A MOSAIC DISEASE OF *SENNA HIRSUTA* INDUCED BY A POTYVIRUS IN NIGERIA

A.T. OWOLABI[1], E. PROLL[2]

[1]Department of Biological Sciences, University of Calabar, P.M.B. 1115, Calabar, Nigeria;
[2]Institute for Resistant Research and Pathogen Diagnostics, Aschersleben, Germany

Received January 15, 2001; accepted March 28, 2001

Summary. – A virus inducing mosaic and severe leaf malformation, isolated from *Senna hirsuta* in Nigeria, was studied. The virus had a rather narrow host range, infecting a few species in *Caesalpinaceae, Chenopodiaceae* and *Fabaceae* families. The virus was widespread in southern Nigeria with prevalence ranging from 74% to 86.4% in some locations. It was transmitted mechanically and in a non-persistent manner by *Myzus persicae, Aphis craccivora* and *A. spiraecola.* There was no evidence of transmission by seeds. Electron microscopy of leaf dip preparations revealed flexuous rod-shaped particles. The viral coat protein had M_r of 32.5 K. The virus reacted positively with a monoclonal antibody (MAb) to peanut stripe virus specific for potyviruses (members of the *Potyvirus* genus) and with antisera to turnip mosaic virus (TuMV), potato virus Y (PVY), TuMV, potato virus A (PVA), potato virus V (PVV) and bean yellow mosaic virus (BYMV), but it failed to react with antisera to celery mosaic virrus (CeMV), bean common mosaic virus (BCMV), soybean mosaic virus (SMV), and clover yellow mosaic virus (ClYMV) in plate-trapped ELISA (PTA-ELISA). No positive reaction was obtained when the virus was tested against any of the antisera in double-antibody sandwich ELISA (DAS-ELISA). This is the first report of natural infection of *Senna* species in Nigeria. The virus, tentatively designated as Senna mosaic virus (SeMV), seems to differ from other viruses previously described from *Senna* species in the literature and indeed other legume potyviruses in Nigeria.

Key words: Senna hirsuta; mosaic; Senna mosaic virus; potyvirus; Nigeria

Introduction

Senna hirsuta (syn. *Cassia hirsuta, Caesalpinaceae* family) is a sub-woody shrub of about 2.5 m tall, growing on roadsides, wastelands and bush regrowths (Akobundu and Agyakwa, 1987). The alternately arranged paripinnate-compound leaves which consist of 4–6 pairs of ovate or ovate-lanceolate leaflets are covered by very dense hairs. The flowers are orange to bright yellow in color and the slightly curved pods contain 20–30 seeds (Hutchinson and Dalziel, 1954).

A review of literature showed that five distinct viruses have previously been described from the *Senna* species (formerly classified as *Cassia* species). Cassia mosaic virus has been isolated from *C. tora* and *C. occidentalis* L. in Papua New Guinea (van Velsen, 1961), while Mathur and

[1]E-mail: owolabi@unical.anpa.net.ng.

Abbreviations: BlCMV = Blackeye cowpea mosaic virus; BYMV = bean yellow mosaic virus; BCMV = bean common mosaic virus; CabMV = cowpea aphid-borne mosaic virus; CasMMV = Cassia mild mosaic virus; CasMV = Cassia mild mottle virus; CasRSV = Cassia ringspot virus; CasSMV = Cassia severe mosaic virus; CasYBV = Cassia yellow blotch virus; CasYSV = Cassia yellow spot virus; CeMV = celery mosaic virus; ClYMV = clover yellow mosaic virus; DAS-ELISA = double-antibody sandwich ELISA; MAb = monoclonal antibody; PTA-ELISA = plate-trapped antigen ELISA; PVA = potato virus A; PVV = potato virus V; PVY = potato virus Y; SDS-PAGE = poly-acrylamide gel electrophoresis in the presence of SDS, SeMV = Senna mosaic virus SMV = soybean mosaic virus; TuMV = turnip mosaic virus

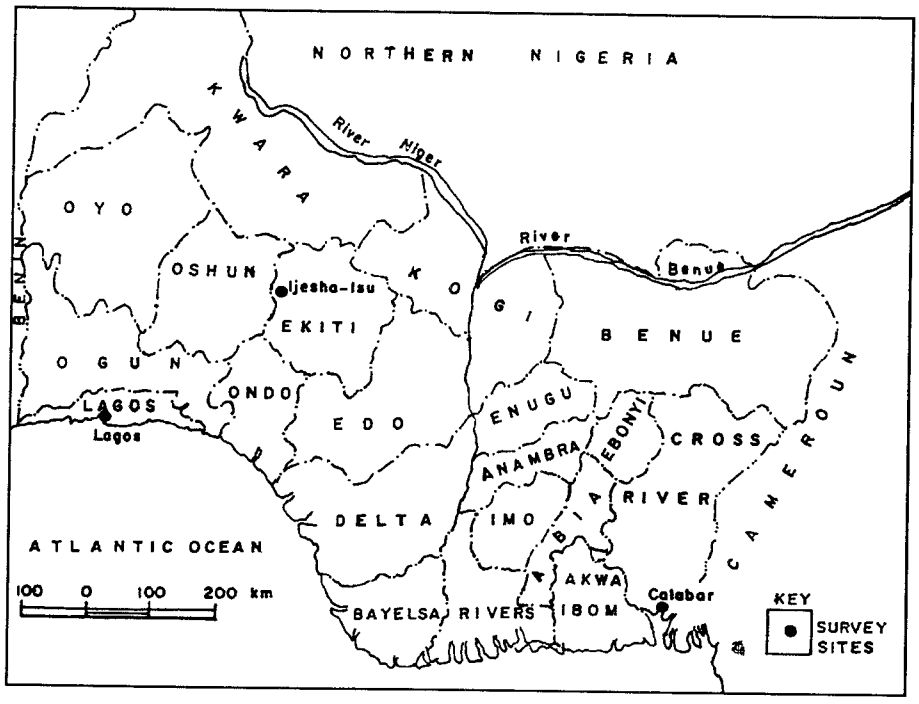

Fig. 1
Map of southern Nigeria
Solid points indicate the towns where surveys were carried out.

Singh (1969) have reported CasRSV on *C. occidentalis* in India. No information is available on particle morphology and serological relationships of these two viruses. CasMMV, isolated from *C. sylvestris* in Brazil, has been described by Lin *et al.* (1979). The virus, which was mechanically transmissible, had 640-nm-long flexuous rod-shaped particles. Dale *et al.* (1984) have described another mechanically transmissible virus isolated from *C. pleurocarpa* in Queensland, Australia. The virus, CasYBV, is characteristic by isometric particles and serologically related to some members of the *Bromoviridae* family. A potyvirus designated CasSMV, transmissible by aphids and having particles of 830 nm in length and a coat protein of M_r of 34.5 K, was isolated from *C. occidentalis* in Yemen (Walkey *et al.*, 1994).

In the rainy season of 1999, a population of *S. hirsuta* was found showing mosaic symptoms in Calabar, south-eastern Nigeria. Other symptoms observed on the infected plants were severe leaf malformation and distortion. Similar observations were made on these plants in Lagos and Ijesha-Isu in the south-western part of the country. This is the first report of a virus disease of *S. hirsuta* in Nigeria.

Materials and Methods

Virus isolates. An attempt was made to assess the spread of the virus in some widely separated locations in southern Nigeria, namely Lagos in Lagos State, Ijesha-Isu in Ekiti State and Calabar in Cross River State (Fig. 1). At each location, not less than 100 plants were sampled and those, which showed the mosaic and leaf malformation symptoms were considered infected. The sampling involved physical examination of plants which in most cases occurred in clusters. The frequency of infection was represented by the percentage of infected plants. Three isolates each from Lagos (designated Lag 1, 2 and 3) and Ijesha-Isu (designated Ije 1,2 and 3), sandwiched between filter paper in polyethylene bags were brought to a greenhouse in Calabar. They were transferred to *Chenopodium quinoa* and/or *Phaseolus vulgaris* and compared to Calabar isolates in terms of host range. Subsequent studies were carried out using the Calabar isolates.

Symptomatic leaf samples, collected in polyethylene bags from naturally infected *S. hirsuta* in Calabar, were ground in 0.03 mol/l sodium phosphate buffer pH 8.0 or 0.1 mol/l potassium phosphate buffer pH 7.5 containing 1% sodium sulphite. The inocula obtained were rubbed onto 300-mesh carborundum dusted *C quinoa* Four single lesion transfers were carried out onto *C. quinoa* on which the virus was subsequently maintained.

Experimental host range tests Inocula prepared from infected leaves of *C. quinoa* were used to inoculate at least 5 seedlings each

of a number of host range plants. Inoculated plants were kept in a greenhouse at 20–22°C for about 4 weeks and were observed for symptom development. Extracts from inoculated plants that did not show symptoms were inoculated back onto *C. quinoa* to detect latent infection. A set of 3 seedlings of each of the host range plants tested were inoculated with the buffer only to serve as controls.

Virus transmission tests. About 500 seeds obtained from naturally infected *S. hirsuta* were mechanically scarified by grinding in a mortar with coarse sand since the seeds of the plant do not germinate readily (Agboola, 1998). The scarified seeds were soaked in water for 24 hrs at room temperature before planting in sterilized soil in seed-trays in insect-proof cages. The resulting seedlings were observed for symptom development until the plants had developed 6 true leaves.

Aphids. M. persicae, A. craccivora and *A fabae* rared on *Capsicum annuun, Ficia fabae* and *A. spiraecola* obtained from its natural host, *Chromolaena odorata* in the wild, were tested for their ability to transmit the virus experimentally using the procedure described by Owolabi *et al.* (1998). *C. quinoa* and *C. capitatum* were the virus source plants while the test plant was *C. quinoa* for *M. persicae* and *A. craccivora.* For *A. spiraecola,* the source and test plants were *S. hirsuta* and *S. occidentalis,* respectively.

Electron microscopy. Leaf dip preparations of viruliferous sap obtained from infected *C. quinoa* were prepared as described by Richter *et al.* (1994). The grids were negatively stained with 2% phosphotungstic acid pH 6.8 and observed under a Zeiss EM-902 electron microscope.

Polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE). Relative molecular mass (M_r) of the virus coat protein was determined by SDS-PAGE as described by Laemmli (1970). Symptomatic leaf tissues of *C. quinoa* by the senna virus were homogenised (1:3 w/v) in distilled water. One hundred microliters of the supernatant were treated with equal volume of SDS dissociation buffer (2% SDS, 0.62 mol/l Tris-HCl buffer, pH 6.8, 5% 2-mercaptoethanol, 5% glycerol and 0.001% bromophenol blue). Fifteen microliters of the extract were electrophoresed using 10% acrylamide in the separating gel and 5% in the stacking gel in a vertical Minigel-Twin electrophoresis apparatus (Biometra, Gottingen, Germany).

Western blot analysis was done according to Richter *et al.* (1994). The proteins, separated by SDS-PAGE, were electroblotted onto nitrocellulose membranes (Hybond-C, 0.45 mm, Amersham) at 200 mA for 30 mins in a Fast Blot B32 apparatus (Biometra, Gottingen) in 20 mmol/l Tris, 192 mmol/l glycine and 20% methanol). Residual binding sites on the blots were blocked by shaking them for 2 x 1 hr at room temperature in 0.14 mol/l NaCl, 2 mmol/l KH_2SO_4, 8 mmol/l Na_2HPO_4, 3 mmol/l KCl and 2% Tween-20 (PBST) containing 5% (w/v) non-fat dry milk. Then the blots were incubated by shaking for 1 hr at room temperature with the appropriately diluted potyvirus-specific monoclonal antibody (MAb) P-3-3H8 (obtained from the Antiserum Bank of the Institute of Pathogen Diagnositics, Aschersleben, Germany) raised against peanut stripe virus. After another two rounds of washing with PBST the membranes were incubated for 1–2 hrs with conjugated sheep anti-mouse IgG diluted 1.1000 in PBS. Bound antibodies were detected by nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyphosphate as substrates. Finally, the blots were treated with 10% sodium hypochlorite. A size marker (M_r of 16.5 K–175 K) and some virus samples of known M_r were added for estimation of M_r.

Serological tests. Potyvirus-specific MAb P-3-3H8 and polyclonal antibody TuMV-314 raised against TuMV, which have been reported to detect most species of potyviruses (Richter *et al.*, 1994) were used to establish the respective genus of SeMV in plate-trapped antigen ELISA (PTA-ELISA). Further serological tests to establish relationships of SeMV to some species of potyviruses were carried out using antisera (IgG) to CeMV, ClYMV, BCMV, BYMV, PVA B.11 strain, PVY N strain, PVV, SMV, and TuMV (obtained from the antisera bank at the Institute for Resistance Research and Pathogen Diagnostics, Aschersleben, Germany). The tests with antisera consisted of DAS-ELISA as described by Clark and Adams (1977) and PTA-ELISA as described by Converse and Martin (1990). ELISA readings were considered positive when they exceeded twice the readings of the corresponding controls (Walkey *et al.*, 1994).

Results

Prevalence of the mosaic disease in S. hirsuta

In Lagos, 95 (86.4%) of 110 plants showed typical mosaic and severe leaf malformation symtoms. In Ijesha-Isu, 89 (74%) of 120 plants showed the symptoms while in Calabar 102 of 125 (81.6%) plants were recorded as infected. The Lagos and Ijesha-Isu virus isolates infected similar host range plants and induced symptoms comparable to those obtained with the Calabar virus isolates (data not shown).

Host range of the virus and symptomatology of the mosaic disease

The results of the host range study indicated that the virus has a very narrow host range, apparently limited to members of the *Caesalpinaceae, Chenopodiaceae* and *Fabaceae* families (Table 1). The virus induced distinct local chlorotic

Table 1. Reaction of test plants to mechanical inoculation with SeMV

Test plant	Symptoms	Back inoculation to *C. quinoa*
Caesalpinaceae		
S. hirsuta		+[a]
S. occidentalis	Mosaic, leaf malformation	+
Chenopodiaceae		
C quinoa	Chlorotic local lesions	+
C capitatum	Chlorotic local lesions, mild mottle	+
C foliosum	Chlorotic local lesions	+
C murale	Chlorotic local lesions	+
Fabaceae		
Glycine max	Mild mottle	+
P vulgaris	Mosaic	+
S. stenocarpa	Mosaic, leaf malformation	

[a] (+) = virus recovered after back inoculation to *C quinoa*

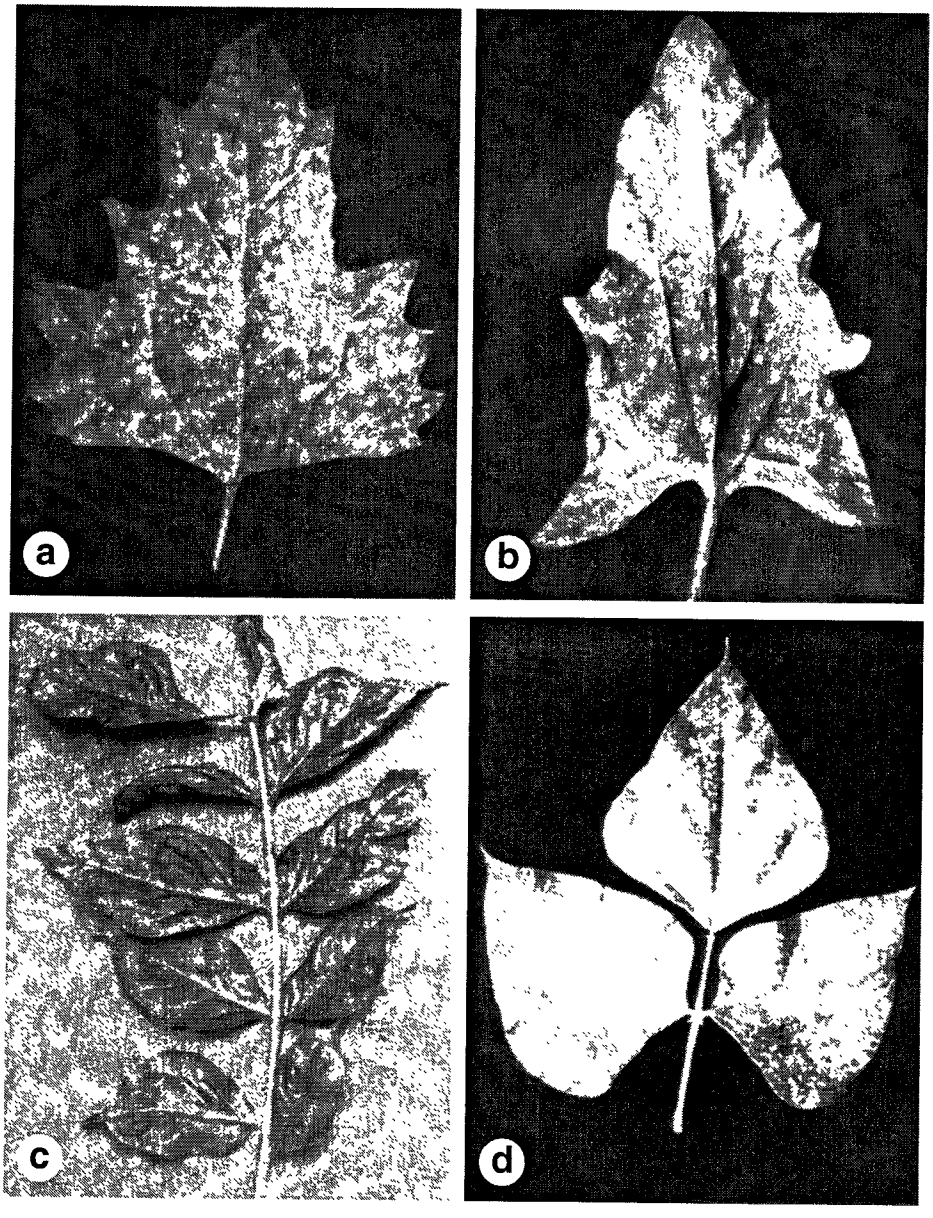

Fig. 2
Symptoms of infection with SeMV
Chlorotic local lesions in *C. quinoa* (a) and *C. capitatum* (b), and leaf malformation and mosaic in *S. hirsuta* (c) and *P. vulgaris* (d)

lesions in *C. quinoa* (Fig. 2a) and *C. capitatum* (Fig. 2b) and inconspicuous local chlorotic lesions in *C. murale* and *C. foliosum*. Systemic symptoms induced by the virus ranged from mild mottle in *C. capitatum* and *Glycine max* to severe mosaic and leaf malformation in *S. hirsuta* (Fig. 2c) and *Phaseolus vulgaris* (Fig. 2d).

The following plants did not show any symptoms and efforts to recover the virus from them yielded negative results: the *Amaranthaceae* family: *Amaranthus hybridus, Celosia argentea* var. TLV 8, purple leaf var., *C. cristata* and *Gomphrena globosa;* the *Chenopodiaceae* family: *Chenopodium foetidum;* the *Cucurbitaceae* family: *Cucumeropsis edulis, Cucurbita moschata, Cucumis sativus, Telfairia occidentalis* and *Luffa cylindrica;* the *Fabaceae* family: *Arachis hypogaea, Cajanus cajan, Cannavalia ensiformis, Medicago sativa, Vigna unguiculata* var. Ife

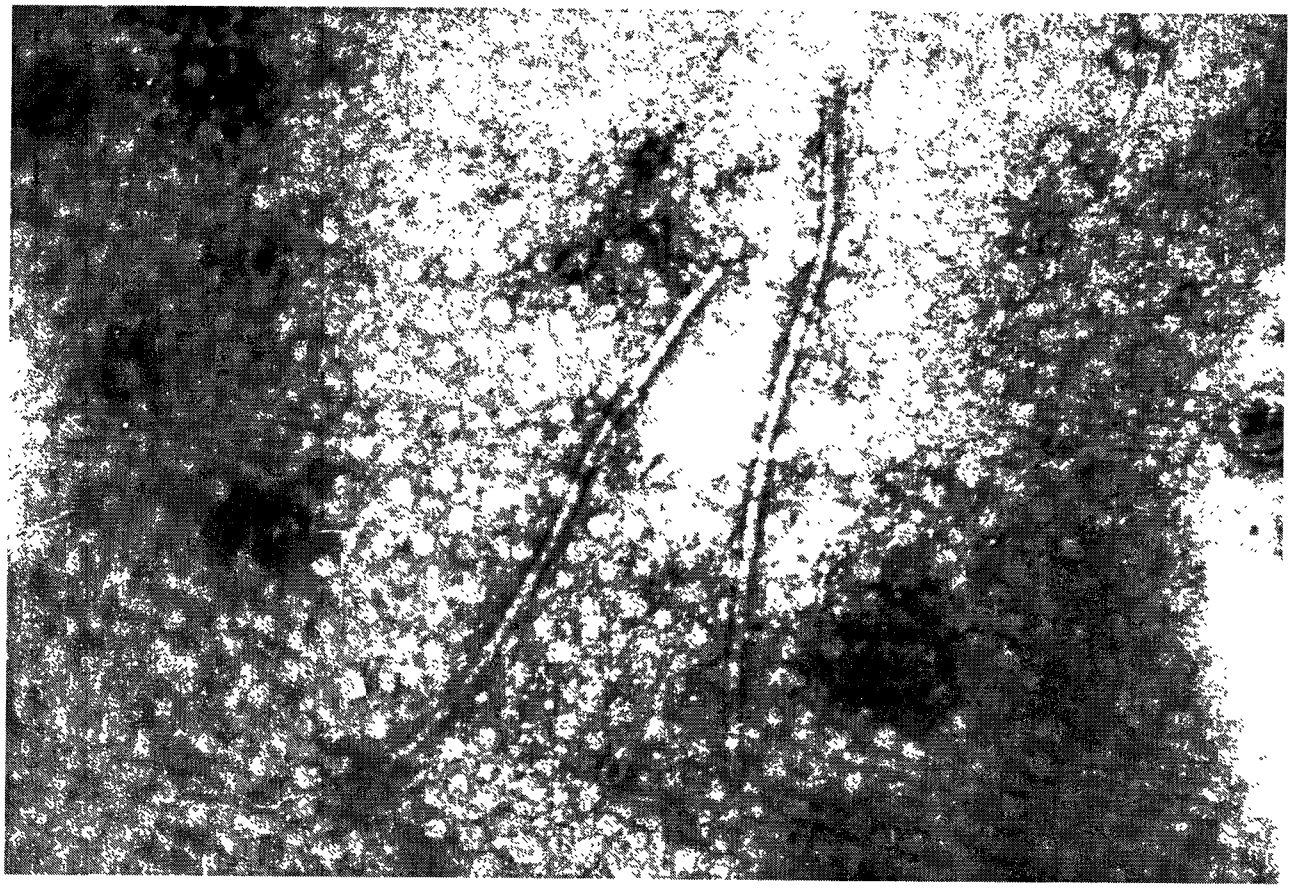

Fig. 3
Virus particle morphology
Flexuous rod-shaped particles of SeMV seen in a leaf dip preparation from *C. quinoa*. Magnification 28,000x

Brown, and accession lines of *V. unguiculata* such as IT 89KD 349, IT 95M-181-1, IT 83S-818, IT 84D-440, and IT 47K-825-15; the *Solanaceae* family: *Datura stramonium, Nicotiana benthamiana, N. occidentalis, N. clevelandii, N. tabacum* var. Samsum, Bell, and Xanthi, *N. glutinosa, N. megalosiphon, P. angulata, Physalis floridana,* and *Solanum melongena*; the *Lamiaceae* family: *Ocimum basilicum* and *O. gratissimum.*

Transmission of the virus

None of the 475 seedlings raised from seeds collected from naturally infected *S. hirsuta* showed any symptoms of infection, while the remaining 25 seeds did not germinate.

The virus was transmitted from *C. quinoa* or *C. capitatum* to *C. quinoa* by the aphids *M. persicae* and *Aphis craccivora* and by the aphid *A. spiraecola* from *S. hirsuta* to *S. occidentalis* in a non-persistent manner.

Morphology and coat protein of the virus

Electron microscopy of the leaf dip preparations showed flexuous rod-shaped particles (Fig. 3). However, the modal length of the particle could not be precisely determined because of insufficient number of the particles in plant extracts even when leaf dip preparations were made from highly symptomatic infected leaf tissues. Nevertheless, the approximate mean length was 750 nm.

The M_r of the virus coat protein as determined by Western blot analysis was 32.5 K.

Serological properties of the virus

The virus reacted positively with the MAb P-3-3H8 and the antiserum to TuMV (TuMV-314) in PTA-ELISA. Also, the virus reacted positively with antisera to PVY, TuMV, PVA, PVV and BYMV in the same test, but failed to react

with antisera to CeMV, BCMV, SMV and ClYMV. The virus did not react with any of these antisera in DAS-ELISA. Resprocal tests were not carried out because no suitable host could be found to ensure propagation and purification of the virus and subsequent antiserum production.

Discussion

The rather narrow host range, the flexuous rod-shaped particles, non-persistent aphid transmission of the virus, the virus coat protein of M_r of 32.5 K and the positive serological reaction with MAb and antiserum to TuMV specific for members of the genus *Potyvirus* (Richter *et al.*, 1994; Owolabi *et al.*, 1998) are strong indications that the virus, tentatively designated as SeMV belongs to the genus *Potyvirus*.

The aphid *A. spiraecola,* which transmitted the virus experimentally and was often found on infected *S. hirsuta* during the surveys, could be implicated as responsible for the widespread occurrence of the virus in nature. The aphid is widely distributed, colonizing its host plant, *Chromolaena odorata* (*Poaceae* family), itself an ubiquitous weed growing throughout the southern belt of Nigeria.

Of the five previously reported viruses infecting *Senna* species (Brunt *et al.*, 1990; Walkey *et al.*, 1994), SeMV reported here is obviously distinct from CasMMV, a carlavirus (Lin *et al.*, 1974) and CasYBV, a bromovirus (Dale *et al.*, 1984). As for cassia mosaic virus (van Velsen, 1961) and CasRSV (Mathur and Singh, 1969), no comparison could be made with SeMV since there is no information regarding their morphology and serological relationship (Brunt *et al.*, 1994). However, evidence from host range and insect transmission did not indicate similarities between these viruses and SeMV. Although, CasSMV is an potyvirus, transmitted by *M. persicae* in a non-persistent manner (Walkey *et al.*, 1994) like SeMV, these viruses differ in their M_r and the type of experimental hosts infected, one characteristic that has been found useful in distinguishing serologically related viruses (Owolabi and Proll, 2000). Unfortunately, no direct and reciprocal serological comparisons could be made because of non-availability of antisera to these viruses.

Viruses of the genus *Potyvirus* infecting legumes in Nigeria include cowpea aphid-mosaic virus, blackeye cowpea mosaic virus (BlCMV), SMV, and cannavalia mosaic virus (Thottappilly, 1992). The failure of SeMV to infect the natural host plants of these viruses, except SMV whose antiserum failed to react with SeMV, seems to indicate that SeMV is a new legume virus in Nigeria.

Although, SeMV differs from all previously described viruses isolated from *Senna* species in the literature and from potyviruses of legumes reported in Nigeria, it remains to be determined if the virus is a new potyvirus. Nucleotide sequence of the virus coat protein gene or of the whole virus genome should be determined for estimating precise relatedness of SeMV to other potyviruses.

The susceptibility of some economically important crops to SeMV suggests that it could represent a potentially serious threat to their production.

Acknowledgements. The authors are grateful to Dr F. Ehrig for the electron micrographs, Mrs S. Giesecke (both from the Federal Center for Breeding Research on Cultivated Plants, Aschersleben, Germany) and Mr J. Ukpong, (Department of Biological Sciences, University of Calabar, Calabar, Nigeria) for the photographs, and the staff of Plant Virology Laboratory, Institute for Resistance Research and Pathogen Diagnostics, Aschersleben, Germany for technical assistance. A. T. is grateful to the Division of Basic Sciences, UNESCO, Paris, France, for a postdoctoral fellowship.

References

Agboola DA (1998): Dormancy and seed germination in some weeds of tropical wastelands. *Niger. J. Bot.* **11**, 79–87.

Akobundu IO, Agyakwa CN (1987): *A Handbook of West African Weeds.* International Institute of Tropical Agriculture, Ibadan.

Brunt A, Crabtree K, Gibbs A (1990): *Viruses of Tropical Plants* CAB International, Wallingford.

Clark MF, Adams AN (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assay (ELISA) for the detection of plant viruses. *J. Gen. Virol.* **43**, 375–383.

Converse RH, Martin RR (1990): ELISA methods for plant viruses. In Hampton R, Ball, E, De Boer S (Eds): *Serological Methods for Dectection and Identification of Viral and Bacterial Plant Pathogens. A Laboratory Manual.* APS Press, St. Paul.

Dale JL, Gibbs AJ, Behneken GM (1984): Cassia yellow blotch virus: a new bromovirus from an Australian native legume, *Cassia pleurocarpa. J Gen. Virol.* **65**, 281–288.

Hutchinson J, Dalziel JM (1954): *Flora of West Tropical Africa.* 2nd ed., Vol. 1, Part 1. Mill-Bank, London.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Lin MT, Kitajima EW, Cupertino FP, Costa CL (1979): Properties of a possible carlavirus isolated from a Cerrado native plant, *Cassia sylvestris. Plant Dis. Rep.* **63**, 501–505

Mathur NM, Singh BK (1969): Ringspot mosaic disease of coffee senna, *Cassia occidentalis* in India. *Indian Phytopathol* **25**, 314–315.

Owolabi AT, Proll E (2000): Differentiation of celosia mosaic virus and asparagus virus-1 based on biological properties *Acta Virol.* **44**, 179–181.

Owolabi TA, Taiwo MA, Thottappilly G, Shoyinka SA, Proll E, Rabenstein F (1998): Properties of a virus causing mosaic and leaf curl disease of *Celosia argentea* L. in Nigeria. *Acta Virol.* **42**, 133–139.

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

Thottappilly G (1992): Plant virus diseases of importance in African agriculture. *J. Phytopathol.* **124**, 265–288.

Van Velsen RJ (1961): Cassia mosaic, a plant virus of *Cassia spp.* in New Guinea. *Papua New Guinea Agric. J.* **14**, 124–127.

Walkey DGA, Spence NJ, Clay CM, Miller A (1994): A potyvirus isolated from *Senna occidentalis*. *Plant Pathol.* **43**, 767–773.