Short Research Article

Survey and serological identification of viruses infecting maize production in Cross River State, Nigeria

Abstract

Aim: The study was designed to survey the agro-ecological zones of the state where maize is cultivated and identify the viruses constituting a constraint to its production.
Place and duration of study: Cross River State in thesouth-south zone of Nigeria. A study wasconducted between April and June, 2022.
Methodology: A total of 102 symptomatic samples were collectedfrom 18 locations in 9 local government areas of Cross River State to determine the disease incidence of the virusand ACP-ELISAwas used for serological identification of the virus.
Results: Out of the 102 samples collected, none reacted positively with either the *Maize chlorotic mosaic virus* (MCMV) or the*Maize dwarf mosaic virus* (MDMV)antiserum.However, 62 of the samples reacted positively to the *Maize streak virus* (MSV) representing 60.8 % of the collected samples. Disease incidence of the virus was highest in the Odukpani local government area with 71 % followed by Akpabuyo local government area with 69.2 % while Bekwarra local government recorded the lowest disease incidence with 30 %. With a moderate incidence of the virus recorded, there is a need for constant surveillance and farmer education to forestall an epidemic that may occur.
Conclusion: MSV was found to infect the crop in all three agro-ecological zoneshowever both southern and central agro-ecological zones recorded higher disease incidence than the northern zone.

Keywords: Disease incidence; Maize streak virus; ACP-ELISA; Maize Lethal Necrosis Disease**;** Surveillance

Introduction

The crop Maize (*Zea mays* L.) has been described by Rashid *et al.*(2020) as being the world's leading cereal crop in terms of production, with 1016 million metric tons(MMT) produced on 184 million hectares (Mha) globally and the third most important cereal crop in the world after rice and wheat (IITA, 2005). Maize, also called corn is known by different vernacular names in Nigeria such as 'oka' in Ibo; 'agbado' in Yoruba; 'masara' in Hausa; and 'ibokpot' in Efik/Ibibio.

It is a delicacy as its cob is eaten boiled, roasted, or prepared into several dishes. The immature and fresh cobs are reportedly rich in vitamins A and C andsince maize is also rich

in carbohydrates, it is considered a staple for millionsof people in Africa and is also a major ingredient in livestock feed formulations (Romney *et al.*, 2003). Iken and Amusa (2004)reportedthat the production of maize is steadily on the rise and many agro-based industries depend upon its production as raw material. The crop production of maize in the country is 12.7 million metric tonnes with a cultivated land of 4.9 million hectares with an average production of 2.1 million metric tonnes per hectare (USDA, 2021; FAO, 2018). This index places Nigeria as the largest producer of Maize in Africa.

Tadele (2017) reported that the yield of maize is constrained by both abiotic and biotic factors. Some of the abiotic constraints to yield include poor farming practices, floods, disease outbreaks,and drought (Morris *et al.,* 1999).Among the biotic factors, those that have been implicated to having good maize yields are insect pests and diseases (Kono *et al.* 2018).

Among the pathogens responsible for reducing crop yields, Fajemisin (2003) noted that viruses are one of the major pathogens limiting crop yields in sub-Saharan Africa. Many maize viruses are known to be transmitted by insect pests, which is the reason for widespread transmission and epidemic of some plant virus diseases. Also, Fajinmi *et al.* (2012) reported that the incidence of viral diseases causes great yield loss in crop production in Nigeria.Virus and viral diseases of maize as reported by Offei *et al.* (2001) in sub-Saharan Africa include *Maize streakvirus* (MSV), *Maize dwarf mosaic virus* (MDMV), *Maize chlorotic mottle virus* (MCMV), *Sugarcane mosaic virus* (SCMV), *Maize chlorotic dwarf virus*(MCDV), *Barley yellow dwarf virus* (BYDV), *Brome mosaic virus* (BMV), *Maize mosaic virus* (MMV), *Maize stripe virus* (MStpV) and *Guinea grass mosaic virus* (GGMV). Maize streak virus (MSV) disease caused by maize streak geminivirus is responsible for sporadic but severe outbreaks in maize (*Zea mays*L.) in sub-SaharanAfrica.

The virus MSV is a geminivirus, indigenous to Africa and is transmitted persistently by leafhoppers of the genus *Cicadulina*(Homoptera: Cicadellidae). The virus is widely

distributed in sub-Saharan Africa from Sudan to South Africa and Kenya to Senegal and in adjacent islands, including Mauritius, Reunion, Madagascar, Sao Tome and Principe (Thottappilly *et al.*, 1993).

Information on the different maize viruses occurring in Nigeria is still not comprehensive, especially ontheir etiology, diversity, and biology, and in Cross River State, virus disease on maize is largely undocumented except by Salaudeen *et al.* (2018). This is important for adequate management measuresfor better productivity for farmers. It is for this reason thatthis study is embarked upon to ascertain and identify the specific viruses infecting maize, their symptomatology and geographic distribution in Cross River State. This is aimed at aiding researchers in their understanding of viruses infecting maize and also contributing todata for the development of resistantvarieties by breeders.

Materials and Methods

Field survey and sample collection

Two maize fields inthe local government area were surveyed between April and June, 2022.Visible symptoms observed include chlorotic patches, severe streaking, venation along midrib and vein, and stunted growth (Fig. 1). The locations were namelyAtan-Onoyom and Okoyong in Odukpani local government area, Ikot Ambai and Esighi in Akpabuyo local government area, Obum and Adim in Biase local government area, Ofodua and Iyamoyong in Obubra local government area,Abijang and Etomi in Etung local government area, Okundi and Bumaji in Boki local government area, Idum and Benkpe in Ogoja local government area, Afrike and Beten in Bekwarra local government area with the last locations being Okuku and Echumoga in Yala local government area (Table 1).

The identification of the disease was based on visual symptoms observed on the plant as described by CIMMYT (2004). A total of 102 leaf samples collected from the field with threefrom each location were pooled together and composite samples were adequately

labelled and preserved in vial bottles containing silica gelbefore being taken to the laboratory for further analyses.

Figure 1: Leaf showing severe streaks of viral infection

Disease incidence

The disease incidence was evaluated using the technique adopted by Mbong *et al*., (2021) and incidence was scored as the presence or absence of virus disease symptoms using a rating scale where low incidence = 1%–20%; moderate incidence = 21%–49%; and high incidence = 50%–100%.

The percent disease incidence was calculated by using the following formula:

$$\text{Disease Incidence (\%)} = \frac{X_0}{X_1} \times 100$$

X_0 = Number of plants suspected to be infected

X_1 = Total number of plants

All the leaf samples collected from the field were subjected to Antigen Coated Plate-Enzyme Linked Immunosorbent Assay (ACP-ELISA) as described by Kumar, (2009), for the presence of the virus using polyclonal antibodies specific for *Maize streak virus* (MSV), *Maize chlorotic mosaic virus*(MCMV) and *Maize dwarf mosaic virus* (MDMV).

Virus detection by enzyme-linked immunosorbent assay (ELISA)

The leaf samples were subjected to antigen coated plate enzyme-linked immunosorbent assay according to the protocol of Kumar (2009). Samples were ground in the sterilized mortar at the rate of 100 mg/mL using cold carbonate buffer pH 7.4 (0.015 M sodium carbonate plus 0.0349 M sodium bicarbonate per litre of distilled water). One hundred microlitres of the leaf extract were added to each well of the microtitre plates (Thermo Scientific "Nunc", Milford, MA). The plates were incubated at 37^0C for 1 hour and washed three times with phosphate-buffered saline-Tween (8g NaCl, 1.1 g Na2HPO4, 0.2 g K2HPO4, 0.2 g KCl, 0.5 mL Tween – 20, 1 L distilled water, pH 7.4) (PBS-T). A blocking solution [3 % (w/v) dried non-fat skimmed milk in PBS – T] was applied at the rate of 200 µL per well. This was followed by incubation of the plates at 37^0C for 30 minutes. The plates were tap-dried on a paper towel. Polyclonal antibodies (PAbs) for MCMV, MDMV and MSV were diluted (1:2000; v/v) with conjugate buffer [half strength PBS-T containing 0.05 % (v/v) Tween-20, 0.02 % (w/v) egg albumin, 0.2 % (w/v) polyvinylpyrrolidone, and 100 µL each were tested against the extract of each sample. Plates were incubated again at 37^oC for 1 hour, washed thrice and 100 µL of the goat antirabbit antibody diluted with conjugate buffer (1:15,000) was added to the wells. The plates were incubated at 37^0C for 1 hour and washed. Afterward, 100 µL of *p*-nitrophenyl phosphate dissolved in substrate buffer (97 mL diethanolamine, 1000 mL H2O, pH 9.8) was added to the well. The plates were finally incubated in the dark at room temperature (37^0 C) overnight. The absorbance of virus concentration was recorded at 405_{nm}using a microplate reader (MRX, Dynex Technologies, Inc., USA). The values of the readings obtained were considered positive when the optical density reading at A_{405nm} was twice the value of the healthy control.

Results and Discussion

Samples were collected from the north (Ogoja, Bekwarra, and Yala), central (Boki, Etung, and Obubra) and southern (Akpabuyo, Odukpani, and Biase) zones of the state with three local government areas per zone (Table 1).

Table 1: Local Government Areas and towns where samples were collected

Local government area (LGA)	Location	Latitude (N)	Longitude (E)
Akpabuyo	Esighi	4^0 49’ 23”	8^0 27’ 20”
	Ikot Ambai	4^0 56’ 00”	8^0 26’ 19”
Odukpani	Atan Onoyom	5^0 19’ 45”	8^0 00’ 22”
	Akpap Okoyong	5^0 06’ 21”	8^0 10’ 39”
Biase	Adim	5^0 44’ 02”	8^0 02’ 18”
	Obum	5^0 45’ 50”	7^0 56’ 44”
Obubra	Ofodua	5^0 58’ 06”	8^0 21’ 17”
	Iyamoyong	5^0 58’ 57”	8^0 15’ 30”
Etung	Abijang	5^0 49’ 16”	8^0 42’ 29”
	Etomi	5^0 57’ 24”	8^0 47’ 49”
Boki	Okundi	6^0 24’ 48”	8^0 47’ 56”
	Nsadop	6^0 19’ 43”	8^0 49’ 26”
Ogoja	Benkpe	6^0 34’ 24”	8^0 55’ 47”
	Idum	6^0 31’ 49”	8^0 53’ 57”
Bekwarra	Afrike	6^0 36’ 38”	8^0 51’ 42”
	Beten	6^0 45’ 26”	8^0 56’ 29”
Yala	Echumoga	6^0 46’ 33”	8^0 47’ 13”
	Okuku	6^0 43’ 12”	8^0 45’ 46”

Incidence of maize viral diseases: A total of 102 samples were collected from 36 maize field locations spread across the state. For the maize fields visited in each location of the 9 local government areas, commonly observed symptoms included broken chlorotic stripes, pale circular patches, necrosis, mild chlorotic mottling, chlorosis, and sometimes severe stunting of the leaves and stems.

Table 2: Disease incidence of Maize streak virus and Maize chlorotic mottle virus on Maize in Cross River state

Local government area (LGA)	Number of samples	Virus	Virus detection	Mean incidence rate (%)
Akpabuyo	13	MSV	9	69.2
		MCMV	0	0.00
Odukpani	14	MSV	10	71.4
		MCMV	-	0.00
Biase	15	MSV	9	60.0
		MCMV	-	0.00
Obubra	11	MSV	6	54.5
		MCMV	-	0.00
Etung	11	MSV	7	63.6
		MCMV	0	0.00
Boki	16	MSV	10	62.5
		MCMV	-	0.00
Ogoja	10	MSV	5	50.0
		MCMV	-	0.00
Bekwarra	10	MSV	3	30.0
		MCMV	-	0.00
Yala	9	MSV	3	33.3
		MCMV	-	0.00

Across the nine local government areas,a total of 62 samples were symptomatic from the 102 samples representing 60.8 % of the samples tested, with no detection of either *Maize lethal chlorotic virus* caused by a synergistic relationship between*Maize streak virus*(MSV) and *Maize chlorotic mottle virus* (MCMV) belonging to the genera of Mastrevirus (Geminiviridae) and Machlomovirus (Tombusviridae) respectively.

The highest mean incidence obtained for MSV was 71.4 % in Odukpani LGA while the next highest mean incidence figures of 50.0 % were recorded in both Odukpani and Ogoja LGAs. The least mean incidence of 30 % of the virus was recorded in Bekwarra LGA.

Using the Were *et al.* (2004) scale for rating incidence, the results revealed a predominantly moderate incidence of the virus in all locations except in the Odukpani and Ogoja local governments areas where a high incidence was recorded.

Serological detection of Maize streak virus and Maize chlorotic mottle virus

Out ofa total of 102 samples collected, the ELISA serological assay was able to detect the presence of *Maize streak virus* (MSV) in 62 samples.The results of the ELISA test on the samples collected from the fields are presented in Table 3.

Table 3: Serological detection of *Maize chlorotic mottle virus* and *Maize streak virus* using enzyme-linked immunosorbent assay

LGA	Location	Mean MCMV ELISA values	Status	Mean MSV ELISA values	Status
Akpabuyo	Ikot Ambai	0.538	-	1.652	+
	Esighi	0.578	-	2.355	+
Odukpani	Atan-Onoyom	0.587	-	0.490	-
	Akpap Okoyong	0.621	-	0.886	+
Biase	Adim	0.332	-	1.572	+
	Obum	0.549	-	0.528	-
Obubra	Ofodua	0.268	-	1.885	+
	Iyamoyong	0.762	-	2.123	+
Etung	Abijang	0.664	-	0.751	-
	Etomi	0.442	-	0.479	-
Boki	Okundi	0.389	-	0.661	-
	Nsadop	0.257	-	0.339	-
Ogoja	Benkpe	0.635	-	0.850	+
	Idum	0.716	-	0.969	+
Bekwarra	Afrike	0.664	-	1.048	+
	Beten	0.558	-	0.746	-

Yala	Echumoga	0.411	-	0.257	-
	Okuku	0.590	-	0.448	-
Disease		0.863	+	1.398	+
Healthy		0.392	-	0.412	-

*Values of virus isolates were considered virus positive when the optical density (OD) reading at A405nm was 2x greater than the absorbance from healthy controls.

The mean values for MCMV in all of the locations tested reacted negatively with the antiserum however for MSV, mean valuesranged from 0.850 to 2.355 at A_{405} nm in nine locations,and spread across six local government areas resulted in a positive reaction with MSV polyclonal antiserum. A negative reaction was obtained for the other locations tested indicating an absence of the virus.

Maize fieldsvisited during the study were predominantly backyard gardens cultivated for subsistence purposeswhile others were small holdings usually less than one and a half hectares in size.As maize is a staple, the northern, central, and southern agro-ecologicalzones of the state are used for the cultivation of the crop,andthe incidence of maize streak disease varied across the different agro-ecological zones.The highest mean incidence was obtained in the southern agro-ecological zone while the least mean incidence was obtained in the northern agro-ecological zone. The high incidence obtained for the southern agro-ecological zone with a prevalent high humidity and temperature is in agreement with the findings of Matthew *et al.* (2005) who reported that viruses were more virulent with a prevalent high temperature and humidity.

Although the presence of MDMV, MSV, and MCMV was tested on the samples, the dominant virus infecting maize in Cross River State was found to be MSV. The absence of MDMV and MCMV on the samples as a result of a negative reaction to the polyclonal antiserum of MCMV and MDMV was a good indication that Cross River State was free from Maize lethal necrotic disease (MLND). This is similar to the findings by Salaudeen *et al.*

(2018) in his survey of viruses infecting the maize crop in Kano, Cross River, Katsina, and Akwa Ibom State.The use of serology as a tool forthe detection of plant viruses has been demonstrated for many viruses including potyviruses and Cucumber mosaic virus (Ekpiken *et al*., 2021; Eyong *et al.*, 2020; Eyong *et al*., 2020a). The results further show that the Maize streak virus is the dominant virus occurring in Nigeria as reported by several authors. Taiwo *et al.* (2006)had earlier reported the infection of the plant by Maize mottle/chlorotic stunt virus (MMCSV) and MSV in Lagos. Akinbode *et al.* (2014) also reported that the prevalent virus disease of maize in Oyo, Osun, Ondo, Ekiti, and Kwara states was MSV, and studies by Fajinmi *et al.* (2019) in Ekiti corroborated these findings.

Conclusion

This study has established the prevalence of MSV in Cross River State and also the absence of MCMV and MDMVwithin maize fields. The disease was found to infect all fields surveyed even though the highest incidence of 71.4 % was recorded in Odukpani local government while the lowest incidence of 30 % was recorded in Bekwarra local government. Clean and resistant seeds that have been certified to be streak resistant are to be deployed to locations with high incidence in order to prevent an epidemic.

To control the disease and prevent a higher incidence of MSV from occurring, numerous options abound. Firstly, continuous surveillance should be maintained such that any form of an outbreak could be easily curtailed. Also, breeders are encouraged to produce disease-resistant and insect-resistant seeds that can be made available to the farmers. Insecticides as well as a host of cultural practices have been suggested for the control of MSV disease or its vectors (Charles, 2014, Redinbaugh and Zambrano 2014; Martin and Shepherd 2009), however, resistance breeding is perceived as the most practical solution for disease control. Breeding for resistance has long been an effort of the International Institute of Tropical Agriculture (IITA) and maize varieties that combine resistance to MSV with other desirable

characteristics have been developed at the Institute. Many IITA open-pollinated varieties and hybrids exhibit reduced virus severity combined with low field disease incidence (Soto *et al*., 1982; Asanzi *et al*., 1994).

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