PROPERTIES OF A VIRUS CAUSING MOSAIC AND LEAF CURL DISEASE OF *CELOSIA ARGENTEA* L. IN NIGERIA

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Summary. – A sap transmissible virus, causing mosaic and leaf curl disease of *Celosia argentea*, was isolated at vegetable farms in Amuwo Odofin, Tejuoso, and Abule Ado, Lagos, Nigeria. The virus had a restricted host range confined to a few species of the *Amaranthaceae, Chenopodiaceae* and *Solanaceae* families. It failed to infect several other species of the *Aizoaceae, Brassicaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Malvaceae, Poaceae* and *Tiliaceae* families. The virus was transmitted in a non-persistent manner by *Aphis spiraecola* and *Toxoptera citricidus* but not by eight other aphid species tested. There was no evidence of transmission by seeds of *C. argentae* varieties.The viral coat protein had a relative molecular mass (M_r) of about 30.2 K. Electron microscopy of purified virus preparations revealed flexuous rod shaped particles of about 750 nm in length. Serological studies were performed using the enzyme-linked immunosorbent assay (ELISA), immunosorbent electron microscopy (ISEM) and Western blot analysis. The virus reacted positively with an universal potyvirus group monoclonal antibody (MoAb) and MoAb P-3-3H8 raised against peanut stripe potyvirus. It also reacted with polyclonal antibodies raised against several potyviruses including asparagus virus-1 (AV-1), turnip mosaic virus (TuMV), maize dwarf mosaic virus (MDMV), watermelon mosaic virus (WMV-2), plum pox virus (PPV), soybean mosaic virus (SoyMV), lettuce mosaic virus (LMV), bean common mosaic virus (BCMV) and beet mosaic virus (BMV) in at least one of the serological assays used. On the basis of host range, mode of transmission, and available literature data, the celosia virus seems to be different from potyviruses previously reported to infect vegetables in Nigeria. The name celosia mosaic virus (CIMV) has been proposed for this virus.

Key words: celosia mosaic virus; potyvirus; Celosia argentea; host range; transmission; aphids; Nigeria

Introduction

Celosia argentea, a member of the *Amaranthaceae* family, is an erect branched annual often reaching a height of 80 – 100 cm. It is widely cultivated in southwestern Nigeria as a vegetable crop for its leaves which are a good source of minerals, proteins, and vitamins (Omueti, 1980).

A number of viruses have been reported to infect *C. argentea* experimentally (Edwardson, 1974), but reports of natural infection of this vegetable with viruses are few.

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Abbreviations: AmMV = amaranthus mosaic virus; AV-1 = asparagus virus-1; BCMV = bean common mosaic virus; BMV = beet mosaic virus; BYMV = bean yellow mosaic virus; CeMV = celery mosaic virus; CMV = cucumber mosaic virus; DAS-ELISA = double antibody sandwich ELISA; ELISA = enzyme-linked immunosorbent assay; EGMV = eggplant green mottle mosaic virus; ESMV = eggplant severe mottle virus; ISEM = immunosorbent electron microscopy; LMV = lettuce mosaic virus; MDMV = maize dwarf mosaic virus; MoAb = monoclonal antibody; PPV= plum pox virus; PTA-ELISA = plate-trapped antigen ELISA; PVMV = pepper veinal mottle virus; PVV = potato virus V; SDS-PAGE = polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate; Soy MV = soybean mosaic virus; TeMV = telfairia mosaic virus; TuMV = turnip mosaic virus; WMV-2 = watermelon mosaic-2 virus

Provvidenti (1975) reported the occurrence of cucumber mosaic virus (CMV) in *C. argentea* in western New York State. In Nigeria, the only record of a virus disease of this vegetable was a partially characterised virus that was mechanically transmissible to several non-amaranthaceous plant species (Atiri and Osemobor, 1991).

In October 1989, a disease causing mosaic and leaf curl of *C. argentea* at a commercial vegetable farm in Amuwo Odofin in Lagos, Nigeria, was observed. Infected plants also showed leaf malformation and stunting. A survey for viruses of leafy vegetables in other parts of Lagos revealed a widespread occurrence of the disease in several other vegetable farms. This paper reports an attempt to fully characterise and identify the virus causing the mosaic and leaf curl disease of *C. argentea* in Nigeria. A preliminary report has been previously published (Owolabi *et al.*, 1995).

Materials and Methods

Virus. Naturally infected *C. argentea* plants were transplanted individually in the greenhouse. Virus inoculum was prepared by triturating young symptomatic leaves in the inoculation buffer (0.03 mol/l potassium phosphate buffer pH 8.0 containing 0.1% sodium sulphite). The inoculum was rubbed onto carborundum dusted leaves of young seedlings of *C. argentea* and *Chenopodium quinoa*. Four successive single lesion transfers were carried out on *C. quinoa* and the virus was subsequently maintained in *Nicotiana benthamiana* in the greenhouse at 20 – 22°C.

Determination of host range of the virus. Virus inoculum obtained from infected leaves of *N. benthamiana* was used for inoculation of leaves of five plants each of 60 plants species belonging to 11 families. Inoculated plants were kept in the greenhouse for 4weeks for symptom development. Extracts from plants that did not show disease symptoms were inoculated back onto *C. quinoa* to detect possible latent infection. A set of five plants of each plant species inoculated with buffer only served as the negative controls.

Virus transmission tests. Seeds collected individually from 15 mechanically inoculated plants of *C. argentea* "TLV 8", "purple" and "narrow-leaved" varieties were sown in sterilized, manure-supplemented soil in seed trays. The resulting seedlings were kept in screen-cages in the greenhouse, watered regularly and observed for symptom development for 8 weeks.

Aphids (Myzus persicae, Aphis craccivora, A. fabae, A. spiraecola, A. nasturti, Brevecoryne brassicae, Rhopalosiphon padi, Toxoptera citricidus, Aulacorthum solani and *Acyrosiphon pisum)* were tested for their ability to transmit the virus. Each aphid species was starved for 2 hrs and allowed acquisition access feeding for 2.5 – 5 mins on detached leaves of *C. argentea, N. benthamiana* or *N occidentalis* floated on water in Petri dishes. Ten to 15 insects were transferred to seedlings of these plants and left overnight, after which they were sprayed with insecticides.

Virus purification and electron microscopy. The virus was purified from frozen *N. benthamiana* leaf tissues harvested 2 – 3 weeks after inoculation according to the procedure of Owolabi *et al.* (1995), except that sodium citrate was used instead of sodium borate in preparing the grinding buffer. The yield of purified virus was estimated spectrophotometrically using an extinction coefficient of 2.8 at 260 nm. Virus particles from purified preparations and leaf dips were negatively stained with 2% phosphotungstic acid pH 6.0 and observed under a Philips TEM – 300 electron microscope.

Polyacrylamide gel electrophoresis was performed in the presence of sodium dodecyl sulphate (SDS-PAGE). M_r of viral coat protein was estimated according to the method of Laemmli (1970). Size markers (M_r 14.2 – 97 K) and virus samples with known M_r were added for comparison.

Antisera. Samples (1.53 mg) of purified virus preparation were mixed with an equal volume of complete Freund's adjuvant (for the first injection only) and administered intraperitonally into 2 mice thrice at weekly intervals. The virus was mixed with incomplete Freund's adjuvant for the subsequent injections. The animals were bled 2 weeks after the last injection and the collected blood was overlaid with 1–2 drops of saline, left overnight at room temperature and centrifuged at 10,000 rpm for 5 mins. The antiserum obtained as well as the universal potyvirus group-specific MoAb (Agdia, Inc. Elkart, IN, USA) and MoAb P-3-3H8 raised against peanut stripe potyvirus (supplied by Dr Vetten, BBA, Braunschweig, Germany) were used to establish the identity of the celosia virus by plate-trapped antigen ELISA (PTA-ELISA).

Serological tests. Reciprocal serological tests were carried out using antisera (IgGs) to AV-1, BCMV, bean yellow mosaic virus (BYMV), potato virus V (PVV), MDMV, SoyMV, TuMV, PPV, WMV-2, beet mosaic virus (BMV), LMV, and celery mosaic virus (CeMV) against the celosia virus. Lyophilized or fresh leaves infected with these viruses constituted the controls.

The assay procedures used were the double antibody sandwich ELISA (DAS-ELISA) described by Clark and Adams (1977), PTA-ELISA, Converse and Martin (1990), Western blot analysis and immunosorbent electron microscopy (ISEM) including decoration (Richter *et al.*, 1994). ELISA readings were considered positive when they exceeded twice the readings of the corresponding healthy controls (Walkey, 1994).

Results

Host range and symptomatology

The result of the host range study showed that the celosia virus had a rather narrow host range infecting a few species in *Amaranthaceae, Chenopodiaceae* and *Solanaceae* families (Table 1). The virus-induced mosaic, leaf curl and malformation in *C. argentea* var. "TLV 8" (Fig. 1a), mosaic and green vein-banding in *N. occidentalis* (Fig. 1b), and chlorotic local lesions in *C. quinoa*. Other local lesion hosts included *C. amaranticolor, C. urbicum, C. morale*, and *Gomphrena globosa*. Besides local lesion induction in *C. quinoa* and *C. amaranticolor*, there was also systemic infection. Other susceptible hosts included *N. benthamiana, N. clevelandii, Chenopodium foetidum, Celosia argentea* (var. "Deutsche", "Purple leaved" and "Narrow-leaved") and

Table 1. Reaction of test plants to mechanical inoculation of celosia virus

Test plant	Symptoms	Back infection of *C. quinoa*
AMARANTHACEAE		
Celosia argentea L.		
„Commercial var."	Mosaic, leaf curl, malformation, stunting	+
„Purple-leaved var"	Leaf curl, mosaic leaf puckering	+
„Narrow leaved var."	Leaf curl, mottle, mosaic, necrotic lesion (inconsistent)	+
Var. „TLV-8"	Chlorotic spots, leaf curl, leaf malformation, mosaic	+
C. trigyna L.	Leaf distortion, shoe stringing, axillary shoot proliferation, stunting	+
Gomphrena globosa L.	Reddish necrotic local lesions	+
CHENOPODIACEAE		
Chenopodium amaranticolor	Necrotic local lesions, systemic chlorotic lesions	+
C. quinoa	Chlorotic local lesions, systemic chlorotic patches, leaf malformation	+
C. foetidum	Mild mottle	+
C. murale	Chlorotic local lesions	+
C. rubrum	Chlorotic local lesions	+
C. urbicum	Chlorotic local lesions	+
SOLANACEAE		
N. benthamiana Domin	Leaf roll, mosaic, leaf malformation	+
N. clevelandii	Leaf puckering, vein yellowing	+
N. occidentalis Wheeler	Mosaic, green vein-banding	+

(+) = positive result.

C. tirgyna. None of the following species developed symptoms and there was no evidence of latent infection: *Aizonaceae –Tetragonia expansa*; *Amaranthaceae – Amaranthus caudatus, A. cruentus, A. hybridus, A. viridis; Chenopodiaceae – Chenopodium capitatum, C. foliosus; Brassicaceae – Brassica pekinensis, B. oleracea* var. *capitata*; *Cucurbitaceae – Citrullus lanatus*, *Colocynthis citrullus*, *Cucumeropsis edulis*, *Citrullus sativus*, cv. "Poinsett",

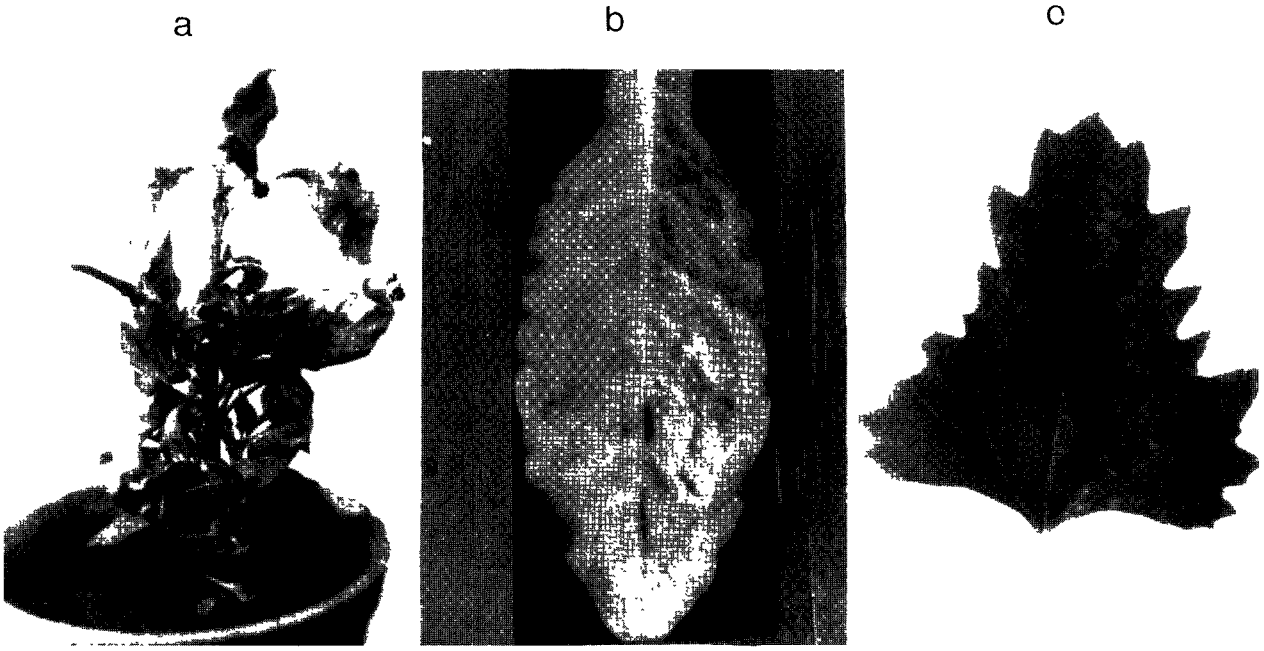

Fig. 1
Symptoms of infection with celosia virus
Mosaic, leaf curl and malformation in *Celosia argentea* var. "TLV 8" (a), mosaic and green vein-banding in *Nicotiana occidentalis* (b), and local lesions on *Chenopodium quinoa* (c).

Fig. 2
Electron micrograph of purified preparation of celosia virus showing flexuous rod-shaped particles

Cucurbita maxima, *C. moschata*, *C. pepo* (var. "Encore", "Consul" and "Corona"), *Luffa acutangula*, *L. cylindrica*, *Telfairia occidentalis; Fabaceae – Cajanus cajan, Canavalia ensiformis*, *Glycine max*, *Phaseolus lunatus*, *P. vulgaris* (cv. "Saxa"), *Vigna mungo*, *V. unguiculata* (cv. "Ife Brown", "K59" and "Mascara"); *Lamiaceae – Ocimum basilicum*, *O. canum*, *O. gratissimum; Malvaceae – Abelmuschus esculentus*; *Poaceae – Zea mays*; *Solanaceae – Capsicum annuum* (var. "Cerasiform" and "Longum"), *C. frutescens, Datura metel*, *D. stramonium*, *Lycopersicum esculentum*, *N. glutinosa*, *N. megalosiphon*, *N. tabacum* (var. "Samsum", "Xanthi" and "White Burley"), *Physalis angulata*, *P. floridana*, *Petunia hybrida*, *Solanum macrocarpon*, *S. melongena*; *Tiliaceae – Corchorus olitoris* L.

Virus transmission

None of the 1,275 seedlings derived from seeds of *C. argentea* var. "TLV 8" systemically infected with the celosia virus showed symptoms; neither did the 347 and 500 seedlings derived from infected "purple" and "narrow leaved" varieties, respectively.

The virus was transmitted by *A. spiraecola* and *T. citricidus* from *C. argentea* to *C. argentea* in a non-persistent manner. Attempts to transmit the virus using *M. persicae*, *A. craccivora*, *A. fabae*, *A. nasturti*, *B. brassicae*, *R. padi*, *A. solani* and *A. pisum* were not successful.

Table 2. Cross-reactivity of antiserum to celosia virus with some potyviruses

Antigen	Serological assay		
	PTA-ELISA[a]	Western blot analysis[a]	ISEM with decoration[b]
Celosia virus	+	+	++
AV-1	–	–	–
TuMV	+	–	–
BCMV	–	–	–
BYMV	–	–	–
PVV	–	–	–
PPV	+	–	–
MDMV	+	–	–
WMV-2	+	–	–
SoyMV	–	–	–
CeMV	+	–	–
LMV	+	–	+
BMV	+	–	+

[a](+) = positive serological reaction; (–) = negative serological reaction.
[b]Degree of decoration. (++) = strong; (+) = light; (–) = none.
Virus acronyms: AV-1 = asparagus virus 1; TuMV = turnip mosaic virus; BCMV = bean common mosaic virus; BYMV = bean yellow mosaic virus; PVV = potato virus V; PPV = plum pox virus; MDMV = maize dwarf mosaic virus; WMV-2 = watermelon mosaic 2 virus, SoyMV = soybean mosaic virus; CeMV = celery mosaic virus; LMV = lettuce mosaic virus; BMV = beet mosaic virus.

Virus morphology and structure

The virus was successfully purified from frozen leaves of *N. benthamiana* harvested 2 – 3 weeks after inoculation with approximate yield of 1.54 mg/kg of leaf tissue. The purified preparations when inoculated onto *C. quinoa*. *N. benthamiana, N. clevelandii* and *C. argentea* var. "Deutsche", exhibited similar symptoms to those observed using sap from infected plants.

Observation of the purified virus preparations under the electron microscope revealed only flexuous rod-shaped particles with mean particle length of about 750 nm (Fig. 2). No other but potyvirus particles were observed in leaf dip preparations.

The M_r of the viral coat protein as determined by Western blot analysis was 30.2 K.

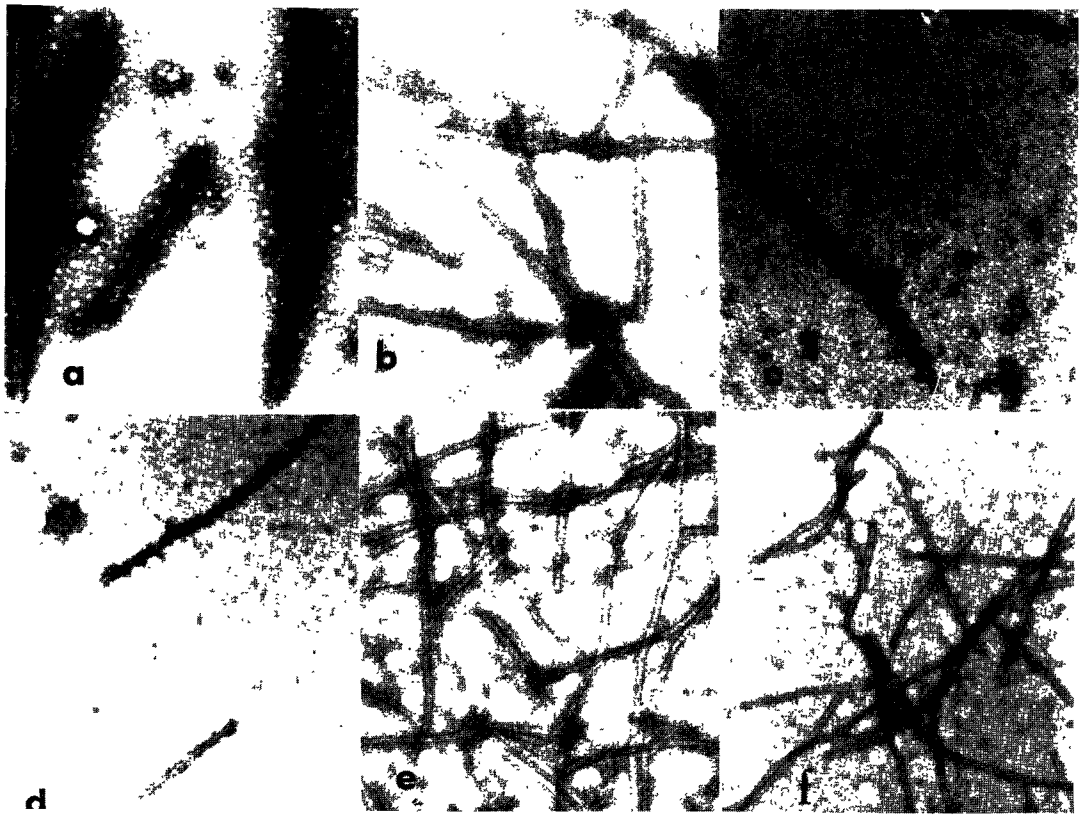

Fig. 3
Reaction of celosia virus particles with homologous and heterologous antisera
Homologous antiserum, magnification 29,000 x (a), heterologous antiserum to AV-1, magnification 28,000 x (b); heterologous antiserum to SoyMV, magnification 24,200 x (c); heterologous antiserum to MDMV, magnification 28,900 x (e); normal serum (f)

Serological studies

In PTA-ELISA, the celosia virus reacted positively with the universal potyvirus group MoAb as well as MoAb P-3-3H8 raised against peanut stripe virus.

The results of serological reactions of the celosia virus antiserum indicated that the celosia virus reacted with its homologous antiserum in PTA-ELISA. TuMV, PPV, MDMV, WMV-2, LMV, CeMV and BMV also reacted positively with the antiserum in the same test (Table 2). In Western blot analysis, the celosia virus reacted exclusively with its antiserum. No cross reactivity was observed with the other viruses tested. The virus was typically decorated by its homologous antiserum (Fig. 3a) but gave a weak decoration with BMV and LMV. Other viruses included in the test showed no reactions.

In reciprocal tests with antisera to some potyviruses the celosia virus cross-reacted with antiserum to AV-1, while other viruses reacted specifically with their homologous antisera in DAS-ELISA. In PTA-ELISA, the virus showed cross-reactivity with antisera to AV-1, MDMV, TuMV, WMV-2, LMV and BMV, while no reactivity was observed with other antisera tested (Table 3). In Western blot analysis, SDS-dissociated coat protein of the celosia virus was detected by antisera to AV-1, TUMV, BCMV, PPV, MDMV and SoyMV. Surprisingly, the virus did not cross-react with antisera to BYMV, PVV, WMV-2, CeMV, LMV and BMV in DAS-ELISA (Table 3). A reciprocal testing with a celosia virus antiserum could not be carried out due to a lack of IgG to it.

The celosia virus was decorated by antiserum to AV-1, SoyMV and MDMV (Fig. 3b, c, d). No decoration of the particles was observed with antisera to TuMV (Fig. 3e), WMV-2, PPV, BCMV, and PVV, and normal serum (Fig. 3f).

Discussion

The symptomatology and sap transmissibility of the agent of the mosaic and leaf curl disease of *C. argentea*,

Table 3. Serological reaction of celosia virus with antisera to some potyviruses

Potyvirus antiserum	Serological assay			
	DAS-ELISA	PTA-ELISA	Western blot analysis	ISEM with decoration
AV-1	+	+	+	+
TuMV	–	+	+	–
BCMV	–	–	+	–
BYMV	–	–	–	–
PVV	–	–	–	–
PPV	–	–	+	–
MDMV	–	+	+	+
WMV-2	-	+	–	–
SoyMV	–	–	+	+
CcMV	–	–	–	–
LMV	–	+	–	–
BMV	–	+	–	ND

(+) = positive serological reaction, (–) = negative serological reaction; ND = not done. For the legend see Table 2

the celosia virus, suggested a viral aetiology for the disease. Aphid transmissibility, particle morphology, a rather narrow host range and reaction of the celosia virus with universal potyvirus MoAb and MoAb P-3-3H8 clearly indicated that the virus belongs to the *Potyvirus* genus of the *Potyviridae* family.

Potyviruses are known to be particularly prevalent in tropical and subtropical countries. Pepper veinal mottle virus (PVMV) (Lana *et al.*, 1975; Ladipo and Robert, 1977; Igwegbe and Waterworth, 1982), telfairia mosaic virus (TeMV) (Shoyinka *et al.*, 1987), amaranthus mosaic virus (AmMV) (Taiwo *et al.*, 1987), eggplant severe mottle virus (ESMV), and eggplant green mottle mosaic virus (EGMV) (Ladipo *et al.*, 1998a, b) have been reported to infect vegetables in Nigeria. The celosia virus seems to differ from all these viruses as their natural hosts were not susceptible to the celosia virus. The inability of *A. craccivora* to transmit the celosia virus seems to differentiate it from PVMV and AmMV which are transmitted by *A. craccivora* (Ogungbenro and Ladipo, 1987; Taiwo *et al.*, 1987). The differences in the host range between the partially characterised virus infecting *C. argentea* as reported by Atiri and Osemobor (1991) and the celosia virus in this study seem to distinguish these two isolates.

The results of immunological studies showed that the celosia virus reacted with heterologous antisera to AV-1, TuMV, WMV-2, MDMV, PPV, SoyMV, LMV and BMV in at least one of the serological assays used in this study. On the other hand, no cross-reactivity was observed with antisera to PVV and BYMV in any of the tests used. The cross-reactivity of the antisera to TuMV, WMV-2, MDMV and LMV which showed reciprocal serological relationship with the celosia virus in PTA-ELISA may not necessarily indicate close relationship as antisera directed against the conserved core region of the coat proteins of potyviruses have been demonstrated to detect viruses differing in biological properties (Shukla *et al.*, 1989a, b; Richter *et al.*, 1994). It is also probable that these antisera were obtained after a long period of immunization, at a time when a mixed population of antibodies lacking the specificity predominated (Regenmortel and Wechmar, 1970, Shephard *et al.*, 1974; Mernaugh *et al.*, 1990). This opinion is supported by the failure of the celosia virus antiserum, obtained 5 weeks after the first injection, to cross-react with any of the viruses in the Western blot analysis.

The positive reaction of AV-1 antiserum with the celosia virus in all the immunological assays including DAS-ELISA, a serodiagnostic tool generally held to be strain-specific (Koenig and Paul, 1982) and capable of distinguishing between virus species (Richter, 1992; Richter *et al.*, 1994), seems to suggest that both viruses involved might be identical. However, the failure of AV-1 to react with the heterologous antiserum to the celosia virus in ISEM with decoration test, Western blot analysis and even PTA-ELISA raised serious doubts about this identity. This type of unidirectional serological relationship has been observed between BYMV and LMV (Holding and Brunt, 1981) and between johnsongrass mosaic potyvirus and WMV-2 (Shukla *et al.*, 1988).

The results of this study indicate that the celosia virus, tentatively named celosia mosaic virus (ClMV), is probably a new member of the *Potyvirus* genus. The intense symptoms induced in all the locally available celosia varieties may render the infected plants unmarketable, thus suggesting that the virus might be of considerable importance. Further investigation on the economical importance and control of the virus is in progress.

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