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STUDIES ON THE INCIDENCE OF RICE YELLOW MOTTLE VIRUS ON RICE PLANTS IN CROSS RIVER STATE, NIGERIA.

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Abstract

Rice yellow mottle virus (RYMV) a member of the genus Sobemovirus causes significant economic losses on rice fields. Cross River State is a major producer of rice in Nigeria. A survey and study to detect the incidence viruses infecting the crop was carried out. Host range studies, disease incidence and detection by serology using DAS- ELISA was made. The result showed that transmission to other plants was unsuccessful and only Oryza sativa used as test plant was infected by the virus. The percentage disease incidence due to RYMV was high with the highest recorded in Obubra local government area (100%) while the lowest was in Obudu local government area (60%). DAS-ELISA detected the virus in all locations with the lowest mean absorbance values being 0.798 and highest 1.421 for Biase and Abi local government areas respectively. This is the first report of the RYMV infecting rice in Cross River State.

Keywords: DAS-ELISA, RYMV, Disease incidence, Cross River State, Rice, Survey

1.0 Introduction

Rice (*Oryza sativa* L.) is an important food crop and also a source of revenue for farmers in Africa. Due to the multiplicity of its uses and its adaptability to different weather conditions, it is a major food crop in the world. Rice is valued for its high nutritional benefits apart from being rich in calories, it is high in fibre, vitamins and minerals and low in cholesterol and sodium, suggesting it is a healthy source of energy. Asia is the largest producer and consumer of rice (Khush, 2005; Sellamuthu *et al*., 2011). In Nigeria, Cross River State ranks ninth among the top rice producing states (Commodity Port, 2022) with the crop eaten majorly as boiled rice or prepared in several ways such as coconut rice, native rice, fried rice, jollof rice and the common rice and stew.

The two widely cultivated species of rice are *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice) for which the former is cultivated throughout the world while the latter is grown partially in western part of Africa (Montcho *et al*., 2017). In terms of production, rice is the world's second most important cereal after wheat with a production of 782 million metric tonnes (FAO 2020) and Nigeria is ranked as the largest producer in Africa with a production capacity of 5.04 million metric tonnes in 2019 (USDA 2020).

In Nigeria, six growing environments have been adopted by rice growers and they include mangrove swamp, upland, rainfed lowland, hydromorphic, irrigation lowland and deep inland water (Longtau, 2003) however, the prevalent method of cultivation is swamp rice.

Several viruses have been reported to infect rice and some of these include *Rice stripe virus* (RSV), *Rice dwarf virus* (RDV), *Rice gall dwarf virus* (RGDV), *Rice ragged stunt virus* (RRSV), *Rice grassy stunt virus* (RGSV), Rice transitory yellowing virus [RTYV), *Rice yellow stunt virus* (RYSV), *Rice black streaked dwarf virus* (RBSDV) and *Rice stripe mosaic virus* (RSMV).

Folarin and Asala (2022) reports that there are primarily three viruses that have previously been identified as infecting the crop in Nigeria and they include Rice stripe necrotic virus (RSNV), Rice yellow mottle virus (RYMV) and Maize streak virus (MSV) with RYMV being predominant.

RYMVwasfirstreportedinNigeriain1976(Raymundo and Buddenhagen, 1976; Rossel *etal.*,1982). It is known to be transmitted by mechanical contact mediated by wind (Sara *et al.*, 2004), farm equipment (Abo *et al.* 1998) such as sickles used in harvesting and also by tight contacts between plants during planting out (Kouassi *et al.* 2005). Other methods of transmission of RYMV include guttation fluid and irrigation water (Abo *et al*. 1998; Abo *et al.* 2000). Cows, donkeys and grass rats had also been indicated as vectors of the virus through trampling and grazing (Sarra *et al.* 2004).

RYMV is a member of the genus *Sobemovirus* and possesses all the characteristic biophysical and biological properties of the members of the genus. The major insect vectors of the virus belong to the genus are Chrysomelidde, which play an essential role in primary infection, while secondary infection is due mainly to plant-to-plant contact (Koudamiloro, 2014). The virus particles are stable in infected dried leaves. Limitations to the spread of the virus include restricted mobility of the insect vectors, a limited host range, and absence of seed transmission. Partially resistant and highly resistant varieties have been identified, but currently the disease is not controlled adequately, and its incidence is increasing significantly in Africa.

Gnanamanickam, (2009) reported that RYMV-infected rice plants usually develop pale yellow mottlleaves, stunted, reduced tillering, nonsynchronous flowering, poor panicle exertion and spikelet discolouration.

This study is aimed at characterizing and detecting viruses infecting rice production in Cross River State.

2.0 Material and methods

2.1 Survey and the Collect of Leave Samples

Survey and the Collect of Leave Samples During May and July 2022, a survey of disease incidence was conducted in Biase, Abi, Obubra, and Obudu, which are local government areas in the Cross River State where rice cultivation is carried out. Some of the rice varieties identified in the field and cultivated by the farmers were FARO-44, MAS 2401, IR-64, IR-119, and UPIA 1. Each farm visited ranged between one and three hectares in size. Two farms in each local government were visited, and 10 samples of both symptomatic and non-symptomatic leaves were randomly collected from both rice fields in each local government. The samples were labelled with the name location and stored in an ice box. Thereafter, the samples were transferred to the laboratory and stored in a freezer at −20˚C for further processing.

2.2 Biological indexing

For biological indexing, mechanical transmission tests were performed using three grass weeds found in rice fields (Imperata cylindrica, Digitariasanguinalis, and Megathyrsus maximus) and the susceptible rice variety IR-64 to confirm RYMV transmission. Leaf samples from fields were ground in a mortar with 0.01 M phosphate buffer and pH 7.0. Carborundum powder (600 mesh) was added to the extracts, which were rubbed onto the leaves of two-week-old seedlings. All experiment was conducted in a screen house under insect proof conditions.

2.3 Serological detection of RYMV

Serological indexing:

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was adopted for the detection of RYMV in rice leaf samples, with some modifications (Pinel-Galzi *et al.* 2018). Microtitre wells of ELISA plates were coated with 100µl of appropriately diluted RYMV immunoglobulin G in coating buffer containing 1.59 g Sodium carbonate (Na2CO3) and 2.93g Sodium bicarbonate (NaHCO3) per litre, pH 9.6. Plates were incubated at 37°C for 2 h after which the contents were decanted and wells washed three times with single strength phosphate buffer saline containing 0.05 % tween 20 (v/v) (PBST). Constituents of PBS-T include 8.0 g Sodium chloride (NaCl), 0.2 g monobasic potassium phosphate (KH2PO4), 1.15 g dibasic Sodium phosphate (Na2HPO4) and 0.2 g Potassium chloride (KCl) per litre, pH 7.4.

ELISA plates were tapped dry and 100µl aliquots of extractedsap of infected rice leaves and macerated insects' guts including the negative and positive controls provided were pipetted into the duplicate wells and incubated overnight in the refrigerator at 4°C. Contents of wells were decanted and wells washed as previously described. One hundred microlitre of appropriately diluted enzyme-labelled antibody(IgG-alkaline phosphatase) in conjugate buffer(1.0 g polyvinyl pyrrolidone [PVP], 0.1g eggalbumin/L PBST) was added to each well and incubated at 37 °C for 2 h. Wells were washed and plates tapped dry before 100 µl of 1mg/mlpara-nitrophenyl phosphate dissolved in substrate buffer (97 ml diethanolamine/L, pH9.8) was added per well and contents incubated at room temperature for 1 h and/or overnight at4 °C, after which the spectrophotometric reading was taken using an ELISA plate reader(model MR-96, MINDRAY, China) at 405nm. Sample was considered positive when the mean of its absorbance values is twice the average value of the healthy control.

3.0 Results

3.1 Biological indexing and percentage incidence

A total of 40 samples were collected from all locations in the four local government areas of the study, out of which 34 (85%) samples were symptomatic while 6 (15%) were asymptomatic. The 34 symptomatic samples and the 6 asymptomatic samples were serologically tested for RYMV with result showing 2 of the 6 asymptomatic and 30 of the symptomatic samples tested positive for RYMV. The mean percentage disease incidence was highest in Obubra local government area (100%) and lowest in Obudu local government area (60%).

Table 1: Percentage incidence of Rice yellow mottle virus (RYMV) on rice plants

Local Government Area	Number of fields visited	Number collected/tested	Number detected	Mean incidence rate (%)
Biase	2	10	9	90
Abi	2	10	7	70
Obubra	2	10	10	100
Obudu	2	10	6	60

For biological detection, typical symptoms of RYMV were induced on the susceptible rice variety IR-64 and associated weeds showing similar symptoms. Transmission was successful with the induction of a characteristic yellow discoloration and mottling of leaves after mechanical inoculation for *Imperata cylindrica* where zero out of 3 of the test plant was infected (Table 2). The use of *O. sativa* as test plant resulted in 7 out of 8 infected while it was zero from *D.sanguinalis and M. maximus* (Fig. 1).

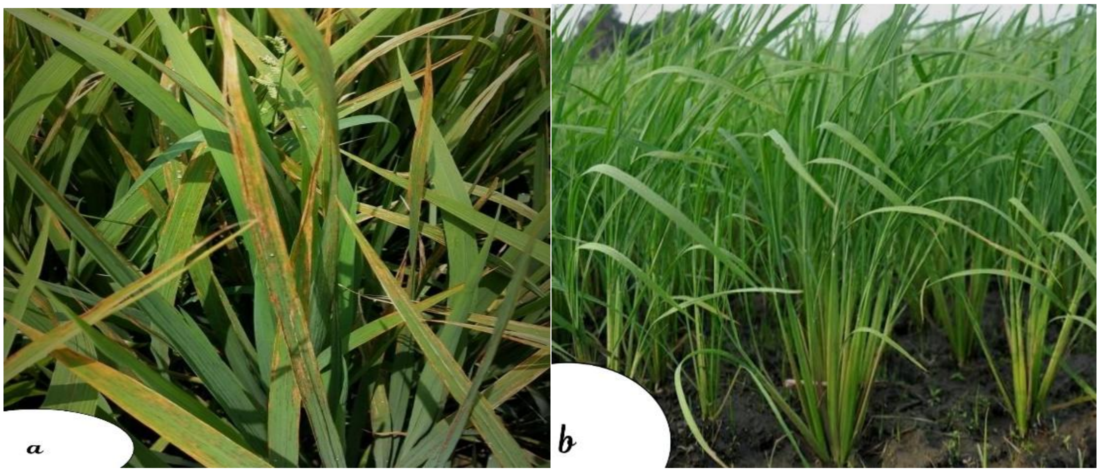

Fig. 1: Symptoms of *Rice yellow mottle virus* in field. (a)yellowing and mottling of leaves (b) Healthy rice plants

Table 2: Mechanicalinoculation of test plants and serological detection of RYMV in rice and weeds found on rice fields

Test plants	Sample number	Sap transmission	DAS-ELISA
I. cylindrica	3	0/3	0/3
O. sativa	8	7/8	7/8
D.sanguinalis	5	0/5	0/5
M. maximus	4	0/4	0/4

3.2 Serological Detection

The leaf samples that were collected during the survey showed a strong positive reaction with the polyclonal antiserum raised against the isolates from all the locations. The healthy control was 0.317 and all samples were twice the value of the healthy control. The mean absorbance value for isolates from Biase local government area was 0.798 and was the lowest while the absorbance for isolates from Abi local government area was 1.421 and it was the highest of the mean absorbance values. The mean values for the isolates from Obubra and Obudu local government was 0.827 and 0.981 respectively. Also, the narrow serological variabilities in optical density among the isolates confirm a prevalent strain of RYMV.

Table 3:

Local Government Area	Suspected symptoms	Number of samples tested	Mean of Absorbance at 405nm	Detection by DAS-ELISA (+/-)
Biase	LM	5	0.798	+
Abi	RT	5	1.421	+
Obubra	LR/LM	5	0.827	+
Obudu	S/RT	5	0.981	+

Healthy control=0.317; LM= Leaf mottling; S=Stunting; LR=Leaf roll; RT=Reduced tillering

4.0 Discussions

The cultivation of rice in Nigeria has been plagued by a number of pest and diseases of which RYMV is said to be described as the primary virus infecting the crop. The symptoms of RYMV observed in the rice field included yellow leaves, yellow stripes, brown or orange discolouration of older leaves, leaf narrowing, leaf mottling, stunting, reduced tillering and in extreme cases plant death. These symptoms were the basis for sample collection however, iron deficiencies also cause yellowing symptoms that can be confused with the symptoms induced by RYMV. This makes diagnosis with serology 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 Nigeria, this virus has been detected in Nigeria including Zamfara state, Ogun state, Niger state, Oyo state (Onwughalu *et al.* 2011; Odedara et al. 2016, Mohammed *et al.* 2019). This is the first report of RYMV occurring on rice plants in Cross River State

All the symptomatic samples from *O. sativa* used for host range studies tested positive for RYMV by DAS-ELISA. These findings corroborate to the findings of Odedara *et al.* (2016) and Eyong *et al.* (2021) that also adopted the use of ELISA for the confirmation of viruses however, what was earlier thought to be suspected symptomatic samples on *I. cylindrica, D. sanguinalis* and *M. maximus* in the field reacted negatively to the RYMV antibodies. This result contradicts the findings of the findings of Traore *et al*. (2008) and Issaka *et al*. (2012) but supports the findings of Allarangaye *et al.* (2007).

The result indicates that although the isolates were collected from different local governments, locations and had different optical density (OD) values which suggested a diversity of the virus strains, they were serologically similar as they all reacted to the RYMV antiserum. This corresponds with the finding of Longue *et al.* (2016) and Mabele *et al.* (2020).

In this study, two of the six asymptomatic samples collected, showed latent infection and the
the presence of latent infection makes it difficult to control the virus because without the use serological diagnostics, it would never have been detected. Also, there was a high incidence of the virus in all locations of the four local government areas where sampling was done. Were et al. (2004) had earlier reported that a percentage incidence greater than 50% can be considered as high. The cultivation of rice in these communities over the years and the cultivation of the crop in nearby states and country presents pool where seeds can be informally sourced thereby creating a genetic pool of diversity of RYMV strains among the rice isolates.

5.0 Conclusion

Prior to this study, there has been no record or data on the incidences of RYMV in Cross River State. It was investigated in this study that infection of rice plants caused by Rice yellow mottle virus occurred in Obudu, Abi, Biase and Obubra local government areas of Cross River State. The high disease incidence encountered could be attributed to the continuous cultivation of susceptible varieties which necessitates the adoption of virus free seeds resistant to RYMV by farmers and stakeholders and a need to employ sanitary measures during seed planting since farmer cultural practices could play a role in disease spread.

There is need for further investigation into the strains of RYMV by embarking on molecular characterization studies ascertain the evolutionary relationship that exist. This will provide knowledge to be used by breeders for the development of more adaptable and resistant rice seeds.

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