DETERMINING THE INCIDENCE AND SEVERITY OF RICE STRIPE NECROSIS VIRUS ON RICE (*Oryza Sativa* L.) IN THE FEDERAL CAPITAL TERRITORY, ABUJA, NIGERIA

[1,2]**Folarin O.O., [2]Asala S.W., [2]Oyerinde, A.A. and [1]Ojo P.O.**

[1]National Agricultural Seeds Council, Abuja, Nigeria
[2]Department of Crop Protection, Faculty of Agriculture, University of Abuja, Abuja, Nigeria
Corresponding Authors' email: oladapo.folarin@gmail.com

Abstract
A survey was conducted in September 2019 in Abuja, to assess the incidence and severity of rice virus disease across 24 rice fields. The samples collected from the survey were used for molecular indexing. All data collected were subjected to statistical analysis using SPSS version 17 and mean separation was done using Duncan Multiple Range Test. Out of 360 leaf samples collected from the survey, Bwari Area Council had the highest incidence (35.5%), while Abuja Municipal Area Council (AMAC) had the lowest (13.3%). Gwagwalada had the highest severity (31.1%) and AMAC recorded the lowest (20%). After testing in the laboratory using Polymerase Chain Reaction (PCR), the samples did not test positive to the virus. This study is the first research on RSNV in the FCT, Abuja and further survey is recommended for the FCT and other parts of Nigeria.

Keywords: Survey, incidence, severity, virus, and PCR

Introduction
Rice (*Oryza sativa*) is the most widely consumed staple food in the world, especially in Asia with 60% of the world's population, where more than 90% of the world's rice is cultivated and consumed. Rice is the third highest produced agricultural crop worldwide (741.5 million tonnes) in 2014 (FAOSTAT, 2018). Rice provides about 19% of global human per capita energy and 13% of per capita protein (Maclean *et al.,* 2013), which makes rice production very important to global food security. Rice has been a staple food accounting for 46 to 85% of the total cereal intake in West Africa during the 1960s (Lancon *et al*., 2002). Despite the slight increase in rice production over the years, Nigeria still remains the highest importer of rice in West Africa with an annual import of 6 million metric tonnes in 2018 (FAOSTAT, 2019). Rice is the most consumed staple food in Nigeria, which makes it important for food security. It is grown in every agro-ecological zone in Nigeria (Okoruwa *et al*., 2006). Rice is used for a variety of food and non-food products. Foods include: cooked rice, breakfast cereals, desserts, rice flour and beer. Rice is also used for *tuwoshinkafa* and *masa*, which are Hausa (a tribe in Nigeria) delicacies. It is also used for making fuel, fertilizer, insulation (Velupillai *et al*., 1996), cooking oil, weaving roofs, hats, baskets and sandals.

Pests and diseases are limiting factors in rice production. Diseases caused by viruses can be devastating when a high percentage of plants are affected early in the cropping season. Early infections may result in severe plant stunting or nearly complex inhibition of flowering and fruit set. Some of the virus diseases that have been reported in Nigeria are Maize Streak Virus (genus Mastrevirus) (MSV), Rice Yellow Mottle Virus (genus Sobemovirus) (RYMV) and Rice Stripe Necrosis virus (RSNV). RSNV belongs to the genus Benyvirus (Lozano, 2009) and was first described in 1977 as a new virus infecting rice in Cote d'Ivoire and was subsequently observed in Nigeria (Fauquet *et al.,* 1988), Liberia, Sierra Leone and Burkina Faso (Sereme *et al*., 2014). It is locally called crinkling rice disease, and was previously thought to be either bacterial in origin or a physiological disorder. Symptoms are characterized by chlorosis, necrosis of the leaf or the whole plant, seedling death, stunting, reduced tillering, severe plant malformation, and crinkling yellow foliar stripes (Oludare, 2015). It's widespread in West Africa, extent of damage it causes and limited report on the disease in Nigeria has made this research necessary to ascertain the incidence and severity of the virus in the Federal Capital Territory.

Materials and Methods
Four Area Councils in FCT namely: Kwali, Gwagwalada, Abuja Municipal Area Council (AMAC) and Bwari, were surveyed in September 2019 to collect rice leaf samples. The fields surveyed per Area Council

depended on the availability of rice farms at the time of survey. Sampling was done using a W-shaped path covering the entire field, for each field surveyed. A total of 24 farmers were interviewed using a content-validated questionnaire that captured bio-data and farm practices. Socio-economic characteristics of the farmers such as sex of farmer, occupation, age, educational qualification, varieties of rice grown, source of seed, years under production, size of farm, presence of pests and diseases, years under production, rice variety most preferred, source of labour for farm operations, farm operations based on cost and production constraints were among the variables considered. An interpreter was used to interpret the questions into local languages where necessary. Eleven farmers' field were surveyed in Kwali Area Council, 8 farmers' field surveyed in Gwagwalada Area Council, two farmers' field surveyed in Abuja Municipal Area Council (AMAC), and three farmers' field surveyed in Bwari Area Council. Field data entry sheet was used to record data and relevant information on sampling and other production variables such as the location of the field, details of the crop grown, types of crop grown in combination with rice on the field, types of symptoms, their severity and the Researcher's identity. Leaf samples were collected from rice plants that showed virus-like symptoms. The leaves were collected from the top, middle and lower portion of each plant. A total of 360 leaf samples were collected. The leaf samples were placed in specimen bottles containing Calcium Chloride ($CaCl_2$) and labeled appropriately based on the locations. Disease incidence of the surveyed fields was estimated by counting the number of plants that expressed virus-like symptoms as described by Odedara et al. (2008). Each symptom type was recorded and expressed as a percentage of the total number of plants (15 per field) that was assessed.

$$\text{Disease incidence (\%)} = \frac{\text{No of symptomatic plants}}{\text{Total no of plants sampled}} \times 100$$

Disease severity was determined by visual assessment and using a modification of the Standard Evaluation System (SES) of International Rice Research Institute (IRRI, 2002) on a scale of 1-9. Where 1 = No symptom observed, 3 = Leaves green but with sparse stripes and less than 5% symptoms on leaves, 5 = Leaves green or pale green with crinkling and 6-25% symptoms on leaves, 7 = Leaves pale, yellow and 26-75% symptoms on leaves, 9 = Leaves turn yellow or orange with more than 75% symptoms on leaves and some plants dead.

$$\text{Disease severity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{No of plants assessed}} \times \text{maximum score (100)}$$

The extraction of Total Nucleic Acid (TNA) was carried out using the CTAB protocol (Chen et al., 2005). A 100mg of sampled rice leaf was grinded in sterile mortar and pestle containing 1000µL CTAB buffer. A 750µL of the homogenate was transferred into sterile 2ml Eppendorf tubes, vortexed briefly and incubated in water bath at 60°C for 10mins. Equal volumes (750µL) of phenol, chloroform and iso-amyl alcohol were added to the tubes at 25:24:1 and carefully mixed by inversion for 5-10 times. The mixture was centrifuged at 12,000g

for 10mins. A 450µL of the supernatant was later transferred into new sterile tubes. A 300 µL of ice cold isopropanol was added to precipitate the DNA/RNA. The tubes were mixed gently and incubated at -20°C for 1 hour, then centrifuged at 12,000g for 10mins to sediment the nucleic acid. The supernatant was decanted gently to avoid disturbing the pellet. A 500µL of 70% ethanol was added into the tubes and centrifuged at 12,000g for 5mins to wash the pellet. The ethanol was carefully decanted without disturbing the pellet and tubes were allowed to dry at 37°C for 30mins to remove final traces of ethanol. The pellets were suspended in 50µL TE buffer and stored in refrigerator at -20°C for further use. Precautionary measures such as use of autoclaved solution, glasses, plastic ware and use of disposable gloves were put in place so as to prevent contamination of reagents and equipment which can be a major cause of poor amplification of nucleic acids.

The quality of the extracted nucleic acid sample was checked by resolving on 1.5% agarose gel electrophoresis. The agarose gel was prepared by mixing 1.5g agarose powder in 100ml TAE (Tris Acetic Ethylene-diamine-acetic) buffer pH 7.4, microwaved for 5 minutes, cooled and 5µL/100ml of DNA stain (EZ Blue light vision, VMR Life Science, USA) was added. The molten gel was then poured into a gel tray fixed with appropriate comb and left to solidify at room temperature for 20 minutes. The solidified agarose gel was placed in electrophoretic tank containing TAE buffer pH 7.4 and 4 µl of each stock nucleic acid sample was pre-mixed with 3 µl loading dye and loaded into a well of the gel. A 100 base pair RNA ladder (Biolab, USA) was loaded in a separate well to calibrate sample movements and verify as a control. The samples were resolved at 120 volts for 40 minutes and gel visualized under UV transilluminator (EZ imager, Bio-Rad, Inc, USA). Also, the quantitation of extracted nucleic acid was analysed using NanoDrop (Wilmington, DE, USA) spectrophotometer 2000.

Rice Stripe Necrosis Virus is an RNA virus, and synthesis of cDNA of the viral genome by Reverse-Transcription (RT) is necessary before the target DNA sequence is amplified. The crude RNA extracts from the leaf samples was used as template for simplified RT-PCR. A 12.5µL reaction mix was prepared in PCR tubes. The mix contained 2.5µL green buffer, 0.25µL dNTPs, 0.75µL $MgCl_2$, 0.25µL each of RSNV primers forward and reverse, 0.06µL Taq RNA polymerase (Promega Corporation, USA), 0.06µL RT, 5.88µL sterile distilled water and 2.0µL RNA template. Extract from known virus-free leaf (cowpea leaf) was used for negative control, while extract from known virus-infected plant (rice leaf) was used as positive control for each reaction set up. The PCR tubes were placed in the thermal cycler (EASTWIN, Inc) for amplification. The amplification profile for the RNA is as follows: 1 cycle of 30 mins at 44°C for denaturation, 1 cycle of 5 mins at 95°C, 95°C for 30sec, primer annealing at 55°C for 1min, 72°C for 1 min for 35 cycles and then finally primer extension was carried out at 72°C for 10mins for 1 cycle. The primer

sequences were as follows: RSNV1-2901F (5'-TGAATTTGGTGCTCTCTTG-3'), RSNV1-3827R (5'-TGTGGCGTTTCCAGACCTAAA-3'), Oludare *et al.*, (2015). Agarose gel electrophoresis was prepared using 2% gel stained with EZ blue light vision. The amplified products were resolved on 1.5% agarose gel at 120volts for one hour and observed under UV light. Incidence and severity was calculated based on the number of plants sampled during the survey. Percentage disease incidence and mean severity were calculated. All the data obtained from the survey were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) version 17. Mean separation was done with Duncan Multiple Range Test.

Results and Discussion
Table 1 shows the location of farm fields with their corresponding Latitude (N), Longitude (E) and Elevations.

Table 1: Surveyed areas, field locations, latitude, longitude and elevation in Federal Capital Territory, Abuja, Nigeria

State	Area Council	Village	Latitude N	Longitude E	Elevation
FCT	Kwali	Yangoji	8°43'14.0"	6°59'28.5"	174m
		Abaji	8°53'31.63"	6°49'5.38"	60m
		Pukafa	8°40'17.2"	6°54'55.3"	87m
		Sheda	8°53'9.0"	7°3'57.0"	198m
	Gwagwalada	UniAbuja	8°58'47.4"	7°10'22.9"	273m
		Tunga-mage	9°59'35.5"	7°02'04.3"	179m
		Dubi	8°59'35.5"	7°02'04.3"	179m
	AMAC	Ido	8°57'60.0"	7°15'10.0"	520m
	Bwari	Bwari	9°17'08.2"	7°22'43.3"	152m

Table 2 shows the distribution of incidence and severity of virus-like symptoms that were observed during the field survey in FCT. From the four locations that were surveyed in Kwali Area Council, Pukafa recorded the highest percentage incidence (46.6%), while Sheda recorded the lowest (19.9%). In Gwagwalada Area Council, Tunga-mage and Dubi recorded the highest percentage incidence (37.7%), while UniAbuja recorded the lowest with 29.9%. One location was surveyed each in AMAC and Bwari Area Council, and each had a percentage incidence of 13.3% and 35.5% respectively. The highest percentage severity in Kwali Area Council was recorded in Pukafa (33.3%), while the lowest was recorded in Yangoji and Sheda (20%). Although, there was no significant difference in the severities observed. Dubi recorded a percentage severity of 37.7%, which was the highest in Gwagwalada Area Council, while UniAbuja recorded the lowest (26.7%). Ido in AMAC Area Council recorded a percentage severity of 20%, and Basango in Bwari Area Council recorded 24.4%.

Table 2: Distribution of incidence and severity of virus-like symptoms of rice observed during the field survey in the Federal Capital Territory, Abuja, Nigeria

State	Area Council	Village	No of farms	Percentage Incidence	Percentage Severity
FCT	Kwali	Yangoji	3	28.9[ab]	20.0[a]
		Abaji	3	28.8[ab]	24.4[a]
		Pukafa	3	46.6[a]	33.3[a]
		Sheda	2	19.9[ab]	20.0[a]
	Gwagwalada	UniAbuja	2	29.9[ab]	26.7[a]
		Tunga-mage	3	37.7[ab]	28.9[a]
		Dubi	3	37.7[ab]	37.7[a]
	AMAC	Ido	2	13.3[b]	20.0[a]
	Bwari	Basango	3	35.5[ab]	24.4[a]

Means in the same column with different alphabets are significantly different at P ≤ 0.05

Good integrity test result was obtained to show the quality of extracted total nucleic acid (TNA) from the selected leaf samples. Using the spectrophotometer to analyze the purity of the extracted nucleic acid, the ratio of the absorbance at 260 nm to the reading at 280 nm (A_{260}/A_{280}) of most of the leaf samples was within 1.7-2.0, while the A_{260}/A_{230} was greater than 1.5 (Table 3), and this indicated good quality TNA. The highest DNA purity value for A_{260}/A_{280} was 2.12 (sample 7), and the lowest 1.58 (sample 9), while for A_{260}/A_{230}, the highest value was 2.43 (sample 4), and lowest 1.09 (sample 1).

Also, after visualizing the extracted nucleic acid in the agarose gel, under the UV transilluminator, the DNA and RNA bands were clearly seen, which indicated that the nucleic acid were isolated in pure form and sufficient amounts (Table 3). The extracted nucleic acid of the leaf samples were then used as templates in RT-PCR amplification. After the amplified product was analyzed on 1.5% agarose gel 1 TAE buffer stained with ethidium bromide and observed under UV light at 120volts for one hour, there were no strong bands observed from the RT-PCR reaction.

Table 3: Quality and quantity check by NanoDrop spectrophotometer 2000

Sample ID	Date and Time	Nucleic Acid	Unit	260/280	260/230	Sample Type
1	14/11/2019 15:27:12	303.9	ng/µl	1.85	1.09	DNA
2	14/11/2019 15:27:43	272.4	ng/µl	2.1	2.18	DNA
3	14/11/2019 15:28:37	1529.7	ng/µl	2.11	2.19	DNA
4	14/11/2019 15:29:10	955.2	ng/µl	2.08	2.43	DNA
5	14/11/2019 15:29:38	283	ng/µl	2.05	2.18	DNA
6	14/11/2019 15:30:13	591.1	ng/µl	2.05	2.26	DNA
7	14/11/2019 15:30:36	2374.3	ng/µl	2.12	2.34	DNA
8	14/11/2019 15:31:02	1241.2	ng/µl	2.05	2.33	DNA
9	14/11/2019 15:31:39	2569.7	ng/µl	1.58	1.04	DNA
10	14/11/2019 15:35:15	4363.3	ng/µl	2.09	2.08	DNA
11	14/11/2019 15:35:42	518	ng/µl	2.06	2.11	DNA
12	14/11/2019 15:36:16	997.6	ng/µl	1.91	1.6	DNA
13	14/11/2019 15:37:12	3769.4	ng/µl	2.06	1.96	DNA
14	14/11/2019 15:38:39	524.8	ng/µl	2.1	2.15	DNA

This research provides information on determining the incidence and severity of RSNV infection on rice in FCT, Nigeria. The samples collected from the survey were indexed for RSNV. From the 360 symptomatic leaf samples collected from the four Area Councils surveyed in the FCT, and indexed for RSNV using molecular diagnosis (RT-PCR), none of the samples tested positive to RSNV, as strong bands were not observed from the reaction. This result was recorded despite some farmers claiming to have planted saved seeds from previous planting season. The negative result recorded after indexing for RSNV, may be attributed to rainfall, as it has been reported that weather conditions can affect distribution of viruses in different ecosystems (Shoyinka *et al*., 1997), thereby contributing to low source of inoculum (Eric *et al*., 2019). The absence of RSNV may also be attributed to resistance by some rice varieties as reported by Correa (2002). Despite not detecting RSNV in the samples tested, infection have been recently reported in several West African countries including Mali, Republic of Benin, Sierra Leone and Burkina Faso (Decroes *et al*., 2017; Tucker *et al*., 2020; Sereme *et al*., 2014), which suggests re-emergence of the virus in West Africa. Surveys conducted at AfricaRice Cotonou, Benin by Oludare (2015) in March 2014, indicated that 106 rice plants out of 119 rice plants were infected with RSNV. The survey was carried out after the death of some rice plants from the AfricaRice research field due to severe attacks in 2012 and 2013; this was the first report of the disease in the Republic of Benin. Other viruses not tested for, may have been responsible for the virus-like symptoms observed on the collected samples. Plants infected with other viruses sometimes may show similar symptoms, and this can limit the accuracy of visual based diagnosis (Gergerich *et al*., 2006; Yang *et al*., 2017). Plants can also show virus-like symptoms as a result of other pathogen infections, nutritional deficiencies, unfavourable weather amongst others (van der Want *et al*., 2006).

Conclusion
Earlier investigation indicated that there was no available research on the survey of incidence and severity of viruses infecting rice in the FCT. This study to determine the possibility of RSNV infecting rice in the FCT is important in order to prevent damages that could limit rice production in the FCT. This research provides the first information on RSNV in the FCT. Based on the results and discussion, and with the knowledge that viruses are not stable, further survey is recommended for the FCT and other parts of Nigeria.

Conflict of Interest
The authors have not declared any conflict of interests.

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