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Characterization and Identification of a Potyvirus Causing Mosaic Disease of *Cucurbita moschata* Duch Ex. Poir in Calabar, South East Nigeria

[1]A.T. Owolabi, [2]F. Rabenstein, [3]M.A. Taiwo, [2]F. Ehrigh and [4]E. Maiss

[1]Department of Botany, University of Calabar, Calabar, Nigeria

[2]Institute of Resistance Research and Pathogen Diagnostics Erwin-Baur St. 27, 06484 Quedlinburg, Germany

[3]Department of Botany and Microbiology, University of Lagos, Lagos, Nigeria

[4]Institute of Plant Pathology and Plant Protection, Leibniz University, Herrenhaeuzer Str. 2, D-30419, Hannover, Germany

Corresponding Author: A.T. Owolabi, Department of Botany, University of Calabar, P.M.B. 1115, Calabar, Nigeria

ABSTRACT

A virus inducing mosaic, green vein-banding and leaf formation in *Cucurbita moschata* was isolated during the 2005-2006 growing season in Calabar, Nigeria. It was characterized based on host range, transmission studies, cytopathology, electron microscopy combined with Immunosorbent Assay (ISEM), serology and coat protein gene sequencing. The virus host range was restricted to Cucurbitaceae and Solanaceae families. There was no evidence of seed transmission. However, the virus was transmitted by *Aphis gossypii, A. spiraecola, Myzus persicae* and *Toxoptera citricida* in a fore-gut (non-persistent manner) but not by *A. craccivora* and *Macrosiphon euphorbiae*. The virus had flexuous rods of about 750 nm in length and induced pinwheels, tubes and laminated aggregates characteristic of the genus *Potyvirus* (Family Potyviridae). The Coat Protein (CP) has a molecular weight of 32.5 kDa as determined by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), followed by Western blotting. Serologically, the virus failed to react with several putative potyviruses in Enzyme-linked Immunosorbent Assays (ELISAs) but weakly decorated by antisera to Papaya Ringspot Virus (PRSV) and Moroccan Watermelon Mosaic Virus (MWMV) in ISEM tests and showed 66% CP sequence homology when compared to that of MWMV. These data suggest that the virus is distinct from both PRSV and MWMV and other potyviruses commonly infecting cucurbits. This is the first report of a potyvirus naturally infecting *C. moschata* in Nigeria. From the result of this study, it seems that the virus is novel in Nigeria and considered distinct from other commonly infecting cucurbit infecting viruses, for which the name Cucurbita mosaic virus has been suggested.

Key words: *Cucurbita moschata*, potyvirus, symptoms, serology, sequence homology

INTRODUCTION

Cucurbita moschata (Duch ex. Lam.) Duch and Poir, commonly called pumpkin, is an edible member of the family Cucurbitaceae. It is cultivated in mixed cultivation as a vegetable crop for its leaves and fruits in the southern parts of Nigeria where it also provides effective cover against soil

erosion and competes with smothering weeds. The young leaves are used as vegetables while the pulp of the slightly unripe fruit is eaten raw or cooked (Dupriez and De Leener, 1989).

A survey of literature revealed a preponderance of information on virus diseases of cucurbits. More than 39 well characterized viruses, distributed in unrelated genera such as *Begomovirus*, *Crinivirus*, *Cucumovirus*, *Polerovirus*, *Ipomovirus*, *Tobamovirus*, *Tospovirus* and *Potyvirus*, are known to infect cucurbits naturally (Antignus *et al*., 2001; Brown *et al*., 2002; Salem *et al*., 2007; Gholamalizadeh *et al*., 2008; Kneirim *et al*., 2010). Among these viruses, the most widely occurring include Cucumber Mosaic Virus (CMV), Squash Mosaic Virus (SqMV), Zucchini Yellow Mosaic Virus (ZYMV), Papaya Ringspot Virus (PRSV) formerly known as Watermelon mosaic virus 1, Watermelon Mosaic Virus-2 (WMV -2) (Yuki *et al*., 2000; Fattouh, 2003; Choi *et al*., 2007; Massumi *et al*., 2007; Yardimci and Ozgonen, 2007) and Moroccan Watermelon Mosaic Virus (Lecoq *et al*., 2001). Other viruses which occur less frequently but also of economic significance include Cucurbit Aphid-borne Yellows Virus (CABYV), Cucurbit Yellows Stunting Disorder Virus (CYSDV), Cucumber Vein Yellowing Virus (CVYV), Cucumber Green Mottle Mosaic Virus (CGMMV) and Zucchini Lethal Chlorotic Virus (ZLCV) (Shim *et al*., 2005; Bananej *et al*., 2006; Safaeizadeh, 2008; Yakoubi *et al*., 2008; Wintermantel *et al*., 2009; Moradi and Jafarpour, 2011; Webster *et al*., 2011).

Several cucurbits are cultivated or found growing in the wild in Nigeria but reports of virus diseases on them are rather few. Nwauzo and Brown (1975) described a mosaic disease of *Telfairia occidentalis* while Shoyinka *et al*. (1987) established that the disease was caused by a potyvirus designated as Telfairia mosaic virus (TeMV). The virus, besides infecting cucurbitaceous plants, was serologically related to but distinct from ZYMV and was distantly related to WMV-2 and BYMV. Atiri (1985) has reported natural infection of *Telfairia occidentalis* by CMV while Igwegbe (1983) reported the occurrence of a virus disease of *Cucumeropsis mannii* (= *C. edulis*). The virus which was readily transmitted by *Myzus persicae*, had flexuous rod-shaped particles and showed no serological relationship with Muskmelon necrotic spot virus, WMV-1 and 2 as well as Moroccan Watermelon Mosaic Virus (MWMV). It induced local lesions in *Chenopodium amaranticolor* and *C. quinoa* but no symptoms on *Luffa acutangula*. A strain of PRPV has also been described from Nigeria (Owolabi *et al*., 2008).

A mosaic disease was observed on *C. moschata* on several farms in Calabar, south eastern part of Nigeria. Elsewhere in the southern part of Nigeria extending from Calabar to Lagos in the south west of the country, similar symptoms on the crop have been observed. Naturally infected plants also showed, leaf malformation, green vein-banding, rugosity and leaf malformation. So far, there has been no previous report of virus diseases of *C. moschata* in Nigeria. The objective of this study was to characterize and identify the causal agent of the disease.

MATERIALS AND METHODS

Virus isolation and maintenance: Young symptomatic leaves obtained from naturally infected *C. moschata* plants during the 2005/2006 growing season were triturated in cold inoculation buffer (0.03 M L^{-1} sodium phosphate buffer pH 8.0) in pre-cooled oven-sterilized mortal. The homogenate was used to inoculate 500-mesh carborundum-dusted 9-day old plants of *Cucumeropsis mannii, Cucurbita moschata* or *C. pepo* in the greenhouse with temperature of 26±2°C. The virus was subsequently maintained in young seedlings of either of the plants by periodic mechanical inoculation.

Determination of host range: About sixty nine plant species or varieties belonging to nine families were tested. Test plants, other than those of cucurbits and legumes, were inoculated at 5-6 days leaf stage, while seedlings of cucurbits and legumes were inoculated at the cotyledonary leaf stage. At least five seedlings of each plant species or variety were mechanically inoculated with the inoculum prepared from virus-infected leaf tissues. All inoculated plants were rinsed with water, kept in the greenhouse at 26±2°C for a period of four weeks for symptom development. In other to ascertain possible latent infection, back-indexing was performed on *C. mannii*. At least three plants of each species or variety were inoculated with buffer only to serve as control.

Screening for resistance: Seeds were collected from fruits of different ecotypes or varieties of *C. moschata* from Cross River, Edo, Imo, Lagos, Ondo and Oyo States, all in the southern belt of Nigeria. The varieties differed in fruit shape (spherical to elongate) and in the colour and hairiness of seeds. About 50–60 seeds from each seed lot were sown and the seedlings were inoculated with the Cucurbita virus isolate.

Virus recovery from floral parts, juvenile fruits of infected plants and seed transmission tests: Virus recovery from floral parts and juvenile fruits of naturally infected plants and mechanically inoculated *C. moschata* was carried out as described by Ladipo (1988).

For seed transmission test, 537 seeds obtained from nine (9) fruits harvested from virus infected *C. moschata* were dried in the sun for a few days before they were planted in seed trays containing sterilized garden soil and kept in the greenhouse, watered regularly and observed for symptom development. Final observation was made when the fourth true leaf was fully developed.

Aphid transmission tests: Nymphs and apterous adults of *Aphis craccivora* Koch, *A. gossypii*, *A. spiraecola* Pach, *Myzus persicae* Sulzer, *Macrosiphon euphorbiae* Thomas and *Toxoptera citricidus* Kirk were tested for their ability to transmit the cucurbita virus isolate. The aphids were starved for 3 h and allowed between 1-3 min acquisition feeding on detached infected leaves of *C. moschata*, or *C. pepo* floated on water in Petri dishes. Ten to fifteen aphids were then transferred to each of five seedlings of *C. pepo* and allowed 3 min inoculation feeding before they were killed with Pirimor or Actellic 50 EC (ICI 10 ml L^{-1}).

Electron microscopy: Crude undiluted extract from infected leaves of *C. pepo* was adsorbed onto zaponlack-carbon-coated grids and washed once in potassium phosphate buffer (K_2HPO_4) pH 7.0 and distilled water. The grids were negatively stained with aqueous 2% uranyl acetate and examined under the Upton-902 electron microscope.

Cytopathology: Small pieces of the cucurbita virus-infected leaf tissues were taken and fixed with 3% (v/v) glutaraldehyde after four changes (×4) of 10 min duration in 0.1 M cacodylate buffer pH 7.0 overnight. The samples were then post-fixed for 2 h with 0.66% osmium tetraoxide in two changes (×2) of 45 min duration in 0.1 M veronate acetate buffer, pH 7.25. This was followed by two times (×2) dehydration of the samples through graded series of alcohol (30, 50, 70 and 90 absolute alcohol) in 1% aqueous uranyl acetate. Thereafter, the samples were embedded in gelatin capsule for 24 h at 40°C and later for 48 h at 60°C. Ultra-thin sections were made using Reichert-Jung ultramicrotome and examined under the Upson-902 electron microscope.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE): Relative molecular mass (Mr) of the virus coat protein was determined by SDS-PAGE as described by Laemmli (1970). Western blotting was performed according to Richter *et al.* (1994).

Serological tests: Serology tests were carried out first to determine the genus to which the cucurbita virus belong using potyvirus specific monoclonal antibody (PTY-Agdia), MoAb P-3-3H8 and polyclonal antibody TuMV-314 known for its reactivity with most potyviruses (Richter *et al.*, 1994) in plate-trapped antigen ELISA (PTA-ELISA) as described by Converse and Martin (1990). Further serological tests to determine degree of relatedness with other potyviruses were performed using antisera (IgG) to Bean common mosaic virus (BCMV), Clover yellow vein virus (CIYVV), Papaya ringspot virus (PRSV), Potato virus Y (PVY), Turnip mosaic virus (TuMV-326), Soybean mosaic virus (SoyMV) and Watermelon mosaic virus 2 (WMV-2) (obtained from the Antiserum Bank of the Institute of Pathogen Diagnostics, Aschersleben, Germany) in DAS-ELISA using the method described by Clark and Adams (1977).

Immune specific electron microscopy: Antisera (IgG) prepared against Moroccan watermelon mosaic virus (MWMV), PRSV BYMV, CIYVV, TuMV, Telfairia mosaic virus (TeMV), WMV (Katabase), WMV-2, Zucchini yellow fleck virus (ZYFV) and ZYMV (supplied by Dr. Vetten) were used in immune specific electron microscopy (ISEM) decoration tests carried out as described by Richter *et al.* (1994).

RNA purification, cDNA synthesis and sequence analysis: The cucurbita virus was propagated in *Cucurbita pepo* under greenhouse conditions and was purified from leaf tissues inoculated 2-4 weeks after inoculation according to method of Wong *et al.* (1994). RNA was extracted from purified virions as described by Maiss *et al.* (1998). Complementary DNA (cDNA) synthesis was performed both by oligo (dT)- and random priming according to Gubler and Hoffman (1983). Double stranded cDNA was dC-tailed and annealed to Pst I cut dG-tailed pBR 322. Cells of *Escherichia coli* strain DH 1 were rendered competent by the dimethylsulfoxide/dithiothreitol procedure according to Hanahan (1983) and transformed with 10 ng of recombinant DNA. Colonies were transferred to nitrocellulose filters placed on LM agar and grown overnight. Lysis of bacteria, DNA denaturation and fixation were carried out as described by Grunstein and Hogness (1975). Clones containing plasmids with cDNA insert were identified and isolated by a modified alkaline lysis method (Birnboim and Doly, 1979). Sequencing was carried using the method of Sanger *et al.* (1977) and sequence data were analyzed by use of computer programme from Schwindinger and Warner (1984).

Sequence alignment was obtained using EMBOSS procedure and distance matrix by EMBLOSUM62. The deduced amino acid sequence data obtained was compared to that of MWMV following weak decoration of the virus under study by its antiserum in ISEM tests.

RESULTS

Host range and symptomatology: The results of the host range studies indicated that the virus had a rather narrow host range. Beside *Chenopodium, quinoa* and *C. amaranticolor* in the family Solanaceae, in which it induced necrotic and chlorotic local lesions respectively (Fig. 1), the cucurbita virus infected mainly cucurbitaceous plants (Table 1). Out of the 69 plant species or

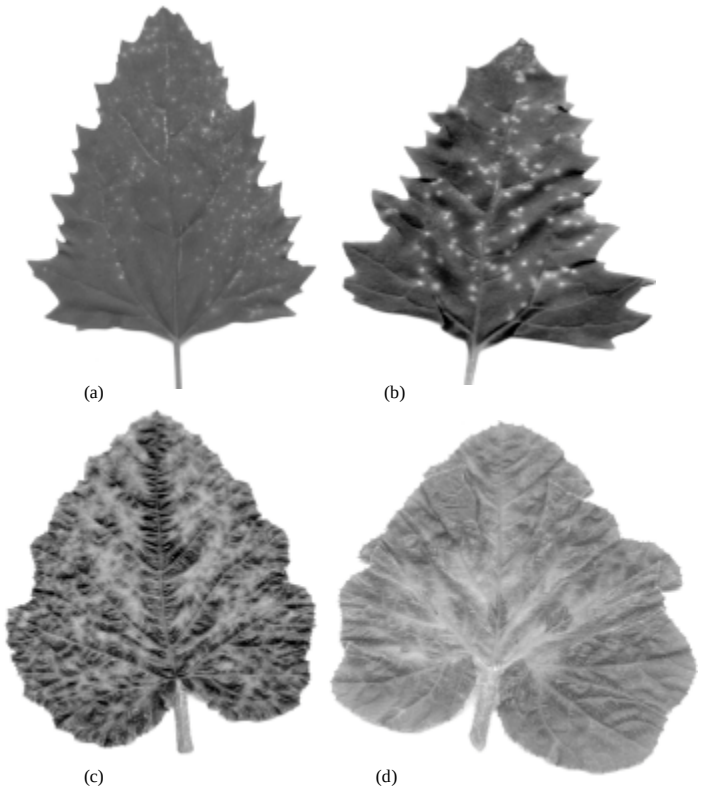

Fig. 1(a-d): Symptoms of infection with the cucurbita virus Necrotic local lesions (a) and (b) in *Chenopodium amaranticolor* and *C. quinoa* respectively, green vein-banding in *Cucurbita pepo* (c) leaf malformation and puckering and (d) in *C. moschata*

varieties belonging to 9 families, the virus infected only 17. Symptoms induced in some of the susceptible cucurbits included mosaic, green-vein banding, leaf malformation, rugosity, reduced leaf size (Fig. 1) or complete defoliation depending on the species or variety. None of the following species developed any symptom and neither was there any evidence of latent infection: Amaranthaceae-*Amaranthus caudatus* L., *A. hybridus* L., *A. viridis.*, *Celosia argentea* L., var. "TLV 8", *C. trigyna* L. and *Gomphrena globosa* L.: Chenopodiaceae-*Chenopodium capitatum, C. foetidum* Shrad, *C. foliosum* Moench Aschers., *C. morale, C. rubrum, C. urbicum*: Cucurbitaceae-*Citrullus vulgaris, Luffa cylindrica* Roem, *Momordica charantia* and *Telfairia occidentalis* Hook: Fabaceae-*Arachis hypogaea, Cajanus cajan* Mill, *Canavalia ensiformis* DC, *Glycin max* (L) Merr., *Phaseolus vulgaris* L. (var. Saxa), *P. lanatus* L, *Sesbania sesban, Vigna mungo* L., *V. unguiculata* (L) Walp. (vars. Ife Brown, Mascara and K59); Lamiaceae-*Ocimum basilicum* L., *O. canum* L., *O. gratissimum* L., Malvaceae-*Abelmuschus esculentus* (L) Moehn; Poaceae-*Zea may* Gaertn Fruct,: Solanaceae-*Datura metel* L., *D. stramonium* L., *Lycopersicum esculentum* Mill., *Nicotiana benthamiana, N. glutinosa* L., *N. occidentalis* Wheeler, *N. clevelandii, N. megalosiphon* Jeurck et Muller Arg. *N. rustica* L., *N. tabacum* L., (vars. Bell, White Burley, Samsum and Xanthi), *Physalis angulata* L., *P. floridana* Rydb, *Solanum macrocarpon* L., *S. melongena* L. *Capsicum frutescens* L., *C. annuum* L., Tiliaceae-*Corchorus olitorius* L.

Table 1: Reaction of test plants to mechanical inoculation to the cucurbita virus isolate

Test plant	Symptoms	Back-indexing on *C. mannii*
Cucurbitaceae		
Adenopus breviflorus	Mosaic	+
Citrullus lanatus (thumb.) Mansf.	Green vein-banding, blistering, leaf malformation, mosaic	
Colocynthis citrullus (L.)	Mosaic	+
Cucumeropsis edulis (L.)	Green vein-banding, leaf malformation, rugosity, defoliation, stunting, mosaic	+
Cucumis sativus (L.) var "Poinsett"	Green vein-banding, leaf malformation, rugosity, mosaic	+
Cucurbita moschata (Duch ex. Lam.) Duch. Ex. Poiret.	Green vein-banding, leaf malformation, rugosity, mosaic	+
Cucurbita pepo (L.)		
var. "Encore"	Vein-clearing, mosaic leaf malformation shoestring	+
var. "Consul"	Vein-clearing, mosaic leaf malformation shoestring	+
var. "Corona"	Vein-clearing, mosaic leaf malformation shoestring	+
C. pepo (unidentified var, from Germany)	Vein-clearing, mosaic leaf malformation shoestring	+
Lagenaria siceraria (Molina) Standley		
"Calabash"	Green vein-banding, mosaic	+
"Bitter gourd"	Green vein-banding, mosaic	+
"Trumpet gourd"	Green vein-banding, mosaic	+
Luffa acutangula (L.) Roxb.	Mosaic	+
Trichosanthes cucumerina L. var. anguina	Green vein-banding, mosaic	+
Solanaceae		
Chenopodium amaranticolor Coste et Reyn	Necrotic local lesions	+
Chenopodium quinoa L.	Chlorotic local lesions	+

Screening for resistance: None of the seedlings derived from the seed lots of the ecotypes or varieties of *C. moschata,* when inoculated with the cucurbita virus isolate showed immunity. All developed typical mosaic and green vein-banding symptoms associated with natural infection of the vegetable crop.

Virus recovery from floral parts, juvenile pods and seed transmission: Virus was recovered from sepals, petals and anthers of flower buds and fully opened staminate and perfect flowers of *C. moschata* but not from juvenile fruits of the plant. Similarly, none of the 537 seedlings of *C. moschata* screened for possible seed transmission of the virus showed any symptoms.

Aphid transmission of the virus: The virus was transmitted by *A. spiraecola, A. gossypii, M. persicae* and *T. toxoptera* in a foregut (non-persistent) manner from *C. moschata* to *C. pepo* and *L. siceraria.* However, all attempts to transmit the virus by *A. craccivora* and *M. euphorbiae* were unsuccessful.

Morphology of the virus particle: Flexuous rod-shaped particles of about 750 nm length were observed under the electron microscope in leaf dip preparations from *C. pepo* infected by the virus (Fig. 2).

Coat protein molecular mass determination: SDS-PAGE analysis followed by Western blotting gave the molecular mass (Mr) of the dissociated coat protein of the virus as 32.5 kDa.

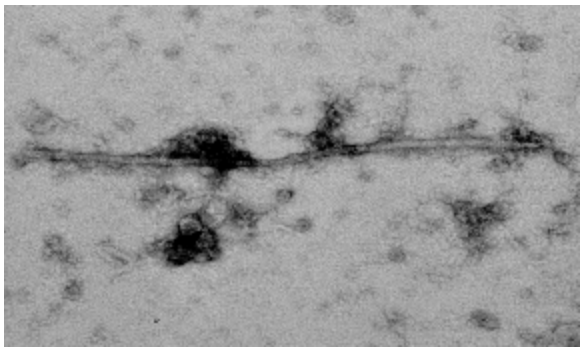

Fig. 2: Electron micrograph of leaf dip preparation showing typical flexuous rod-shaped particle of the Cucurbita virus. Bar = 39.5 nm

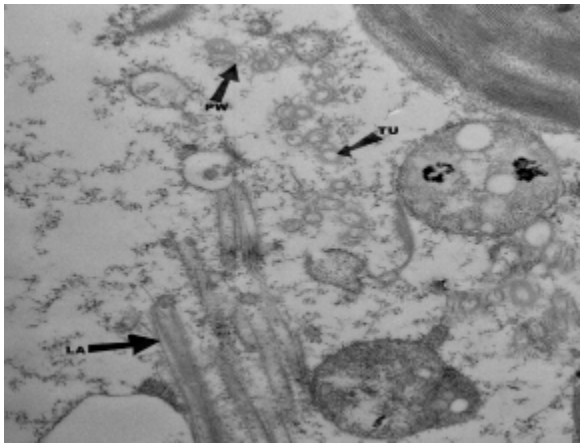

Fig. 3: Pinwheel (PW), Tube (TU) and Laminated Aggregate (LA) induced by the Cucurbita virus in *Cucumis sativus*

Cytopathology: Examination of ultra-thin sections obtained from *C. pepo* showed the presence of pinwheels, tubes and laminated aggregates (Fig. 3).

Serological properties of the virus: The virus reacted positively with the potyvirus specific monoclonal antibody (PTY-Agdia), MAb P-3-3H8 and antiserum to TuMV (TuMV-314) in PTA-ELISA. However, the virus failed to react with antisera to BCMV, BYMV, CIYVV, PRSV, PVY, MWMV, TuMV-326, SoyMV and WMV-2 in DAS-ELISA.

Immune specific electron microscopy (ISEM): The virus was weakly decorated by antisera to MWMV and PRSV in ISEM – decoration tests. On the other hand, antisera raised against BYMV, CIYVV, TuMV, WMV (Katabase), WMV-2, ZYFV and ZYMV gave no decoration at all.

(A)

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tccaaacttcctgagcacagattagaggccatcacggctgcgattattgaatcctggggatatcctgatctaac
gcagcatattcgcaagttctatcaatgggttttggaacaagctccgtataacgaactggcgcgcattggaagag
ccccatatgtctcagaagccgggctcagaaatctgtacacttcacagcgaggtagcccagttgatttggaggca
tatgtcacagcatattttcaaaacgaaacgggagatacacctgaacttgtggtttatcatcaagctggcgagac
tcaagatgctggtgatatagtaagaagaaagaaaaagaagaaaaagaacaaaaagaaaagaaggaggctgctga
aaccgcagctaaagcaagcggagacactaagaaggcaagtgtcaaaggaaaggaaaaggatgtggatgttggaa
cgtctggcacattcacgatacccaaaattaagaccttcaccgataggatgattttgccaaagagcaatggaaaa
ttagcgcttaatttggaacatctcttggtttacaatccaactcaagtgcagctgtcaaacacccgatcaacaca
acgccaatttgacaagtggtatgaggggattatgaatgactatggattgaacagcagcgagatgccaattctcc
taaacggtttgatggtttggtgcattgaaaatggaacctcaccaaacgttaatggagtttgggttatgatggat
ggagaagagcaaatcgaatatcctatcaagccattgttggatcacgccagtccaacttttagacagattatggc
acacttcagcaacgcg
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(B)

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1       SKLPEHRLEAITAAIIESWGYPDLTQHIRKFYQWVLEQAPYNELARIGRAPYVSEAGLRN    60
61      LYTSQRGSPVDLEAYVTAYFQNETGDTPELVVYHQAGETQDAGDSSKKKEKEEKEQKEKK    120
121     EAAETAAKASGDTKKASVKGKEKDVDVGTSGTFTIPKIKTFTDRMILPKSNGKLALNLEH    180
181     LLVYNPTQVQLSNTRSTQRQFDKWYEGIMNDYGLNSSEMPILLNGLMVWCIENGTSPNVN    240
241     GVWVMMDGEEQIEYPIKPLLDHASPTFRQIMAHFSNA   277
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Fig. 4 (a-b): The 831 nucleotide bases of the genome of the Cucurbita virus isolate Sequence alignment of the amino acids of the N-terminal region of the coat protein of the Cucurbita virus isolate

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                *           *           *    *                  **    ** *
CurV    1       SKLPEHRLEAITAAIIESWGYPDLTQHIRKFYQWVLEQAPYNELARIGRAPYVSEAGLRN     60
MWMV    105     AKLPEHRLEAISAAIIESWGYPELTNEIRKFYQWVLEQAPYSDLALKGKAPYVSEAGLRN    164

                      **   * * *  **  *   *    *       *****  *** *** * * * * * *    *
CurV    61      LYTSQRGSPVDLEAYVTAYFQNETGDTPELVVYHQAGETQDGDSSKKKEKEEKEQKEKKK    120
MWMV    165     LYTSQRGSPQELERYITHYFKSESGDCPELMVYHQADNLKDAGQGVGEKEKKEKEKEKEK    224

                **************************  * *         *    **   *    * *    *****  **
CurV    121     EAAETAAKASGDTKKASVKGKEKDVDVGTSGTFTIPKIKTFTDRMILPKSNGKLALNLEH    180
MWMV    225     DKKSSDDTGGSSSQDQGRKDKDKDVDVGTTGTFRVPKVKTFNDKMILPRVRGRIALNLEH    284

                  *   * **     *  *   *  *****   ***  ***   *            *
CurV    181     LLVYNPTQVQLSNTRSTQRQFDKWYEGIMNDYGLNSSEMPILLNGLMVWCIENGTSPNVN    240
MWMV    285     LLQYNPNQIDLSNTRATQNQFDRWYDGVKSDYGLDDEEMAIVLNGFMVWCIENGTSPNIN    344

                   *   **  *  **   ***     *     *
CurV    241     GVWVMMDGEEQIEYPIKPLLDHASPTFRQIMAHFSNA      277
MWMV    345     GVWTMMDNGEQVEYLLKPMIEHASPTLRQIMAHYSNA      381
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Fig. 5: Sequence alignment of the amino acids of the N-terminus of the coat protein of the Cucurbita virus here represented as CurV with that of Moroccan watermelon mosaic virus (MWMV). Stars (*) indicate points of differences between the sequences

RNA purification, cDNA synthesis and sequence analysis: The result of the data generated from the sequence analysis of the genomic RNA shows that it consisted of 831 nucleotide bases (Fig. 4a). The coat protein sequence analysis of the N-terminus of the Cucurbita virus isolate was composed of 277 amino acids (Fig. 4b). Alignment of the amino acid composition showed 66% homology compared to that of MWMV (Fig. 5).

DISCUSSION

The characterization and identification of the aetiological agent of a mosaic inducing virus in *C. moschata* was studied. The diagnostic tools used included host range, mode of transmission, electron microscopy, cytopathology, serology and coat protein sequencing. The virus had a limited host range, was vectored by some aphids in a fore-gut manner and characterized by flexuous rod particles. The virus also reacted positively with potyvirus group monoclonal antibody (PTY-Agdia), P-3-3H8 MAb and potyvirus antiserum TuMV reputed for detecting potyviruses (Richter *et al.*, 1994). The virus also induced pinwheels, laminated aggregates and tubes. These characteristics are consistent with the properties of the genus Potyvirus (Gulya *et al.*, 2002; Desbiez *et al.*, 2007; Gholamalizadeh *et al.*, 2008) and the virus obviously belongs the genus *Potyvirus* (Family Potyviridae).

On the basis of host range, the virus under investigation differs from the WMV-like virus described by Igwegbe (1983) from Nigeria. The WMV-like virus isolate infected *C. amaranticolor* and *C. quinoa* with reactions far too erratic according to Igwegbe (1983) whereas, the cucurbita virus isolate elicited conspicuous and consistently, chlorotic local lesions and necrotic ringspots on *C. quinoa* and *C. amaranticolor*, respectively. In addition, *Colocynthis citrullus, Cucurbita pepo* and *Luffa acutangula* which were not susceptible to the WMV isolate were readily infected by the cucurbita virus reported in this study. Equally important is that no serological relationship was detected using antisera to all available serotypes of WMV either in DAS-ELISA or in ISEM decoration tests.

The cucurbita virus is also considered different from TeMV described by Shoyinka *et al.* (1987). Whereas TeMV infected several other plant species from six families, the virus from *C. moschata* comparatively has a restricted host range, mostly infecting cucurbitaceous plants. Besides, *T. occidentalis,* the natural host of TeMV and *N. benthamiana* which showed severe reaction to the virus were not infected by the cucurbita virus. The virus was also not decorated with antiserum to TeMV. Based on ISEM decoration tests, PRSV and MWMV are the only members of the family Potyviridae infecting cucurbits that appeared distantly related to but distinct from the cucurbita virus isolate.

Potyviruses with sequence homologies ranging between 38-71% (average 54%) are considered distinct members while that between strains of the virus ranged from 90-99% (average 95%) (Shukla and Ward, 1988). The cucurbita virus isolate has 66% amino acid sequence homology with MWMV, indicating that they are distinct viruses.

CONCLUSION

This is the first report of a virus naturally infecting *C. moschata* in Nigeria. It seems that the virus is novel in Nigeria and also considered distinct from other viruses commonly infecting cucurbits for which the name Cucurbita mosaic virus (CuMV) is suggested.

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