F) in this study. The Sep A isolates were present in Gombe, Kaduna, Kano, Niger, Sokoto and Zamfara states. Sep B isolates were common in Borno, Gombe, Kaduna and Kano states. Sep C isolate was identified only in Gombe state. Sep D isolate were detected in Borno, Sokoto and Zamfara states, while Sep E isolates were identified in Borno and Zamfara states. Sep F isolates were identified in Kano, Niger and Sokoto states. However, serological typing sometimes gives ambiguous result and could be less discriminating than molecular typing (Kanyeka *et al.*, 2007). This may probably explain the fact that some of the isolates grouped under the same serological profiles had no reaction with the same specific primer, but had similar reaction with other isolates grouped under different serological profiles in molecular typing in this study. It could also explain the fact that within a set of isolates of related strains in the same host plant, many possibilities of interaction exist (Mathews, 1991). Hierarchical classification of all the RYMV isolates into three main serogroups (SG1, SG2 and SG3) and six subgroups (Sg1a, Sg1b, Sg2a, Sg2b, Sg3a and Sg3b) in this study indicates the existence and levels of serodiversity among RYMV isolates in northern Nigeria. 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 (Boccard and Baulcombe, 1993). Similarly Sere *et al.* (2005) suggested that as a result of possible interaction between different strains of the same isolate in the same host plant, which varies from one ecological zone to another, diverse serological variability tends to exist between isolates of RYMV in West Africa. High serological profile or serotype diversity of RYMV found in this study may be associated with occurrence of resistance breaking isolates (Traore *et al.*, 2006), which urgently calls for deliberate effort to study the potential problem for future development of resistant cultivar to RYMV in the surveyed areas. Similarly hierarchical classification of RYMV isolates into three main serogroup and six subgroup obtained from cross reactivity of four monoclonal antibodies derived from serological profiles data in TAS-ELISA, could be useful for epidemiological studies to assess isolate identity and interaction

in the fields. The high viral load in some of the surveyed sites in Gombe, Kano, Kaduna, Niger and Zamfara states, implies that these are suitable locations for strategic studies aimed at designing management interventions against RYMV disease.

5. CONCLUSION

A panel of four monoclonal antibodies (MAbs) was used to study the immunological profile of *Rice yellow mottle virus* (RYMV) genus Sobemovirus. Serological profiles of 35 representative isolates of RYMV from Borno, Gombe, Kaduna, Kano, Niger, Sokoto and Zamfara states in Northern Nigeria. All the RYMV isolates were classified into three major serogroups (SG1, SG2 and SG3) and further separated into six subgroups (Sg1a, Sg1b, Sg2a, Sg2b, Sg3a and Sg3b). The results demonstrate a significant serological variability among RYMV isolates in Northern Nigeria.

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