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Strains of Moroccan Watermelon Mosaic Virus Isolated from *Lagenaria breviflorus* and *Coccinia barteri* in Calabar, Southeastern Nigeria

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ABSTRACT

Virus infection of *Lagenaria breviflora* and *Coccinia barteri* were observed in Calabar, Nigeria during the 2005/2006 growing season. The viruses causing the diseases were characterized in this study. Diagnostic tools were host range, transmission studies, cytopathology, electron microscopy, Immunosorbent Electron Microscopy (ISEM), serology and coat protein gene sequencing. Evidence from biological, serological and sequence data confirmed that both viruses belong to the genus *Potyvirus*, family *Potyviridae*. Both were mechanically transmissible and also transmitted by *Myzus persicae* and *Aphis gossyppi* in a fore-gut manner. They also induced cytoplasmic inclusions in infected leaf tissues. The Lagenaria virus isolate has a coat protein molecular weight of 32.5 kDa and 35.0 kDa for Coccinia isolate. Of the two viruses only Lagenaria virus isolate showed cross reactivity with MWMV in DAS-ELISA. Both viruses reacted negatively with antisera to some notable cucurbit viruses in the same test, showed weak to moderate decorations with antisera to PRSV, TuMV and TeMV in immunosorbent electron microscopy (ISEM) tests. The Coccinia virus was, however, strongly decorated by antiserum to MWMV but no decoration with the Lagenaria virus. Comparison of the amino acid sequence data of the N-terminal regions of the coat proteins to that of MWMV reported from Sudan indicated 92% and 93% identities for the Coccinia and Lagenaria viruses, respectively. It is suggested that the virus isolates reported in the study be considered strains of the MWMV Sudan isolate. This is the first report of the occurrence of MWMV in Nigeria.

Key words: *Lagenaria breviflora, Coccinia barteri,* morrocan watermelon mosaic virus, serology, sequence data

INTRODUCTION

Lagenaria bleviflora (Benth.) Roberty (*Adenopus breviflorus*) is a member of the Cucurbitaceae family. It is characterized by glabrous stems and leaves which are distinctly 5-lobed. Arising from the axils of the leaves are branched tendrils. The plant is monoecious but the male and female flowers are borne separately on the same plant. The fruits are roundish, streaked and flattened at

both ends (Burkill, 2004). Economically, the seeds are considered a good source of most essential amino acids comparable to soybean (Oshodi, 1996) and oil (85.1% unsaturated fatty acid and 65.3% linoleic acid) suggesting potential uses in soap making, shoe polish, shampoo and edible purposes (Akintayo and Bayer, 2002).

Coccinia barteri (Hork.f) Kay is also a member of the Cucurbitaceae family. It is herb characterized by unbranched tendrils which arise from the axils of leaves by which it attaches itself to supports. The leaves are variable in shape, more or less deeply 3-5 lobed, shining, glossy and dark green in colour often with white blotches. The male and female flowers are borne in a raceme and the fruits are streaked and ellipsoidal (Holstein and Renner, 2010). It is a pot-herb and of medicinal importance in Cross River State of Nigeria.

Globally, Zucchini Yellow Mosaic Virus (ZYMV), Papaya Ringspot Virus (PRSV) and Watermelon mosaic virus-2 and Cucumber Mosaic Virus (CMV) are considered among the most economically important viruses infecting cucurbits (Yardimci and Korkmaz, 2004; Lecoq *et al.*, 2001; Fattouh, 2003; Salem *et al.*, 2007; Massumi *et al.*, 2007; Gholamalizadeh *et al.*, 2008; Safaeizadeh, 2008). Others, though of limited distribution but of no less importance, are Zucchini Lethal Chlorotic Virus (ZLCV) (Yuki *et al.*, 2000), Watermelon leaf mottle virus (De Sa *et al.*, 2000), Zucchini green mottle mosaic virus (Choi, 2001), Melon Necrotic Spot Virus (MNSV) (Choi *et al.*, 2003), Kyuri green mottle mosaic virus (Ko *et al.*, 2006), Zucchini yellow fleck virus (Desbiez *et al.*, 2007), Cucurbit Yellow Disorder Virus (CYSDV) (Wintermantel *et al.*, 2009), Cucumber Green Mottle Mosaic Virus (CGMMV) (Shim *et al.*, 2005; Ko *et al.*, 2007; Liu *et al.*, 2009), Cucurbit Aphid-borne Mosaic Yellows Virus (CABYV), (Kneirim *et al.*, 2010) and Cucumber Green Mottle Virus (CGMV) (Moradi and Jafarpour, 2011).

Moroccan Watermelon Mosaic Virus (MWMV), first isolated in Morocco (Fischer and Lockhart, 1974; McKern *et al.*, 1993) was reported to have caused severe damage to cucurbits in all commercial cucurbit producing regions. The virus has also been reported from southwest Spain (Quiot-Douine *et al.*, 1990) and Italy (Roggero *et al.*, 1998). In African the virus has been reported from South Africa (Van Der Meer and Garnett, 1987), Sudan (Lecoq *et al.*, 2001), Democratic Republic of Congo (Arocha *et al.*, 2008) and Tunisia (Yakoubi *et al.*, 2008).

The southeastern corner of Nigeria is rich in both cultivated and wild cucurbit species. Among the cultivated ones are *Cucurbita moschata* (Duch ex Lam) Duch and Poir, *Cucumis sativus* L. *Lageneria siceraria* (Mol) Standl, *Cucumeropsis manni* (= *C. edulis*) and *Colocynthis citrullus* L. cultivated for their leaves as pot-herbs and fruits and seeds. Among the wild species are *Lagenaria breviflora* and *Coccinia barteri* which are importantly in traditional medicine. Virus infection of some of these cucurbits is widespread but largely unreported. So far, a watermelon mosaic-like virus isolated from *C. edulis* (= *C. manni*) (Igwegbe, 1983), Telfairia mosaic virus (TeMV) reported on *Telfairia occidentalis* (Shoyinka *et al.*, 1987) and a strain of PRSV from *Cucumis sativus* (Owolabi *et al.*, 2008) are the potyviruses that have been reported naturally infecting cucurbits in Nigeria.

In 2005-2006 growing season, virus-like induced symptoms characterized by mosaic, leaf malformation and conspicuous green-vein banding on *Lagenaria breviflora* while vein-clearing and sometime yellow mosaic, green vein-banding which sometimes may be masked by white blotches on the leaves, were observed on *Coccinia barteri*. This paper reported the biological, serological and molecular characterization of these virus isolates tentatively designated LbreV and CbarV for the *L. breviflora* and *C. barteri* virus isolates, respectively.

MATERIALS AND METHODS

Virus isolation and propagation: Symptomatic leaves obtained from both *L. breviflora* and *C. barteria* were collected from the field in sealed polyethylene bags. The infected leaf tissues were triturated in cold 0.03 mol L sodium phosphate buffer pH 8.0 in pre-cooled oven-sterilized pestle and mortar. The inocula were mechanically transferred onto healthy seedlings of a range of test plants in the greenhouse (23±2°C) and the inoculated plants were left for symptom development. Three serial local lesion transfers were made for LbreV which elicited chlorotic local lesions in *Chenopodium quinoa*. No local lesion hosts were identified for CbarV. However, preliminary serological tests using double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) confirmed that symptoms observed on *C. barteri* was not due to mixed infection. Both viruses were propagated in *C. sativus* and or *C. pepo* by periodic sap inoculation.

Host range and symptomatology: For host range studies, inocula derived from infected leaf tissues of the propagation hosts were inoculated by rubbing on 600-mesh carborundum dusted leaves of at least 25 plant species and cultivars belonging to the Amaranthaceae, Chenopodiaceae, Solanaceae and Cucurbitaceae families. The plants were kept in the greenhouse at 23±2°C. Symptoms were recorded for a period spanning 4 weeks. All inoculated plants without visible symptoms were assayed for virus presence by back-indexing on *C. manni*.

Aphid transmission tests: Myzus persicae, Aphis craccivora, A gossypii and *Macrosiphon euphorbiae* reared on *Ficia faba* and *A. spiraecola* obtained from its natural host (*Chromolaena odorata*) were starved for 1 h and allowed acquisition feeding time of about 3-5 min. Ten aphids were then transferred to each of five healthy seedlings of *C. pepo* in insect-screened cages, kept and sprayed with an insecticide (Pirimor). The plants were then kept in the greenhouse and symptom development was monitored for about 3 weeks.

Electron microscopy: The particle morphology of the virus isolates was determined by leaf-dip serology. Virus particles were trapped onto grids pre-coated with polyclonal antiserum (TuMV-314) or monoclonal antibody (MoAb) P-3-3H8, followed by negative staining with 2% phosphotungstic acid, pH 6.0. The grids were then examined under the Upson-902 electron microscope.

Cytopathology: Small pieces from symptomatic leaf tissues of *Lagenaria breviflora* and *Coccinia barteri* virus-infected leaf tissues were taken and fixed with 3% (v/v) glutaraldehyde after four changes (x4) 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 (x2) of 45 min duration in 0.1 veronate acetate buffer, pH 7.25. This was followed by two times (x2) 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.

Gel electrophoresis and immunoblotting: Symptomatic leaf tissues of *C. pepo* for both viruses were homogenized (1:3 w/v) in distilled water. One hundred microlitres were treated with equal volume of Sodium Dodecyl Sulphate (SDS)s dissociation buffer (2% SDS, 0.62 mM Tris-Hcl buffer

pH 6.8, 5% (v/v) 2-mercaptoethanol, 5% (v/v) glycerol and 0.001% (v/v) bromocresol blue) by boiling for 5 min. Proteins were separated by SDS-polyacrylamide gel electrophoresis (PAGE) in a Vertical Minigel-Twin electrophoresis apparatus (Biometra, Gottingen, Germany) as described by Laemmli (1970). Western blotting was carried out as described by Richter *et al.* (1994).

Virus purification and antisera production: The viruses were purified from infected zucchini squash (*Cucurbita pepo*) leaf tissues 2-3 weeks post inoculation by differential centrifugation as described by Owolabi *et al.* (1998). Briefly, the procedure involved grinding tissues in high salt buffer, precipitating with polyethylene glycol and isolating viral zones after sucrose density gradient centrifugation.

Antisera to the viruses were produced by injecting rabbit intramuscularly with 1 mg/1 mL purified viral suspensions emulsified with (1/1) Freund's incomplete adjuvant followed by two booster injections with Freund's incomplete adjuvant at weekly interval. The animals were bled starting 2 weeks after injection. Immunoglobulins G (IgGs) and alkaline phosphate conjugates were prepared using standard procedures and used for serological tests.

Serology tests: Serological relationships between the virus isolates used in this study and some definitive potyviruses were investigated in plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA) as described by Converse and Martin (1990) and DAS-ELISA (Clark and Adams, 1977). Polysterene microtitre plates were used and the reaction volumes consisted of 100 µL per well. Appropriate dilutions of a panel of 15 antisera mostly immunoglobulins (IgGs) (obtained from the antiserum bank, Institute of Pathogen Diagnostics, Aschersbelen, Germany) and prepared as recommended were used in the ELISA tests. Symptomatic leaf samples were ground in extraction buffer at 1:10 (w/v). ELISA readings were considered positive when they exceeded twice the readings of the corresponding controls (Walkey *et al.*, 1994).

Immune specific electron microscopy: Antisera (IgG) to (MWMV), PRSV, Beet Yellow Mosaic Virus (BYMV), ClYVV, TuMV, (TeMV), WMV (*Katabase*), WMV-2, Zucchini Yellow Fleck Virus (ZYFV) and ZYMV obtained from Biologische Bundesanstalt, Brauschweig, Germany, were used in Immunospecific Electron Microscopy (ISEM) decoration tests carried out as described by Richter *et al.* (1994).

RNA purification, cDNA synthesis and sequence analysis: RNA was extracted from purified virions of both isolates by incubation for 30 min in 10 mM Tris-HCl pH 7.6, 100 mM NaCl. 0.5% SDS and 200 µg mL^{-1} proteinase K at 30°C followed by two phenol extraction and ethanol preparation as described by Maiss *et al.* (1988). 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 place 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 the use of computer program from Schwindinger and Warner (1984).

Sequence alignment was obtained using EMBOSS procedure and distance matrix by EMBLOSUM62. The deduced amino acid sequence data obtained were compared to that of MWMV.

RESULTS

Host range and symptomatology: Both virus isolates somewhat had narrow host ranges. The host range of CbarV was restricted to the cucurbitaceous plants as non-cucurbits tested were not susceptible. Besides infecting a good number of the cucurbit test plants, the LbreV induced chlorotic local lesions in *Chenopodium amaranticolor* (Fig. 1) and *C. quinoa* though inconspicuous (Table 1). The susceptible cucurbits reacted differently to the two virus isolates. For example, LbreV induced conspicuous and severe mosaic leaf malformation and green vein-banding in *C. pepo, C. moschata* and *L. siceraria*, while infection of these cucurbits species by CbarV was characterized by mild mottle except in *L. siceraria* that showed severe leaf malformation. The following plant species were not susceptible to both viruses and there was no evidence of latent infection. Amaranthaceae: *Celosia trigyna* Linn., *Gomphra globosa* Linn.; Chenopodiaceae: *Chenopodium foetidum, C. foliosus C. murale* Linn. *C. rubrum, C. urbicum* and *C. capitatum.*; Cucurbitaceae: *Colocynthis citrullus* Mill. Gard. *Luffa aegyptica* Mill.; Cruciferae: *Brassica oleracea V. capitata* Linn.; Solanaceae: *Nicotiana benthamiana* Domim. *N. occidentalis* Wheeler, *N. tabacum V. kamsum* Linn., *Solanum melogena Linn., S. nigrum* Linn. and *Physalis angulata* Linn.

Aphid transmission tests: Both viruses were transmitted in the fore-gut manner (non-persistent) by *M. persicae* and *A. gossyppii* but not by *A. craccivora*. CbarV was also transmitted by *M. euphorbiae* but failed to transmit LbreV.

Electron microscopy and cytopathology: Leaf dip preparations of *C. edulis* infected by the CbarV and LbreV isolates revealed flexuous rod shaped particles resembling those of potyviruses (Fig. 2a, b). LbreV induced laminated aggregates while CbarV produced laminated aggregates and tubes in thin sections made from *C. edulis* (Fig. 2c, d).

Electrophoresis and western blotting: The results showed that the molecular weight (Mr) of LbreV was about 32.5 kDa while that of the CbarV was approximately 35.0 kDa (Fig. 3).

Table 1: Reactions of some host range plants to Lagenaria and Coccinia virus isolates

	Symptom	
Test plant	Lagenaria virus isolate	Coccinia virus isolate
Chenopodiaceae		
Chenopodium amaranticolor	Chlorotic local lesions	No symptom
C. quinoa	(inconspicuous) Chlorotic local lesions	No symptoms
Cucurbitaceae		
Cucumropsis edulis Naud.	Severe mosaic, leaf malformation	Mosaic, veinal chlorosis
Cucurbita pepo Linn.	Severe mosaic, green vein banding, leaf malformation	Mild mosaic, leaf malformation
C. moschata Linn.	Mosaic, leaf malformation	Mild mottle
Cucumis sativus Linn.	Mosaic, leaf malformation, reduced leaf size.	Leaf edge yellowing, angular veinal chlorosis
Lagenaria breviflora	Mosaic, green vein-banding, severe leaf malformation	Mosaic
L. siceraria (Molina) Standl.	Mosaic, leaf malformation	Green vein-banding, leaf malformation
Trichosanthes cucumerina	Leaf malformation	No symptom

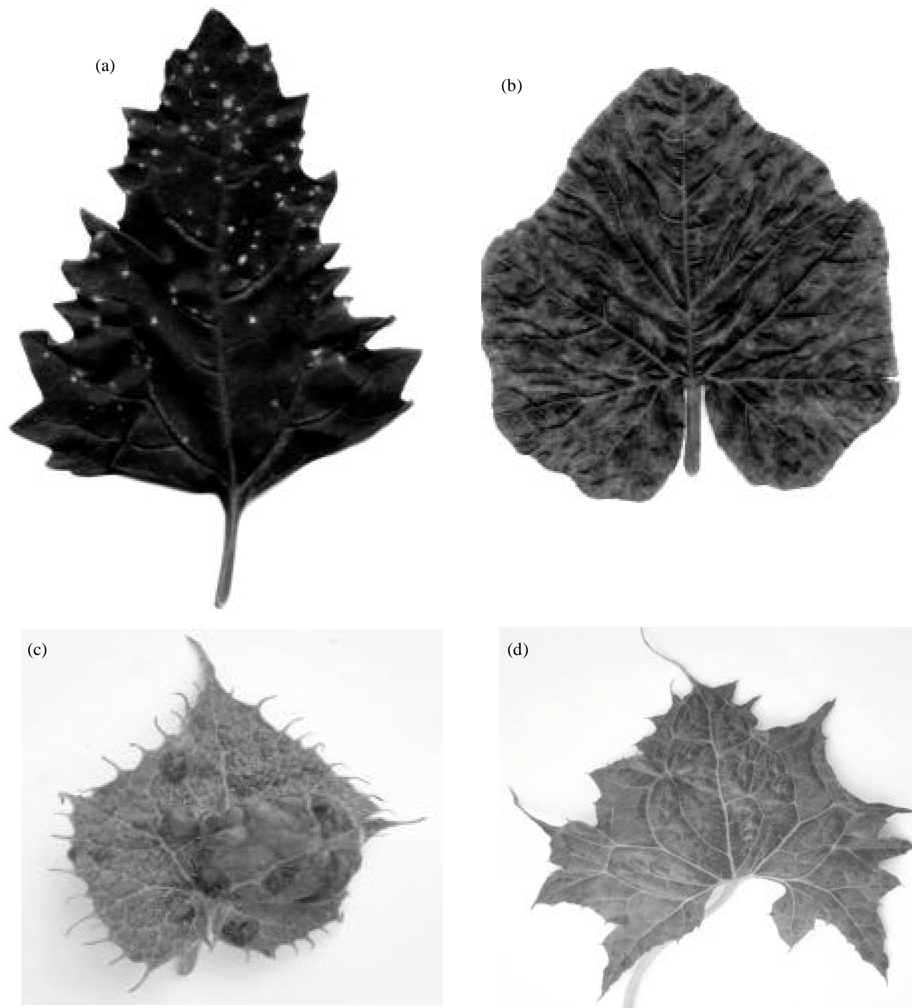

Fig. 1(a-d): Chlorotic local lesions induced by the Lagenaria virus isolate in *C. Amaranticolor* (a) and mosaic and dark green-vein banding in *Cucurbita pepo* (b). Leaf malformation induced by the Coccinia virus isolate in *Cucumeropsis mannii* (c and d)

Serological tests: Both LbleV and CbarV showed strong homologous reactions with their, respectively antisera with values of 2.00 and 1.47 compared to 0.02 and 0.01 when reacted with antiserum to PRSV. LbreV reacted positively with antibody to CbarV (0.41) but there was no reciprocal reaction between CbarV and antiserum to LbreV (Table 2). Both virus isolates reacted positively with potyvirus specific MoAb P-3-3H8 and polyclonal antibody TuMV-314 in PTA-ELISA. Of the two viruses only LbreV showed cross reaction with MWMV in DAS-ELISA. Both failed to react with antisera to BCMV, ClYMV, PRSV, TuMV-326, Soybean Mosaic Virus (SoyMV) and WMV-2 in the same test. However, in ISEM decoration tests, the LbleV was moderately decorated by anitisera to PRSV, TuMV and TeMV, very weakly decorated by antisera to WMV (kalabase), WMV-2 and ZYFV but no decorations were observed with MWMV and ZYMV antisera. On the other hand, CbarV was strongly decorated by MWMV antiserum, moderately decorated by antisera

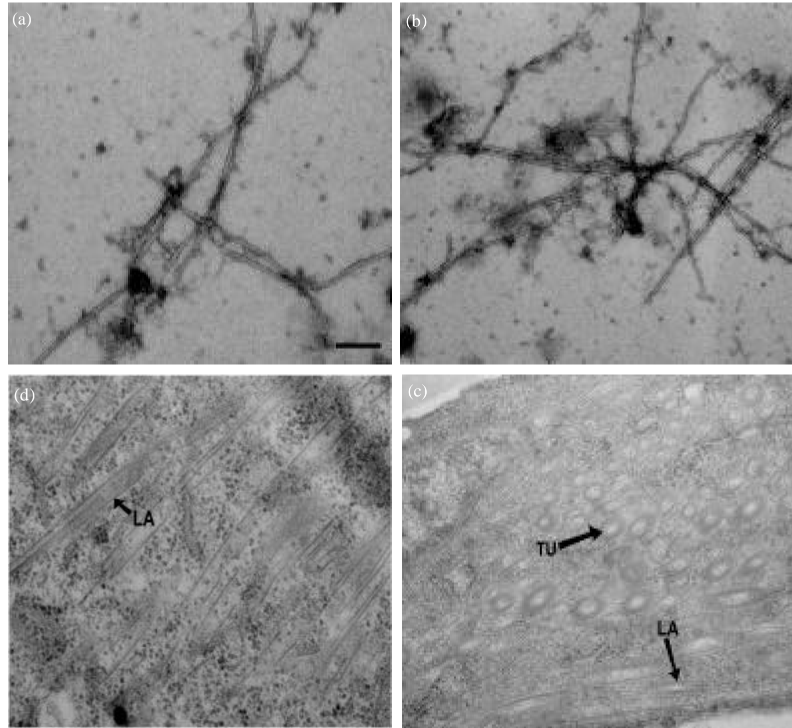

Fig. 2(a-d): Flexuous rod particles of *Lagenaria breviflora* virus isolate (a) and *Coccinia barteri* virus (b) stained with uranyl acetate. Laminated aggregates (LA) (c) laminated aggregates and tubes (TU) (d) induced by the Lagenaria and Coccinia virus isolates, respectively. Bars = 38.5 nm

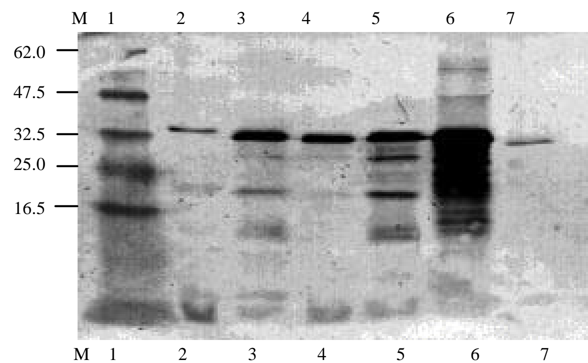

Fig. 3: Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting of some potyviruses. Pre-stained protein marker (M) (16.5-175 kDa). Lane 1: Coccinia barteri virus, Lane 2: Papaya rinspot virus (Owolabi *et al.*, 2008); Lane 3: Lagenaria breviflora virus; Lane 4: Cucurbita mosaic virus (In press); Lane 5: Watermelon mosaic virus-2 (Hungary); Lane 6: Potato virus Y; Lane 7: healthy sap from Nicotiana benthamiana. Viruses were detected after incubation with P-3-3H8 monoclonal antibody.

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         * * *  * *     * * *     *       20 *          *       * * *   *   * * *40          * * * *        *          60      *                        80
LbreV :  ADDPKDVGQGVADKKE - - - -  KKEKEKEKEKEQGKKSSDDTGGSSSQTQNQKGKEKDVDVGTTGTFRVPKVKTFNDKMILP

MWMV:    ADDVRDASQGVNEKKE - - - -  KKEKEKEREKEKEVKT SDETGGSSSQ ERGKKDKDKDVDVGTTGTFRVPKVKTFNDKMILP

CbarV:   ADNPRDAGQGTGDKKDGKEKEKEKEKEKEKEKEQDTKSSDDAGGSSSQGQGNK- - DKDVDVGTTGI FRVPKVKTFNDKMILP

         *          *                 100                            * 120    *                          140             *     *       160
LbreV:   KVRGR IALNLEH LLQYNPNQIDLSNTRATQNQFDRWYDGVKSDYGLDDEEMAIVLNGFMVMCIENGTSPN I NGVWTMMDD

MWMV:    KVRGR IALNLEH LLQYNPNQIDLSNTRATQNQFDRWHDGVKNDYGLDDEEMAIVLNGFMVMCIENGTSPNVNGVWTMMDN

CbarV:   RVRGRVALNLEHLLQYNPNQIDLSNTRATQNQFDRWYDGVKSDYGLDDEEMAIVLNGFMVMCIENGTSPN I NGMWTMMDN

                           180                          200                          220                         *     240
LbreV;   GEQVEYLLKPMIEHASPTRLRQIMAHYSNAAEAYIAKRNATERYMPRYGQKRNLRDISLARYAFDFYEMTSKTPEWAREAH

MWMV:    GEQVEYLLKPMIEHASPTRLRQIMAHYSNAAEAYIAKRNATERYMPRYGQKRNLRDISLARYAFDFYEMTSKTPE RAREAH

CbarV    GEQVEYLLKPMIEHASPTRLRQIMAHYSNAAEAYIAKRNATERYMPRYGQKRNLRDISLARYAFDFYEMTSKTPE RAREAH

                           260        *     *
LbreV:   MQMKAAAIRGANTRLFGIDGRHTVEDVDRDMHSLLGMRK  :      285

MWMV:    MQMKAAAIRGANTRLFGIDGRHTVDDVERDMHSLLGMRK  :      287

CbarV:   MQMKAAAIRGANTRSFGIDGRHTVEDVDRDMHSLLGMRK  :      285
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Fig. 4: Sequence alignment of the N-terminal regions of the coat protein of Lageneria breviflora virus (LbreV), Coccinia barteri virus (CbarV) and MWMV (Sudan isolate). Stars (*) indicate points of differences in the amino sequences of the viruses

Table 2: Serological relationships between the Lagenaria (LbreV) and Coccinia virus (CbarV) isolates and papaya ringspot virus (PRSV) using polyclonal antisera (PAS) raised against the viruses

	PAS-CurV	PAS-LbreV	PAS-CbarV	PAS-PRSV-ASL-281[a]
CuMV[b]	1.69†	0.23	0.04	0.02
LbreV	0.17	2.00	0.41	0.01
CbarV	0.05	0.02	1.47	0.02
PRSV-DSMZ[c]	0.05	0.01	0.06	2.05
Healthy Sap	0.03	0.01	0.01	0.03
Buffer	0.03	0.01	0.01	0.02

[a]Antiserum was obtained from Institute of Pathogen Diagnostics, Ashersleben. [b]Antiserum against Cucurbita mosaic virus (a potyvirus from Nigeria. In press). [c]Antiserum was obtained from Institute of Biochemistry and Plant Virology, Braunchweig, Germany, † = Readings were made at E450 nm

to PRSV, very weakly decorated by antisera to TuMV and TeMV but no decoration at all with antisera to WMV (katabase), WMV-2, Zucchini Yellow Fleck Virus (ZYFV) and ZYMV.

Sequence data: Comparing the amino acid (aa) sequences of the N-terminal regions of LbreV and CbarV with the corresponding region in MWMV showed greater variability at the proximal end (Fig. 4). Sequence alignment of the amino acid sequences of the conserved regions of LbreV and CbarV indicated 92 and 93% sequence identities, respectively when compared to that of MWMV while the difference in the sequences of both viruses study differed by 7%.

DISCUSSION

The characteristics of the viruses isolated from *L. breviflora* and *C. barteri* reported in this study are consistent with those of other members of the genus *Potyvirus* previously reported in the literature. The viruses are transmitted in a fore-gut manner by aphids and have flexuous rod-shaped particles (Lecoq *et al.*, 2001; Owolabi *et al.*, 2008). They induced tubes and laminated aggregates, have coat protein molecular weights of 32.5 and 35.0 kDa which fell within the range of 30-47 kDa for potyviruses (Hull, 2002). The virus isolates also reacted serological to potyvirus specific monoclonal antibodies and decorated to varying degrees by antisera to some putative members of the genus in ISEM decoration tests. They also share high degree of sequence homologies with a putative member of the genus (Quiot-Douine *et al.*, 1990; Shukla *et al.*, 1994; Lecoq *et al.*, 2001).

Hitherto, non-cucurbit potyviruses such as Amaranthus mosaic virus (Taiwo and Owolabi, 2004), Eggplant severe mild mottle and Eggplant green mosaic viruses (Ladipo *et al.*, 1988a, b) and a mosaic- inciting virus isolated from *Senna hirsuta* (Owolabi and Proll, 2001) have been described from Nigeria. Records of cucurbit viruses included a watermelon-like virus (Igwegbe, 1983), CMV (Atiri, 1985), TeMV (Shoyinka *et al.*, 1987) and a strain of PRSV (Owolabi *et al.*, 2008). These viruses differ widely in their host ranges compared to the virus isolates reported in this study. Besides sharing properties such as particle morphology (flexuous rods), transmission by aphids in a fore-gut manner, induction of cytoplasmic inclusions which are basic to members of the genus *Potyvirus* (Hull, 2002), no further comparison could be made with LbleV and CbarV for lack of antisera to these viruses.

Serological data obtained reveal lack of relationships between LbleV and CbarV and most of the potyviruses commonly associated with cucurbits whose antisera were used in this study. The weak to moderate decoration of LbleV by antisera to PRSV, TuMV and TeMV and in the case of CbarV by MWMV is, however, suggestive of some degree of relatedness to these viruses. That both viruses differ from PRSV is confirmed by lack of reactivity with the polyclonal antisera prepared against LbleV and CbarV. Also, Coat protein sequence data for TeMV (Maiss *et al.*, 1988,) suggest that it is more related to Cowpea Aphid-borne Mosaic Virus (CABMV) than to any of the cucurbit viruses and therefore different from the viruses reported in this study.

Biological, serological and molecular characterization of both LbleV and CbarV indicate that both viruses unambiguously differ from each other. Host range studies show that the former induced chlorotic local lesions in *C. amaraticolor* and *C. quinoa* which are not susceptible to the latter. Results from this study also indicate differences in the symptoms induced even in plants susceptible to both viruses. CbarV was transmitted by *M. euphorbiae* but failed to transmit LbreV. The virus isolates also differ in their molecular weights. Serologically, each virus reacted specifically with it antiserum and gave no cross reactivity with antiserum of the other in DAS-ELISA. Furthermore, only LbleV shows cross reaction with MWMV in DAS-ELISA while the particles of CbarV were decoration by antiserum to MWMV but failed to decorate LbleV.

Yakoubi *et al.* (2008) have reported the existed of three clusters of MWMV. Isolates from Tunisia (MWMV-Tn) said to be closely related to those from Spain, Italy and France formed one cluster with the type strain from Morocco. The second cluster comprised the isolates from Cameroun, Niger Republic and Democratic Republic of Congo which showed wide divergent from others MWMV strains (75-80% identity) while the third cluster included isolates from Swaziland and South Africa. The Sudan isolate considered to be at the fringe of MWMV species (Lecoq *et al.*, 2001) presented only 75-80% amino acid sequence identity in the CP with the other isolates. From the

result of this study, sequence alignment of amino acid composition of the N-terminal regions of the CP of both LbleV and CbarV with that of MWMV Sudan isolate showed 92 and 93% sequence identity.

Shukla *et al.* (1994) have suggested that virus isolates with less than approximately 40% sequence identity should be regarded as belonging to different genera. Isolates with 55 to 75% sequence identity, they posited, should be considered as different species while isolates presenting 74 to 88% sequence identity should be regarded as different entities within a related subset of a species. They also suggested that viruses with 90% amino acid sequence identity should be regarded as strains of the same virus. Consequently, these virus isolates reported in this study should be considered strains of MWMV.

CONCLUSION

The differences observed in the biological characteristics (host range and insect transmission) and immunological attributes of the virus isolates in this study apparently stem from differences in their sequence data. Our results further emphasize the need to combine biological, serological and molecular characteristics in delimiting strains of virus species. To our knowledge, this is the first report of MWMV strains in Nigeria.

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REFERENCES

Akintayo, E.T. and E. Bayer, 2002. Characterization and some possible uses of *Phikenetia conophora* and *Adenopus brevilorus* seeds and seed oils. Bioresour. Technol., 85: 95-97.

Arocha, Y., N. Vigheri, B. Nkoy-Florent, K. Bakwanamaha and B. Bolomphety *et al.*, 2008. First report of the identification of *Moroccan watermelon mosaic virus* in papaya in Democratic Republic of Congo (DRC). Plant Pathol., 57: 387-387.

Atiri, G.I., 1985. An isolate of Cucumber mosaic virus from fluted pumpkin in Nigeria. J. Phytopathol., 114: 268-273.

Birnboim, H.C. and J. Doly, 1979. A rapic alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acids Res., 7: 1513-1523.

Burkill, H.M., 2004. The Useful Plants of West Tropical Africa. Royal Botanic Gardens, London..

Choi, G.S., 2001. Occurrence of two tobavirus diseases in cucurbits and control measures in Korea. Plant Pathol. J., 17: 243-248.

Choi, G.S., J.H. Kim and J.S. Kim, 2003. Characterization of Melon necrotic spot virus isolated from muskmelon. Plant Pathol. J., 19: 123-127.

Clark, M.F. and A.N. Adams, 1977. Characteristics of microplate method of enzyme-linked innunospdent away for the detection of plant virus. J. Gen. Virol., 34: 475-483.

Converse, R.H. and R.R. Martin, 1990. ELISA Methods for Plant Viruses. In: Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens: A Laboratory Manual, Hampton, R., E. Ball and S. DeBoer, (Eds.). The American Phytopathological Society, St. Paul, MN., USA. pp: 179-196.

De Sa, P.B., E. Hiebert and D.E. Purcifull, 2000. Molecular characterization and coat protein serology of Watermelon leaf mottle virus (Potyvirus). Arch Virol., 145: 641-650.

Desbiez, D., I. Justafre and H. Lecoq, 2007. Molecular evidence that zucchini yellow fleck virus is a distinct and variable potyvirus related to papaya ringspot virus and moroccan watermelon mosaic virus. Arch. Virol., 152: 449-455.

Fattouh, F.A., 2003. Double infection of a cucurbit host by zuccchini yellow mosaic virus and cucumber mosaic virus. Plant Pathol. J., 2: 85-90.

Fischer, H.U. and B.E.L. Lockhart, 1974. Serious losses in cucurbits caused by watermelon mosaic virus in Morocco. Plant Dis., 58: 143-146.

Gholamalizadeh, R., V. Vahdat, T. Keshavarz, A. Elahinia and K. Bananej, 2008. Occurrence and distribution of ten viruses infecting cucurbits in Guilan province, Iran. Acta Virologica, 52: 113-118.

Grunstein, M. and D.S. Hogness, 1975. Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene. Proc. Natl. Acad. Sci. USA., 72: 3961-3965.

Gubler, U. and B.J. Hoffman, 1983. A simple and very efficient method for generating cDNA libraries. Gene, 25: 263-269.

Hanahan, D., 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol., 166: 557-580.

Holstein, N. and S.S. Renner, 2010. *Coccinia* (Cucurbitaceae) gains two new species from East Africa, three new synonyms and one new combination. Kew Bull., 65: 435-441.

Hull, R., 2002. Matthew's Plant Virology. 4th Edn., Academic Press, London, San Diego, ISBN: 81-8147-403-1, Pages: 1001.

Igwegbe, E.C.K., 1983. Properties of a virus causing severe mosaic of *Cucumeropsis edulis* in Nigeria. Plant Dis., 67: 315-317.

Kneirim, D., T.C. Deng, W.S. Tsai, S.K. Green and L. Kenyon, 2010. Molecular identification of three distinct *Polevirus* species and a recombinant cucurbit aphid borne yellows virus strain infecting cucurbits in Taiwan. Plant Pathol., 59: 991-1002.

Ko, S.J., Y.H. Lee, K.H. Cho, S.H. Lee and H.S. Choi *et al.*, 2006. Incidence and distribution of virus diseases on cucumber in Jeonnam Province during 1999-2000. Plant Pathol. J., 22: 147-151.

Ko, S.J., Y.H. Lee, M.S. Cho, J.W. Park, H.S. Choi, G.C. Lim and K.H. Kim, 2007. The incidence of virus diseases of melon in Jeonnam Province during 2000-2002. Plant Pathol. J., 23: 215-218.

Ladipo, J.L., D.E. Lesemann and R. Koenig, 1988a. Host range, serology and cyptopathology of eggplant and tomato strains of eggplant severe mottle virus, a new potyvirus from Nigeria. J. Phytopathol., 122: 359-371.

Ladipo, J.L., D.E. Lesemann and R. Koening, 1988b. Further studies on a virus causing a green mosaic disease of eggplant in Nigeria. J. Phytopathol., 121: 159-165.

Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of the bacteriophage T4 head. Nature, 247: 680-685.

Lecoq, H., G. Dafalla, C. Desbiez, C. Wipt-Schiedel and B. Delecolle *et al.*, 2001. Biological and molecular characterization of *Moroccan watermelon mosaic virus* and a potyvirus isolate from Sudan. Plant Dis., 85: 547-552.

Liu, Y., Y. Wang, X. Wang and G. Zhou, 2009. Molecular characterization and distribution of *Cucumber green mottle mosaic virus* in China. J. Phytopathol., 157: 393-399.

Maiss, E., E. Breyel, A. Brisske and R. Casper, 1988. Molecular cloning of DNA complementary to the RNA-genome of Plum pox virus (PPV). J. Phytopathol., 122: 222-231.

Massumi, H., A. Samei, A.H. Pour, M. Shaabanian and H. Rahimian, 2007. Occurrence, distribution and relative incidence of seven viruses infecting greenhouse-grown cucurbits in Iran. Plant Dis., 91: 159-163.

McKern, N.M., P.M. Strike, O.W. Barnett, C.W. Ward and D.D. Shukla, 1993. *Watermelon mosaic virus*-morocco is a distinct potyvirus. Arch. Virol., 131: 467-473.

Moradi, Z. and B. Jafarpour, 2011. First report of coat protein sequence of *Cucumber green mottle virus* in cucumber isolated from Khorasan in Iran. Int. J. Virol., 7: 1-12.

Oshodi, A.A., 1996. Amino acid and fatty acid compositions of *Adenopus breviflorus* benth Seed. Int. J. Food Sci. Nutri., 47: 295-298.

Owolabi, A.T. and E. Proll, 2001. A mosaic disease of *Senna hirsuta* induced by a potyvirus in Nigeria. Acta Virol., 45: 73-79.

Owolabi, A.T., F. Rabentein and F. Ehrig, 2008. A strain Papaya ringspot virus naturally infecting cucumber (*Cucumis sativus* L.) in Calabar, South Eastern Nigeria. Nig. J. Bot., 21: 97-108.

Owolabi, A.T., M.A. Taiwo, G.A. Thottappilly, S.A. Shoyinka, E. Proll and F. Rabenstein, 1998. Properties of a virus causing mosaic and leaf curl disease of *Celosia argentea* L. in Nigeria. Acta Virol., 42: 133-139.

Quiot-Douine, L., H. Lecoq, J. B. Quiot, M. Pitrat and G. Labonne, 1990. Serological and biological variability of virus isolates of *Papaya ring spot* virus. Phytopathology, 80: 256-263.

Richter, J., E. Proll, F. Rabenstein and A. Stanarius. 1994. Serological detection of members of the *Potyviridae* with polyclonal antisera. J. Phytopathol., 142: 11-18.

Roggero, P., G. Dellavalle and V. Lisa, 1998. First report of *Moroccan watermelon mosaic* potyvirus in zucchini in Italy. Plant Dis., 82: 351-351.

Safaeizadeh, M., 2008. Comparative biological and molecular variability of *Zucchini yellow mosaic virus* in Iran. Asian J. Plant Pathol., 2: 30-39.

Salem, T.Z., S.M. El-Gamal and A.S. Sadik, 2007. Use of helper component proteinase gene to identify a new Egyptian isolate of *Watermelon mosaic potyvirus*. Int. J. Virol., 3: 107-116.

Sanger, F., S. Nicklen and A.R. Coulson, 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA., 74: 5463-5467.

Schwindinger, W.F. and J.R. Warner, 1984. DNA sequence analysis on the IBM-PC. Nucl. Acids Res., 12: 601-604.

Shim, C.K., K.S. Han, J.H. Lee, D.W. Bae, D.K. Kim and H.K. Kim, 2005. Isolation and characterization of watermelon isolate of cucumber green mottle mosaic virus (CGMMV-HY1) from watermelon plants with severe mottle mosaic symptoms. Plant Pathol. J., 21: 160-171.

Shoyinka, S.A., A.A. Brunt, S. Phillips, D.E. Lesemann, G. Thottappilly and R. Lastra, 1987. The occurrence, properties and affinities of Telfairia mosaic virus, a potyvirus prevalent in *Telfairia occidentalis* (Cucurbitaceae) in South Western Nigeria. J. Phytopathol., 112: 13-24.

Shukla, D.D., C.W. Ward and A.A. Brunt, 1994. The Potyviridae. CAB International, Wallingford, UK.

Taiwo, M.A. and A.T. Owolabi, 2004. Comparative study of Amaranthus leaf mottle virus genus Potyvirus and Amaranthus mosaic virus. L. J. Sci. Res. Dev., 9: 63-74.

Van der Meer, F.W. and H.M. Garnett, 1987. Purification and identification of a South African isolate of Watermelon mosaic virus-Moroccan. J. Phytopathol., 120: 255-270.

Walkey, D.G., A.N.J. Spencer and A. Miller, 1994. A poytvirus isolated from *Senna occidentalis*. Plant Pathol., 43: 767-773.

Wintermantel, W.M., L.L. Hladky, A.A. Cortez and E.T. Natwick, 2009. A new expanded host range of cucurbit yellow disorder virus include three agricultural crops. Plant Dis., 93: 685-690.

Yakoubi, S., C. Desbiez, H. Fakhfakh, C. Wipf-Scheibel, M. Marrakchi and H. Lecoq, 2008. Biological characterization and complete nucleotide sequence of a Tunisian isolate of Morocco watermelon mosaic virus. Arch. Virol., 153: 117-125.

Yardimci, N. and S. Korkmaz, 2004. Studies on spread and identification of zucchini yellow mosaic virus disease in the north-west mediterranean region of turkey by biological indexing and double-stranded RNA analysis, Plant Pathol. J., 3: 1-4.

Yuki, V.A., J.A.M. Rezende, E.W. Kitajima, P.A.V. Barrosa, H. Kuniyuki, H.G.A. Groppo and M.A. Pavan, 2000. Occurrence, distribution and relative incidence of five viruses infecting cucurbits in the State of Sao Paulo, Brazil. Plant Dis., 84: 516-520.