Asian Journal of Biochemistry, Genetics and Molecular Biology

Volume 16, Issue 6, Page 19-31, 2024; Article no.AJBGMB.113399
ISSN: 2582-3698

Biological, Serological and Molecular Characterization of *Cucumber mosaic virus* (CMV) Infecting *Cucurbita moschata* (Duchene ex. Poir) (Summer Squash) in Itu and Uyo Local Governments Areas, Akwa Ibom State, Nigeria

Daniel A. Usanga [a], Ayodeji T. Owolabi [b] and Oduba I. Eyong [c*]

[a] *Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria.*
[b] *Department of Plant and Ecological Studies, University of Calabar, Calabar, Nigeria.*
[c] *Department of Plant Science and Biotechnology, University of Cross River State, Calabar, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/AJBGMB/2024/v16i6379

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/113399

Original Research Article

Received: 19/12/2023
Accepted: 23/02/2024
Published: 23/04/2024

**Corresponding author: E-mail: eyongoduba@gmail.com;*

ABSTRACT

Cucurbita moschata is an edible member of the family Cucurbitaceae. The plant is commonly observed showing typical virus-like symptoms during the growing season in Akwa Ibom State, Nigeria. A total of 7 leaf samples showing typical virus-like symptoms were collected from C. moschata in different locations in Itu and Uyo Local Government, Akwa Ibom State. All the virus isolates used in this study were sap transmissible with a wide host range. The virus isolates irrespective of the place of collection were transmitted in a fore-gut manner by A. spiraecola. Aphis citricida did not transmit the viruses. All the virus isolates reacted positively to cucumovirus polyclonal antibody in antigen coated plate-ELISA (ACP-ELISA). Gel electrophoresis of the cDNA of the virus isolates produced 500 base pairs typical of Cucumber mosaic virus. The gene sequence analysis revealed that all the isolates had sequence homology of 95% and above with Cucumber mosaic virus (CMV) and are therefore considered strains of CMV. The results also showed that CMV isolates investigated in this study shared common ancestry, though belonging to different clusters. The results also indicated that CMV could be widespread and therefore a threat to C. moschata production in Itu and Uyo Local Government Areas of Akwa Ibom State. This is the first report of CMV infecting C. moschata in Akwa Ibom State, Nigeria. Further work should be carried out to cover the entire state.

Keywords: Cucurbitaceae; cucumovirus; ACP-ELISA; moschata; Gel electrophoresis.

1. INTRODUCTION

Cucurbita moschata (Duchene ex. Poir), commonly called summer squash, is an edible member of the family Cucurbitaceae [1], believed to have originated from Latin America [2]. Carine [3] described *C. moschata* as an annual dicotyledonous creeping crop that trails along the ground or climbs by tendrils, growing up to a height of five (5) metres. The stem is usually mildly strong, cylindrical or pentangular with petioles, measuring up to 12 – 30 cm. The leaf may be circular, kidney-shaped or heart-shaped and often deeply indented, weakly lobed, wavy or toothed. According to Carine [3], the flowers are large, yellow, bell-shaped, five-lobed and may be up to 12 cm long. The fruit may be round, oblate, oval, oblong, or pear-shaped, deep yellow, orange, pale green or white in colour and weighing up to 45 kg, with a hollow center containing pulpy loose fibers and numerous seeds. *Cucurbita moschata* is cultivated for its fruits which are reputed for good shelf life and high nutritive value. The fruits, leaves and seeds have been reported to contain βeta-carotene, vitamin E, riboflavin, ascorbic acid, calcium, iron, protein, thiamine, fat, phosphorous, niacin and fiber [4]. The flowers, leaves and young stems are often blanched, boiled or stir-fried or added to soups and stew [5]. The fruit could be baked, fried, boiled, mashed or dried while the leaves play an important role as leading leaf vegetable in some African countries [6]. Aliu et al[7] also reported that oil extracted from the seeds is used in margarine production, soup making and as fuel. Products from *C. moschata* have also been reported to have numerous medicinal uses [8], attributed to the presence of triterpene glycosides called cucurbitacins [9]. Extracts from the plant have been used in the suppression of antibodies in certain allergic disorders, as feeding stimulant in the control of western corn rootworm, *Diabrotica virgifera* [10] and crushed fresh seeds of the plant have been used as anthelmintic, anti-inflammatory, anti-diabetic, and anti-carcinogenic [3,11,12].

In Nigeria, several viruses have been reported infecting cucurbits. *Telfairia mosaic virus* (TeMV) has been reported from different parts of the country [13,14]. Anno-Nyako [15], Shoyinka and Thottappilly [16]. The virus was transmitted by *Aphis spiraecola* in a fore-gut borne manner. Owolabi et al.[17], reported infection of cucumber by *Papaya Ringspot virus* (PRSV). A virus tentatively named *Cucurbita mosaic virus* was also reported on *Cucurbita moschata* in Calabar, Cross River State [18]. The virus identified as a potyvirus was transmitted by *A. spiraecola, A. gossyppi, Myzus persicae* and *Toxoptera citricida* in a fore-gut borne manner and had a sequence homology of 66% with MWMV. Owolabi et al. [19], have also reported two potyvirus isolates, considered to be strains of *Morroccan watermelon mosaic virus*, from *Lageneria breviflorus* and *Coccinia barteri* in southern Nigeria. Eyong et al. [20] have also reported what they considered a new species of virus called Telfairia severe mosaic virus from *Telfairia occidental is* in Calabar with a sequence identity

of 75% with *Moroccan watermelon mosaic virus* (MWMV). *Cucumber green mottle virus* (CGMMV), *Melon necrotic spotvirus* (MNSV), PRSV and CMV have also been reported infecting cucumber, watermelon, *Lagenaria siceraria* and *T. occidentalis* in Ogun and Oyo States and derived savannah agro-ecology in Nigeria [21-24].

Several viruses belonging to several genera have been reported to infect *C. moschata*. Yoon et al[25] reported *Squash leaf curl virus Phillippines* (SLCV-Ph), *Squash leaf curl virus* (SLCV) a begomovirus, family *Geminiviridae, Kyuri green mottle mosaic virus* (KGMMV) a tobamovirus, family *Virgaviridae*, *Lettuce infectious yellows virus* (LIYV) a crinivirus, family *Closteroviridae* and *Squash leaf curl virus* (SqLCV) a torradovirus, family *Secoviridae* infecting the crop. *Zucchini green mottle mosaic virus*, a tobamovirus, belonging to the family *Virgaviridae* have been isolated from the vegetable [26].

A survey of some farms in Itu and Uyo LGAs of Akwa Ibom State revealed virus-like symptoms, such as mosaic and leaf malformation on *C. moschata*. From available literature, no report of virus infection of this vegetable crop has been documented in these areas. The purpose of this research is to identify viruses infecting the crop in these LGAs.

2. MATERIALS AND METHODS

2.1 Buffer Preparation

The buffer used for virus isolation and propagation in this study was 0.03M disodium hydrogen orthophosphate ($Na_2HPO_4.2H_20$), pH 8.0. This was prepared by dissolving 4.26 g of $Na_2HPO_4.2H_20$ in one litre of distilled water. The pH was adjusted by adding few drops of 0.5M sodium dihydrogen phosphate (NaH_2PO_4) until the desired pH 8.0 was obtained and the buffer was kept cold before use.

2.2 Sources and Isolation of Viruses

Infected leaf samples of *C. moschata,* showing typical virus-like symptoms, were collected in Ziploc air-tight polyethylene bags from Ikot Ekang, Ikot Ekpuk, Nkim Itam and Ikot Andem in Itu Local Government area and from Edem Akai, Aka Offot and Ediene Obio Imo in Uyo Local Government area. Virus inocula were prepared by triturating the symptomatic leaf tissues of the samples in cold inoculation buffer, in a sterilized pestle and mortar. The homogenate from each infected leaf sample was inoculated onto carborundum-dusted 9-day old seedlings of *C. moschata, Cucumis sativus* and *Cucumerupsis mannii* in the screen house of the Botanical Garden of the University of Calabar, Calabar in order to identify a readily susceptible propagation host.

2.3 Host Range Studies

For the host range studies, 33 plant species belonging to 8 families were tested. These were Cucurbitaceae - *Cucurbita moschata. Cucurbita maxima, Citrullus lanatus. Cucumis melo, Cucumis sativus, Cucurbita pepo, Cucumeropsis mannii, Luffa cylindrica, Telfairia occidentalis, Lagenaria siceraria, and Trichosanthes cucumerina; Solanaceae - Nicotiana tabacum, Lycopersicon esculentum, Capsicum annuum, Nicotiana rustica, Nicotiana tabacum*, *Solanum melongena* and *Datura stramonium; Fabaceae - Phaseolus vulgaris, Arachis hypogaea, Vigna sinensis*, *Vigna radiata, Vigna unguiculata,* and *Dolichus lablab. Amaranthaceae - Amaranthus hybridus, Chenopodium album, Spinacia oleracea, and Amaranthus viridis; Lamiaceae – Ocimum gratissimum;* Malvaceae - *Abelmoschus esculentus,* Poaceae - *Oryza sativa and Sorghum bicolor; Asteraceae - Helianthus annuus.* The seeds of the plants were sown at the rate of five seeds per bag in 16 cm diameter polyethylene bags, ¾ filled with sterilized humus soil. Inocula prepared from the symptomatic leaf tissues were then transferred mechanically on carborundum (600-mesh) dusted leaves of the test plants. The cucurbitaceous and fabaceous plants were inoculated at the 2-leaf stage and others at 4-5 leaf stage. Inoculated leaves were then rinsed with water and left for symptoms development.

2.4 Insect Transmission Tests

The ability of *Aphis spiraecola* and *A. citricida,* two common aphid species in Calabar, Cross River State, Nigeria obtained *from Chromolaena odorata and Citrus sinensis, their natural host respectively was tested. The insects were starved for one hour in a transparent plastic container over which a piece of gauze was firmly secured and then allowed acquisition access feeding period of between 2-5 min on C. moschata virus infected leaves. A paint brush was used to transfer 5 aphids to 10 young*

seedlings of C. moschata, (the test plant) inside insect proof screen cages for inoculation access feeding of 10 min. The aphids were then killed by spraying with Pirimor – an aphicide.

2.5 Serological Tests

The virus isolates were reacted with cucumovirus polyclonal antibody in antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) as described by Kumar & Legg [27]. Symptomatic leaf samples weighing 0.1g were triturated in 1mL of coating buffer (0.015M Na_2CO_3 + 0.0349M $NaHCO_3$ + dH_2O) and dispensed into each well of the ELISA plates. After incubation at 37°C for 1 hour, the plates were washed 3 times with PBS-Tween 20 for 3 minutes. Cross adsorption was made by grinding 1 g of healthy plant sample in 20 mL of conjugate buffer (1/2 PBS + 0.05% Tween 20 + 0.02% egg albumin + 0.2% PVP). Cucumovirus polyclonal antiserum diluted at 1:3000 in the adsorption solution and 100µL of the antiserum were added to wells of the ELISA plates and again incubated at 37^0C for 1 hour. The ELISA plates were then washed 3 times with PBS-Tween 20. 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 37^0C for 1 hour. The plates were again washed 3 times with PBS-Tween 20. One hundred-µL of $0.001 g \cdot mL^{-1}$ 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 hour. For all incubations, the ELISA plates were covered to avoid edge effects and to maintain uniform temperature. Healthy plant samples were used as controls. After one (1) hour, absorbance was measured at A_{405nm} using an ELISA plate reader (Micro Read 1000 ELISA plate analyzer, U.S.A). A sample was considered positive when the ELISA reading was at least twice the reading for the healthy control [28].

2.6 Ribonucleic Acid (RNA) Extraction from Infected Leaf Samples

Total RNA was extracted from the infected leaf samples using the cetyltrimethyl ammonium bromide (CTAB) protocol described by Abarshietal.[29]. One hundred milligrams of each infected leaf sample were ground in 1 ml extraction buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2% hexade-cetyltrimethyl ammonium bromide (CTAB)) in sterile mortar and 0.4% β-mercaptoethanol, added just before use. Each of the homogenates was poured into a new 1.5 ml Eppendorf tube. The tubes were vortexed briefly, incubated at 60^0C for 10 minutes and allowed to cool to room temperature. Then 0.75 ml (750 µl) of phenol: chloroform: isoamyl alcohol (25:24:1) was added to each tube containing the homogenate. Each tube was then vortexed vigorously and then centrifuged at the speed of 12,000 relative centrifugal force (rcf) for 10 minutes. The supernatant was then transferred to a clean 1.5 ml Eppendorf tube and nucleic acids were precipitated by adding 0.6 volumes (300 µl) of ice cold (-20^0C) isopropanol. The mixture was then centrifuged at 12,000 rcf for 10 minutes at 4 ^{0}C to precipitate the nucleic acid. The supernatant was discarded and the nucleic acid pellet washed in 500 µl of 70% ethanol and centrifuged at 12,000 rcf for 5-10 minutes. The supernatant was decanted and the resultant nucleic acid pellet was air-dried at room temperature. Nucleic acid pellet was then re-suspended in 50 µl sterile distilled water and used as a template source for reversed transcriptase polymerase chain reaction (RT-PCR). Nucleic acid extracts from the leaves of healthy plants were used as negative controls.

2.7 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The total nucleic acid extracted from infected leaf tissues was re-suspended in 50 mL sterile distilled water, quantified by gel electrophoresis then stored at -20^0C. The presence of CMV was verified with RT–PCR using primer pairs CMV F4 (5'GCCGTAAGCTGGATGGACAA3') and CMV R4 (5'CCGCTTGTGCGTTTAATGGCT 3'). PCR assays were performed in a final reaction volume of 12.5µl containing 5x PCR buffer (New England BioLabs, inc.), 10µM each dNTP, 10µM each of upstream and downstream primer, 5 units Taq DNA polymerase (New England BioLabs, inc.) and 2.5 ng μl^{-1} of viral RNA. PCR assays for the CMV virus were performed in an Eppendorf Master cycler (USA Scientific, Inc.). Cycling conditions were: one cycle of 42^0C for 30 minutes, one cycle of 94^0C for 5 minutes, followed by 36 cycles with each cycle of 94^0C at 30 seconds, 55^0C for 30 seconds and 72^0C for 30 seconds. This was followed by a final extension at 72^0C for 5 minutes. The PCR reaction products were separated on 1.5% agarose gel stained with ethidium bromide, visualized with UV trans-illuminator.

Subsequently selected amplicons were purified and sequenced.

2.8 Amplicon Purification and Sequencing

The RT-PCR amplicon for each sample was purified by adding 95 % ethanol to 40 µl of the amplicon in a new 1500 µl Eppendorf tube and the solution was kept in –80^{0}C for 10 minutes. The tube was centrifuged at 12,000 rcf for 10 minutes and the supernatant discarded. Five hundred µl of 70 % ethanol was added and centrifuged at maximum speed for 5 min. The supernatant was discarded and the tube was left at room temperature to dry. Thereafter, the purified cDNA was dissolved in 30 µl of sterile distill water. The product was sequenced at Bioscience Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan.

2.9 Sequence Analysis

The sequence data of the virus under study were obtained using Bio Edit software followed by sequence analysis using NCBI (www.ncbi.nlm.nih.gov) blast search. The molecular evolutionary genetics analysis (MEGA) software version 6.0 [30] was used for pair-wise comparisons of CMV sequences of the virus isolates with other selected CMV sequences obtained from the gene bank. The same software was also used for the phylogenetic analyses.

2.10 Phylogenetic Analysis

Phylogenetic tree was constructed from the muscle-aligned sequences using MEGA version 6[31] and neighbour-joining method with selected viruses of the same genus. Parameter model was used for estimating the distances and conducting the bootstrap analysis. Evolutionary divergences were estimated using MEGA version 4 [30].

3. RESULTS

3.1 Host Range and Symptomatology

The results of the host range study are presented in Table 1. The results indicated that all members of the cucurbit family used in this study were susceptible to all the virus isolates. Symptoms induced on tested plant species included mosaic, leaf malformation, leaf deformation, reduction in leaf size, enations, blistering, green-vein banding, leaf puckering, and veinal chlorosis (Fig 1). The isolates from Ikot Ekang, Aka Offot, Nkim Itam induced veinal chlorosis on *C. moschata* while isolates from Ikot Ekang, Edem Akai, Ikot Ekpuk, Nkim Itam, Ediene Obio Imo, and Ikot Andem induced leaf blistering on *Cucurmeropsis mannii.* The isolates from Ikot Ekpuk, Aka Offot, Nkim Itam, Ediene Obio Imo, and Ikot Andem elicited enations on *Cucurbita pepo* and *C. manni*. All the isolates caused mosaic, leaf reduction and deformation on *Cucumis sativus*. The isolates from Ikot Ekang, Aka Offot, Nkim Itam caused mosaic on *Capsicum annum* while isolates from Ediene Obio Imo, Ikot Andem, Ikot Ekpuk, and Edem Akai induced leaf deformation in the same vegetable. Edem Akai isolate elicited leaf puckering on *C. moschata.* All the virus isolates were also found to induce mosaic symptom on *Lycopersicum esculentum* and *Capsicum annum* while leaf malformation and deformation were induced on *Vigna unguiculata.* All the isolates caused only *green-vein banding on Spinacia oleracea*. None of the plant species in the other families tested (Lamiaceae, Malvaceae, Poaceae and Asteraceae) was susceptible to the virus as none of them showed symptoms.

3.2 Insect Transmission Test

All the virus isolates obtained from infected *C. moschata* samples were transmitted by *A. spiraecola* and *A. citricida* at varying degrees in a fore-gut (non-persistent) manner. Transmission efficiency ranged from 60% - 90% with *A. spiraecola* proving to be more efficient compared to *A. citricida*.

3.3 Serology

The results obtained from this test showed that all the seven (7) virus isolates reacted positively with cucumovirus polyclonal antiserum in ACP-ELISA, as all the values obtained were twice that of the control. The isolates from Ikot Ekang, Ikot Andem, Ikot Ekpuk, and Nkim Itam, from Itu LGA, had optical density (OD) readings of 2.739, 2.619, 2.634 and 1,873 respectively while isolates obtained from Aka Offot, Edem Akai and Ediene Obio Imo in Uyo LGA had optical density (OD) readings of 1.992, 2.168 and 2.861 respectively (Table 2).

3.4 Gel Electrophoresis

Gel electrophoresis of the amplified cDNA of all the virus isolates formed bands corresponding to 500bp on the ladder which was the approximate base pair for CMV amplification. Lane D represented positive control and lane B was the buffer.

TABLE 1.Reactions of some plants to the *Cucurbita moschata*virus isolates

Plants species	Ikot Ekang isolate	Edem Akai isolate	Ikot Ekpuk isolate	Aka Offot Isolate	NkimItam isolate	EdieneObio Imo Isolate	Ikot Andem Isolate
Amaranthaceae							
Spinacia oleracea	GVB	GVB	GVB	GVB	GVB	GVB	GVB
Cucurbitaceae							
Cucurbita moschata	Mo, VC	Mo, LP	Mo	Mo, VC	Mo, VC, LC	Mo	Mo, LC
Cucurbita maxima	Mo	Mo	Mo	Mo	Mo	Mo	Mo
Citrullus lanatus	Mo	Mo	Mo	Mo	Mo	Mo	Mo
Cucumis melo	Mo	Mo	Mo	Mo	Mo	Mo	Mo
Cucumis sativus	Mo, LR	Mo, LR, LD	Mo, LR, LD	Mo, LR, LD	Mo, LR, LD	Mo, LD	Mo, LR
Cucurbita pepo	LB	LB	En, LB	En, VC	LB, En	En	LB, VC
Cucumeropsis manni	LB	Mo, LD	En, LB	Mo, LM	En, LB	En, LB	En, LD
Luffa cylindrical	Mo	Mo	Mo	Mo	Mo	Mo	Mo
Telfairia occidentalis	Mo	Mo	Mo	Mo	Mo	Mo	Mo
Lagenaria siceraria	Mo	Mo	Mo	Mo	Mo	Mo	Mo
Trichosanthes cucumerina	Mo	Mo	Mo	Mo	Mo	Mo	Mo
Fabaceae							
Lycopersicon esculentum	Mo	Mo	Mo	Mo	Mo	Mo	Mo
Solanaceae							
Capsicum annuum	LD	Mo, LD	Mo, LD	Mo	Mo	LD	LD
Vigna unguiculata	LD	LM, LD	LM, LD	LM, LD	LM, LD	LM, LD	LM, LD

GVB = Green Vein Banding Mo = Mosaic; LP = Leaf Puckering; VC = Veinal Chlorosis; LC = Leaf-Edge Chlorosis; LR = Leaf Roll; LRT = Leaf Reduction; En = Enation; LB = Leaf Blistering; LD = Leaf Deformation; LM = Leaf Malformation

Table 2. Antigen coated plate (ACP) enzyme -linked immunosorbent assay (ELISA) for detection of *Cucumber mosaic virus* (CMV)

S/N	Samples	Location	OD reading at A405nm against Virus Polyclonal Antibody
			CMV
1.	*C. moschata* virus isolate	Ikot Ekang – Itu	2.739*
2.	*C. moschata* virus isolate	Ikot Andem – Itu	2.619*
3.	*C. moschata* virus isolate	Ikot Ekpuk – Itu	2.634*
4.	*C. moschata*virus isolate	NkimItam – Itu	1.873*

S/N	Samples	Location	OD reading at A405nm against Virus Polyclonal Antibody
5.	*C. moschata* virus isolate	Aka Offort – Uyo	1.992*
6.	*C. moschata* virus isolate	Ediene Obio Imo – Uyo	2.861*
7.	*C. moschata* virus isolate	Edem Akai – Uyo	2,168*
8.	Healthy Control		0.786
9.	Infected Control		3.182

**Sample was considered positive when the Optical Density (OD) reading at A_{405} nm was 2x greater than the absorbance for healthy control*

Lane M = 100bp DNA ladder
Lane 1. *Cucurbita moschata*virus isolate from Ikot Ekang – Itu
Lane 2. *C. moschata* virus isolate from Edem Akai – Uyo
Lane 3. *C. moschata*virus isolate from Ikot Ekpuk – Itu
Lane 4. *C. moschata* virus isolate from Aka Offot – Uyo
Lane 5. *C. moschata* virus isolate from Nkim Itam – Itu
Lane 6. *C. moschata* virus isolate from Ediene Obio Imo – Uyo
Lane 7. *C. moschata* virus isolate from Ikot Andem – Itu
Lane D. Positive control
Lane B. Buffer

Plate1. Amplification of cDNA bands of isolates testing for *Cucumber mosaic virus* (CMV) of approximately 500 base pairs

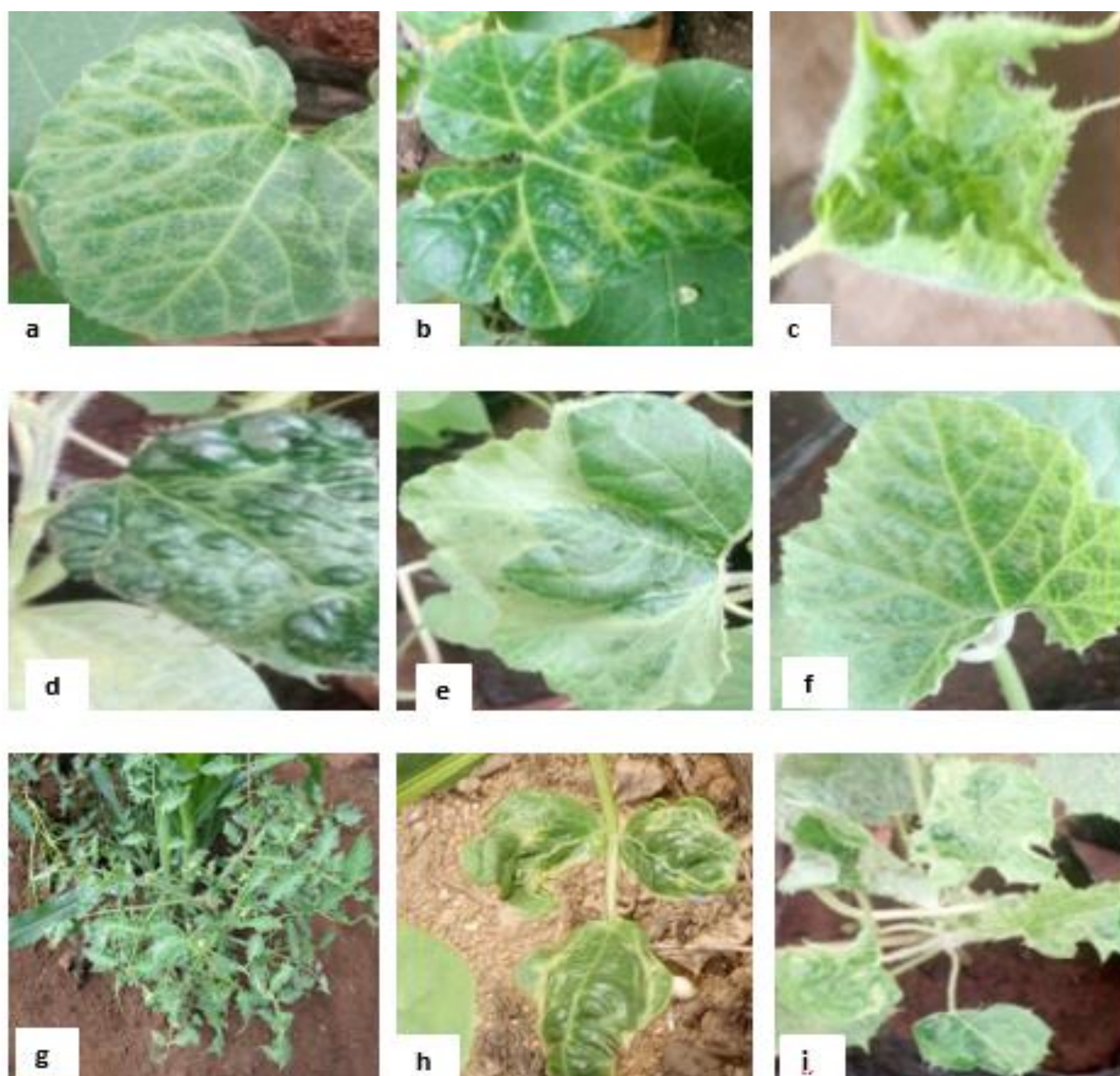

Fig. 1. (a) Veinal chlorosis on *C. moschata* (b) mosaic and leaf puckering on *C. moschata* (c) leaf roll, malformation and deformation on *Cucumis sativus* (d) enation and blistering on *Cucurbita manni* (e) leaf-edge chlorosis on *C. moschata* (f) mosaic and veinal chlorosis on *C. moschata* (g) mosaic on *L. esculentum* (h) leaf deformation and malformation on *V. unguiculata* (i) enation and leaf blistering on *C. manni.*

3.5 Gene Sequence and Sequence Alignment

The result of gene sequences of RNA (nucleic acid) of all virus isolates compared to the CMV sequences obtained from the gene bank shared a sequence homologue of between 95 - 98% (Table 3).

The phylogenetic tree revealed two major clusters and each with its own subclusters (Fig. 2). The first major cluster had 3 subclusters, the first comprising of CMV NkimItam, and CMV Ikot Andem showing 98% similarity and 61% group consensus with the third CMV Ikot Ekang strain, the second subcluster had CMV Aka Offor and CMV Ediene Obio Imo with similarity of 61% between the two strains and the third subcluster had CMV Ikot Ekpu showing 99% similarity with a gene bank strain FJ89160 CMV Nigerian coat protein gene. The second major cluster had CMV isolate from Edem Akai with percentage similarity of 96% with MH178110.1 CMV virus isolate ER nig viral helicase 1 gene sourced from the gene bank. The high percentage similarity and group consensus between MH178110.1 and CMV

Table 3. Summary of Gene sequence alignment of *Cucumber mosaic virus*

Virus isolate	Name of virus	% homologue	CMV sequences*
Ikot Ekang isolate	*Cucumber mosaic virus*	95%	CMV Ug92-RNA1 replicase gene (MG021460.1)
Edem Akai isolate	*Cucumber mosaic virus*	97%	CMV ER nig helicase 1 gene (MN178110.1)
Ikot Ekpuk isolate	*Cucumber mosaic virus*	98%	CMV nig Coat Protein gene (FJ896160.1)
Aka Offot isolate	*Cucumber mosaic virus*	96%	CMV ER nig helicase gene (MN178110.1)
Nkim Itam isolate	*Cucumber mosaic virus*	97%	CMV GTCMV (MN481937.1)
Ediene Obio Imo isolate	*Cucumber mosaic virus*	97%	CMV ATCMV (MN481936.1)
Ikot Andem isolate	*Cucumber mosaic virus*	97%	CMV GTCMV (MN481937.1)

**Cucumber mosaic virus sequences obtained from gene bank Phylogenetic relationship of isolated viruses with other CMV*

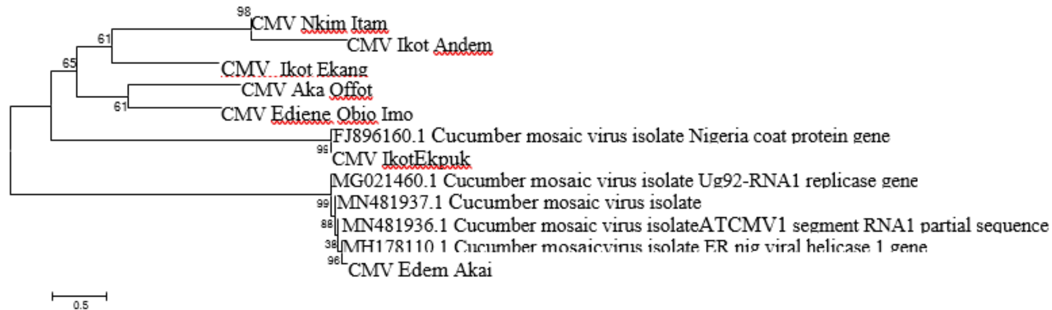

Fig. 2. The phylogenetic relationship of the CMV isolates with related CMV strain based on GenBank Data Library. The phylogenetic tree was constructed using MEGA 6.0 software.

Edem Akai and between CMV Ikot Ekpuk and the gene bank strain FJ89160 CMV Nigerian coat protein gene indicated that they were the same with the identified gene bank strains causing cucumber mosaic diseases in the study locations. Fig. 2: Phylogenetic relationship of the CMV isolates with CMV strains sourced from GenBank Data Library. The phylogenetic tree was constructed using MEGA 6.0 software.

4. DISCUSSION AND CONCLUSION

The result of this study indicated that all the virus isolates obtained from *C. moschata* belong to the genus *Cucumovirus,* family *Cucumoviridae*. The study showed that all the virus isolates from *C. moschata* has a fairly wide host range as all the plants tested from the different families were susceptible to them. Cuccumber mosaic virus (CMV) is reported to have a host range of more than 1,200 species in 521 genera from 100 families [31]. Previous reports by Arogundade et al. [24] and Ayo-John et al. [22,23] on the same virus also confirm this. If the virus could infect other cucurbits beside the one studied, this is a pointer to how economically important the virus could be. With regards to insect transmission, all the isolates were transmitted mechanically by *A. spiraecola* in fore-gut manner corroborating previous report on transmission of the CMV by the aphid species [22,23]. The ubiquity of the *A. spiraecola* and the ability of the aphid to transmit the virus isolates could have been responsible for their widespread in Akwa Ibom. ACP-ELISA has been a very veritable tool for the detection and identification of plants viruses into the genus taxon. The positive reaction of all the virus isolates with cucumovirus polyclonal antiserum further affirmed they belong to the genus *Cucumovirus.*The production of 500 base pair amplicons using cucumovirus specific primers by all the virus isolates in this study further confirmed that they were all CMV isolates. Yeturu, et al. [33] had reported the detection of CMV using cucumovirus specific primers with the resultant production of amplicons of 500 base pairs. Characterization and identification of plant viruses using gene sequence data has become the ultimate in recent times [34-39]. A virus identity becomes unassailable if the degree of homology of its sequence is established after comparison with sequences of previously characterized members of the genus to which the virus in question belongs. King et al. [40] have suggested that virus sequences with less than approximately 76% sequence identity should be regarded as belonging to different species while isolates with 76-89% sequence identity should be considered as different viruses and while sequence presenting 90-100% sequence identity should be regarded as strains of the same virus. All the viruses isolated from *C. moschata* in this study from Ikot Ekang, Ikot Ekpuk, Nkim Itam and Ikot Andem in Itu LGA and isolates from Edem Akai, Aka Offot and Ediene Obio Imo in Uyo LGA had sequence identity above 95% and are therefore considered as different strains of *Cucumber mosaic virus*. This assertion is further confirmed by phylogenetic tree which revealed that the CMV reported in this study shared to a common ancestry [41]. While it could be argued that all the variants are from a common ancestor especially since they are from the tropics, the CMV isolates from cluster 1 are somewhat distantly related to those in cluster 2 and can therefore be categorized into subgroup I isolates.

The result of this study confirms, for the first time, the occurrence of CMV in Itu and Uyo LGAs of Akwa Ibom State [42,43].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pitrat, M. Viruses and virus diseases of vegetables in the Mediterranean Basin. Advances in Virus Research. 2012;84:1-29.
2. Jeffrey C. Systematic of the Cucurbitaceae. In: Bates DM, Robinson RW, Jeffrey C, Eds, biology and utilization of the Cucurbitaceae, Cornel University Press, Ithaca. 1990;3-9.
3. Carine Marie-magdelein & harry archimede Pumpkin (*Cucurbita moschata* Duchesne ex. Poir.) seeds as an anthelmintic agent. In: PreedyNR, WatsonRR, Patel VB(eds). Nuts and seeds in health and disease prevention. San Diego: Academic Press. 2011;933-939.
4. Grubben GJH, Chigumira NF. *Cucurbita moschata* Duschene. Record from Plant Resources of Tropical Africa/Resources Vegetable de l' Afrique Tropicale,(PROTA4U.PROTA) Wageningen, Netherlands/Backhuys Publishers; 2004.
5. Hallen V, Kumari R, Bhardway P, Singh L, Zaidi, A. Biological and molecular characterization of *Cucumber mosaic* virus subgroup 11 isolate causing severe mosaic in cucumber. Indian Journal of Virology. 2013;24:27-34.
6. Chigumira NF. Survey of literature on mandate vegetable species of the SADC plant genetic resources centre, Lusaka Zambia, occurring in Zimbabwe. Regional Vegetables. IPGRI Crop Working Group Report. 2000;98-99.
7. Aliu S, Rusinovci I, Salihu S, Fetahu S, Kika A, Zeka D. Nutritive value and seed chemical variability of pumpkin (*Cucurbita pepo* L.) populations. Journal ofFood, Agriculture & Environment. 2016;14(2): 28-33.
8. Joshi VK. Panesar PS. Pumpkin wine. Science and technology of fruit wine production. Londres, U.S.A. Academic Press; 2017.
9. Rehm S, EnslinPR, Meeus ADJ, Wessels JH. Bitter principles of the Cucurbitaceae. VII. The distribution of bitter principles in this plant family. Journal of the Science of Food and Agriculture. 1957;8: 679-686.
10. Tallamy DW, Hibbard BE, Clark TL, Gillespie JJ. Western corn rootworm, Cucurbits and Cucurbitacin. In: Vidal, S., Kuhlmam U. Edwards, C. R.(eds.). Western Corn Rootworm: Ecology and Management. Wallingford. CAB International. 2005;67-39.
11. Ahmad G, Khan AA. Pumpkin: Horticultural importance and its roles in various forms; a Review. International Journal of Horticulture and Agriculture. 2019;4(1):1-6.
12. Yadav M, JainS, Tomar R, Prasad GBKS, Yadav H. Medicinal and biological potential of pumpkin: an updated review. Nutrition Research Reviews. 2010;23:184-190.
13. NwauzoEE, Brown WM. Telfairia mosaic virus in Nigeria. Plant Disease Reporter. 1975;59:430-432.
14. Atiri GI. An isolate of *Cucumber mosaic* virus from fluted pumpkin in Nigeria. Journal of Phytopathology. 1985;3:4-5.
15. Anno-NyakoFO. Seed transmission of *Telfairia mosaic* virus in fluted pumpkin in Nigeria. Journal of Phytopathology. 1988; 121;85-87.
16. ShoyinkaSA,Thottappilly G. Occurrence of *Telfairia mosaic* virus in fluted pumpkin in Nigeria. African Crop Science. 1988; 6(1):69-78.
17. Owolabi AT, RabensteinF,Ehrig F. A strain of *Papaya ringspot virus* naturally infecting cucumber (*Cucumis sativus* L.) in Calabar, South Eastern Nigeria. Nigerian Journal of Botany. 2008;21(1):97-108.
18. OwolabiAT, Rabenstein F, Taiwo MA,Ehrigh F,Maiss E. Characterization and identification of a potyvirus causing mosaic disease of *Cucurbita moschata* Duch Ex. Poir in Calabar, South East Nigeria. International Journal of Plant Pathology. 2011;2(4):165-176.
19. OwolabiAT, Rabenstein F, Ehrigh F, Maiss E, Vetten. Strain of *Morroccan* watermelon mosaic virus isolated from Lagenaria breviflorus and Cocciniabarteri in Calabar, southern Nigeria. International Journal of Virology, 2012;8(3):258-270.

20. Eyong OI, Owolabi AT, Mofunanya AAJ, Ekpiken EE. Biological, serological and molecular characterisation of a new virus species infecting Telfairia occidentalis in Calabar, Southern Nigeria. Journal of Experimental Agriculture International. 2020;42(1);23-33.
21. Ayo-John EI, Emily I, Mohammed, Wasiu A, Oyagbinrin Samuel T. Virus symptoms and viruses associated with two cucurbit crops grown in a derived savannah agro-ecology in Nigeria. Albanisan Journal of Agricultural Science. 2008;45:56-78.
22. Ayo-John E, Ekpo EJA, Hughes JD. Characterization of three cucumber mosaic virus (CMV) isolates, genus Cucumovirus, infecting host crops in Nigeria. Tropical Agriculture. 2012;89(3):151-160.
23. Ayo-John E, Patience M, Olorunmaiye O, Odedara O, Dada E, Kolade O. Assessment of field-grown cucurbit crops and weeds within farms in South-West Nigeria for viral diseases. Notulae Scientia Biologicae. 2014;6(3):321-325.
24. Arogundade O, Olusegun S, Balogun, Kehinde T. Occurrence and distribution of *Cucumber mosaic* virus in cucumber in Ibadan. Nigeria Virology Journal. 2012;9:79.
25. Yoon JY, Min BE, Choi SH, Ryu KH. Completion of nucleotide sequence and generation of highly infectious transcripts to cucurbits from full-length cDNA clone of Kyuri green mottle mosaic virus. Arch Virological. 2001;146(11):2085-2096.
26. Yoon JY, Min BE,Choi SH. Genome structure and production of biologically active *In vitro* transcripts of cucurbit-infecting Zucchini green mottle mosaic virus. Phytopathology. 2002;92(2):156-163.
27. Kumar PL, Legg J. Methods for the diagnosis of plant virus disease. International Institute of Tropical Agriculture (IITA), Ibadan, Laboratory manual; 2009.
28. Walkey DGA, Spence NJ, Clay CM, Miller A.A potyvirus isolated from Senna occidentalis. Plant Pathology. 1994;43: 767-773.
29. Abarshi MM, Mohammed IU, Wasswa P, Hillocks RJ, Holt J, Legg JP, Seal SE, Maruthi MN. Optimization of diagnostic RT-PCR protocols and sampling procedures for the reliable and cost-effective detection of Cassava brown streak virus. Journal of Virology Methods. 2010;163(2):353-359.
30. Tamura K, Dudley J, Nei M, Kumar S. Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution. 2007;24(8):1596-1599.
31. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. Molecular evolutionary genetics analysis version 6.0.Molecular Biology and Evolution. 2013;30(12);2725-2729.
32. Yoon JY, PalukaitisP, Choi SK; 2019. Available:http://doi.org/10.1094/9780890546109.004
33. Yeturu S, Mendez K, Garrido P, Serrano S, Garrido A. Serological and molecular identification of Cucumber mosaic virus (CMV) infecting banana crops in Ecuador. Revista Cientifica Ecuatoriana, 2016;3:17-22.
34. Ahmed H, Ricardo F, Thierry Candresse, and Marina (Next generation sequencing and genome editing in plant virology. F, Microbiology. 2016;7:1325
35. Maina S, Zheng L. Rodoni Targeted genome sequencing (Ta-seq) approaches to detecting plant viruses. Viruses. 2021;13(4):583.
36. Nam-Yeon Kim, Hyo-Jeong Lee, Hong-Sup Kim, Su-Heon Lee, Jae-Sun Moon and Rae-Dong, Jeong. Identification of plant viruses infecting pear using RNA sequencing. Plant Pathology Journal. 2021;37(3):258-267.
37. Hakan F. Occurrence of *Alfalfa mosaic* virus (AMV). Diseases on cucumber crops in Northern Cyprus. Acta horticulturae, 2012;960:341-346.
38. Desbiez C, Lecoq H. Zucchini yellow *mosaic* virus. Plant Pathology. 2012;109:76-82.
39. Usher L, Sivparsad B, Gubba A. Isolation, identification and molecular characterization of an isolate of Watermelon mosaic virus 2 occurring in KwaZulu-Natal, South Africa. International Journal of Virology. 2012;18:10-17.
40. King AM, Lefkowitz E, Adams MJ, Carstens EB. Virus taxonomy: 9th report of the international committee on taxonomy of viruses. San Diego, Academic Press; 2011.

41. Higgins G. Vegetable: Cucumber mosaic virus – Umass extention. Center for Agriculture, Food and the Enviroment; 2018.
Available:https://ag.umass.Edu
42. Tang W,Eisenbr and G. Chinese drugs of plant origin: Chemistry, pharmacology and use in traditional and modern medicine. Berlin, Springer – Verlag; 1992.
43. Whitaker TW, Davis GN. Cucurbits: Botany, cultivation, and utilization. New York, Etats-Unis: Interscience Publishers. 1962;162- 170.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/113399