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Detection and Serological Characterization of Rice Yellow Mottle Virus in Central African Republic

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Abstract

Serological and biological detection of Rice Yellow Mottle Virus (RYMV) in leaf samples belonging from cultivated rice species and wild grasses revealed 201 positive detections. All these isolates occurred exclusively on cultivated rice species (*Oryza sativa* L. and *O. glaberrima* Steud). A relationship was found between cultural practices and presence of RYMV in fields ($\chi^2 = 108.83$, df = 1, $P < 0.001$). The serological characterization using Monoclonal antibodies (Mabs A and D), showed homogenous reaction with Mabs A alone. These results indicated that Ser1 serotype is present in the south of Central African Republic.

Keywords

RYMV, Detection, Serological Characterization, Central African Republic

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereals in the world. It is an important food crop and a source of revenue for farmers in Africa. Rice has gradually become one of the major cereal productions in Central African Republic (CAR) where it contributes to fight against food insecurity. However, rice production is severely affected by *Rice Yellow Mottle Virus* (RYMV), the most important viral disease of rice in Africa. Member of genus Sobemovirus, RYMV was the first reported in Kenya in East Africa [1]. RYMV was found in most countries where rice is growing and also in the islands of Zanzibar and Madagascar [2] [3] and more recently in Chad, Cameroon, Central African Republic and Democratic Republic of Congo [4].

The virus induced symptoms that include yellowing and mottling, reduced tillering and sterility of flowers. RYMV is mechanically transmissible and is naturally transmit-

ted mainly by chrysomelid beetles. It can also be transmitted by mammals such as cows, grass rats or donkeys [5]. Disease caused yield loss ranges from 25% to 100% depending on the rice genotype, date of infection and the virus isolates.

Variability in RYMV isolates has been reported and revealed five main serotypes in West, Central Africa (Ser1 to Ser3) and East Africa (Ser4 and Ser5) [6] [7]. For this study, we investigate the presence of RYMV in several sites around Bangui and further south which benefit from forest climate (up to 1500 mm rainfall per year). Natural and experimental hosts of the virus are taken into account to establish the role of wild grasses in the virus epidemiology in the country. We also analyze the variability of isolates at serological level to compare later with molecular typing.

2. Material and Methods

2.1. Survey and the Collect of Leave Samples

Between 2012 and 2015, a survey was performed in the south of CAR in several localities bordering Bangui city and other locations further in the south at 75 km from the capital (**Figure 1**). Leaves samples were harvested from cultivated rice and wild grass including *Oryza* species in lowland, upland and irrigated conditions (**Table 1**) and processed as indicated by Konate *et al.* [8]. Wild grasses were identified using a document entitled "weeds of rice in West Africa" [9]. The visual diagnostic of RYMV are yellowing and mottling of leaves as well as severe stunting in some rice genotypes [10]. Thus, leaves were collected based on these typical symptoms (**Figure 2**) of RYMV using plastic bags. Samples were labeled with name location and ecological condition and they were stored in the ice box. Thereafter, samples were transferred to the laboratory and stored in the freezer at −20˚C for further processing.

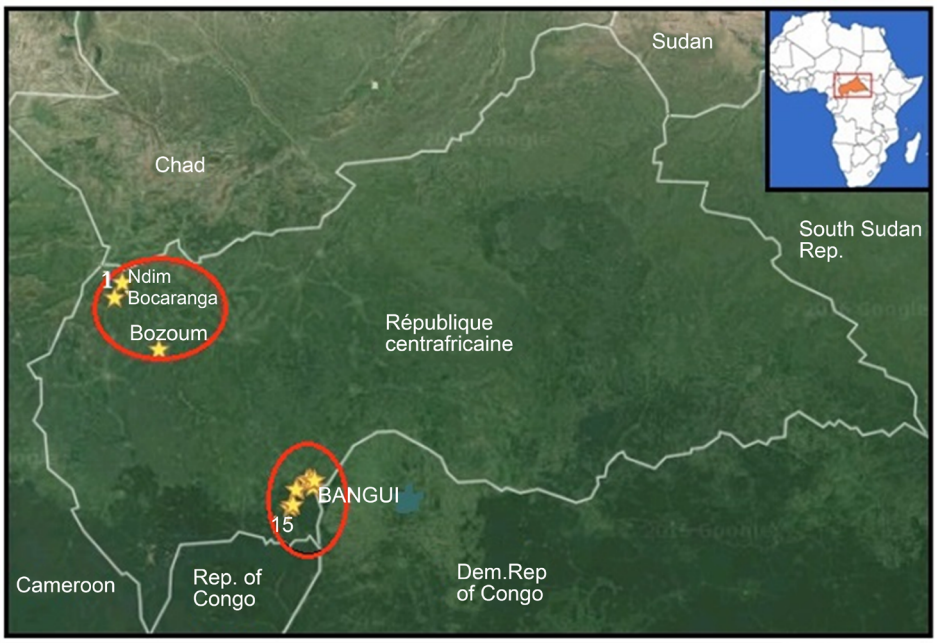
2.2. Biological Tests

Typical symptoms of rice yellow mottle disease could be wrongly attributed to iron or nitrogen deficiency. Therefore, the mechanical transmission test was done using the susceptible rice variety IR64 to confirm RYMV transmission. Leaf samples from fields were ground in the mortar at the ratio of 1:10 (w/v) with 0.01 M phosphate buffer and pH 7.0. The powder of carborundum (600 mesh) was added to the extracts, which were rubbed onto leaves of 2 week-old seedlings [11] [12] [13]. All experiment was conducted in a greenhouse under insect proof conditions.

2.3. Serological Detection of RYMV

The presence of RYMV in the collected samples was confirmed using DAS-ELISA (double antibody sandwich enzyme_linked immunosorbent assay) method [14]. A polyclonal antibody raised against isolates from Madagascar known to react strongly with isolates from west and Central Africa was used as coating antibody [6]. It was kindly offered by the Doctor Denis Fargette (IRD, Montpellier, France). All procedures of the test were as previously described [8].

Leaf samples (1 g) were ground in 10 ml PBS buffer (Phosphate buffered saline pH



Legend

Sample collection areas

Sites prospected

N°	Locality	Geographic coordonates: Latitude	Longitude
1	Ndjim	7.257498	15.77207
2	Bocaranga	7.042452	15.66628
3	Bozoum	6.305508	16.32624
4	PK10	4.435499	18.543291
5	Ngola	4.438952	18.511487
6	Ngoulanga	4.457199	18.476802
7	Sabiri	4.451576	18.441966
8	M'poko	4.441282	18.464645
9	Goundou	4.437069	18.470723
10	Sakaï	4.420801	18.478575
11	Guita	4.346611	18.534001
12	Bimon	4.314091	18.299898
13	kapou	4.313722	18.286822
14	Boyali	4.097309	18.226095
15	Pissa	4.057718	18.189164

Figure 1. Map of the part of the country where survey was done**.** Sites prospected are numbered from 1to 15 with corresponding geographic coordinates.

7.4) containing 0.05% of tween 20 (PBS-T) and 2% polyvinylpyrolidone (PVP) or extraction buffer. The resulting extracts were centrifuged for 10 min at 8000 rpm at 4˚C. The procedure was the following: immunoplates were coated with 5 µg/ml IgGs anti-RYMV in 5 mM carbonate buffer, pH 9.6 for 2 hours at 37˚C. After washing three times with PBS-T, the plates were incubated with 3% skimmed milk in PBS-T for 30 minutes. Thereafter, extracts were deposited to each well and incubated for 2 hours at 37˚C. IgGs anti-RYMV conjugated with alkaline phosphatase diluted in extraction buffer containing 1% skimmed milk were incubated for 2 hours. The substrate (p-nitrophenylphosphate) was diluted (1g/ml) in 10% diethanolamine, pH 9.8. Absorbance was recorded at 405 nm after 1 h substrate incubation using a multiscan

Table 1. Biological and serological detection of RYMV in rice and wild grass.

Plant Species	Sample number	Suspected symptoms[a]	Mechanical transmission	Serological detection
Acroceras zizanioides	8	YM	0/8[b]	0/8
Echinochloa cholona	13	GM	0/13	0/13
Eragrostis japonica	3	YM	0/3	0/3
Imperata cylindrica	23	YM	0/23	0/23
Leersia hexandra	22	M	0/22	0/22
Sacciolepis africana	8	M	0/8	0/8
Leptochloa caerulescens	5	GM	0/5	0/5
Paspalum vaginatum	3	M	0/3	0/3
Oryza eichingeri	28	M	0/28	0/28
Oryza sativa/*glaberrima*	208	YM	201/208	201/208
Rottboellia exaltata	3	M	0/3	0/3

[a]YM, yellowing mottle; GM, green mottle; M, mosaic; [b]Number of positive samples out of total number.

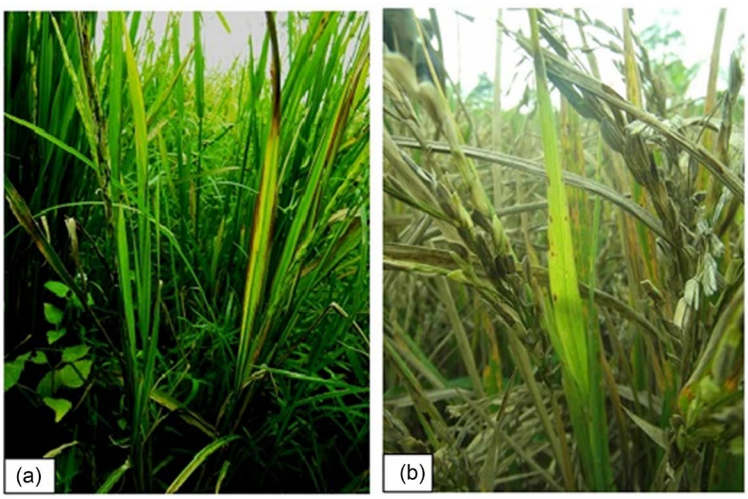


Figure 2. Symptoms of Rice Mottle Disease in field. (a): necrosis; (b): mottle yellowing and sterility of flowers (Picture, R. D. S. Longué, 2015).

microplate reader Metertech $\sum$960 model. For the whole procedure, each step was followed from washing the plate three times with PBS-T excepted blocking step (skimmed milk incubation step).

2.4. Serological Typing

For immunological typing, triple antibody sandwich-ELISA (TAS-ALISA) procedure was performed to determine the serological profiles of RYMV isolates which were detected positive with both biological and serological detection. So, 45 isolates chosen according to sites of provenance were used. Two monoclonal antibodies were used (Mab A and Mab D) to distinguish Ser1 from Ser2 [6] [7]. Thus, the procedure was the same

as described above with two differences: after leave extracts incubation, monoclonal antibodies were incubated followed by the incubation of Goat anti mouse IgGs conjugated with phosphatase alkaline (GAM-PAL).

2.5. Statistical Analysis

In order to test a possible relationship between cultural practices and RYMV occurrence in rice fields, the Chi-square independence test with Yates continuity's correction was used using R statistical software https://www.r-project.org/.

3. Results

3.1. Identification of RYMV in Plant Samples

For biological detection, typical symptoms of RYMV were induced on susceptible rice variety IR64 only by samples belonging from cultivated rice (**Table 1**). Up to 96% (201 out of 208) of leaves samples induced the characteristic yellow discoloration and mottling of leaves (as shown in **Figure 2**) after mechanical inoculation between 7 and 10 day after inoculation (DAI). By contrast, all samples collected from wild grasses did not infected IR64 for 30 DAI. In addition, the immunological detection using polyclonal antibodies showed positive reactions with all leave samples which were positive at biological test. The positive reaction including positive control ranged from 0.851 and 2.35. Absorbance between 0.028 and 0.2 were attributed to negative samples as they corresponded to the negative control. Negative samples were those from wild grasses. Failure to detect RYMV in these samples suggested that symptoms observed on collected wild grasses were not due to infections by RYMV.

Taking account of the ecology from which samples were collected and cultural practices, we found that infection rates depended on rice growing practices i.e. whether rice was directly seeded or seedlings were transplanted from pre-established seedbeds. Infection rates were significantly higher with transplantation (94.2%) than with direct seeding (22.8%) according to Chi-square test ($\chi^2 = 108.83$, df = 1, $P < 0.001$). This indicates that cultural practices, such as transplantation, likely play a role in spread of the disease in the field. However, as shown in **Table 2**, in both upland and lowland rice fields where direct seedlings were performed, few positive samples were detected.

3.2. Serological Characterization

The serological profile of RYMV isolates tested with monoclonal antibodies was homogenous. Indeed, all tested isolates were reacted strongly with Mab A and failed to react with Mab D. The virus titers estimated through absorbance values ranged from 1.063 to 2.628 with an average of 1.909 ± 0.360 (average ± standard deviation, n = 45) for Mab A and from 0.018 to 0.296 ± 0.069 with an average of 0.115 (average ± standard deviation, n = 45) for Mab D (**Figure 3**). The positive controls of both Mabs had similar absorbance with average of 1.774 ± 0.525 and 1.675 ± 0.346 for Mabs A and D respectively. This suggested that all isolates belonged to Ser1 serotype, thus RYMV is not diversified at serological level into this region.

Table 2. Identification of RYMV according to cultural practices and agro-ecosystem.

Locality	Number of samples	Ecology	Cultural practice	Detection[a]
Bimon	8	Lowland	Direct seeding	–
Bocaranga	3	Upland	Direct seeding	–
Bozoum	4	Irrigated	Transplantation	–
Boyali	5	Upland	Direct seeding	–
Kapou	10	Lowland	Direct seeding	–
Ndjim	2	Lowland	Direct seeding	–
Ngola	87	Irrigated	Transplantation	+
Ngoulanga	5	Lowland	Transplantation	+
Pissa	6	upland	Direct seeding	–
PK10	4	Lowland	Transplantation	+
Sabiri	8	Lowland	Transplantation	+
Sakaï	59	Irrigated	Transplantation	+

[a] + and – for positive and negative reactivity respectively.

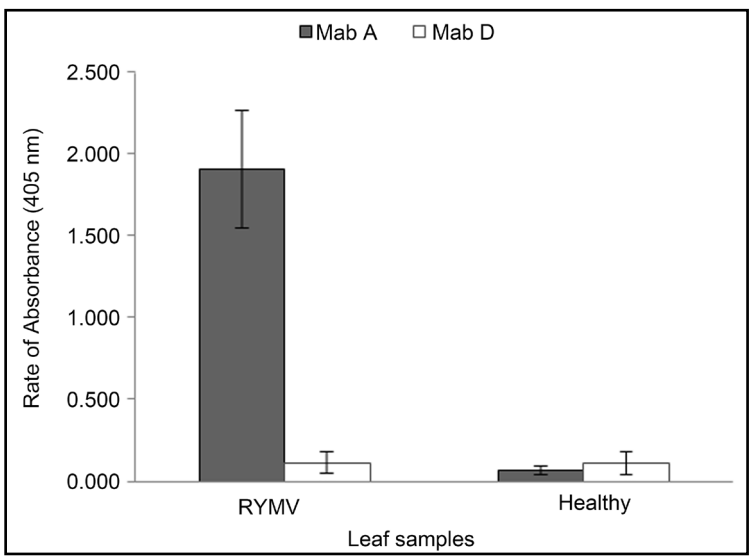


Figure 3. Serological profile of isolates of RYMV from CAR using monoclonal antibodies A and D.

4. Discussion

In the Central African region (Cameroon and Chad), RYMV was identified for the first time in 2001 as described by Traoré *et al.* [15]. Afterward, RYMV was observed in irrigated rice field around Bangui city in Central African Republic a ten years ago [4].

In this study, we investigated the presence of RYMV into several different rice growing sites and analyzed the diversity at serological level using both polyclonal and monoclonal antibodies.

Our results revealed that 201 leaf samples from cultivated rice exclusively, were detected positive in DAS-ELISA test with absorbance values between 0.851 and 2.35. By contrast, not any sample from wild grasses was positive by serological detection (0.024 < A405 nm < 0.3). This was confirmed by mechanical transmission test where no sample could reproduce symptoms of yellowing and mottling. Indeed, only leaf samples which were detected positive at serological level induced characteristic symptoms of rice yellow mottle disease.

Contrary to other reports [8] [12] [16], our results indicated that *Echinochloa colona* (from Poacea family) is not susceptible to RYMV, confirming the results reported in Est Africa [17]. Moreover, it also has been demonstrated that this species is not host by inoculation to seedlings [18]. However, some wild grasses were found to be host of RYMV such as *Leersia hexandra* [16] unlike our results. The symptoms observed on *Imperata cylindrical* were more similar to those of rice yellow mottle disease. The non-detection of RYMV in this species suggested that these symptoms may be probably due to *Imperata yellow mottle virus*, a *Sobemovirus* closely related to RYMV [19] which were found in West Africa [20].

Apart from rice cultivated species (*Oryza sativa* L. and *O. glaberrima* Steud), the natural host range of RYMV includes a few wild rice species and other Poaceae family. Therefore, the non-detection of RYMV from *O. eichingeri* (forest wild rice species) which is the main wild rice species found into lowland and irrigated system suggested that these symptoms observed belonged to other disease. There is probably that this rice species be resistant to RYMV, because the inoculated seedlings and the back inoculation to susceptible rice cultivar have never induced symptoms (data no shown). Nevertheless, inoculation tests in Kenya with RYMV did not infect this forest wild rice *O. eichingeri* [1].

Like wild grasses may be important in the epidemiological aspects, we suggested that artificial host range could be investigated.

Our result shows clearly that the main natural host of RYMV remains the cultivated rice. In addition, the good observation of symptoms in field can be the first detection of rice yellow mottle disease which may be confirmed by serological detection.

5. Conclusions

Results obtained in this study show that 201 isolates of RYMV were detected. All isolates detected positively proceed from samples collected to cultivated rice exclusively. RYMV was only found in the south of the country in the irrigated and lowland rice crops. In addition, farmer cultural practices could play important role in the spread of the disease. At the northwest of the country where most rice is growing, RYMV is suspected but an important survey could be done for identification of the virus in this area.

At the natural hosts level, none species were identified as host of RYMV, because all samples collected from wild grasses were negative in both biological and serological tests. Consequently, it would be interesting to look artificial hosts to better guide the natural hosts identification.

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