International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 8, Issue 6 -2021

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.06.025

Serological, molecular and phylogenetic analysis of *Rice Yellow Mottle Virus* (RYMV) isolates collected in Southern-Benin

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Abstract

The rice yellow mottle is the best known viral disease of rice in Africa. It causes significant economic losses in farmer's fields. The serological properties of Rice Yellow Mottle Virus (RYMV) isolates, collected in Southern Benin, were assessed by immunological tests with polyclonal and monoclonal antibodies (Pabs and MAbs). The isolates CP (Capsid protein) portions, obtained by RT-PCR, were sequenced and their amino-acids sequences were analyzed and used for phylogenetic analysis. Three different RYMV pathogenic groups, including three resistance breaking (RB) isolates (Be20, Be21 and Be27) which over came allele *rymv1-5*gene, were identified. Two serotypes Ser1/strain S1 and Ser2/ strain S2 were also distinguished. The molecular properties of the isolates CP gene and the phylogenetic characteristics indicated that the Southern Benin RYMV strain is heterogeneous. The strain S1-Benin linked to the West Central African lineage(S1-WCA) is related to strain from Togo and Niger, whereas, the strain S2 Benin is the stumps of the West African lineage (S1-WA) and is related to Mali, Burkina Faso and Ivory Coast RYMV strains. These results reported the virus pathogenicity level and showed the relationships between RYMV strains in all Dahomey gap countries and in West and Central Africa.

Keywords: serotypes, RYMV strains, RB isolates and phylogenetic analysis

Introduction

Rice is the most important cereal crops in developing country and is the primary food grain consumed by almost half of the world's population (Khush, 2005). In Benin, it is becoming more and more important in both urban and rural communities. The annual rice production is not sufficient to ensure the consumption which is 80,000 tons (Danvide and Assigbe, 2003). Therefore, the country is face at the need to import 50,000 tons of rice each year, in order to fill the gap. As in many other countries in Africa, efforts are developed in Benin to increase the local production because the recent crisis in rice availability and price indicated clearly that the African Sub-Saharan (SSA) countries cannot rely upon importation to fill the gap between their need and their current production (Seck *et al.*, 2012). However, both the development of new area and the intensification of rice cultivation are susceptible to bring new problems among which the diseases are important (Sere *et al.*, 2012). One of the most important diseases is due to *Rice yellow mottle virus* (RYMV), genus *Sobemovirus*.

Specific to African continent, not detected outside this continent and first described in Kenya in 1966 by Bakker, (1970), RYMV is now present in all the rice-growing areas in Africa (Kouassi *et al.*, 2005; Ndikumana *et al.*, 2012;Rakotamala*et al.*, 2019) causing yield losses comprised between 1 - 100% (Oludaré *et al.*,2016).

The virus variability and the rice crop resistance have been very studied in Africa. According to the serological properties and the CP gene, six (6) strains, with different geographical distribution and pathogenic characteristics were described. The strains S1, S2 and S3 are present in West and Central Africa and S4, S5 and S6 localized in East Africa (N'guessan *et al*, 2000 ; Pinel *et al*., 2000; Fargette *et al.* 2002 ; Abubakar *et al*, 2003 ; Traoré *et al*., 2005). The strain S1 is subdivided in clads S1-WA from West Africa and S1-CAfrom Central Africa (Traoréet al., 2005; Longuéet al., 2017). Recently a new monophyletic group, named strain Sg of RYMV, was reported in Senegal (Tall et al., 2020). This strain regrouped the isolates from the south of Senegal and Gambia, in West Africa (WA).

Three major resistance genes RYMV1, RYMV2 and RYMV3 were identified in the two rice species cultivated in Africa, *Oryza sativa* and *Oryza glaberrima* (Albar *et al.*, 2003; Thiémélé *et al*., 2010; Pidon *et al*., 2017). The resistance of the gene RYMV1 was controlled by recessives genes rymv1-2 in O. sativa cv. Gigante (Ndjiondjop *et al*., 1999), rymv1-3, rymv1-4 (Albar *et al*., 2006) and rymv1-5 (Thiémélé *et al*., 2010) in O. glaberrima accessions Tog 5681, Tog 5672 and Tog 5674, respectively. The Gene RYMV2 identified as a CPR5-1(Constitutive expressor of pathogenesis Related genes-5) is observed in varieties Tog7291 and Tog5672 (Thiéméléet al., 2010; Pinel-Galziet al., 2016). The resistance linked to this gene is recessive and conferred by a null allele observed in seven O. glaberrima accessions. RYMV3 is a dominant gene, identified and mapped in Tog5307 variety (Pideon *et al*., 2017). This gene also carries resistance of Tog5672 variety.

RYMV was first recorded in Benin in 1999 (Danvi and Assigbé, 2003). Studies on pathological and serological diversity of RYMV and on the rice varieties resistance were conducted on isolates most collected in the Northern and Central part of the country (Bancole, 2006; Hadonou, 2006 ; Oludaré *et al.*, 2016). Therefore, less or none molecular and phylogenetic analysis were conducted with large set of RYMV isolates from southern-Benin.

Objectives: The present study focused to study the serological, molecular and phylogenetic characteristics of RYMV isolates collected in this geographical part (Southern Benin), in order to report the virus pathogenicity and diversity levels, and show the relationships between RYMV strains in all Dahomey gap countries and in West and Central Africa.

Materials and Methods

Samples collection

Five (5) locations in Southern-Benin: Lokossa, Kpinnou, Songhai, Dangbo and Calavi were covered by field survey and samples collection. Leaf samples were collected based on the typical symptoms attributed to rice yellow mottle, then stored in a cooler with ice until their delivery to the laboratory. The samples were then stored in freezer at -20 ° C.

Isolates propagation: summary biological characterization

Extracts of leaf samples collected in the field were propagated on the susceptible variety IR64 and four resistant varieties Gigante, Tog5681, Tog 5672 and

Tog 5674, bearing the resistant genes *rymv1.2*, *rymv1.3*, *rymv1.4*and*rymv1-5*respectively, using the method of mechanical transmission according to N'guessan*et al.*, (2001). Twenty-eight days after inoculation, leaves from each RYMV isolates bearing typical yellow mottle symptoms were denumbred, harvested and used for RYMV sero-diversity and phylogenetic studies.

RYMV isolates serological characterization

The Antigen Coating Polyclonal Elisa Test (ACP-ELISA) was first conducted to ascertain the presence of the virus in the samples collected. Samples were tested with a pool of polyclonal antibodies (Pabs) prepared from ten (10) distinguished Pabs (Table 1) developed by Africa Rice Center (Afolabi *et al.*, 2009); this pool having a broad detection spectrum.

Table 1: Polyclonal antibodies (Pab) used

PAb Code	Origin of RYMV isolates used to develop the Pabs	
	Site	Country
M-1	Niono4	Mali
M-2	Niono8	Mali
M-3	M'Peniesso	Mali
BF-1	Banzon	Burkina-Faso
BF-2	Kafirguela	Burkina-Faso
IITA	IITA	Nigeria
Ng-1	Saga	Niger
Ng-2	Kollo	Niger
CI-1	M'Bé	Côte d'ivoire
CI-2	Danané	Côte d'ivoire

The serological properties of twenty one (21) RYMV samples collected in Southern Benin were analyzed, by ACP ELISA according to the technique described by Séré et *al.*,(2007).Thereafter, Triple Antibodies Sandwich Elisa (TAS-Elisa) was developed as described by Fargette *et al.*, (2002) with eleven (11) isolates from different localities in Southern-Benin, using four (4) monoclonal antibodies (Mab A, Mab D, Mab G and Mab M) obtained from IRD (Institute for the research Development) in France.

RYMV isolates molecular characterization

The RNA extraction was performed with the RNeasy plant mini-kit Qiagen kit, according tothe method of Pinel*et al.*, (2000). The virus isolates, in sheets of rice were first soaked in liquid nitrogen and then lysed using a ball mill (RestschTissuelyser II Qiagen) and homogenized in the presence of a strongly denaturing buffer containing guanidine isothiocyanate (GITC). This buffer inactivates RNases and ensures isolation of the RNA intact after rupture and lysis of cell membranes. Total RNA was selectively attached to the membrane of gel in a mini RNeasy column.

The total RNA was converted into cDNA by using an enzyme M-MLV-RT (Promega) according to the method developed by Pinel*et al.*, (2000).The reaction amplification of the capsid protein is produced with the Dynazyme polymerase (Ozyme) of cDNA in a PTC100 thermocycler (MJ Research, Inc..,), from the product of RT and a reaction mixture. The quantity and quality of DNA were estimated by electrophoresis on agarose gel containing ethidium bromide in a vat of migration I-Mupid (Cosmo Bio. Ltd.). A photo documentation of the gel is carried out using the software Gel Doc 2000 (Biorad).

The capsid proteins gene (CP) of four isolates typed Ser1 (Be36) and Ser2 (Be27, Be23, Be20) were sequenced, after extraction of total viral RNA, and amplification by RT-PCR**.** In the case of this study, the sequencing of the capsid protein gene of RYMV isolates was performed by the company MilleGen, according a DNA sequencing technique which is an adaptation of the method developed by Sanger *et al.*, (1977), replacing radioactive DNA marking with fluorescent marking.

RYMV isolates phylogenetic analysis

The CP sequences were analyzed, including twenty two (22) sequences reported in previous studies. Indeed, the CP gene antigen sites were useful to identify the country RYMV strain, it's genetic diversity and the relationship between it's isolates and those from other countries, as reported by Nguessan *et al.*, (2000) and Hebrard *et al.*, (2008).

The genetic diversity was assessed by distance parameters described by Tamura *et al.*, (2007) and phylogenetic trees were constructed using the MEGA4 software. It's a distance method with maximum likelihood estimates of transition/transversion ratio and of the alpha parameter of the gamma shape.

Results

RYMV detection in samples collected

During the prospection fifty-nine (59) samples were collected into five (5) localities of Southern Benin (Table 2). These samples were originating from cultivated rice (44 samples) and adventices (15 samples). Twenty-two (22) samples were positive (Table 3), with an optical density (DO) doubly greater than the DO of the negative control; indicating the presence of RYMV in the tested samples (Table 3).

Table 2: Inventory of RYMV samples collected

Locality		Number of samples		Ecology
Name	GPS position	Rice	Adventices	
Calavi	N6°27'01.0872'' E2°20'48.552''	8	6	Lowland
Dangbo	N6°35'01.3884'' E2°33'07.0704''	4	5	Lowland
Lokossa	N6°38'41.3124'' 1°43'11.3556''	15	4	Lowland
Lokossa / Kpinnou	N6°38'41.3124'' 1°43'11.3556''	7	0	Lowland
Lokossa / Songhai	N6°38'41.3124'' 1°43'11.3556''	10	0	Lowland
Total		**44**	**15**	

Table 3: Inventory of RYMV isolates obtained by ELISA

Locality	Number of samples collected	Number of RYMV isolates
Calavi	14	6
Dangbo	9	6
Lokossa	19	8
Lokossa / Songhai	7	1
Lokossa / Kpinnou	10	1
Total	**44**	22

Identified isolates pathogenicity

The tested varieties mechanical inoculations permitted to obtain symptoms (Figure 1) of rice yellow mottle for all positive ELISA samples (Table 4); ranging virus isolates in: (i) wild type isolates, caused symptoms only on sensitive variety, (ii) resistance breaking isolates (RB), virulent on resistant varieties and (iii) isolates ELISA negative which product typical symptoms on sensitive variety. The second class of isolates (ii) included three isolates (Be20, Be21 et Be27) which were able to induce symptoms on Tog 5674 carrying the resistant gene *rymv 1.5*.The remaining isolates were not pathogenic on the four (4) resistant accessions.

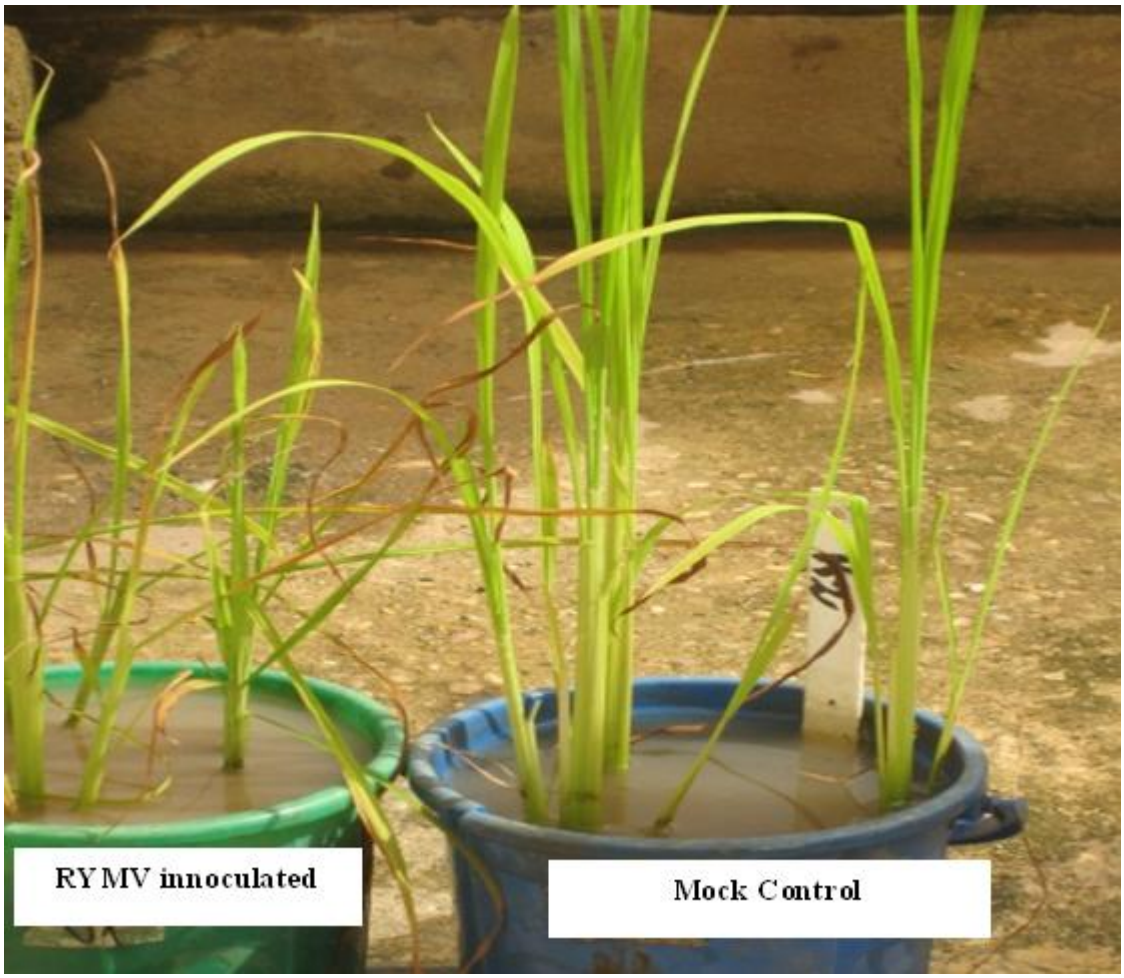

Figure 1.RYMV typical symptoms on infected IR64 variety (at left)

Table 4: RYMV isolates pathogenicity

Isolates code	Origin		Isolates pathogenicity				
	Host	Locality	IR64	Gigante	Tog5681	Tog5672	Tog5674
Be36	Nerica42	Calavi	+	-	-	-	-
Be37	Nerica42	Calavi	+	-	-	-	-
Be20	Nerica1	Lokossa	+	-	-	-	+
Be21	Weeds	Lokossa	+	-	-	-	+
Be22	11-B	Calavi	+	-	-	-	-
Be23	FKR19	Calavi	+	-	-	-	-
Be24	75-1-127	Calavi	+	-	-	-	-
Be25	Rice	Dangbo	+	-	-	-	-
Be26	Weeds	Dangbo	+	-	-	-	-
Be27	Rice	Kinnou	+	-	-	-	+
Be28	Rice	Songhai	+	-	-	-	-

+ Sensible *- Resistant*

RYMV serological profiles

The RYMV isolates analysis with Pabs revealed the presence of three serogroups Sgb1, Sgb2 and Sgb3 in Southern Benin (Fig. 2 and Table 5) with overlapping geographical distribution. Immunoassay with monoclonal antibodies distinguished two serotypes Ser1 and Ser2 (Table 6);Indeed, all the isolates tested reacted with the Mabs G, E, M whereas some isolates reacted with the Mabs A and others with the Mabs D (Table 6 and 7). However, no Serotype Ser3 isolate was identified. The Ser3 / S3 strain reacts with all Mabs, the S2 responds to Mabs G, E, M and D without reacting with the Mab A, and the S1 strain responds to Mabs A, G, E, M without reacting with the Mabs D. These reaction profiles showed that the isolates tested belong to two different serotypes Ser1 / strain S1and Ser2 /strain S2.

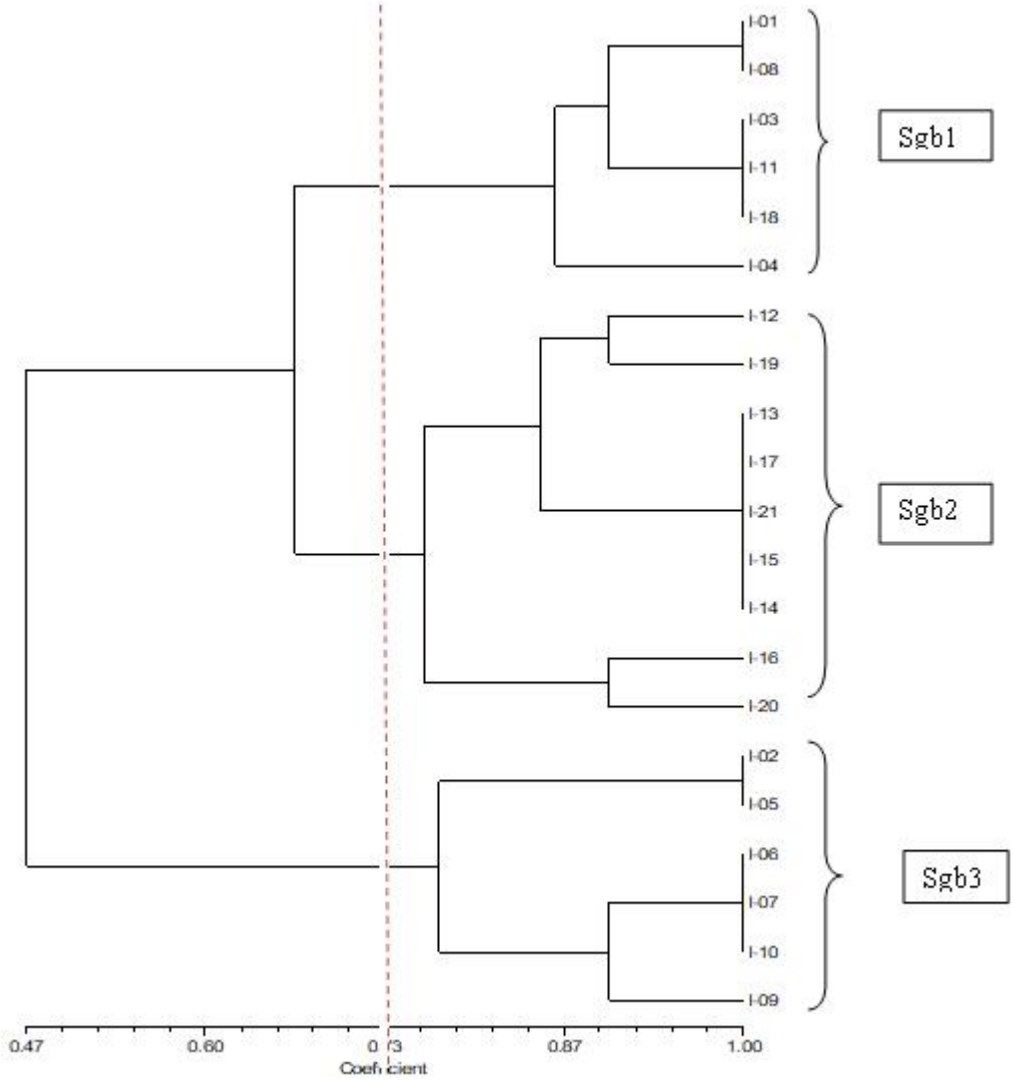

Figure 2: Dendrogram showing the RYMV serological diversity of Southern-Benin isolates

Table 5: Tested isolates serogroups

Codes	Isolates origin		Serogroups
	Geographical	Host	
I01 = Be20	Lokossa	Nerica1	
I08 = Be27	Lokossa	Rice	
I03 = Be21	Lokossa	Weeds	
I11 = Be23	Calavi	FKR19	Sgb1
I18 =Be37	Calavi	Nerica42	
I04 = Be33	Lokossa	Weeds	
I12 = Be24	Calavi	75-1-127	
I19 = Be42	Dangbo	Weeds	
I13 = Be43	Dangbo	Weeds	
I17 = Be36	Calavi	Nerica42	
I21 = Be44	Dangbo	Mauvaises herbes	Sgb2
I15 = Be32	Calavi	Nerica-L-16 Riz	
I14 = Be33	Lokossa	Weeds	
I16 = Be48	Lokossa	Weeds	
I20 = Be42	Dangbo	Weeds	
I02 = Be39	Lokossa	Nerica1-11-B	
I05 = Be22	Calavi	Rice	
I06 = Be25	Dangbo	Rice	Sgb3
I07 = Be28	Lokossa	Weeds	
I10 = Be26	Dangbo	Rice	

The rice ecology of Southern Benin is therefore characterized by the presence of two RYMV serotypes, including three RB isolates (Be20, Be21 and Be27) all related to S2 serotype and strain (Table 6).The Ser2 serotype /strain S2 was wide spray in Soutern Benin; it covered four locations on five. However, the study revealed that there was no evidence of Ser1 and Ser2 mixtures, even though the collection is made on nearby sites. Ser1 serotypes are characterized by profiles 4 4 0 4 and 4 2 0 4 and Ser2 serotypes by profile 4 0 4 4 (Tables 6 and 7).

Table 6 : List of isolates tested by TAS Elisa and their respective serotypes

Code	Host	Locality	Ecology	Serotypes
Be_{36}	Nerica42	Calavi	Upland	S1
Be_{37}	Nerica42	Calavi	Upland	S1
Be_{20}	Nerica1	Lokossa	Irrigated	S2
Be_{21}	Weeds	Lokossa	Irrigated	S2
Be_{22}	11-B	Calavi	Lowland	S1
Be_{23}	FKR19	Calavi	Lowland	S2
Be_{24}	75-1-127	Calavi	Lowland	S1
Be_{25}	Rice	Dangbo	Lowland	S1
Be_{26}	Weeds	Dangbo	Lowland	S2
Be_{27}	Rice	Lokossa (Kpinnou)	Lowland	S2
Be_{28}	Rice	Lokossa (Songhai)	Lowland	S1

Table 7: Reaction profiles of isolates tested showing the strains identified

Isolates	Reaction profiles				Strains
	M	A	D	G	
Be36	4	4	0	4	S1
Be37	4	4	0	4	S1
Be20	4	0	4	4	**S2**
Be21	4	0	4	4	**S2**
Be22	4	4	0	4	S1
Be23	4	0	4	4	**S2**
Be24	4	4	0	4	S1
Be25	4	2	0	4	S1
Be26	4	0	4	4	**S2**
Be27	4	0	4	4	**S2**
Be28	4	4	0	4	S1

RYMV Phylogeny

The phylogenetic tree of RYMV-Benin established (Figure 3) from the CP gene sequences indicates that the Be36 / serotype1 isolate belongs to the S1 strain of the West African Central Lineage (S1-WCA), when the isolates Be27, Be23 and Be20 identified as Ser 2 were linked to the RYMV strain S2.So, the Southern-Benin area is colonized by two strains of RYMV: the strain S2, first time confirmed both by molecular and phylogenetic analysis, and the strain S1, lineage S1-West-Central African (S1-WCA).

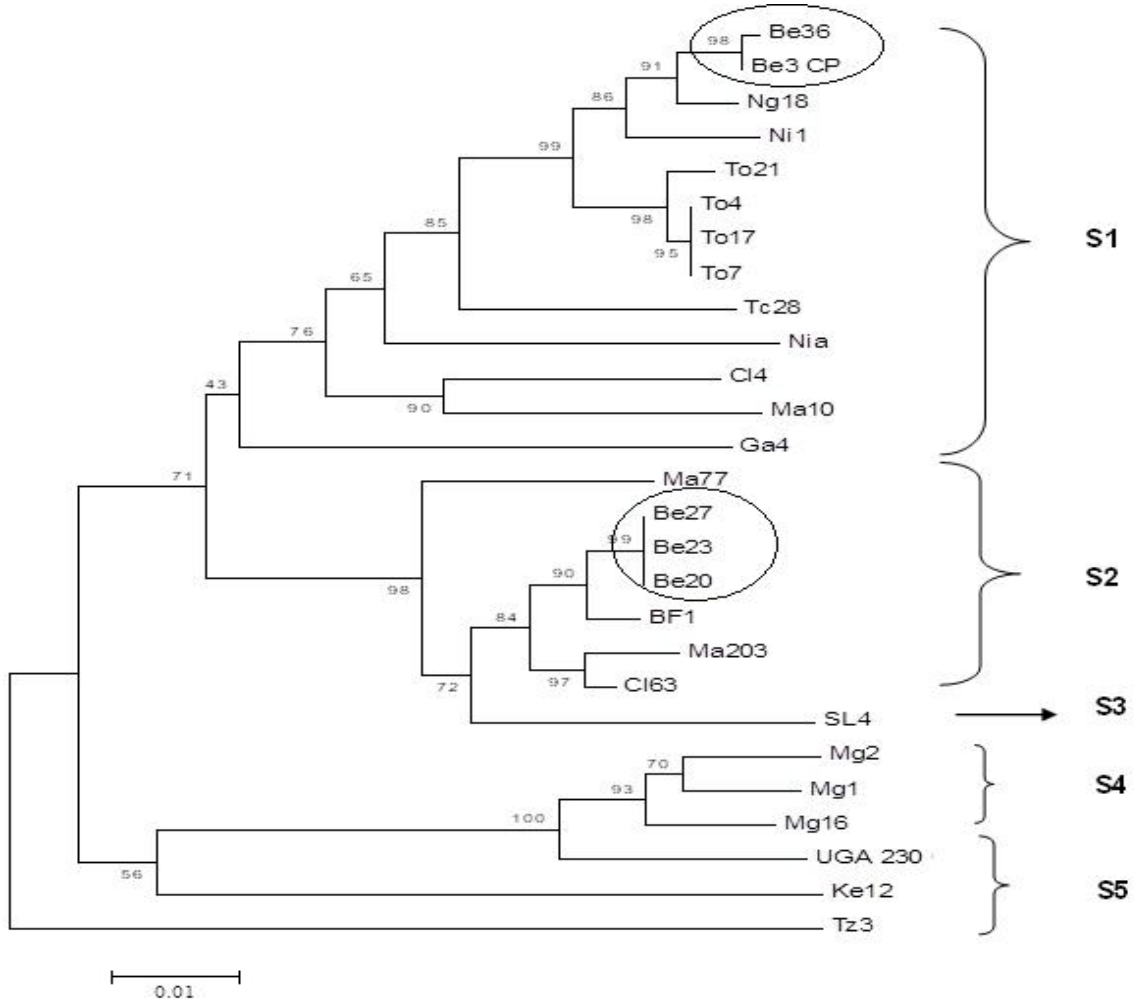

Figure 3: Phylogeny of RYMV in Benin as compared African strains

Discussion

Serological and biological diversity of studied isolates

The RYMV detection in his natural hosts is not always easy to achieve. Indeed, the virus infects some hosts without inducing visible symptoms, and the only presence of symptoms is sometimes insufficient as a diagnostic tool. For example, certain mineral deficiencies, especially iron deficiencies, cause yellowing symptoms that can be confused with the symptoms induced by RYMV. The virus diagnosis serology was then often used as a method of choice because the RYMV is highly immunogenic and has no known serological relationship with any other virus (Pinel *et al.*, 2000).

In this study, results revealed that the use of data from ELISA is very useful in the identification and classification of RYMV-Benin isolates. Immunoassay with monoclonal antibodies by triple antibody sandwich (TAS) ELISA showed that the RYMV isolates tested belong to two different serotypes Ser1 and Ser2. No serotype Ser3 isolate was identified and there was no evidence of Ser1 and Ser2 mixtures, even though the collection is made on nearby sites. Similarly results were obtained with isolates of Northern Benin (Bancolé, 2006 ; Hadonou, 2006) and all Benin regions (Oludaré *et al.*, 2016) ; indicating that in Benin, the RYMV is widely distributed and very diversified in all rice cultivating areas. Indeed, Oludaré*et al.*, (2016) reported by TAS-ELISA the existence of two main serogroups S1 and S2, with the prevalence of S1. The isolates related to S2 serogroup were from only one locality (Lokossa) in southern Benin and were based only on the serological properties. The present study revealed the presence of S2 serogroup in three locations of southern Benin (Lokossa, Dangbo and Kinpinu); confirming the S2 presence in Lokossa and indicating the RYMV strains circulation as reported in East Africa (Ndikumana *et al.*, 2014) and West-Central Africa (Issaka *et al.*, 2012a). This serogroups distribution was confirmed both by molecular and phylogenetic analysis. The S2 serogroup spatial distribution is widely then reported by Oludaré *et al.*, (2016) with isolates of less locations. These diversity results complete and contribute to well know the RYMV Benin characteristics.

Preliminary serological studies conducted by Bancolé (2006) with the use of Pabs polyclonal antibodies, revealed a high serological diversity in Northern Benin. Previously, Sorho, (2006) identified three serological groups with isolates belong to Serotype1. Furthermore, our results indicate that isolates from the same locality or even range are serologically different, which explains the fact that many interactions exist in a set of related strains in our communities of Southern Benin (Be22 and Be23 isolates case). Similarly, serological similarities have been observed between isolates in different localities (Be24 and Be28); thus, confirming the potential for RYMV infection, transmitted under natural conditions by insect vectors (Bakker, 1971; Hammond *et al.*, 1999). Vector insects play an important role in the spread of yellow mottle virus (RYMV).

Serological typing with polyclonal (Pabs) and monoclonal (Mabs) antibodies performed by N'guessan *et al.*, (2000) on a wide range of isolates (125 in total) revealed the presence of five (5) serological groups of RYMV (Ser1, Ser2, Ser3, Ser4 and Ser5) in the different rice fields of Africa. Subsequently, a sixth serotype, named Ser-sa, was observed by Traoré *et al.*, (2001) in the savannah area of West Africa. The 6 serotypes determined are divided into three (3) distinct geographic poles. Ser1, Ser2, Ser3 and Ser-sa were found in forest and savannah areas of West and Central Africa, while the other two (2) serotypes (Ser4 and Ser5) are found in East Africa and Madagascar (N'guessan *et al.*, 2000; Fargette *et al.*, 2002). This work also showed the existence of two major serogroups in Côte d'Ivoire: Ser1, in the north of the country (savannah strain) and Ser2, in the central and west part of the country (forest strain). Thereafter, Séré *et al.*, (2005, 2007) on the indices of serological differentiation and phylogenetic analyses of 178 RYMV isolates against 26 polyclonal antibodies produced by Africa Rice's laboratory (Afolabi *et al*., 2009) showed that in Côte d'Ivoire there are 3 serogroups and 6 subgroups; which indicates the existence of several RYMV serotypes in Côte d'Ivoire.

The pathogenic results reported the existence of three types of RYMV isolates in the studied area, confirming the ELISA tests results. It also detected isolates that overcame the resistant variety Tog5674 carrying allele *rymv1-5* gene. This isolates group includes three RB isolates (Be20, Be21 and Be27),

all related to S2 serotype and strain. However, Oludaré *et al.,* (2016) reported that none isolate from all Benin was able to overcome the resistance conferred by the gene /allele *rym1-5*. This allele gene resistance breaking by only the Ser2 isolates would indicate a link with the capsid protein (PC) properties.

Some isolates which ELISA was negative showed RYMV typical symptoms. This phenomenon, which could be explained by a low viral load in the samples, was also observed by Issaka *et al.,* (2012b) on samples from Niger Republic. The lack of detection of the virus in the other samples suggests that the symptoms observed are physiological in origin or caused by other pathogens infecting rice. None isolates overcame the resistances of Gigante, Tog5672 and Tog5681, thus suggests that the alleles *rymv1-2*, *rymv1-3* and *rymv1-4* were very efficient and useful to be deployed in Southern Benin, in order to control the RYMV disease. In some cases, the isolates pathogenesis resulting from inoculation is high, i.e., they are very virulent. In other cases, the isolates have weak pathogenic properties, i.e., they are less virulent (Fargette*et al.* 2002). According to the work carried out by Bancolé (2006) on the pathogenesis of the RYMV from Northern Benin, isolates have been able to overcome the high resistance of Gigante, and were very virulent. That is not the case of southern-Benin isolates which may be less aggressive than the Northern ones.It would be interesting to study this difference between isolates from the North and the South Benin, in order to find out if this difference is not related to climatic conditions.

The RYMV isolates pathogenic variability was first reported by Lecomte *et al.,* (1993), who obtained the aggressively of geographically isolates from different rice varieties. Since then, two (2) to three (3) pathogroups of the virus (highly pathogenic, moderately pathogenic and low pathogenic) have been identified in Africa contrasting rice ecologies; indicating some biological diversity of RYMV (Konaté *et al.*, 1997; N'guessan *et al.*, 2001; Onasanya *et al.* 2006; Issaka *et al.,* 2012b).Results on the molecular properties of the isolates capsid protein confirmed the serological results obtained by Fargette *et al.*, (2002) with several isolates.

RYMV phylogeny in Southern Benin

The molecular properties of the capsid proteins gene of isolates Be36 / Ser1 and Be27, Be23, Be20 (all Ser2) confirmed the serological results. The isolates phylogenetic tree indicated two main strains in Southern Benin, S1 and S2. The Benin S1 strain belongs to the West and Central African lineage (S1-WCA) and it's corresponding isolate Be36 is closely related to isolates from Niger and Togo. The isolates Be27, Be23 and Be20 belonged to the West African lineage S2 strain are related (degree of kinship 99%) to isolates from Burkina Faso, Mali and Ivory Coast. These results are in contradiction with Sorho (2006) results which concluded thatSer1 serotype is found mainly in savannah and Sahel areas in northern Ivory Coast, Togo, Benin and Niger. When, Ser2 had not been found among Dahomey gap isolates (Benin, Niger, Togo). Therefore, the larger spraid of the strain S2 identified in this study is confirmed for the first time in Benin, towards molecular and phylogenetic characteristics.

Serological and molecular properties show that the RYMV-Benin strain is heterogeneous. The presence of the S2 strain in the agro-ecological areas of Southern Benin shows that the virus migrated under the combined effects of several ecological factors (insect vectors, weeds, etc.).Advances in sequencing have led to a flood of data that lends itself particularly well to phylogenetic analysis. DNA (or RNA) or protein sequences (thus the species from which they originate) are therefore the basis of molecular phylogeny (Philippes *et al.,* 2002). Molecular phylogeny (or cladistic) analysis allows the relationship between individuals to be reconstructed under a phylogenetic tree (Astier *et al.* 2001).The gene sequences of the capsid protein allow an isolate to be assigned to a specific strain. Serotypes are defined in relation to reactions with monoclonal anti-body. The strains are so through molecular typing, even though molecular typing gives the same results as immunological typing (Fargette *et al.*, 2002).

The genetic variability of RYMV was first suspected by Fauquet and Thouvenel (1977) who had shown that the isolate in Niger is serologically different from that of Nigeria (although the two countries are bordering). Thus, the Ivory Coast isolate was serologically related to that of Kenya (in East Africa). Mansour and Baillis (1994) confirmed the existence of the genetic variability of RYMV and reported, on the basis of the typical reaction profiles of five isolates (from Niger, Nigeria, Ivory Coast, Sierra Leone and Kenya), the existence of three serological groups. Three serological groups (RYMVI, RYMVII and RYMVIII) with a different but partially overlapping geographic distribution were also identified from polyclonal antibodies (Konaté *et al.*, 1997).

Conclusion

Pathogenicity trials with Southern Benin isolates indicated that they may be less aggressive than the Northern ones. It revealed the overcoming of the allele *rymv1-5* gene resistance by the isolates belonged only to the S2 RYMV strain. It's reported for the first time the geographical distribution and the large spray of this strain (S2) in Southern Benin, towards molecular and phylogenetic analyses. Also, the relationship between RYMV isolates from Dahomey gap, on the one hand, and those from Central Africa, on the other hand, has been established. These results could help to prepare deployment of resistance genes / alleles in Benin rice ecologies and particularly in Southern Benin and all Dahomey gap countries. Future molecular studies should focus on a broad sampling of North and South Benin, in order for better understanding of the movement of the virus from East to Central and West Africa.

Conflict of Interest Statement

The authors declare that there no conflict of interest.

Acknowledgments

Africa Rice Center (ARC) and Institute of Research for Development had supported financially the researches. We thank them for contribution. We thank also M. George SOTON (support staff at Africa Rice) and Agnès-PinelGalzi (IRD Montpellier), for their technical and laboratory aids.

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DOI:10.22192/ijarbs.2021.08.06.025

How to cite this article:
Issaka Souley, Bachabi Louisiane, Naroua Illiassou, Basso Adamou, Souley Mariama Abdoulaye M., Sere Yacouba. (2021). Serological, molecular and phylogenetic analysis of *Rice Yellow Mottle Virus* (RYMV) isolates collected in Southern-Benin. Int. J. Adv. Res. Biol. Sci. 8(6): 224-236.
DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.06.025