Original Research Article

Serological detection of viruses infecting Okra in Cross River State, Nigeria

ABSTRACT

Okra (*Abelmoschus esculentus* L.) also known as lady's finger in England is an important vegetable crop of the family Malvaceae growing throughout the tropical and warm temperate regions of Asia, Africa, and the USA for food, fibre, Gum, starch, spices, and medicinal purposes. The viral disease is a major constraint to okra production worldwide. A visit to some okra farms during the 2022 planting season revealed evidence of wide spread virus infection with infected crops exhibiting mosaic, mottling, and leaf malformation resulting in poor yield. Thirty (30) infected leaf samples were collected from different okra fields and tested using virus-specific antisera which included Cucumber Mosaic Virus (CMV), Okra Leaf Curl Virus (OkLCV), Okra Mosaic Virus (OkMV), and Black eye Cowpea Mosaic Virus (BiCMV). The results revealed that out of the 30 samples tested, 15 tested positive for CMV which constitutes 50% of the total virus identified followed by OkMV and BiCMV which infected 6 samples for each virus constituting 20 % of virus detected, and OkLMV infected only 3 samples constituting 10 % of total virus detected. This study has revealed that CMV is widespread in Cross River State Nigeria. Reports abound on the detection of okra viruses in northern Nigeria. Furthermore, this is the first report of wide-scale detection of viruses infecting okra in southern Nigeria

Keywords: Okra, ELISA, antisera, virus, mosaic, Mottling

INTRODUCTION

"Okra (*Abelmoschus esculentus* L) also called lady's finger in England is an important Due to higher consumer demand and better appreciation in terms of revenue, it is grown throughout the tropical and warm temperate regions of Asia, Africa, and the USA. In Nigeria, it is a source of revenue for local and peasant farmers who grow it to raise income to support their families. It is used industrially as a fibre and for food as a vegetable. Gum, starch, spice, and medicinal products can be obtained from it" [5].

"There is a recent outbreak of severe leaf curl disease of okra which has posed a serious threat to the okra cultivation by taking an epidemic proportion in Nigeria. Okra leaf curl disease is emerging and serious disease okra in Nigeria. The yellow vein mosaic disease was first reported in India" [13], "subsequently reported in other parts of the world" [20]. "The prominent symptoms of plants infected with these viruses are vein clearing followed by its yellowing which will finally lead to a yield loss of about 96% by the reduced size of leaves and fruits" [18].

Over 27 viruses have been reported to infect this crop resulting in significant yield losses. Among the viruses, *Okra mosaic virus* (OkMV; genus *Tymovirus*) has been reported to be economically important causing one of the most important diseases of the crop [15]. "It elicits mosaic and yellowing symptoms interspersed with green islands on okra leaves and infected fruits develop chlorotic flecks" [2]. "A yield loss of 10 to 80% is caused by the virus [3] and loss incidence may reach 100% before harvest" [7]. "In Tanzania, OkMV incidence of 30 to 89% has been reported" [15]. "The virus is not seed transmitted (Koenig and Givord, 1974), but it is mainly transmitted by the beetles of *Podagrica*spp". [15].

"An earlier study the on the viral disease of okra and its casual gent revealed that the diseases are caused by a complex consisting of a begomovirus, Bhendi yellow vein mosaic virus (BYVMV), and a beta satellite molecule" [11]. However, "a DNA-β component was successfully amplified using a pair of universal primers specific to a highly conserved region found in all DNA-βsequences. In addition, it has been observed that the progenies of the cloned BYVMV DNA and DNA-β were transmitted by white flies to okra for BYVMV disease symptoms" [11]. Few of these viruses have been characterised and reported in Nigeria, for example. [4]. and [19] have reported the presence of Okra mosaic virus infecting okra in northern Nigeria. The gradual increase in the incidence of severe viral disease of okra plants is also highly observed in farmer's field in Cross River State, Nigeria but there is no information on the emerging viral disease associated with okra reported Southern Nigeria, the present study is designed to diagnose and identify viruses infecting okra in Cross River State southern Nigeria. ELISA test was employed as a diagnostic tool in this study.

MATERIALS AND METHODS

Collection of sample

A survey of viral diseases in different Okra farms was conducted during the 2022 farming season. Farms surveyed included Abanwam, Erei, Ikot-Efanga, Isong Inynag, Old Netim, Oban, Biakpan, Betem, Umon, Okurike, Adim in Southern Senatorial zone, Boje, Buda, Buentsebe, Alankwu, Abo, Agbokim, Etomi, Itaka, Nde in Central Senatorial zone and Basang, Becheve, Utanga, Ekajuk , Mbube, NkumIborr, Gabu, Ijiraga, Echumofana and Okpoma in Northern zone.

Thirty (30) symptomatic okra leaf samples were collected into Ziploc air tight polyethylene bags to keep them fresh to ensure the viability of the viruses and later transported to the laboratory of National Institute of Horticulture Ibadan Nigeria, for ELISA testing.

Serological tests

Antigen coated plate enzyme linked immunosorbent assay (ACP-ELISA) as described by [14] was employed to test for the presence of virus RNA. Symptomatic leaf samples of 0.1g were triturated in 1mL of coating buffer (0.015M $Na_2 Co_3$ + 0.0349M $NaHCo_3$ + dH_2O) and dispensed into each well of ELISA plate. After incubation at 37°C for 1h the plate was washed 3 times with PBS-Tween for 3 min between each wash. Cross adsorption was made by grinding 1 g of healthy plant sample in 20mL of conjugate buffer (1/2 PBS + 0.05% Tween 20 + 0.02% egg albumin + 0.2% PVP). The samples were tested against Cucumber Mosaic Virus (CMV), Okra Leaf Curl Virus (OkLCV), Okra Mosaic Virus (OkMV) and Black eye Cowpea Mosaic Virus (BiCMV) antisera. The antisera were diluted at 1:3000 in the adsorption solution and 100µL of each antiserum polyclonal antisera were added to wells of the ELISA plates and again incubated at 37°C for 1hour. The ELISA plates were then washed 3 times with PBS-Tween. One hundred-µL of protein, A-alkaline phosphatase conjugate diluted in the ratio 1:15000 in conjugate buffer (1/2 PBS + 0.05% Tween 20 + 0.02% egg albumin + 0.2% PVP + 0.02g $NaNO_3$) was added per well and the plates incubated at 37°C for 1h. The plates were again washed 3 times with PBS-Tween. One hundred-µL of 0.001g·mL^{-1} of *p*-nitrophenyl phosphate substrate in substrate buffer (97mL diethanolamine + 800mL H_2O + 0.2g $NaNO_3$ and HCl to give pH 9.8) was added per well and incubated at room temperature for 1 h. For all incubations, plates were covered with ELISA cover plates to avoid edge effects and to maintain uniform temperature. Healthy plant samples were used as controls. After 1 h absorbance was measured at A_{405nm} using an ELISA plate reader (Micro Read 1000 ELISA plate analyser, U.S.A) after 1 h of incubation. The samples were

considered positive when the ELISA reading was at least twice the reading for the healthy control [14].

RESULTS AND DISCUSSION

A total of 30 symptomatic leaf samples were collected from 30 okra field locations spread across different villages in Cross River State, one sample from each community. Commonly observed symptoms included Mosaic, mild chlorotic, mottling, and leaf malformation. All the samples tested positive for either of the four virus antiserum (CMV, OkMV, OkLCV, and BiCMV). The results revealed that out of the 30 samples tested, 15 tested positive for CMV which constitutes 50% of the total virus identified followed by OkMV and BiCMV which infected 6 samples for each virus constituting 20 % of virus detected,and OkLMVinfected only 3 samples constituting 10 % of total virus detected. The ELISA results showed that CMV was the most predominant virus infecting half of the total samples (Table 1).

Table1: Serological detection of Cucumber Mosaic Virus (CMV), Okra Leaf Curl Virus (OkLCV), Okra Mosaic Virus (OkMV) and Black eye Cowpea Mosaic Virus (BiCMV) in symptomatic Okra leaf Samples

S/N	Sample	Locations	Different Virus antisera/Status							
			CMV ELISA Value	Status	OkMV ELISA value	Status	OkLCV ELISA value	Status	BiCMV ELISA value	Status
1	Okra	Erei	1.749*	+	0.712	-	0.612	-	0.401	-
2	Okra	Ikot-Efanga	0.612	-	0.890*	+	0.612	-	0.612	-
3	Okra	Isonglnynag	0.186	-	0.500	-	0.500	-	1.612*	+
4	Okra	Old Netim	1.105*	+	0.712	-	0.712	-	0.712	-
5	Okra	Oban	1.749*	+	0.712	-	0.632	-	0.212	-
6	Okra	Biakpan	0.611	-	0.132	-	1.749*	+	0.632	-
7	Okra	Betem	1.749*	+	0.512	-	0.612	-	0.412	-
8	Okra	Umon	0.400	-	0.124	-	1.149*	+	0.112	-
9	Okra	Okurike	0.500	-	1.749*	+	0.741	-	0.549	-
10	Okra	Adim	0.449	-	1.749*	+	0.249	-	0.441	-
11	Okra	Boje	0.700	-	0.241	-	0.442	-	1.749*	+
12	Okra	Buda	0.760	-	0.552	-	0.352	-	1.270*	+
13	Okra	Buentsebe	1.289*	+	0.752	-	0.552	-	0.652	-
14	Okra	Alankwu	0.240	-	0.541	-	0.341	-	1.949*	+
15	Okra	Abo	1.370*	+	0.241	-	0.541	-	0.641	-
16	Okra	Agbokim	0.701	-	0.601	-	0.521	-	1.949*	+
17	Okra	Etomi	0.970*	+	0.160	-	0.060	-	0.260	-
18	Okra	Itaka	1.610*	+	0.110	-	0.210	-	0.310	-

29	Okra	Nde	1.570*	+	0.281	-	0.012	-	0.470	-
20	Okra	Basang	0.121	-	0.970*	+	0.170	-	0.100	-
21	Okra	Becheve	0.480	-	0.701	-	1.470*	+	0.170	-
22	Okra	Utanga	0.100	-	0.470	-	0.410	-	0.770*	+
23	Okra	Ekajuk	1.210*	+	0.180	-	0.320	-	0.260	-
24	Okra	Mbube	0.170	-	1.210*	+	0.170	-	0.270	-
25	Okra	NkumIborr	0.100	-	0.970*	+	0.100	-	0.210	-
26	Okra	Gabu	1.271*	+	0.101	-	0.402	-	0.611	-
27	Okra	Ijiraga	0.270	-	0.270	-	0.270	-	0.970*	+
28	Okra	Echumofana	1.170*	+	0.470	-	0.220	-	0.373	-
29	Okra	Okpoma	1.470*	+	0.770	-	0.610	-	0.272	-
30	Okra	Abanwan	1.070*	+	0.270	-	0.270	-	0.270	-
	Healthy		**0.405**		**0.405**		**0.405**		**0.405**	
	Diseased		**1.894**		**1.894**		**1.894**		**1.894**	

***Values of virus isolates were considered virus positive when the optical density (OD) readings at A_{405nm} was 2x greater than the absorbance from healthy controls.**

Farmers are continually developing a stronger interest in okra production given its potential as an economic crop and its ability to grow optimally in the absence of fertilizers and also its ability to produce promising yields within a short period of time. However, there are several factors such as pathogens, pests, and very important diseases such as viruses that hinder the realization of these intended objectives. Most plant viruses depend on vectors for their survival and spread, a most effective way of controlling viruses could therefore be by the use of herbicides, synthetic insecticides, and cultural control of weeds that would interfere with vector landing feeding.

Okra fields visited during the study were mostly backyard gardens cultivated for subsistence purposes while others were small holdings usually less than one and a half hectares in size. Okra is an important vegetable crop and is now widely cultivated in different parts of the world including Nigeria. It is used industrially as a fibre and for food as a vegetable. Gum, starch, spice, and medicinal products can be obtained from it. The result of this study has revealed a widespread distribution of CMV infecting okra across the three senatorial zones in the state. *Cucumber mosaic virus* has been reported to possess widespread infection of plants across almost all plant families [13]. This explains the reason for 50 % infection of the total samples tested. The wide spread of CMV is also attributed to the prevalent high humidity and temperature which is in agreement with the findings of [16] who reported that viruses were more virulent with a prevalent high temperature and humidity. BiCMV and

OkMV also revealed widespread infection of samples across the three senatorial zones but with few infected samples.

A report by [5, 21] also suggests that the epidemiology of BiCMV and OkMV can be hinged on the premise of early rains with intermittent dry and wet conditions which isthe characteristic weather condition in these areas and can favour the spread of OkMV and BiCMV, while abundant vectors and alternate host can be reliable agents in the transmission of these viruses.

The use of ELISA has been reported as one of the most reliable methods in plant virus detection [14]. [8] have further reported thatthis method provides a simple and rapid means of detecting and identifying viruses in crude sap extracts with the aid of virus antisera. [9, 10] have employed the use of ACP-ELISA in the detection of plant viruses infecting cucurbits in Cross River State. Finally, the endemic nature of okra mosaic disease in Cross River State Nigeria may be due to weeds such as *Urenalobata*, *Physalis angulate,*and *Sida acuta,* hosting the virus, which was present in and around okra fields [6, 12] and may be an important source of these viruses. Efforts should be made to develop environmentally friendly control measures to contain the menace caused by these diseases.

CONCLUSION

A survey of viral diseases infecting Okrawas conducted between January to June 2022**.** Samples were tested against Cucumber Mosaic Virus (CMV), Okra Leaf Curl Virus (OkLCV), Okra Mosaic Virus (OkMV), and Black eye Cowpea Mosaic Virus (BiCMV) antisera. The results revealed that out of the 30 samples tested, 15 tested positive for CMV which constitutes 50% of the total virus identified followed by OkMV and BiCMV which infected 6 samples for each virus constituting 20 % of virus detected, and OkLMVinfected only 3 samples constituting 10 % of total virus detected. The ELISA results showed that CMV was the most predominant virus infecting half of the total samples. This study has revealed that CMV is widespread in Cross River State Nigeria. Reports abound on the detection of okra viruses in Northern Nigeria, however, this is the first report of wide-scale detection of viruses infecting okra in southern Nigeria.

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