IDENTIFICATION OF OKRA MOSAIC VIRUS FROM *INDIGOFERA SPICATA* IN NIGERIA

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Summary. – Okra mosaic virus (OMV, tymovirus group) was isolated from *Indigofera spicata* plants growing at the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria. Its identity was established on the basis of particle morphology, analysis of viral coat protein and nucleic acid, and serology. In reciprocal agar gel diffusion tests, the virus isolate from *I. spicata* and an OMV isolate from okra in Ibadan (OMV-Ibadan isolate) were found to be serologically identical. However, because the isolates differ in symptom induction in various host plants, the name OMV-*Indigofera* isolate is suggested. This is the first report on the occurence of OMV in *I. spicata.*

Key words: okra mosaic virus; *Indigofera spicata;* serology

Introduction

I. spicata Forsk (syn: *I. hendecaphylla* Jacq./ *I. endecaphylla* Jacq.) is a perennial plant widespread in Nigeria. Apart from use as a source of indigo dye, the plant is a valuable cover crop and fodder plant.

During the past 4 years, *I. spicata* plants growing on the lawn of the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, have exhibited a mild to severe mosaic, yellowing, and stunting symptoms.

This paper presents the symptomatology, host range, purification, and some physico-chemical and serological properties of an OMV isolate from *I. spicata.*

Materials and Methods

Viruses. The original isolate of OMV (referred to in this paper as the *Indigofera* isolate), recovered from naturally infected *I. spicata* plants which had shown mosaic symptoms, was maintained by regular sap inoculation of cowpea plants (*Vigna unguiculata* cv. Ife Brown). For host range studies and routine sap inoculation, leaves were homogenized in 10 mmol/l potassium phosphate buffer pH 6.0, and the extract was rubbed on leaves of test seedlings previously dusted with carborundum powder. Leaves were then rinsed with water and the plants were maintained in an insect-proof screenhouse for observation. For comparative studies, an Ibadan isolate of OMV from okra *Abelmoschus esculentus* was used.

Electron microscopy. Leaf samples were crushed on a glass microscope slide in 0.1% (w/v) sodium sulphite in distilled water and a drop of the mixture was placed on carbon-reinforced, formvar-coated grids, dried, and then stained with 2% (w/v) uranyl acetate pH 6.0. Purified preparations were stained similarly or with 0.2% potassium phosphotungstate pH 6.8. The samples were examined in a Philips EM 201-C electron microscope.

Virus purification was carried as follows. Systemically infected cowpea leaves were homogenized (1 g/2 ml) in 100 mmol/l potassium phosphate buffer pH 6.0. One volume of ice-cold chloroform and one volume of n-butanol were added to two volumes of the homogenate and the mixture was stirred for 5 mins. The resulting emulsion was centrifuged at 10,000 x g for 10 mins and the virus was precipitated from the aqueous phase with 8% polyethylene glycol and 20 mmol/l NaCl and pelleted at 10,000 x g for 15 mins. After resuspending the pellet in 50 mmol/l potassium phosphate buffer pH 6.0, and low-speed centrifugation, the virus was sedimented (125,000 x g, 2.5 hrs, 5°C) through a 20%-sucrose cushion. The resulting pellet was dissolved in 50 mmol/l

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Abbreviations: IITA = International Institute of Tropical Agriculture; OMV = okra mosaic virus; SDS-PAGE = polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate

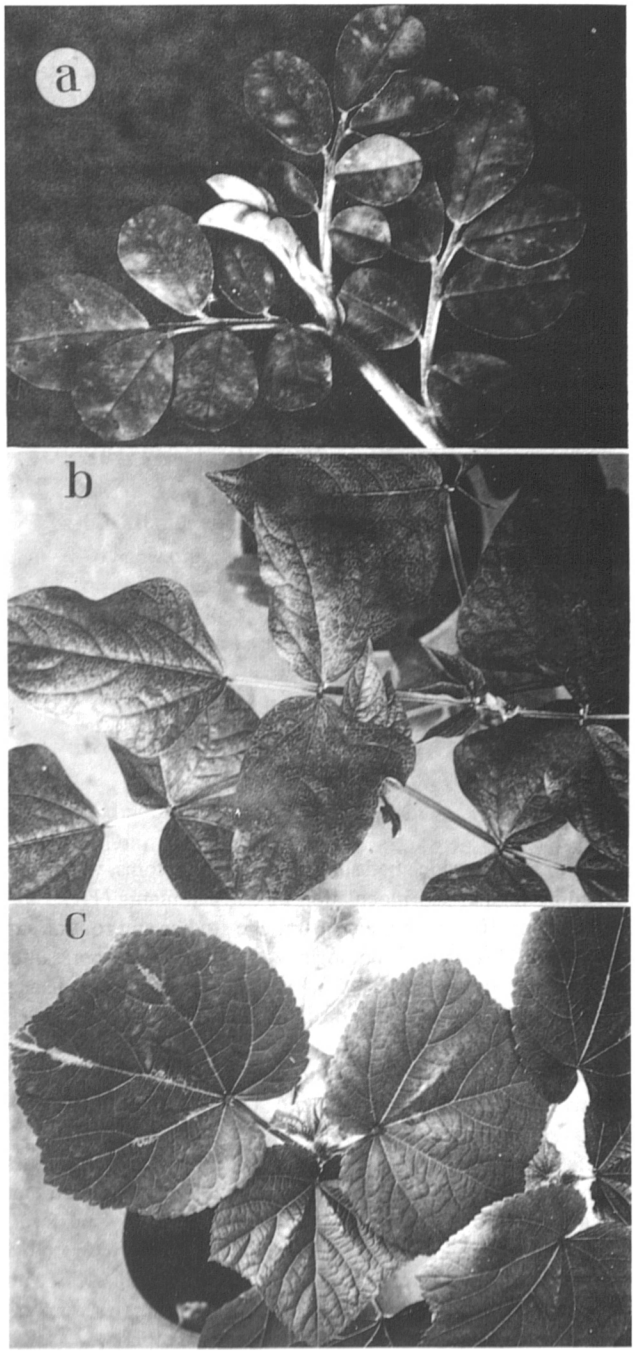

Fig. 1
Symptoms induced by the *Indigofera* isolate on representative plant species
(a) Naturally infected *I. spicata*; (b) Infection resulting from sap-inoculation in *Vigna unguiculata;* (c) Infection after sap-inoculation of *Abelmoschus esculentus*.

potassium phosphate buffer pH 6.0 and the suspension was centrifuged at 10,000 x g for 30 mins. The virions were further purified by linear sucrose density gradient (10-40%) centrifugation, and the light-scattering band was recovered and concentrated by ultracentrifugation. The pellets were dissolved in the abovementioned buffer and the suspension was clarified by low-speed centrifugation. Virus concentration was estimated spectophotometrically using the extinction coefficient E (0.1%, 260 nm) = 9.0 (Brunt *et al.*, 1990).

Antisera. Antiserum against the *Indigofera* isolate was produced by injecting rabbits with virus at a concentration of 1 mg/ml. Three intramuscular injections with incomplete adjuvant and two intravenous injections were given at weekly intervals and the blood was collected 10 days after the last injection.

The antibody titer as determined by the agar gel diffusion test was 1:1024. Forty four antisera to different isometric viruses (belonging to 12 plant virus groups) including members of the tymovirus group were from a collection maintained at the IITA Virology Unit. These were originally obtained from various sources including American Type Culture Collection (Rockville, USA), Deutsche Sammlung von Mikroorganismen (Braunschweig, Germany) and several antisera to tropical plant viruses were produced by the author at IITA.

Agar gel diffusion method. Serological tests were performed by agar gel diffusion method using 0.9% agarose in 0.85% sodium chloride with 0.1% sodium azide. The agar gel bands were stained with 0.05% Coomassie Blue in 50% methanol and 7.5% acetic acid, and destained in 50% methanol and 7% acetic acid.

Viral coat protein and RNA. The molecular mass of the viral coat protein was estimated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE). To subject virion protein subunits to electrophoretic analysis, they were dissociated from virions in purified virus preparations (Laemmli, 1970). Samples were loaded onto a prepared gel (stacking gel was 4% and the separating gel 12%) with size markers (Sigma) and electrophoresis was performed in a vertical gel apparatus (Minigel, Bio-Rad, USA) at 100 V for 1 hr.

Nucleic acid was extracted from purified virus preparations by the phenol-SDS method (Wyatt and Kuhn 1977). Agarose gel electrophoresis of RNA was performed according to Sehgal (1990).

Immunoblot analysis. Purified virus preparations were subjected to SDS-PAGE (see above). Gels were blotted onto nitrocellulose membranes using a Bio-Rad Trans-Blot Module. The blots were probed with specific antisera (one strip with antiserum to the *Indigofera* isolate and the other one with antiserum to OMV) according to Donald *et al.* (1993).

Results and Discussion

Symptomatology and host range

Field-infected *I. spicata* plants exhibited stunting *and* yellowing, and mild to severe foliar mosaic (Fig. 1a). Similar symptoms were produced in screenhouse inoculations.

When a sap from infected *I. spicata* leaves was inoculated to cowpea *(Vigna unguiculata* cv. Ife Brown and

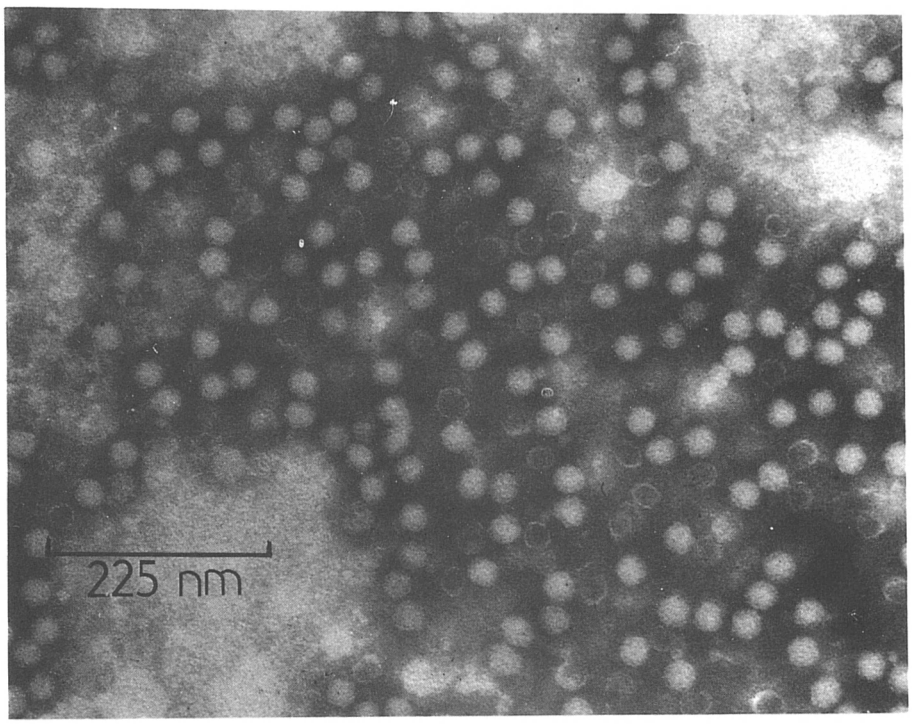

Fig. 2
Electron micrograph of purified virions of the *Indigofera* isolate

breeding line IT-82E-10) and *Nicotiana benthamiana*, typical vein clearing, mosaic, and mottle symptoms were readily induced. Based on viral characteristics, including analyses (described later herein) of viral coat protein and nucleic acid and serology, the causal virus was identified as OMV.

OMV was first found in Cote d'Ivoire (Givord and Hirth, 1973). OMV causes a mosaic disease in okra (*Abelmoschus esculentus* = *Hibiscus esculentus, Malvaceae*) and natural infection with OMV occurs also in some other members of the *Malvaceae* (Brunt *et al.*, 1990). OMV has also been recorded in Nigeria (Lana *et al.*, 1974; Bozarth *et al.*, 1977; Igwegbe, 1983) in okra. It belongs to the tymovirus group (Givord and Koenig, 1974; Koenig and Givord, 1974; Koenig and Lesemann, 1981), and it has been reported to be transmitted by the flee-beetle *Podagrica decolorata* in Cote d'Ivoire (Givord and den Boer, 1980). In Nigeria, *P. sjostedti, P. uniforma* and *Syagrus calcaratus* are reported as vectors of OMV (Lana and Taylor, 1976).

The *Indigofera* isolate was transmitted by sap inoculation to the following plant species: *Legumisosae – Indigofera spicata, Vigna unguiculata, Arachis hypogea*; *Malvaceae – Abelmoschus esculentus*; *Chenopodiaceae – Chenopodium amaranticolor*; *Solanaceae – Nicotiana benthaminana, N. clevelandii*. In all the abovementioned species, the symptoms were vein clearing and mosaic (Fig. 1b).

In comparative host range studies, the Ibadan isolate of OMV generally induced similar symptoms in *I. spicata* and in the abovementioned plant species. However, the symptoms caused by the *Indigofera* isolate were milder than those caused by the Ibadan isolate of OMV, especially on okra plants (Fig. 1c). Furthermore, in *Chenopodium amaranticolor*, the OMV caused systemic chlorotic spotting symptoms, but the *Indigofera* isolate caused only chlorotic local lesions.

The following species were not susceptible to infection by either the *Indigofera* isolate or OMV: *Glycine max, Pisum sativum, Rynchosia* sp., and *Lycopersicon esculentum*.

Electron microscopy

Leaf dip preparations from cowpea and *I. spicata* stained with 2% uranyl acetate showed numerous isometric particles. This indicated that the virus concentration in these plants was high. No other types of particles were seen in electron microscope. In purified preparations, virions had an average diameter of 28 nm (Fig. 2) using tobacco mosaic virus as the internal size standard. The purified preparations also showed empty particles. The overall morphology

of the *Indigofera* isolate was similar to that of the Ibadan isolate of OMV.

Sedimentation analysis

The analysis of virus preparations by sucrose density gradient centrifugation showed two components. The absorbance of the bottom component was typical of a nucleoprotein, with a maximum at 260 nm and a minimum at 242 nm. The 260/280 nm absorbance ratio was 1.60. The absorbance profile of the top component was typical of a protein, with a maximum at 280 nm and minimum at 250 nm.

When sucrose density gradient-purified virus preparations were inoculated on *I. spicata*, cowpea, and okra seedlings, symptoms were similar to those observed on field-infected leaf samples (Fig. 1).

Serology

Antisera to 44 different isometric viruses (belonging to 12 plant virus groups), including several that affect legumes in Nigeria, (e.g., cowpea mosaic, cowpea mottle, cucumber mosaic, and southern bean mosaic), gave no reaction when tested against purified *Indigofera* isolate virions or crude extracts from the naturally infected *I. spicata* plants.

The results of the agar gel diffusion test showed that the *Indigofera* isolate and Ibadan isolate of OMV were identical (Fig. 3). Extracts from infected plants and purified preparations of the *Indigofera* isolate and OMV reacted with both antisera, as did 50 field- infected *I. spicata* samples. The precipitin line, developed in the homologous reaction, produced a confluent band with the heterologous antigen (Fig. 3).

In order to confirm the relationship between the *Indigofera* isolate and Ibadan isolate of OMV, a four-well agar gel diffusion test (Ball, 1990) was performed (Fig. 4). The results clearly indicated that both isolates formed confluent bands with both antisera. Furthermore, in reciprocal agar gel diffusion tests, antiserum to the *Indigofera* isolate had a titer of 1/1024 with either isolate. In addition, as reported earlier by Koenig (1976), serological tests using antisera to other members of the tymovirus group showed that belladonna mottle, desmodium yellow mottle, clitoria yellow vein, and cacao yellow mosaic viruses are related to OMV.

Viral coat protein

A single protein with an apparent M_r of 20,000 was detected when SDS-disrupted virions of the *Indigofera* isolate were analyzed in SDS-PAGE (Fig. 5a). This protein co-migrated with the coat protein of the Ibadan isolate of OMV.

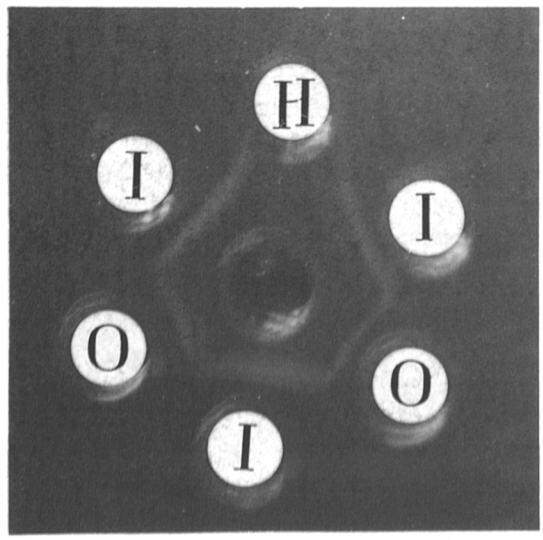

Fig. 3
Serological reactions of the *Indigofera* isolate and Ibadan isolate of OMV with an antiserum to the *Indigofera* isolate

Well H contained sap from healthy *Indigofera*. Wells I contained sap from *Indigofera* isolate-infected plants, while wells O contained sap from Ibadan isolate of OMV-infected plants.

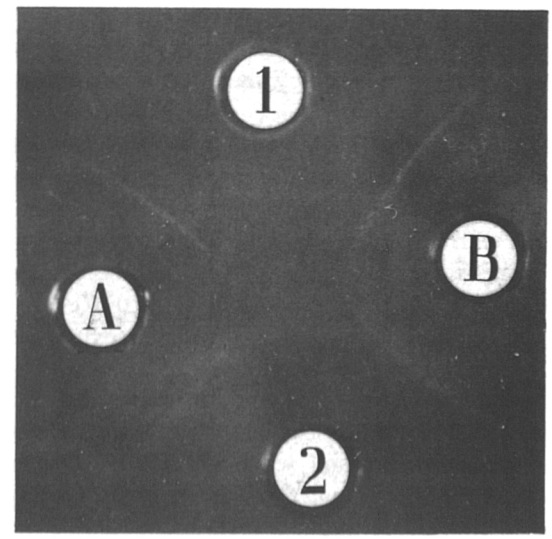

Fig. 4
Serological reactions of purified preparations of the *Indigofera* isolate and Ibadan isolate of OMV with homologous and heterologous antisera
The well 1 was loaded with antiserum to the *Indigofera* isolate while the well 2 was loaded with antiserum to the OMV; both antisera were diluted 1:20. Wells A and B contained the OMV and *Indigofera* isolate, respectively.

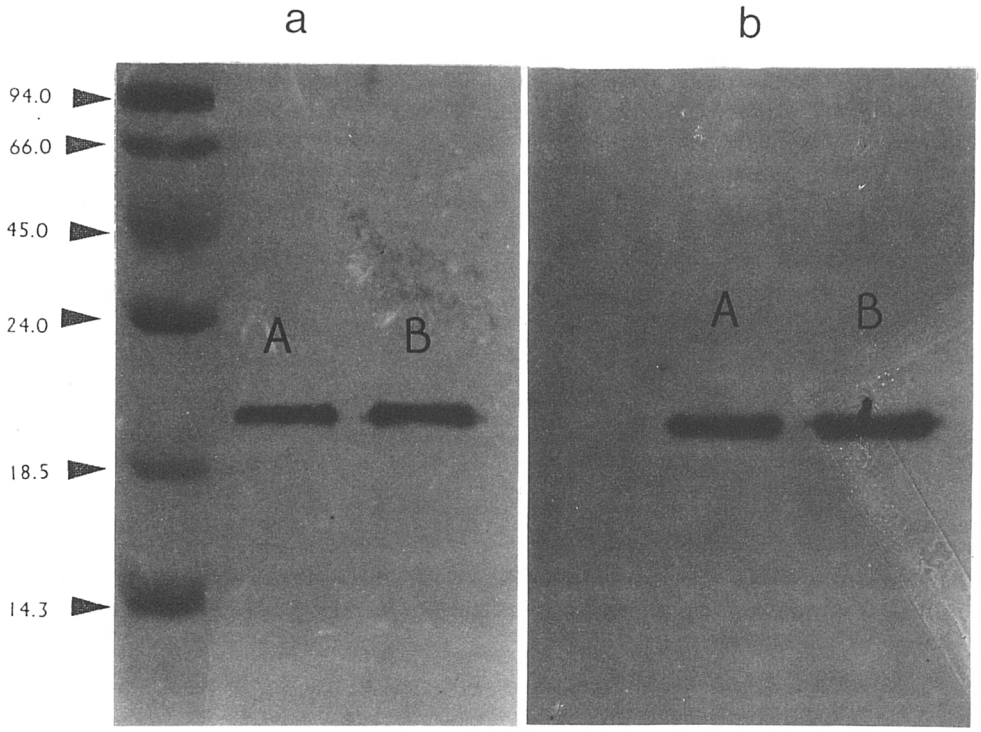

Fig. 5
SDS-PAGE (a) and immunoblot analysis (b) of the *Indigofera* isolate (A) and OMV (B).

Fig. 5b illustrates an immunoblot analysis of the *Indigofera* isolate with antibodies to the *Indigofera* isolate. Identical results were obtained with antibodies to OMV (data not shown). The M_r values of the coat proteins, specifically detected by the antibodies, were estimated to be 20 K for both isolates in agreement with the results obtained by SDS-PAGE.

Viral nucleic acid

Virion nucleic acids of the *Indigofera* isolate and Ibadan isolate of OMV were resolved into a single species of RNA with M_r of 1.9 x 10^6 in agarose gel electrophoresis. The nucleic acid was rapidly depolymerized by pancreatic RNase at low and high salt concentrations, indicating that it was a single-stranded RNA (data not shown).

The results clearly established that an OMV is the causal agent of mosaic in *Indigofera*. Since there are slight differences in the host range and symptomatology, based on the limited studies done so far, the *Indigofera* isolate should not be designated OMV but the *Indigofera* isolate of OMV.

Although the *Indigofera* isolate was transmitted easily by sap inoculation of cowpea in the experimental studies, extensive surveys and virus monitoring tests indicate that it does not occur naturally in cowpeas in Nigeria (Thottappilly and Rossel, 1992). At present, the *Indigofera* isolate does not appear to pose any threat to cowpea, which is an important crop in Africa. Lana and Taylor (1976) reported that the chrysomelid beetle, *Podagrica uniforma,* transmitted OMV to cowpea. The chrysomelid beetles are present abundantly at Ibadan. Since *Indigofera* is a perennial plant, it could serve as a primary virus source, which could be transmitted to okra, also an important crop in Nigeria.

One hundred and twenty IITA cowpea breeding lines were tested for their sensitivity to infection with the *Indigofera* isolate. Several cowpea lines were resistant to the virus; such lines included IT83D-326-2, IT82D-789, IT82D-927, and TVX 4677-010E. However, these lines were segregating, with about 80% of resistant and 20% of susceptible plants (data not shown).

There are thus very good sources of resistance to the *Indigofera* isolate among IITA's cowpea breeding lines, in the event this virus should pose a threat to cowpea production.

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