SOME PROPERTIES OF A COWPEA SEVERE MOSAIC VIRUS ISOLATE FROM TABASCO, MEXICO[1] /

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Resumen

Se presentan los resultados de una caracterización parcial del virus del mosaico severo del caupí (cowpea severe mosaic virus) identificado en el Estado de Tabasco, México. El virus (CSMV--Tabasco) infectó 14 genotipos de caupí, incluyendo 6 variedades cultivadas en Tabasco, las cuales reaccionaron produciendo lesiones locales en las hojas inoculadas, seguidas por un mosaico sistémico. los genotipos Macaibo y PI-186465 se comportaron como inmunes al virus. Otros hospedantes susceptibles fueron Canavalia ensiformis, Glycine max. Lupinus albus. Phaseolus lunatus P. vulgaris, Vigna radiata, Chenopodium quinoa. Nicotiana benthamiana. N. rustica. N. tabacum y Vinca rosea Se encontró un punto térmico de inactivación entre 60 y 65°C, un punto máximo de dilución entre 10^{-5} y 10^{-6} y un envejecimiento in vitro de 11 días a 22-24°C. En muestras de virus purificadas se observaron numerosas partículas poliédricas de alrededor de 25 nm de diámetro. En pruebas serológicas en doble difusión en agar, se encontró que el CSMV--Tabasco está estrechamente relacionado con el CSMV--Arkansas y con otros aislamientos del virus de Puerto Rico, El Salvador y Venezuela; sin embargo, se distingue de algunos de ellos por la formación de espolones en las bandas de precipitación

Introduction

Cowpea severe mosaic virus (CSMV) (5) is an important beetle-transmitted pathogen of cowpea (*Vigna unguiculata* (L.) Walp.) throughout warm temperate and tropical zones of the American continent, where it has long been known (4). CSMV has been recorded in the United States (21), El Salvador (7). Costa Rica (10, 24). Venezuela (6). Suriname (12), Brazil (14). Cuba (13), Puerto Rico (19). Trinidad (4), and likely it is present in other Latin American countries. Field incidence of CSMV may reach 100% of infected plants (4, 10, 14, 24) and severe infections can result in a 50-90% reduction in the yield (6, 14, 25). At present, CSMV is confined to North, Central and South America and associated lands in the western hemisphere (5, 9). Cowpea (yellow) mosaic virus (CPMV) refers to another comovirus occurring primarily in Africa (8, 9). In 1979, in the Tabasco state in Mexico, CSMV was isolated and identified from field collected cowpea plants showing foliar mosaic and distortion symptoms (17). Identification was based on sap inoculation, beetle transmission and by reaction with a CSMV-antiserum from Brazil. This paper reports additional data on the Tabasco isolate of CSMV, as well as its serological relationship to the CSMV-Arkansas and to other isolates of the virus from several Latin American countries.

Materials and methods

Host reactions: Sixteen cowpea and seven bean genotypes, as well as other legumes and non-legumes,

were tested in the greenhouse for their reaction to the virus The plants were mechanically inoculated with sap of infected cowpea and observed 2-3 weeks for symptoms. The presence of the virus was determined by the Ouchterlony gel diffusion test (18) and by inoculation to healthy cowpea plants

Physical properties: Thermal inactivation point, dilution end point and longevity *in vitro* (at 22-24°C), were determined in sap of infected Monarch cowpea plants, 8-10 days after inoculation, according to the suggested procedures for legume viruses (2) *Canavalia ensiformis* DC was used as a test plant

Purification: The virus was purified using a modification of the Steere's chloroform-butanol method (22) Infected tissue of Monarch cowpea, 8-10 days after inoculation. was homogenized in a Waring blender with 1 5 ml of 0 1 M phosphate buffer, pH 7 2 containing 0 1 M ascorbic acid, and 1.0 ml of the chloroform and n-butanol mixture (1:1) per gram of tissue The resulting extract was held overnight at room temperature and centrifuged at 5 000 g for 10 min to remove cellular debris The supernatant was subjected to three alternate high (80 000 g for 90 min the first and 60 min the second and third) and two low (5 000 g for 10 min) speed centrifugations. The high speed pellets were resuspended in 0 01 M phosphate buffer pH 7 2

Electron microscopy: Purified preparations of the virus were mixed with an equal volume of 2% phosphotungstic acid, pH 5 7 and applied to formvar coated grids The virus was observed in a JOEL JEM-100 CX electron microscope at 33 000 and 66 000 magnifications

Serology: Antiserum to the virus was developed in rabbits, by 4 subcutaneous infections at weekly intervals with an emulsion of purified virus and Freund's incomplete adyuvant The animals were bled about 2 months after the last infection, by nicking the marginal ear vein The antiserum was recovered, mixed with an equal volume of glycerol and kept in a freezer The CSMV isolates from Tabasco, Arkansas, Puerto Rico, El Salvador and Venezuela, each in sap from infected Monarch cowpea, were reacted in various combinations with antisera for both CSMV Tabasco and CSMV-Arkansas, using the Ouchterlony gel diffusion test for determining their relationship

Results

Host reactions: The results of host reactions are summarized in Table 1 Fourteen cowpea genotypes were susceptible to the virus and most reacted with

the production of chlorotic local lesions on the inoculated primary leaves, followed by the development of a severe mosaic, with distortion and blistering on the trifoliate leaves The accessions Macaibo and PI-186465 remained symptomless under our test conditions and no infection was detected in repeated attempts by serology or back inoculation

All the bean cultivars tested were susceptible to the virus and reacted with the production of either necrotic or chlorotic local lesions on the inoculated primary leaves The Top Crop bean showed no systemic symptoms, but the infection was detected on the trifoliate leaves.

Table 1. Reaction of plant species following mechanical inoculation with the Tabasco isolate of cowpea severe mosaic virus[a]

Species inoculated	Symptoms produced[b]
Legumes	
Canavalia ensiformis D C.	LLn
Glycine max (L) Merr	
Lee	S
Lupinus albus L.	S
Phaseolus lunatus L	
Henderson	LLc, S
Phaseolus vulgaris L.	
Black Turtle	LLn
Bountiful	LLc
Kentucky Wonder	LLn
Pinto	LLn
Pinto 3	LLnc
Top Crop	LLn
Viva Pink	LLn
Vigna radiata (L.) Wilczek	LLc, S
Vigna unguiculata (L.) Walp.	
Blackeye	LLc, S
Chinese Red	LLc, S
Crimson	LLc, S
Criollo "carita"	LLc, S
Criollo "castilla"	LLc, S
Criollo "castilla punto cafe"	LLc, S
Criollo "negro"	S
Criollo "pucuy"	LLc, N, S
Criollo "sin tiempo"	LLc, S
Georgia 21	LLc, S
Macaibo	--
Monarch	LLc, S
PI 186465	--
PR-Black	LLc, S
PI 293466	LLc, S
PI 293514	LLc, S

Table 1. Continuation . . .

Species inoculated	Symptoms produced[b]
Vicia faba L. Bush Fava	--
Non Legumes	
Chenopodium quinoa Willd	LLc
Cucumis sativus L. Model	–
Cucurbita pepo L. Small Sugar	--
Nicotiana benthamiana Domin.	LLc, S
Nicotiana rustica L.	LLc
Nicotiana tabacum L.	–[c]
Vinca rosea L.	S

a   Virus was transmitted mechanically by rubbing infected sap on carborundum-dusted leaves with a cheese-cloth pad.

b   Indicates no reaction observed; LLc = chlorotic local lesions; LLn = necrotic local lesions; S = systemic reaction as a severe mosaic or mild mottle; N = necrosis and collapse of the epycotil

c   Systemic infection was detected by back inoculation to healthy cowpea plants.

The other hosts susceptible to the virus were: soybean (*Glycine max* (L.) Merr., "Lee"). jack bean (*Canavalia ensiformis* DC.), white lupine (*Lupinus albus* L.), lima bean (*Phaseolus lantus* L. "Henderson"), mung bean (*Vigna radiata* (L.) Wilczek). *Chenopodium guinoa* Willd., *Nicotiana benthamiana* Domin., *Nicotiana rustica* L., *Nicotiana tabacum* L. and *Vinca rosea* L. No symptoms were observed and no infection was detected on broad bean (*Vicia faba* L. "Bush Fava"), cucumber (*Cucumis sativus* L. "Model") and squash (*Cucurbita pepo* L. "Small Sugar").

Physical properties: The *in vitro* properties of the virus were as follows: thermal inactivation, infection at 60°C, but none at 65°C. dilution, infection at 10^{-5}, but none at 10^{-6}. longevity *in vitro*, infection at 11 days, but none at 12 days

Electron microscopy: The electron microscopic observations of purified preparations of the virus showed polyhedral particles of about 25 m in diameter (Figure 1)
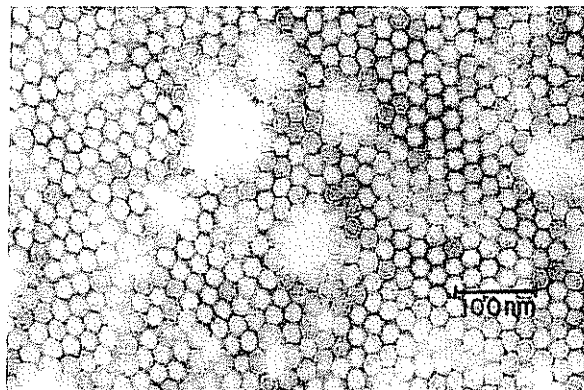

Fig 1   Electron micrograph of a purified preparation of CSMV-Tabasco, stained with 2% phosphotungstic acid, pH 5 7

Serology: The antiserum prepared to the virus had a titer of 1:512 in gel double-diffusion tests No reaction was detectable with healthy cowpea sap. The better reactions were obtained when the antiserum was diluted at 1:16 and 1:32 Results of the serological tests are shown in Figure 2. All the CSMV isolates produced strong lines of precipitation with both CSMV antisera, indicating a close serological affinity among them When using CSMV-Tabasco antiserum, its homologous antigen formed spurs with the isolates of Puerto Rico and Venezuela, but not with those of Arkansas and El Salvador (Figure 2a) When the CSMV-Arkansas antiserum was used, however, its homologous antigen formed spurs with all the isolates tested, including that of Tabasco (Figure 2b) Additionally, some heterologous isolates formed spurs with one another using both antisera
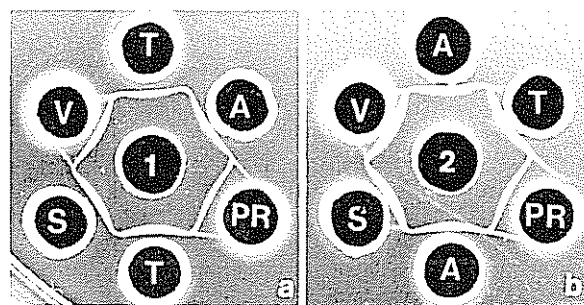

Fig. 2.   Agar double-diffusion tests showing reactions of CSMV-Tabasco and CSMV-Arkansas with other CSMV isolates. Center wells contain antiserum to: 1- CSMV-Tabasco; 2- CSMV-Arkansas. Peripheral wells contain sap of cowpea plants infected with CSMV isolates from: T- Tabasco; A- Arkansas; PR- Puerto Rico; S- El Salvador; V- Venezuela

Discussion

The results reported here confirmed the presence of the cowpea severe mosaic virus in the State of Tabasco in Mexico. The host reactions, as well as the physical properties of this isolate (CSMV-Tabasco), are similar to those reported by other investigators for various CSMV isolates from different countries (4, 6, 13, 19, 21). Slight differences, however, were noted In host reactions, for example, the CSMV-Tabasco was found to infect Top Crop bean and *Vinca rosea*, whereas the Arkansas isolate of the virus failed to infect these hosts (21). The isolates of CSMV from Trinidad (4), Cuba (13) and Puerto Rico (19) did not infect *Nicotiana tabacum*, which, although no symptoms were observed after inoculation with CSMV-Tabasco, the virus was recovered by back inoculation to healthy cowpea plants

Of the sixteen cowpea genotypes tested, only the cultivar Macaibo from Brazil and the line PI-186465 from Nigeria were immune to CSMV-Tabasco Both genotypes have been reported as resistant to several CSMV isolates from diverse geographical origins (8, 14, M T. Lin, personal communication); therefore, they could be a useful source of resistance in cowpea breeding programs against CSMV The six cowpea cultivars grown in Tabasco state (Carita, Castilla, Castilla Punto Cafe, Negro, Pucuy, and Sin Tiempo) were susceptible to the virus

Nicotiana benthamiana, N. rustica and *Lupinus albus*, three plant species previously not tested as hosts of CSMV (3, 4, 6, 13, 19, 21), were susceptible to CSMV-Tabasco

There were also some differences in physical properties of CSMV-Tabasco to those reported for other CSMV isolates (4, 6, 13, 19, 21); but such small differences often depend on the source, assay hosts, and experimental conditions (5), and are probably not significant

The Ouchterlony gel diffusion tests showed that CSMV-Tabasco is closely related, but not identical serologically, to CSMV-Arkansas and to other CSMV isolates tested from Puerto Rico, El Salvador and Venezuela When CSMV-Tabasco antiserum was used, no serological difference were found between the Tabasco isolate and those of Arkansas and El Salvador However, when using CSMV-Arkansas antiserum, Arkansas and Tabasco isolates appeared to be different serologically, as shown by a fine spur beyond the point of coalescence of the precipitation bands between the two isolates (Figures 2a-b). These reactions indicate that the Arkansas isolate contains additional antigenic determinants not present in the

Tabasco isolate Homologous antisera for isolates from Puerto Rico, El Salvador and Venezuela were not available for comparason.

Spur formation between CSMV isolates with different geographical origins has been noted (9). Fourteen isolates of CSMV collected in central Brazil were separated by immunodoffusion into two serologically distinct groups, which also showed slight differences in host range (16).

CSMV causes an important and widespread beetle-transmitted disease of cowpea in many parts of tropical America (4, 5, 6, 10, 12, 13, 14, 19, 25) Natural infections of this virus also occur in *Phaseolus vulgaris* L. (1), soybeans (4, 24), winged bean (11), pigeon pea (4), and several tropical leguminous weeds, such as *Macroptilium lathyroides* (L.) Urb. (1, 4), *Centrosema pubescens* Benth., *Calopogonium mucunoides* Desvaux (15) and *Vigna vexillata* (L.) A. Rich (24), which have been regarded as reservoirs of the CSMV in the field (1, 8, 14, 15, 24) In Tabasco state in Mexico, field incidence of CSMV often reaches 50-95% (17) The symptoms shown by most of the cowpea genotypes after inoculation with CSMV-Tabasco indicate that it is a severe isolate of CSMV Differences in aggressiveness among CSMV isolates from diverse geographical origins have already been emphasized (8, 23). Studies on the epidemiology and importance of the CSMV-Tabasco are in progress

Summary

Host reactions, physical properties and serology of a cowpea severe mosaic virus isolate from Tabasco, Mexico (CSMV-Tabasco) are presented Fourteen cowpea genotypes, including six cultivars grown in Tabasco, were susceptible to the virus and reacted with the production of local lesions on the inoculated primary leaves, followed by the development of a severe mosaic on the trifoliate leaves; the cowpea accessions Macaibo and PI-186465 were immune to CSMV-Tabasco infections Other hosts susceptible to the virus were: *Canavalia ensiformis, Glycine max, Lupinus albus, Phaseolus lunatus, P. vulgaris, Vigna radiata, Chenopodium guinoa, Nicotiana benthamiana, N. rustica, N. tabacum* and *Vinca rosea* Thermal inactivation was between 60 and 65°C; dilution, between 10^{-5} and 10^{-6}; longevity *in vitro* at 22-24°C, 11 days Electron microscopic observations of purified preparations showed polyhedral particles of about 25 nm in diameter Agar double-diffusion serology demonstrated that CSMV-Tabasco is closely related, but not identical, to CSMV-Arkansas and to other CSMV isolates from Puerto Rico, El Salvador and Venezuela

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Reseña de libros

HODGSON, J. M. Soil sampling and soil description (reprinted). Oxford University Press, Great Britain 1982. 241 p.

El tomar muestras de suelos y describir el sitio y el suelo de donde provienen, siempre ha sido un trabajo de especialistas. Desafortunadamente en nuestras latitudes los "especialistas", en muchos casos, aprenden por prueba y error y muchos otros toman muestras de suelos sin conocer del asunto. En este sentido podría mencionarse que en la literatura se encuentra mucha contaminación con información poco significativa por su representabilidad.

Hodgson presenta una obra simple y de fácil manejo; por su aporte fundamental consiste en proporcionar un complemento, primeramente publicado en 1978, a los esfuerzos iniciales del Departamento de Agricultura de los Estados Unidos (Handbook 18) y de la FAO (Guías para la descripción de perfiles de suelos).

La presentación del material es excelente e incluye (i) una introducción histórica de este campo, (ii) un capitulo sobre el equipo necesario para la preparación del sitio de muestreo y la ejecución en este paso, (iii) la escogencia y caracterización del sitio, (iv) la descripción del suelo, (v) las formas de anotar la información del sitio y del suelo, (vi) la manera de tomar muestras (disturbadas y sin disturbar) y (vii) un capitulo comparando los métodos empleados en diversas partes del mundo, que ocupa la mitad del texto.

Tanto la presentación como la claridad del contenido del libro permiten a personas que se inician en la ciencia del suelo estimar la necesidad de efectuar un trabajo cuidadoso. Desde el punto de vista didáctico, le ahorra al maestro el copilar y resumir un material que de por si es de difícil acceso. Hodgson insiste en que "no existe un sistema ideal para describir sitios y suelos", es más, duda de que sea necesario un sistema único; pero fundamenta su libro en la necesidad de un lenguaje internacional que facilite la comunicación entre científicos.

En términos generales el capitulo final, en el cual se comparan los sistemas empleados en paises y organizaciones seleccionadas, es el más interesante por la gran cantidad de información contenida. La comparación de sistemas de clases textuales incluida, representa un bello ejemplo del ingenio humano y una razón del porqué se debe tender hacia la uniformidad de criterios.

Como las otras monografías sobre cartografía de suelos de Oxford University Press (ver Turrialba 33(4):405. 1983), la presente es una contribución valiosa para las personas que laboran en este campo del saber.

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