Field occurrence and identification of Southern Bean Mosaic Virus (Cowpea strain) in Nigeria*

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COMPENDIO

Durante un reconocimiento sobre la incidencia de virus en campos comerciales de caupí (Vigna unguiculata) *en Nigeria Occidental en 1973, se encontró, en tres lugares diferentes, un virus transmisible por semilla, identificado serológicamente como Mosaico Sureño de Frijol - Raza Caupí (SBMV-CS) En todos los casos el virus se presentó como una infección mezclada con el Virus del Mosaico Amarillo del Caupí (CYMV) Se separaron mediante las diferencias en el ámbito de sus hospedantes y sus propiedades físicas.*

La inoculación de uno de los aislamientos del SBMV-CS a diferentes hospedantes indicó que el ámbito del virus estaba restringido a las leguminosas. Las reacciones de 25 cultivares de caupí a la inoculación manual con el virus varió desde inmune hasta infección severa que conducía a la muerte de las plantas. El virus fue transmitido mediante las semillas de por lo menos una variedad local.

Las propiedades del virus en la savia cruda fueron: inactividad termal (10 min. de exposición) a 90C (ocasionalmente a 95 C) pero no a 85 C; punto final de dilución, 10^{-6} — 10^{-7}; y senectud in vitro, *15 días.*

Las preparaciones purificadas de virus contenían partículas esféricas de 28,0 ± 1,0 nm de diámetro las que sedimentaban como un componente único en una gradiente de densidad de sucrosa o ultracentrifugación analítica a S 20, W = 114.

El antisuero preparado contra el virus reaccionaba con la raza de frijol, la raza de caupí, y la raza de Ghana de caupí de SBMV. La formación de espolones indicó que el organismo aislado está relacionado pero distinto a los aislamientos previamente aislados de SBMV.

Introduction

ALTHOUGH many viruses have been reported as infecting cowpeas in Nigeria (3, 10, 11, 12, 18), positive identification based on morphological and serological data was not achieved in most cases. Consequently, confusion still exists regarding the identities of cowpea viruses which cause widespread disease in Nigeria. The characterization of virus isolates by morphology and serology and their subsequent identification are fundamental aspects of the virus research program of the Plant Pathology Division of the Institute of Agricultural Research and Training.

Since Shepherd and Fulton (14) first described the cowpea strain of southern bean mosaic virus (SBMV-CS) and the disease it induced in affected hosts, the virus has been reported in the crop in many other parts of the world (2, 7, 8). In the late season (August - December) 1973 while conducting a survey of virus incidence in the Western State of Nigeria, SBMV-CS was

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identified serologicaly and found to occur in mixed infections with cowpea yellow mosaic virus (CYMV) (1,3) in three commercial farms located in widely separated areas. This paper reports the isolation and characterization of the virus and its separation from its more commonly occurring partner, CYMV.

Virus strains and culture. Virus isolates collected from many areas of the Western State of Nigeria were cultured in cowpea *(Vigna unguiculata* cv: 'Victor Brabham 892 A') in insect-proof glasshouses. Infective sap extracted in 0.1 M phosphate buffer, pH 7.0 was manually inoculated on to the primary leaves of seedlings which had been dusted with Carborundum. One isolate (Oyo 4) collected from Oyo, 37 miles north of Ibadan, was selected for detailed studies and subsequent comparison with other isolates.

Other viruses used in these experiments were SBMV bean strain (SBMV-B) originally obtained from I. R. Schneider, U.S.D.A., Beltsville, Md., U.S.A.; cowpea yellow mosaic (CYMV) (Shoyinka, unpublished data); cowpea severe mosaic virus (CSMV) from N. Vakili, U.S.D.A., Mayaguez, Puerto Rico; and cowpea mottle virus (CMeV) (15).

Host range.- All plants inoculated throughout the investigation were started from seeds and grown in a glasshouse at 23 C - 30 C. The glasshouse was kept insect-free by a twice-weekly application of Dimethoate (Rogor 40) insecticide. At least 5 plants of each species or cultivar were inoculated in this investigation. Reisolations to 'Brabham 892A' cowpea seedlings were made from both inoculated and non-inoculated leaves of test plants about 2 weeks after inoculation. When necessary, tests were repeated with certain host range species if initial results were inconclusive.

In-vitro properties - Physical properties were determined with crude infective cowpea sap without addition of distilled water or buffer.

Virus purification.- After the initial identification in Nigeria, the virus was sent to the second author for more detailed studies. There the virus was manually inoculated to seedlings of *Vigna unguiculata* cv. 'Calif. Blackeye Nº 5' (George Taite Seed Co., Norfolk, Va.) in which it produced a very mild systemic mottle.

Two to 3 weeks after inoculation, the systematically infected leaves were ground in a Waring Blender containing an equal weight of 0.1 M potassium phosphate buffer, clarified by low-speed centrifugation at 10,000 rpm for 10 min. and subjected to 3 cycles of differential centrifugation. The final high-speed pellets were resuspended in buffer and further purified by centrifugation on sucrose density gradient in the Beckman Ti-15 (Beckman, Palo Alto, Calif.) zonal rotor.

Details of subsequent analysis by sucrose density gradient centrifugation, fractionation of grandients, analytical ultracentrifugation, ultraviolet spectroscopy, and electron microscopy have been previously described (6).

Serology.- Density gradient purified virus was mixed 1:1 with Freunds complete adjuvant (Difco, Detroit, Mich.) and injected intramuscularly into rabbits at intervals of 3 weeks. Rabbit normal sera was withdrawn prior to the first injection.

Ouchterlongy immunodiffusion tests (4) by the senior author were conducted in 100X 15 mm plastic petri dishes containing 20 ml of 0.8% Ionagar Nº 2 (Colab, Glenwood, Ill.) and 0.02% (wt./vol.) sodium azide. Wells were made in the agar with a Shandon gel punch (Shandon Scientific Co., London, U.K.) equipped with 7 mm diameter bores spaced 1.0 cm apart. Antigens were placed in the peripheral wells and the antiserum in the central well.

Serological reactions carried out by the second and third authors were made on glass slides using Gelman immunodiffusion equipment (Scentific Products, Chicago, Ill.) in 1.0% Agar Noble dissolved in 0.85% saline and 0.01% sodium azide. Test antigens consisted of leaf extracts of systemically infected leaves diluted 1:1 with 0.85% saline or purified virus at a concentration of approximately 0.2 mg/ml. In addition to the antiserum prepared against the virus, the following antisera were used: SBMV-CS from C. W. Kuhn, Univ. of Georgia, Athens, Ga., U.S.A.; SBMV-CS PVAS-11 from the American Type Culture Collection (ATCC), Rockville, Md.; SBMV-B PVAS-2 from ATCC; CSMV and CYMV which were passed onto us from Agrawal (1) via H. A. Wood of Boyce Thompson Institute, Yonkers, N. Y.; and CMeV (15).

Results

Host range and symptoms.- Inoculations of various cultivars of *V. unguiculata* with isolates from different geographical origins resulted in different symptoms which were classified visually as shown in Table 1.

Initial symptoms of the disease included inconspicuous chlorotic spots which appeared on the inoculated primary leaves 5 - 7 days after inoculation. Some varieties reacted by clearing of the main and secondary veins. The initial symptoms were followed by a mild to severe systemic mottle or mosaic, depending on the variety. In very susceptible varieties, other symptoms such as leaf distortion, reduction in size of leaves, and dwarfing accompanied the severe mosaic manifestations.

Virus was not recovered from plants of the following symptomless species after repeated attempts: *Cucumis sativus, Lycopersicon esculentum, Datura stramonium, Physalis peruviana, Chenopodium quinoa, C. amaranticolor, C. murale, Nicotiana tabacum* cv. 'White Burley', *N. clevelandi, N. rustica, N. glutinosa, Petunia hybrida, Canavalia ensiformis, Capsicum frutescens* cvs 'Tabasco' and 'Rodo', *Zinnia elegans, Phaseolus vulgaris* cvs. 'UK Commercial', 'French Dwarf', and 'Prince', *P. lunatus* (Acc. 64009), *P. lathyroides, P. acutifolius* var. *latifolius, P. aureus, P. mungo,* and *Glycine max.*

Stability in crude juice.- The *in vitro* properties in crude juice were thermal inactivation point between 90

Table 1.—Reaction of some cowpea varieties to infection by a Nigerian isolate of southern bean mosaic virus (cowpea strain).

Immune	Mild	Moderate	Susceptible	Very Susceptible
Kano 1696	New Era G8	Nigeria B4	Nigeria B7	Prima
	Victor	Ima	Blackie	Early Ramshorn I
	TVu 410	Farin Juda	Nigeria A104	Early Ramshorn II
	TVu 470		Debora	L 7
			Ife Brown	DS 213
			Victor Brabham 892A	Kano 2479
				Igbirra
				Faru 13
				Albino Bulk F3
				Mala
				Banikido-3

C - 95 C after 10 min. exposure, a dilution end-point 10^{-6} — 10^{-7}, and longevity of 15 days at laboratory temperatures of 22 - 24 C. The separation of CYMV from SBMV-CS was achieved by inoculating *C. amaranticolor* and subsequent local lesion transfer of CYMV. SBMV was separated by heating sap to 85 C for 10 min., inoculating cowpea leaves, and subsequent local lesion transfer of SBMV.

Purification and serology.- Preliminary results using leaf-dip electron microscopy and sucrose density gradient analysis of a partially purified preparation indicated that we were dealing with a single component spherical virus and that no rod-shaped particles were present. After purification by differential and zonal centrifugation, the virus was concentrated and analyzed by sucrose density gradient centrifugation. A single band was obtained (Fig. 1).

The virus sedimented at S 20,w = 114 in the analytical ultracentrifuge. The ultraviolet spectrum was typical for nucleoprotein with maximum absorbancy at 260 nm and minimum absorbancy at 242 nm. The ratio of absorbancy at 260/280 was 1.67. An electron micrograph of the preparation showed only virus particles which measured 28.0 ± 1.0 nm diameter (Fig. 2).

Results of the serological tests are shown in Fig. 3. Fig. 3A indicates that the antiserum against SBMV-CS (N) reacted with its homologous antigen and with the strain, SBMV-B; however, a spur is formed between the 2 lines of reaction. The normal sera (NS) of the rabbit did not react with SBMV-CS(N). Fig. 3B shows the

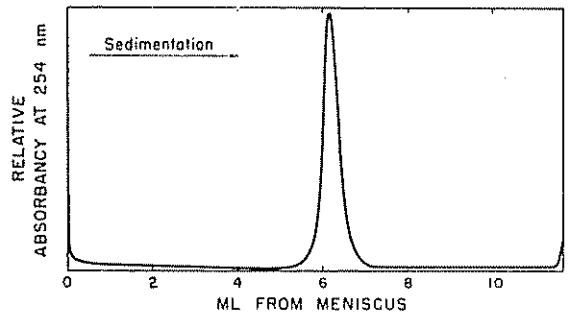

Fig. 1.—*Ultraviolet absorbancy scanning patterns of SBMV-CS(N) following centrifugation for 2.5 hr at 39,000 rpm on 10-40% sucrose density gradients.*

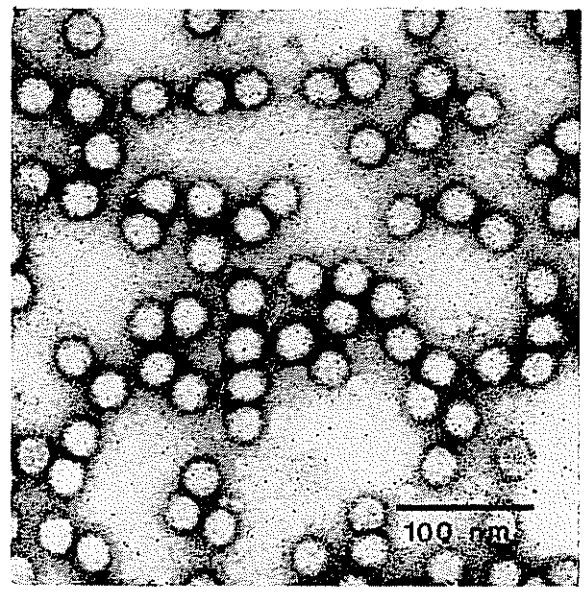

Fig. 2.—*Electron micrograph of SBMV-CS(N) negatively stained with 1% uranyl acetate.*

positive reactions of SBMV-CS(N) and SBMV-B with antisera to SBMV-B, and negative reactions of SBMV-B antiserum with CYMV, CSMV, and CMeV The spurs in these tests are reciprocal to those in Fig 3A Fig 3C shows spur formation in the reaction between antiserum to the cowpea strain of SBMV, (SBMV-CS) with SBMV-B and SBMV-CS(N) No reaction occurred with CSMV, CYMV or CMeV. Negative reactions were also obtained between all SBMV strains and antisera to CSMV, CYMV, and CMeV.

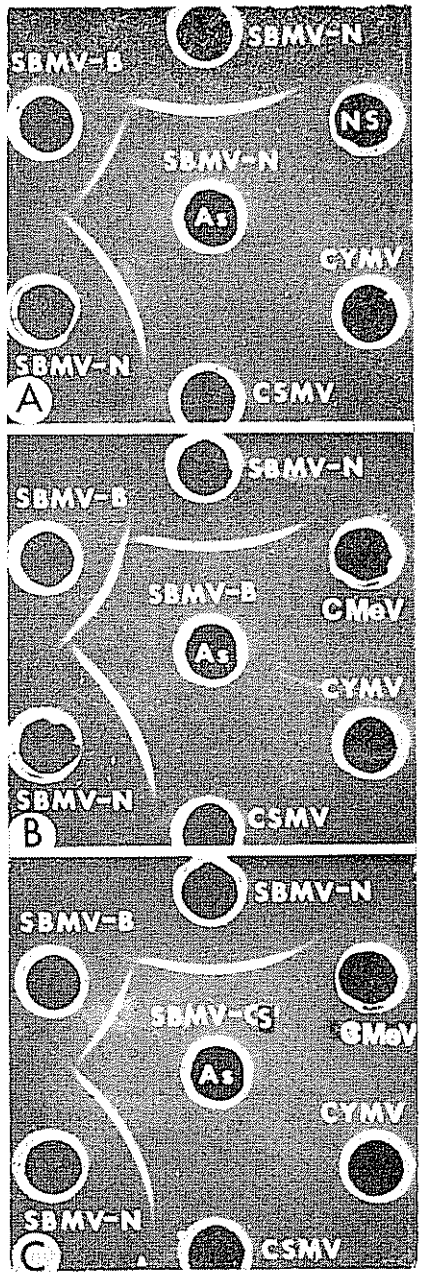

Fig 3.—*Ouchterlony double-diffusion serological tests of cowpea viruses and antisera. Center wells contain the antisera indicated. A. SBMV-N from the Nigerian strain SBMV-CS(N) prepared in this research; B. SBMV-B (ATCC, PVAS-2); C. SBMV-CS (ATCC, PVAS-11). Outer wells contain normal sera (NS) or the antigens indicated: SBMV-B, bean strain of southern bean mosaic virus; CSMV, cowpea severe mosaic virus, CYMV, cowpea yellow mosaic virus, CMeV, cowpea mottle virus.*

Discussion

That SBMV-CS(N) is related to other strains of southern bean mosaic virus reported from other parts of the world seems certain. Its physical properties and serological reactions indicate that it is a strain of SBMV. A comparison of host ranges and physical properties of strains of SBMV reported in literature is given in Tables 2 and 3.

The various particle diameters listed for SBMV in Table 3 probably represent differences in preparation for electron microscopy, accuracy in measurement, and calibration of the magnification of the electron microscope. Probably the best estimate of the diameter of SBMV is 28.6 nm made by Leonard *et al.* (9) using small angle scattering of X-rays. The sedimentation coefficients reported by all workers are essentially the same. Serological results indicated strong positive relationships between the bean strain (SBMV-B), the cowpea strain (SBMV-CS), and SBMV-CS(N) although there was evidence that they were not identical.

Table 2.—A comparison of the host range of southern bean mosaic virus (SBMV) isolates reported in literature with SBMV-CS(N).

Species	Virus Strain			
	SBMV-CS [a] (14)	SBMV-CS [a] (7)	SBMV-GH [a] (8)	SBMV-CS(N) (This paper)
Vigna unguiculata (V sinensis)	S[b]	L,S	L,S	L,S
V sesquipedalis	L,S	+	—	—
V cylindrica	—	+	—	—
Glycine max	S	+	0	0
Pisum sativum	S	0	0	0
Phaseolus vulgaris	0	0	L,T	0
P aureus	—	—	L	0
Cyamopsis tetragonoloba	L,S	0	—	—

[a] Literature citation

[b] L = local lesions; S = systemic infection with symptoms; T = systemic infection without symptoms; 0 = not susceptible under conditions of test; — = not tested; + = positive reaction without details

Table 3.—Comparison of properties of southern bean mosaic virus isolates reported in the literature with SBMV-CS(N).

Virus Isolate	Physical Properties[b]			Particle Diameter (nm)	S 20,w	Mode of Transmission			Serological Relationships
	TI	DEP	LIV (Days)			Sap	Seed	Vector	
SBMV-CS(14)[a]	85 C	10^{-5}	15	33-35 26[c]	115	+	+	Cerotoma trifurcata[d]	SvBMV SBMV-CS SBMV-B
SBMV-CS (7)[a]	90 C	10^{-7}–10^{-8}	19	33		+	+		SBMV-CS
SBMV-GH (8)[a]	90 C	10^{-7}–10^{-8}	7	30	115	+	+		SBMV-B SvBMV
SvBMV (19)[a]	92 C	10^{-7}	165	26 3 ± 2.2[c]		+	—		SBMV-B SBMV-CS
SBMV-CS (N) (This paper)	90–95 C	10^{-5}–10^{-7}	15	28 0 ± 1.0	114	+	+	Ootheca mutabilis	SBMV-CS SBMV-GH SBMV-B

[a] Literature citation

[b] TI = Thermal inactivation point; DEP = Dilution end-point; LIV = Longevity *in vitro*

[c] Grogan and Kimble (5)

[d] Walters (17)

Our results have been confirmed by Hamilton (personal communication) who found that a cowpea strain of SBMV obtained from Ghana (SBMV-GH) was serologically related, but not identical, to SBMV-CS(N) in agar double-diffusion tests. The host range of SBMV-CS(N) differs from other strains of SBMV in the following ways: whereas the Ghanaian isolate (8), SBMV-GH, and severe bean mosaic virus isolate (SvBMV) (19) infected both bean and cowpeas, SBMV-CS(N) infected only cowpeas, as did cowpea strains reported by Shepherd and Fulton (14) and Kuhn (7). The failure of SBMV-CS(N) to infect hosts other than *V. unguiculata* may be due to strain and environmental differences. The physical properties of SBMV-CS(N) are in agreement with the earlier reports (7, 8, 13, 14, 19). Differences in host reactions seem to reflect strain effects which is confirmed by serological evidence. This may also support the evolution theory suggested for strains of the virus (8). The transmissibility of SBMV-CS(N) through seeds (3 out of 56 of a local white cowpea variety) and by the beetle, *Ootheca mutabilis*, which is also the vector of CYMV (D. J. Allen, personal communication) agrees with results obtained by Walters (16, 17) who reported that a beetle, *Cerotoma trifurcata*, transmitted cowpea mosaic virus and SBMV in the U.S.A. The occurrence of mixed infections of CYMV and SBMV in widely scarttered locations in southern Nigeria and the transmission of both viruses by the same vector pose a serious threat to current efforts to boost legume production and reduce protein malnutrition in this area which traditionally has low animal-protein supply.

Summary

During a survey for virus incidence in commercial cowpea fields in Western Nigeria in 1973, a seed-transmissible virus identified serologically as southern bean mosaic (SBMV-CS) was found at three different locations. In all instances the virus occurred as a mixed infection with cowpea yellow mosaic virus (CYMV). They were separated by employing the differences in their host ranges and physical properties.

Inoculation of one of the isolates of SBMV-CS to different hosts indicated that the host range of the virus was restricted to the Leguminosae. Reactions of twenty-five cowpea cultivars to manual inoculation with the virus varied from immune to severe infection leading to death of plants. Te virus was transmitted through the seeds of at least one white-seeded local cowpea variety.

Properties of the virus in crude sap were: thermal inactivation (10 min exposure) at 90 C (occasionally at 95 C) but not at 85 C; dilution end-point, 10^{-6} — 10^{-7}; and ageing *in vitro*, 15 days.

Purified virus preparations contained spherical particles 28 0 ± 1.0 nm diameter which sedimented as a single component in sucrose density gradient or analytical ultracentrifugation at S20,w = 114.

Antiserum prepared against the virus reacted with the bean strain, the cowpea strain, and the Ghana cowpea strain of SBMV. Spur formation indicated that the isolate was related but distinct from previously studied isolates of SBMV.

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Notas y Comentarios

Publicaciones

Ciencia e Prática. Desde 1977, está apareciendo la revista *Ciencia e Prática,* de periodicidad semestral, órgano de la Escola Superior de Agricultura de Lavras, Minas Gerais. Contiene artículos originales de investigación (unos nueve en cada número) sobre temas diversos como café, manzano, zanahoria, pera, cebolla, cítricos, algodón, trigo, irrigación, genética y ecología. La dirección es: E.S.A.L., Caixa Postal 37, 37.200 Lavras, MG Brasil.

Publicaciones

Revista Grama. En La Paz, Bolivia, ha aparecido, en el primer semestre de 1979, la *Revista Grama,* órgano de la Carrera de Bibliotecología y Ciencias de la Información de la Universidad Mayor de San Andrés, destinada a ser "vehículo eficaz de comunicación de ideas, fruto de distintas investigaciones y experiencia". El primer número contiene nueve artículos de temas relacionados con la bibliotecología, los bibliotecarios y su acción en Bolivia. La dirección es: Programa UMSA-OEA, Casilla Nº 3279, La Paz. El director es Constantino Tancara.