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Incidence and Distribution of Rice Yellow Mottle Virus Disease in Kebbi State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author IUM designed the study, wrote the protocol and draft the first and the final manuscript, Authors YAB and RI conducted the field survey and performed laboratory analysis, Author AM performed the statistical analysis, Authors AAA and MAY managed the analyses of the study. Author ASM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was conducted to assess the incidences of Rice Yellow Mottle Virus disease (RYMVD) in Kebbi State Nigeria, a field survey was conducted in four rice-growing areas of the State. Rice fields were selected randomly at 2 km interval, severity of the disease was assess using arbitrary five-point scale and disease incidence was assessed according to the proportion of the plants showing symptoms. Thirty plants were assessed in each field visited. Symptoms occurred in varying levels of incidence. The presence of RYMV in the collected samples was confirmed using Reverse Transcription Polymerase Chain Reaction (RT-PCR). Mottle/yellowing symptom was found more on the plants assessed (46%) followed by leaf curling (21%), leaf necrosis (09%), leaf deformation (11%) and irregular patches (13%). RYMVD was found highly distributed in the State with average incidence of 54.38%. The highest incidence was recorded in in Yauri (67.50%) followed by Argungu (55.00%), Bagudo (52.50%) and the lowest was recorded in Suru (42.50%).

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The average symptom severity across all the four Local Governments visited was 2.8, the highest was recorded in Yauri (3.2), followed by Argungu (2.9), Bagudo (2.7) and Suru 2.3. The information obtained in this study would assist rice breeding programs to develop durable RYMV rice resistant cultivars and guide in the identification of RYMVD hot spot locations for seed multiplication trials in Kebbi State.

Keywords: Rice; survey; incidence; severity; variety; symptoms.

1. INTRODUCTION

Rice is a seed from the grass species *Oryza sativa* (Asian rice) or *Oryza glabberima* (African rice). Rice is a staple food for over 180 million people in Nigeria, of which in the last decade alone rice consumption was at an annual rate of 10% of the total energy food consumed in the country [1]. The per capita consumption of rice has grown from 3 kg in the 1960s to an estimated 38 kg in 2014 [2]. Rice is one of the most widely produced cereal crop worldwide (742 million tons) after maize (1 billion tons) and sugarcane (2 billion tons) [3]. It is regarded as one of the cash crops that serve as source of income for farmers in Kebbi State in particular and in Nigeria at large [4]. Rice is grown as upland crop where there is no standing water and rainfall is the sole source of moisture and is also grown during dry season using irrigation system [5]. Kebbi State has a very big potential in terms of rice production.

However, this potential is being threaten by the menace of *Rice yellow mottle virus* (RYMV), which is contributing to low yields of rice in the State [6]. Yield of 3 tonnes per ha have been reported in Africa compared to 5 tonnes per ha in Asia [1]. RYMV virus disease was first reported in 1966 at Otonglo near Lake Victoria, Kenya [7] and since then the virus spread to many countries in Sub-Saharan Africa. RYMV is commonly found where rice is grown especially in Africa [8,9,10,11]. Therefore, the study was designed to understand the epidemiology of Rice Yellow Mottle Virus Disease in Kebbi State.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was carried out in the Molecular Biology Laboratory of Kebbi State University of Science and Technology, Aliero in the Faculty of Agriculture. (Lat. 12^{o} 18' N and Long. 4^{o} 29' E).

2.2 Field Survey

A survey of rice Farms (fields) in Kebbi State was conducted for virus-like symptoms on rice leaves in four LGAs (Argungu, Bagudo, Suru and Yauri). In each field, 30 plants were examined for the virus-like symptoms and five categories (asymptomatic, mild, severe, moderate and very severe) of rice leaves showing RYMV symptoms were collected from the plants as representative samples. The information of each field was collected (i.e. name of the rice variety, age of the crop, farm size and the pictures of the rice farm).The coordinate (in term of latitude and longitude; and altitude) of each field were taken with the use of a Global Positioning System (GPS) receptor. The leave samples collected were placed and packed in a herbarium.

2.3 Disease Incidence

The percent disease incidence was calculated by expressing in percent, the total number of affected plants per total number of plants sampled using the formula below:

$$RYMVD\ incidence\ (\%) = \frac{No.\ of plants\ with\ symptoms}{Total\ no.\ of plants\ sampled} x\ 100$$

2.4 RYMV Disease Severity Score

RYMVD severity were expressed using an arbitrary scale indicating the extent of symptom development [12,8,5,7,13] [14,15,16] as follows: 1 = no visible RYMVD symptoms, 2 = mild foliar symptoms on some leaves, 3 = pronounced foliar symptoms but no die-back, 4 = pronounced foliar symptoms which might include slight die-back of terminal branches, and 5 = severe foliar symptoms and plant die-back [14,15,16].For each plant show symptoms of RYMVD were counted and recorded.

2.5 Extraction of RYMV RNA

Total RNA of RYMV was extracted from rice leaves collected from farmer's field (field survey) using the Rneasy Plant Mini Kit (Qiagen, Germany) [17]. Viral suspension was collected in 2 mL Eppendorf tube with sterile steel beads, frozen in liquid nitrogen and were grounded with high speed TissueLyser II mechanical shaker for 1 min at 30 rpm. The RTL lysis buffer was added,

mixed by vortexing then incubated in water bath at 56ºC for 2 min and centrifuged at 7000 rpm for 7 min. Tissues are separated by 225 µL of 100% ethanol followed by spinning at 10,000 rpm for 1 min, and then the supernatants are transferred into 2 mL Eppendorf tubes. Proteins of RYMV were removed by adding 700 µL RW1 and 500 µL RPE buffer, respectively and separately, centrifuged as above then the supernatant liquid were discarded and transferred into sterile 2 mL tubes. Ribonucleic acid ware washed in 500 µl RPE buffer by spinning at 13,000 rpm for two minutes. Nucleic acids were eluted by 30 µL RNase free water directly to the spin column membrane and placed into clean sterile 1.5 mL tubes then centrifuged at 10,000 rpm for 1 min at 25ºC. The obtained RNAs are stored in the freezer at -20ºC for RT-PCR amplification.

2.6 cDNA Synthesis

The RNA extracted was converted into complementary DNA (cDNA) by reverse transcription using reverse transcriptase as an enzyme to generate cDNA templates for carrying out PCR amplification (RT-PCR) analysis.

2.7 RT-PCR

The cDNA templates generated (from cDNA synthesis), with set of specific degenerated primers (genus/family) for rice viruses, a suitable buffer solution and a thermo-stable DNA polymerase were used for exponential amplification using the same methodology as in conventional PCR. A dye fluorescein**,** fluorophore was added to the mixture in a thermal cycler that contains sensors for measuring the fluorescence of the fluorophore after it has been excited at the required wavelength allowing the generation rate to be measured for one or more specific products. The data generated were analyzed using computer software to calculate relative gene expression (mRNA copy number) in the samples [18].

2.8 Specific Primer Test for RYMV

The primer set of (RYMV_R and RYMV_F)5′CTCCCCCACCCATCCCGAGAATT3′(reverse primer) and 5′CAAAGATGGCCAGGAA3′ (forward primer) were used for PCR tests and have amplified the 720 nucleotide CP gene of RYMV [19].

2.9 Agarose Gel Electrophoresis

PCR products were processed by agarose gel electrophoresis using the protocol of [12]. Photographs were then taken of the gel picture using a gel documentation system. Gel picture taken were compared to the photographs included for each protocol for the presence of right size bands.

2.10 Statistical Data

Statistical data analysis was carried out using R-Software (PC-Window, 2009 version). The data for symptom severity scores were processed by analysis of variance (Anova).

3. RESULTS

3.1 Assessment of RYMVD Phenotypes

The most predominant RYMV symptom associated with all the varieties of rice cultivated in Kebbi State were mottle/yellowing, chlorotic, leaf curling and deformation (Fig. 1). Other symptoms observed were spikelet distortion, necrosis and sterility of flowers, stunted growth, and distortion. Mottling with the irregular patches of distinct light and dark areas was also observed in farmer's fields. On the fields, symptoms induced by RYMV such as necrotic lesions, leaf yellowing and brown blotches which are also known as brown streaking were among the several symptoms encountered during the survey. These symptoms occurred in varying levels of incidence. Mottle/yellowing had highest number of plants (46%) followed by leaf curling (21%) and leaf necrosis (9%), leaf deformation (11%) and irregular patches (13%) (Table 1).

3.2 RYMVD Incidence and Severity

RYMVD incidence varied among the four LGAs but it occurred in all the locations surveyed in Kebbi State, Nigeria at an average of 54.38%.The highest incidences were registered in Yauri (67.5%) followed by Argungu and Bagudo with (55.00) and (52.5%) respectively. While on the other hand Suru had the lowest disease incidence (42.5%) (Table 2). Disease incidence and severity were high in Kebbi State with mean values of 54.38% and 2.8, respectively. Mild to severe symptoms were prevalent in sampled rice fields ranging between 2.3 and 3.2 in each location. Moreover, high severity of RYMVD was recorded in Yauri (3.2).

3.3 Effect of Rice Variety on Incidence and Severity of *Rice Yellow Mottle Virus*

The highest incidence of RYMVD on variety with (63.89%), whereas the variety UT-Rice and

Jemila had the lowest incidence (each 37.30%) with an overall average of all the varieties (50.56%). Disease severities on all the varieties range from 2.0 to 3.9 with an average mean of 2.8. (Table 3).

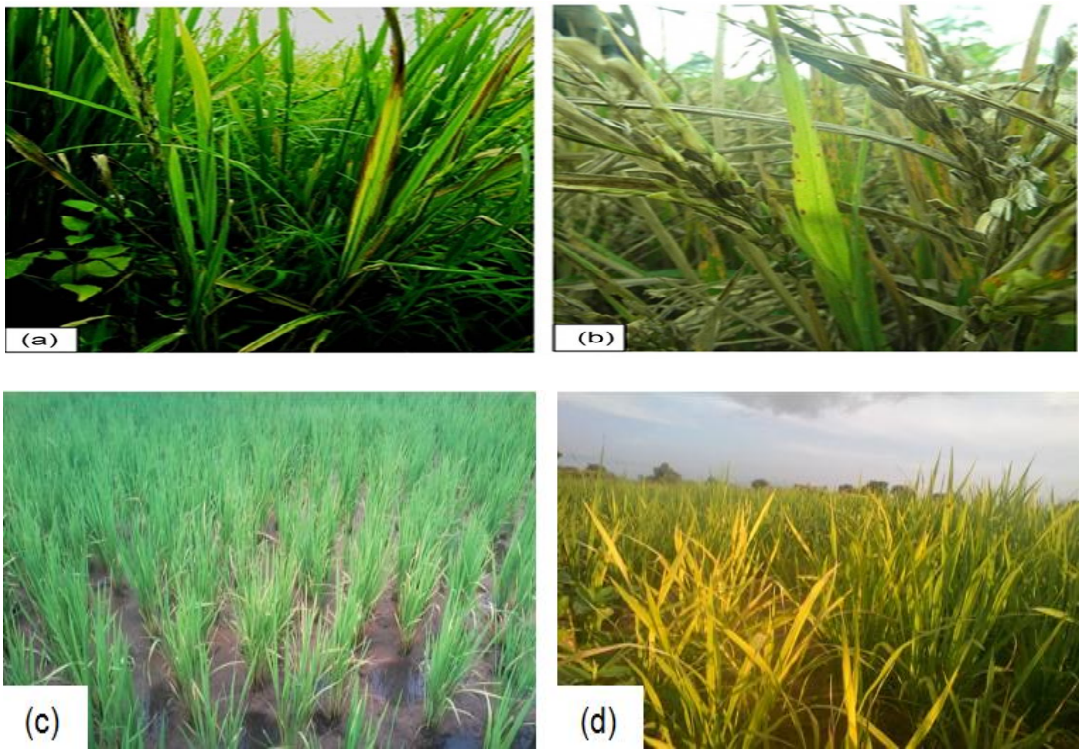

Fig. 1. Shows RYMVD disease symptoms observed in the field; symptomless (a) necrosis; (b) mottle/yellowing and sterility of flowers (c) Leaf deforming; (d) chlorosis and mottle yellowing

Table 1. Assessment of RYMVD symptom phenotypes on rice plants in Kebbi state

LGAs	% Symptoms types observed				
	Mottle/yellowing	Leaf curling	Leaf necrosis	Leaf deformation	Irregular patches
Argungu	2	1	1	0	4
Bagudo	1	10	2	5	1
Suru	3	5	6	5	4
Yauri	40	5	0	1	4
Total (%)	**46**	**21**	**9**	**11**	**13**

Table 2. Percentage incidence and mean symptoms severity of RYMVD for each location

Location	% Disease incidence	Mean symptom severity
Argungu	55.00	2.9
Bagudo	52.50	2.7
Suru	42.50	2.3
Yauri	67.50	3.2
Mean	54.38	2.8
SE±	**3.09**	**0.1**

Table 3. Incidence and severity of *rice yellow mottle virus*

Varieties	% Disease incidence	Mean symptom severity
Haji kusa dogo	58.34	3.0
Haji kusa guntu	52.09	2.7
Jemila	37.50	2.0
Semira	50.00	2.8
Sippi 44	63.89	3.3
UT Rice	37.50	2.0
Yarkundu	50.00	2.0
Faro 44	62.50	3.9
Jumbo	43.75	2.8
Unknown	50.00	3.0
SE±	**2.93**	**0.2**

Table 4. Effect of locations and rice variety on incidence and severity of *rice yellow mottle virus*in Kebbi State

Locations	Varieties	% RYMVD Incidence	% RYMVD Severity
Argungu	Dangote	37.50	25.00
Argungu	Faro 44	58.33	50.00
Bagudo	Faro 44	62.50	43.75
Yauri	Faro 44	66.67	50.00
Suru	Haji kusa dogo	50.00	25.00
Yauri	Haji kusa dogo	66.67	58.33
Suru	Haji kusa guntu	37.50	25.00
Yauri	Haji kusa guntu	75.00	75.00
Argungu	Jemila	50.00	25.00
Suru	Jemila	25.00	25.00
Bagudo	Jumbo	43.75	43.75
Argungu	Semira	50.00	50.00
Bagudo	Semira	50.00	35.75
Suru	Semira	50.00	50.00
Argungu	Wita	75.00	75.00
Suru	Wita	50.00	50.00
Yauri	Wita	66.67	50.00
Argungu	Unknomn	50.00	50.00
Suru	UT-Rice	37.50	25.00
Suru	Yar kundu	50.00	25.00
Mean±SE		**52.60±2.96**	**42.80±3.61**

3.4 Effect of Locations and Rice Variety on Incidence and Severity of *Rice Yellow Mottle Virus*

Variety Dangote is only found in Argungu with very low incidence and severity 37.50% and 25.00% respectively with an overall mean percentage incidence of 52.60%. The overall percentage disease severities were in the range of 25.00% to 75.00% with an overall percentage mean severity of 42.83% (Table 4). In Argungu, Bagudo and Yauri, Faro 44 was found with higher percentage incidence and low to moderate percentseverity (58.33%, 62.50% and 66.67%) and (50.00%, 43.75% and 50.00%) respectively. Haji kusa dogo were found in both Suru and Yauri with (50.00% and 66.67%) and (25.00% and 58.33%) percentage incidence and severity. Haji kusa guntu were also found in both Suru and Yauri with (37.50% and 75.00%) and (25.00% and 75.00%) percentage incidence and severity. Jemila were found in Argungu and Suru with low disease incidence and severity, Jumbo variety was present in only Bagudo. In Argungu Bagudo and Suru, there is variety called Semira with 50.00% disease incidence.

4. DISCUSSION

The findings suggest that the surveys conducted so far provided an exhaustive assessment of

RYMV phenotypes in the study area. Then, analysis of the pattern of symptoms diversity should be conducted. Coalescent analysis should reveal the major demographic events which shaped RYMV symptoms diversity. Accordingly, it would be interesting to test whether the differences in disease incidence among LGAs reflect massive extinction of the inoculum. Selection pressure may also counter select severe or intermediate strains in different locations as was experienced by [9]. The extinction processes may be due to lack of infection of host, or lack of transmission by vector. The phenotypes of RYMVD observed in this study were like the ones observed in field by [20] whose observations were mottled and yellowing symptoms of varying intensities depending on the genotype (Table 1). The findings suggest that the surveys conducted so far provided an exhaustive assessment of RYMV phenotypes in the study area. Then, analysis of the pattern of symptoms diversity should be conducted. Coalescent analysis should reveal the major demographic events which shaped RYMV symptoms diversity. Accordingly, it would be interesting to test whether the differences in disease incidence among LGAs reflect massive extinction of the inoculum. Selection pressure may also counter select severe or intermediate strains in different locations as was experience by [9]. The extinction processes may be due to lack of infection of host, or lack of transmission by vector. It was also observed in some farmers' field visited during the survey symptoms which include stunting, grain or spikelet sterility, and grain discoloration as was earlier described by [13] in his study. RYMVD were present in all the field visited with the overall disease incidence of (54.38%) the highest disease incidence was recorded in Yauri (67.50%) followed by Argungu (55.00%) and Bagudo (52.50%) and the lowest disease incidence was recorded in Suru (42.50%). This result indicated that the RYMVD is prevalence in all the location visited. RYMVD mean severity value was higher in Yauri (3.2) followed by Argungu (2.9) and Bagudo (2.7) and the lowest mean severity were recorded in Suru (2.3) indicating that the mean severity for Yauri (3.2) is severe symptoms while Argungu, Bagudo and Suru shows mild symptoms (Table 2).The highest incidence of disease occurred in variety Wita (63.89%) whereas the variety UT-Rice and Jemila had the lowest incidence (37.30%) with an overall average of (50.56%). Semira, Yarkundu and Unknown varieties shows similar percentage disease incidence with (50.00%). this is contrary to the findings of Dennis *et al,* (2011) whose recorded mean percentage incidence 72% and the highest percentage incidence of 75% within their varieties tested. Within the four locations visited, RYMVD leaf symptoms severity was mild (2.0) in three of the varieties namely: UT-Rice, Yarkundu and Jemila. Variety Wita (3.3) and Faro 44 (3.9) are close to very severe symptoms, this was like the findings of [21], who found that Faro 44 is vulnerable to virus. The rest of the varieties are severe with an overall mean severity of (2.8) (Table 4).The study revealed that Rice Yellow Mottle Virus is widely spread in most of the rice growing areas of Kebbi States of Nigeria. The prevalence of RYMVD in rice farms of the four locations with significance different in the disease incidence and the diseases symptoms severity. Further studies should focus at characterizing the rice yellow mottle virus diversity by sequencing for thorough understanding of the existing types of virus strains in study area. There is therefore the need to sensitize the farmers regarding the effects of RYMVD and mean of obtaining the planting materials (seedlings).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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